ENHANCING THE USE OF OPIOIDS IN PAIN MANAGEMENT: ANTINOCICEPTIVE POTENTIATION WITH OPIOID AGONIST/ANTAGONIST COMBINATIONS

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Abstract

While opioids are the most effective and widely used class of drug for the management of moderate to severe pain, their use may be limited by adverse effects that are unpleasant and potentially dangerous. Research is increasingly directed towards strategies to improve the use of opioids in pain management, investigating methods by which the analgesia afforded by an opioid may be enhanced, while minimising adverse effects. One approach that has produced promising findings in animal studies and some clinical reports is the combination of an opioid agonist and "ultra-low" (nanomole) doses of an opioid antagonist. A recent animal study reported that antinociception may be significantly enhanced with the combination of the partial opioid agonist/antagonist buprenorphine and ultra-low doses of the antagonist naloxone. The central aim of the studies described herein was to investigate the effect of this drug combination on response to experimental nociceptive stimuli and the incidence and severity of adverse effects among healthy volunteers.

The first study established normative responses to two commonly used nociceptive tests, the cold pressor and electrical stimulation tests, in 100 healthy volunteers. The effect of buprenorphine on nociceptive test performance had not previously been determined, therefore a dose-ranging study of buprenorphine was conducted to establish a dose-response relationship. The subsequent two studies investigated the effect of a range of buprenorphine:naloxone IV dose ratios (5:1, 10:1, 12.5:1, 15:1, 20:1 and 25:1) on nociception and adverse effects among healthy volunteers. These studies are the first to investigate the combination of buprenorphine and ultra-low dose antagonist in humans, and the first to assess the agonist:antagonist combination in an experimental model of human nociception. Antinociception was significantly enhanced with the combination of buprenorphine and naloxone in the 12.5:1 and 15:1 ratios. Moreover, this enhanced

antinociception occurred *without* a simultaneous increase in adverse effects and indeed with a *reduction* in the severity of some effects. An agent that produces greater analgesia and reduces adverse effects has the potential to overcome some of the barriers that limit the use of opioids in pain management. The current findings indicate that further investigation of this drug combination is warranted.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Abbreviations, prefixes and symbols

5HT	5-hydroxytryptamine (serotonin)
AC	Adenyl cyclase
ANOVA	Analysis of variance
APD	Action potential duration
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
BUP	Buprenorphine
$Ca2^{+}$	Calcium
cAMP	Cyclic adenosine monophosphate
CCI	Chronic constriction injury
CGRP	Calcitonin gene related peptide
CL	Clearance
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CP	Cold pressor
CPTHR	Cold pressor threshold
CPTOL	Cold pressor tolerance
CTX	Cholera toxin
CV	Coefficient of variation
DB	Double blind
DCN	Dorsal column nuclei
DF	Dorsal funiculus
Df	Degrees of freedom

DLF	Dorsolateral funiculus
DRC	Dose response curve
DRG	Dorsal root ganglion
DRN	Dorsal raphe nuclei
ES	Electrical stimulation
ESTHR	Electrical stimulation threshold
ESTOL	Electrical stimulation tolerance
FPQ-III	Fear of Pain Questionnaire-III
GRK	G-protein coupled receptor kinase
hr(s)	Hour(s)
IM	Intramuscular
IP	Intraperitoneal
IQR	Interquartile range
IT	Intrathecal
IV	Intravenous
K	Kurtosis
kg	Kilogram
K_i	Inhibition constant
K^+	Potassium
L	Litres
LCN	Lateral cervical nucleus
LRN	Lateral reticular nucleus
LSN	Lateral spinal nucleus
MDvc	Medial dorsal thalamus
mg	Milligram
min(s)	Minute(s)

ML	Median lemniscus
ml	Millilitre
MPE	Maximum possible effect
MTP	Maximum tolerated pain
N/OFQ	Nociceptin / Orphanin FQ
NCF	Nucleus cuneiformus
ng	Nanograms
NGF	Nerve growth factor
NH&MRC	National Health & Medical Research Council
NLX	Naloxone
NM	Nanomolar
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NON-N	Nonnociceptive (neurons)
NorBUP	Nor-buprenorphine
NPY	Neuropeptide Y
NRM	Nucleus raphe magneus
NRPG	Nucleus reticularis paragigantocellularis
NS	Nociceptive specific (neurons)
NSAIDs	Non-steroidal anti-inflammatory drugs
NTX	Naltrexone
ORL1	Opioid receptor like
PAG	Periaqueductal grey
PBN	Parabrachial nucleus
PCA	Patient-controlled analgesia
PCP	Phencyclidine

PKC	Protein kinase C
PKC	Protein kinase C
PM	Picomolar
PO	Oral
PTX	Pertussis toxin
RCT	Randomised controlled trial
S	Skewness
sec(s)	Second(s)
SC	Subcutaneous
SCL	Superior colliculus
SD	Standard deviation
SEM	Standard error of the mean
SP	Substance P
SSRI	Selective Serotonin Reuptake Inhibitor
STAI	State Trait Anxiety Inventory
$T_{1/2}$	Half-life
T_{max}	Time to maximum plasma concentration
VAS	Visual analogue scale
Vd	Volume of distribution
VLF	Ventrolateral funiculus
VMH	Ventromedial hypothalamus
VMPo	Ventromedial posterior thalamus;
VPI	Ventroposterioinferior thalamus;
VPL	Ventroposterolateral thalamus
VPM	Ventroposteromedial thalamus
WDR	Wide dynamic range

1. INTRODUCTION

1.1. Background

Pain has been described as "a more terrible lord of mankind than even death itself" (Schweitzer, 1932, in Melzack and Wall 1996). Pain serves an essential protective role in our lives, alerting us to tissue damage and often provoking a reflex reaction to prevent further damage, or motivating us to seek medical attention. Notwithstanding, pain can be a chronic, debilitating affliction associated with stress, anxiety and depression. Pain is the most common reason for seeking medical advice, and the treatment of pain has been touted as the greatest challenge of medicine (Melzack and Wall 1996).

In the last 45 years a virtual explosion has occurred in the area of pain management. Prior to 1960, pain was regarded by clinicians and patients alike as simply an unpleasant but inevitable consequence of disease or injury. It was viewed as a symptom that would be resolved with the appropriate treatment of the disease or healing of the injury. Since that time, the specialisation of pain medicine has emerged, pain research has flourished, the original biomedical concept of pain has given way to the broader biopsychosocial approach, considerable progress has been made in elucidating the molecular biology of pain, and standards of clinical training and patient care have been established (Loeser 2000).

Despite these advances, it is recognised that the management of pain is often inadequate (NHMRC 1999; Kamming et al. 2004; Primm et al. 2004; Viscusi 2004). Findings indicate

widespread unsatisfactory management of both acute (Wilder-Smith et al. 2002; Shang et al. 2003; Stomberg et al. 2003; Rupp and Delaney 2004) and chronic pain (Lister 1996; Davies and McVicar 2000). Moreover, it has been demonstrated that the under-treatment of pain has significant negative implications for the health, overall wellbeing and course of recovery for patients. Unsatisfactory treatment of pain has been shown to increase morbidity following trauma and surgery (Wattwil 1989), and lead to negative affective states, frustration, stress, anxiety and craving for medication to relieve pain (McCaffery and Vourakis 1992). Findings also indicate that the perception of pain is only one of a range of related physiological responses triggered by the activation of nociceptors (sensory fibres stimulated by noxious, or potentially noxious, stimuli - see discussion in section 1.4.1). For example, nociception (see section 1.2) has been implicated in the secretion of stress-related hormones involved in tissue breakdown; cardiovascular responses such as tachycardia, ischemia, hypertension and ventricular arrhythmias; slowing of peristalsis; and immune impairment (Carr 1993; NHMRC 1999). Inadequate pain control has been described as "unethical, clinically unsound, and economically wasteful" (Phillips 2000).

In recent years there has been an increasing international focus on the problem of inadequate pain management, with an increase in basic and clinical pain research, as well as government and institutional initiatives to draw attention to the problem and the promulgation of therapeutic guidelines. The United States Congress declared January 1st 2001 to be the beginning of "The Decade of Pain Control and Research". This sentiment has been echoed in Europe, with the European Federation of the International Association for the Study of Pain (IASP) Chapters convening from 2001 an annual "European Week Against Pain". The problem has also been recognized in Australia, with the National Health and Medical Research Council endorsing in 1998 the first Australian multi-

disciplinary report on the management of acute pain, with the acknowledgement that acute pain must "rank with the more serious causes of contemporary morbidity in our society, and be one of the most expensive" (NHMRC 1999).

1.2. The terminology of pain

In order to understand the complex phenomenon of pain and the issues involved in pain management, an understanding of pain-related terminology is crucial. A distinction must be drawn between the terms "pain" and "nociception". Sir Charles Sherrington first proposed the term *nociception*, which was derived from the *perception* of *noxious* stimuli, in the early 1900s. Nociception is the process by which noxious stimulation in the periphery is transmitted to the central nervous system, while pain is the subjective experience. Nociception is not pain (Loeser and Cousins 1990), and can occur in the absence of the perception of pain, just as pain may be perceived in the absence of nociception (Compton and Gebhart 1998). Thus, we speak of *nociceptors*, receptors that are preferentially sensitive to noxious or potentially noxious stimuli, rather than speaking of "pain receptors", as it is only when this sensory input reaches the brain that it is perceived as pain. Similarly, we refer to *nociceptive stimuli*, stimuli that activate sensory receptors to a potentially injurious degree, rather than "painful stimuli" which would imply that the stimulus *per se* is directly responsible for the experience of pain. This distinction is important given that there are many complex processes involved in the actual experience of pain.

1.3. The development of pain theories

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue

damage, or described in terms of such damage" (Merskey et al. 1979). Our understanding of the mechanisms involved in the perception of pain has developed greatly over time. With the evolution of pain theories, a gradual shift in focus from the periphery to the central nervous system (CNS) is evident, as the brain becomes regarded as a functional component of the pain experience, rather than merely a passive recipient of sensory input.

1.3.1. Specificity theory

Traditionally, pain was explained by the Specificity theory, first described in its most basic form by the French scientist and philosopher Descartes in 1664 (Melzack and Wall 1996). It was held that the pain system was a direct channel from the skin to the brain. When exposed to a noxious stimulus, specific skin receptors carried a message directly to a pain centre in the brain. Descartes illustrated this concept by comparing it to the ringing of a bell in a church - the rope is pulled down below and the bell rings above. Descartes proposed that a noxious stimulus to the foot activates particles in the foot, which are then transmitted up the leg and body to the brain. The individual then feels the pain and responds to it.

This theory remained relatively unchanged until the 19th century when physiology developed as an experimental science. Various physiologists and physicians throughout the late 1800s refined the theory, notably von Frey (1894, in Melzack and Wall 1996), whose research formed the basis of the "modern" Specificity theory. It was proposed that free nerve endings were "pain receptors" which, upon stimulation, would generate pain impulses. These impulses were carried by A-delta and C fibres to a pain centre in the thalamus. An integral part of the theory was the notion of physiological specificity. It was purported that these receptors specifically responded to painful stimuli and that there exists

a direct connection between the skin where the stimulus is applied and the pain centre in the brain where the pain is "felt". Hence, stimulation of this receptor will *always* produce this effect, and *only* this effect.

It became apparent that this theory was unable to explain the complex phenomenon of pain. A considerable amount of clinical, psychological and physiological evidence refuted the theory (see Melzack and Wall 1996). Clinically, pathological pain syndromes such as phantom limb pain and peripheral neuralgias could not be reconciled with the theory. Surgical lesions both in the periphery and the central nervous system were unsuccessful in permanently eradicating these pains, despite lesions having been made at almost every level. In many cases, pain was still felt when a stimulus was applied below the level of the lesion.

Psychological evidence further refuted the notion of a direct relationship between stimulus intensity and pain perception. A great deal of research has demonstrated that pain is not only a function of sensory input, but is also determined by a variety of psychological variables. Pavlov illustrated perhaps the most famous case of this in his conditioning experiments. When a painful stimulus was applied to a dog, pain behaviours would be elicited. However, when this stimulus was paired with a positive reinforcer, in this case the provision of food, there was no evidence of pain behaviours. Instead, the stimulus provoked salivation and excitement for the anticipated reward (Pavlov 1928). HK Beecher further demonstrated the psychological component of pain with soldiers wounded in battle on the Anzio beachhead. In treating these wounded soldiers it became apparent to Beecher that the men did not complain of pain from their wounds, and often would decline the offer of analgesic medication despite extensive injuries, which, under normal circumstances,

would be very painful (Beecher 1946; Beecher 1959). The lack of pain experienced by these soldiers was interpreted as a consequence of the absolute relief at having escaped from the battlefield alive. This observation further suggested that the experience of pain could be significantly mediated by psychological and situational factors.

1.3.2. Pattern theory

In response to the deficits of the Specificity theory, several alternative theories emerged, which were collectively termed the "Pattern theory". The principle that is common to these theories, that both stimulus intensity and central summation are critical in the experience of pain, was first proposed by Goldscheider in 1894 (Melzack and Wall 1996). Following observations from earlier studies of pathological pain, in particular demonstrations of temporal and spatial summation, Goldscheider concluded that mechanisms of central summation in the dorsal horn were fundamental to understanding pain. From this model, several theories were proposed, all of which incorporated the notion of patterns of sensory input in the experience of pain.

The Simple Pattern theory proposed by Weddell (Weddell 1955) and Sinclair (Sinclair 1955) was based on the earlier work of Nafe (Murchison 1934), which asserted that pain is associated with patterns of nerve impulses rather than separate specific transmission pathways. Excessive peripheral stimulation of non-specific receptors activates a pattern of nerve impulses that is interpreted by the brain as pain. This theory, however, overlooked the established phenomenon of physiological specialisation*.

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* *An important distinction must be drawn between the notion of physiological specificity and specialisation. Specificity asserts that a receptor or fibre serves one specific modality alone, a concept purported by the flawed Specificity theory outlined above. Specialisation, on the other hand, is the notion that receptors or other components of a sensory system are highly specialised, such that activation results in characteristic*

To account for the summation observed in pain syndromes such as phantom limb pain, Livingstone (Livingstone 1943) proposed the existence of circuits in the dorsal horn. Some years later another theory emerged asserting that small diameter, slow conducting fibres carry the sensory impulse patterns that produce pain. Under normal circumstances, these fibres are inhibited by larger diameter, rapidly conducting fibres. A shift in the ratio of large-to-small fibres in favour of small fibres, though, would produce an increase in transmission, summation, and pain (Noordenbos, 1959, in Melzack and Wall 1996).

Despite the progress that had been made, there lacked a single unifying theory. Each of the theories proposed could explain certain aspects of the pain experience, but could not adequately address others. It has been noted, however, that while the pattern theories were generally poorly defined and inadequate in their capacity to explain the experience of pain, they did provide the foundation for the next major step in our understanding of this complex phenomenon (Melzack 1993).

1.3.3. Gate Control theory

A major revolution in our understanding of the mechanisms of pain occurred in the 1960s with the emergence of Melzack and Wall's "Gate Control" theory (Melzack and Wall 1965). This was the first pain theory that implicated the brain as an active component involved in the transmission and modulation of nociception. It was proposed that there are three spinal cord systems that receive nerve impulses following stimulation of the skin: the

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patterns of neural signals. Other sensory input or cognitive processes, however, may alter the quality of the experience.

cells of the substantia gelatinosa in the dorsal horn, the dorsal-column fibres that project towards the brain, and the first central transmission cells (T cells) in the dorsal horn.

This theory holds that the experience of pain is determined by interaction between three systems. (i) The cells of the substantia gelatinosa of the dorsal horn, which have a "gate keeper" function, modulating the synaptic transmission of nerve impulses from peripheral fibres before they reach the T cells; (ii) The dorsal-column system, which acts as a "central control" that, when exposed to afferent impulses, triggers certain brain processes that exert an influence on the gate control system; and (iii) the T cells, which activate brain mechanisms associated with perception and response.

Even in the absence of evident stimuli, the spinal cord constantly receives nerve impulses, which are carried predominantly by small fibres. These continuous incoming impulses keep the "gate" in an open position. Upon stimulation of the skin, many more fibres will be activated, including the larger diameter fibres. As these larger fibres are generally inactive in the absence of a significant stimulus, the activity that follows from stimulation will result in a proportionally greater increase in large fibre activity than small fibre activity. This barrage of large-fibre impulses results in a partial closing of the gate, and a consequent reduction in the firing of T cells. If either the stimulus is prolonged, or there is an increase in stimulus intensity, output from the T cells will increase. This is due in the first instance to the adaptation of the large fibres, and the consequent increase in small fibre activity, which partially reopens the gate. In the second instance, an increase in stimulus intensity creates an increase in the number of active receptor fibres. The positive and negative effects of the small and large fibres counteract each other causing the gate to open further and the output of T cells slowly rises.

Three features of sensory input, then, are involved in the experience of pain: the ongoing activity in the absence of a stimulus, the activity resulting from the stimulus, and the relative proportion of large and small fibres activated.

1.3.4. Neuromatrix theory

While the Gate Control Theory incorporated many aspects of the pain experience, it did not account for long-term changes in the response of the nervous system to noxious stimuli. Several pieces of evidence have led to the proposition of the Neuromatrix theory. Firstly, research indicated that a nociceptive stimulus of moderate intensity could permanently alter spinal cord function, leading to possible development of chronic pain following injury (Dubner and Ruda 1992). It was also demonstrated that environmental influences could alter response to noxious stimuli (Rainville et al. 1996) and that pain behaviours could be elicited by certain environmental cues and by the expectation of pain. Furthermore, there remained the question of phantom limb pain and other cases in which pain is experienced in the absence of input from the periphery. It became apparent that learning plays a considerable role in the pain experience. The Neuromatrix theory was developed to account for these factors. The theory proposed that a pattern-generating mechanism exists in the brain, which holds an image of self, created by genetics and memories of previous experiences (Melzack 1990; Loeser and Melzack 1999; Melzack 1999). Sensory input feeds into the neuromatrix, as well as information from other areas of the brain that are involved in cognitive and affective activities. From the combined input from the periphery and other brain regions, the neuromatrix then produces patterns of nerve impulses, which result in the experience of pain. A variety of factors such as stress, past experience and expectation may moderate the relationship between the periphery and the neuromatrix, such that pain may be generated in the absence of peripheral input.

Our understanding of the mechanisms involved in the perception of pain has developed considerably since the initial theory proposed by Descartes in the 1600s, and our knowledge of this complex phenomenon continues to evolve. The physiological mechanisms associated with the experience of pain may be divided into two categories according to the source of the experience: nociceptive pain, which is produced by exposure to noxious stimuli, and neuropathic pain, which is associated with damage to sensory fibres, or to the CNS itself (Millan 1999). The following section will outline our current understanding of the mechanisms involved in nociceptive pain, or nociception.

1.4. Neurobiological mechanisms of nociception

Contrary to the early interpretation of pain as the result of activation of a direct channel from the skin to the "pain centre" of the brain, we now understand that between the exposure of the skin or other tissue to "noxious" stimuli and the conscious experience of pain, there is an intricate sequence of mechanisms involved in the peripheral receipt, central transfer and supraspinal integration of nociceptive input. Furthermore, the subjective experience of pain is determined by the modification and integration of nociceptive signals in the periphery, the spinal cord, and the higher centres (Dray 1997). In order to understand the complex events that lead to the perception of pain, one must consider three vital components of the pain projection system: the fibres that respond to noxious or potentially damaging stimuli, the peripheral and CNS systems that are activated, and the mechanisms by which various components of the process may be sensitised or suppressed.

1.4.1. The detection of noxious stimuli in the periphery

First hypothesised by Sherrington in the early-1900s and described by Perl and colleagues in the 1960s, nociceptors are primary afferent fibres that are preferentially sensitive to noxious or potentially noxious stimuli (Sherrington, 1906, in Melzack and Wall 1996). Nociceptors have naked sensory endings in peripheral tissues, and have a higher threshold than other nerves, such that they are only activated by noxious stimuli that are likely to result in some tissue damage. Nociceptors have been described in skin, joints, muscle and some visceral structures (Willis 1995). Unlike most other afferent fibres, which are subject to adaptation (a decreased response with repeated stimulation), nociceptors are sensitised by repeated stimulation, which may involve a decrease in the threshold for activation, increased and prolonged firing to a suprathreshold stimulus, and an increase in spontaneous activity (Levine et al. 1993).

Cutaneous afferent fibres involved in the transmission of nociceptive information are classified as C, Aδ or Aβ according to their diameter, structure and conduction velocity (Millan 1999). The speed of neural transmission is related to the size and myelination of the nerve fibre (Markenson 1996; Millan 1999). C-fibres are thin, unmyelinated fibres with slow conduction velocity (< 2 m/s). Aδ-fibres are myelinated, and of intermediate diameter and conduction velocity (12-30 m/s), while Aβ-fibres are large, myelinated and have a faster conduction velocity (30-100 m/s). While all three classes of cutaneous fibres can transmit non-nociceptive information, in the absence of tissue or nerve injury only C- and Aδ-fibres transmit nociceptive messages. Under these conditions, Aβ-fibres are responsive only to innocuous, low-intensity mechanical stimuli such as touch and vibration (Markenson 1996; Millan 1999). Activation of Aδ-fibres will elicit sharp localised pain, whereas C-fibres will induce dull, burning, aching pain (Ochoa and Torebjork 1989;

Handwerker and Kobal 1993; Belemonte and Cervero 1996). Generally, when the skin is exposed to noxious stimulus, Aδ-fibres will elicit a first phase of pain which is sharp and localised; this will be followed by a second wave of dull pain elicited by activation of the C-fibres (Meyer et al. 1994; Belemonte and Cervero 1996). It should be noted, however, that the threshold for activation of individual nociceptors is often well below the threshold for pain (Handwerker et al. 1984), therefore, individual nociceptors may reach a moderate level of activation before the conscious perception of pain.

Several classes of both C- and Aδ-fibre exist, however their characterization is complicated by a number of factors, including method of detection, species differences, and inconsistencies in terminology (Millan 1999). In terms of C-fibres, chemoreceptors, thermoreceptors, low threshold mechanoreceptors, and high threshold polymodal receptors (responding to thermal, chemical and mechanical stimulation) have been described (Meyer et al. 1994). Rapidly-conducting Aδ-fibre mechanoreceptors activated by high intensity stimuli (such as pinching) have been described and termed "Type I" nociceptors. These fibres are weakly responsive to high intensity heat, cold and chemical stimuli, but have been shown to become sensitised to heat following repetitive thermal stimulation (Handwerker and Kobal 1993; Meyer et al. 1994). Aδ-fibres demonstrating a lower threshold to noxious thermal stimuli have been termed "Type II" nociceptors (Treede et al. 1990; Beydoun et al. 1996).

1.4.2. Activation of nociceptors in non-cutaneous tissue

As mentioned above, nociceptors have also been described in muscle, joint and some visceral tissue (Willis 1995). There are several differences between the processing of nociceptive information from cutaneous and non-cutaneous tissue. While noxious

stimulation of cutaneous tissue is generally associated with first (Aδ-fibre) and second (C-fibre) phases of pain, these phases are not as distinct in nociceptive input from other tissue. For example, muscle pain mediated by both Aδ- and C-fibres is experienced as dull, aching and cramp-like (Millan 1999). A further difference is that transmission of nociceptive input from viscera is often associated with an unpleasant autonomic component, such as hypotension, nausea and perspiration, which is indicative of the involvement of sympathetic and parasympathetic pathways.

1.4.3. Ascending transmission of nociceptive signals

Nociceptive information is transmitted synaptically to interneurons of the spinal cord and dorsal horn (Willis and Coggeshall 1991). The fibres carrying nociceptive impulses enter the spinal cord via the dorsal roots, ending in the grey matter of the dorsal horn. The dorsal horn comprises six laminae. Nociceptive afferent fibres primarily terminate in the superficial region of the dorsal horn, generally in laminae I and II. The cells of laminae II form the substantia gelatinosa. The cells of the substantia gelatinosa are predominantly short inhibitory interneurons, which project to lamina I and V, and regulate transmission between the primary afferent fibres and the spinothalamic tract transmission neurons, hence, the "gate keeper" function proposed by the Gate Control theory as described above.

Dorsal horn neurons with nociceptive responses have been classified into several groups, although the criteria for each category varies between laboratories and according to the neuron under investigation (Willis 1995). The taxonomy includes "wide dynamic range" (WDR) neurons, which respond maximally to noxious stimuli but also respond to innocuous stimuli, "nociceptive specific" (NS) neurons, which respond exclusively to noxious stimuli, and non-nociceptive neurons (NON-N) (Price and Dubner 1977). More

recent studies have identified neurons in the marginal layer (I) of the dorsal horn that respond specifically to cold, as well as polymodal neurons responding to thermal and mechanical stimuli (Dostrovsky and Craig 1996; Zhang and Craig 1997), indicating that the aforementioned taxonomy may be too simplistic to account for the encoding properties of dorsal horn neurons (Morgan 1998).

Following integration in the dorsal horn, nociceptive information is transmitted via projection neurons to the higher centres in the brain. The anatomy and organisation of ascending pain projection pathways is complex (Millan 1999). The ventrolateral funiculus channel of the spinothalamic tract innervating the thalamus has long been considered the most important in the transmission of nociceptive input to the higher centres, though this now appears to be an oversimplification (Millan 1999). Several other pathways are involved in the transmission of nociceptive information, including neurons belonging to the spinoreticular, spinomesencephalic and spinocervical tracts and postsynaptic dorsal-column pathway (Willis 1995). There has been suggestion that a specific pain pathway exists (Perl 1998), though this notion is controversial (Besson 1999). An overview of ascending nociceptive pathways is displayed in Table 1-1.

Tract	Laminae of origin	Cell types	Tissue input	Ascending pathways	Principal sub-cortical targets	Axon types	Phylogenetic distribution	Possible roles
Spinothalamic tract	I II IV V/VI VII/VIII LSN	NS WDR Non-N	Skin Viscera Joints/muscle	Mainly VLF DLF (I, LSN) Mainly contralateral	Thalamus: VLF → VPL/VPM DLF → VMPo/VPI/MDvc Also PAG and collaterals → Reticular structures	Unmyelinated Small and large myelinated	All mammals Prominent in primates	Discriminative-sensory (VLF) Motivational-affective Descending inhibition
Spinoreticular tract	I V/VI VII/VIII X	NS WDR Non-N	Skin Viscera Muscle	Mainly VLF Mainly contralateral but ipsilateral (I-V) via dorsal columns to DRN	RF of brainstem → LRN, medial thalamus and DRN	Small and large myelinated	All vertebrates	Motivational-affective (?) Descending inhibition
Spinomesencephalic tract	I-II IV/V VII X LSN	NS WDR Non-N	Skin Viscera Joints/muscle	Mainly VLF DLF (I, LSN) Mainly contralateral	Midbrain and PAG Deep SCL, NCF and PBN Thalamus	Unmyelinated Small and large myelinated	All vertebrates	Motivational-affective Autonomic, motor
Spinoparabrachio-amygdaloid tract	I II	NS	Skin Viscera Joints/muscle	DLF-LF Mainly contralateral	PBN → amygdala and Stria terminalis	Unmyelinated Small, myelinated	Mammals	Motivational-affective Autonomic
Spinoparabrachio-hypothalamic tract	I II	NS	Skin Viscera Joints/muscle	DLF-LF Mainly contralateral	PBN → hypothalamus (VMH)	Unmyelinated Small, myelinated	Mammals	Motivational-affective Endocrine
Spinohypothalamic (spinotelencephalic) tract	I V X LSN	NS WDR Non-N	Skin Viscera	VLF Mainly contralateral	Hypothalamus and thalamus. Also pons, amygdala, striatum (bilateral)	Unmyelinated Small, myelinated	Mammals	Sleep, autonomic and endocrine function Thermoregulation
Spinocervical tract	I III/IV V	WDR Non-N	Skin Joints/muscle	DLF Ipsilateral – then contralateral (from LCN)	Relay LCN → contralateral thalamus and midbrain Some LCN cells → spinal cord	Small and large myelinated	All vertebrates Prominent in carnivores and primates	Discriminative-sensory Motivational-affective Autonomic (?)
Postsynaptic dorsal column pathway	III-V VI VII	NS WDR Non-N	Skin Viscera Joints/muscle	DF (and DLF) Ipsilateral – then contralateral (from DCN)	Relay DCN of caudate medulla: via ML → contralateral thalamus Also SCL and spinal cord	Small and medium myelinated	Not fish Prominent in mammals	Discriminative-sensory (VPL) Motivational-affective (VMPo)

DCN dorsal column nuclei; **DF** dorsal funiculus; **DLF** dorsolateral funiculus; **DRN** dorsal raphe nuclei; **LCN** lateral cervical nucleus; **LRN** lateral reticular nucleus; **LSN** lateral spinal nucleus; **MDvc** medial dorsal thalamus; **ML** median lemniscus; **NCF** nucleus cuneiformus; **Non-N** non-nociceptive; **NS** nociceptive-specific; **PAG** periaqueductal grey; **PBN** parabrachial nucleus; **SCL** superior colliculus; **VLF** ventrolateral funiculus; **VMH** ventromedial hypothalamus; **VMPo** ventromedial posterior thalamus; **VPI** ventroposterioinferior thalamus; **VPL/VPM** ventroposterolateral/ventroposteromedial thalamus; **WDR** wide dynamic range; →symbolises subsequent, second order projection. (Adapted from Millan, 1999)

Table 1-1. Ascending pathways transmitting nociceptive information.

1.4.4. Chemical modulators and transmitters in the nociceptive pathways

Chemicals play a vital role in the transmission and modulation of nociceptive information at all stages of the pain projection system. In the periphery, chemicals are involved in the stimulation of nociceptive fibres and also contribute to pain associated with inflammation and ischaemic changes, which can persist long after the noxious stimulus has been removed. Excitatory amino acids and Substance P are released by the primary afferent fibres at their terminals in the dorsal horn. Nociception is modulated in the dorsal horn by the release of these modulators, both from primary afferent fibre terminals and from other sources, such as intrinsic neurons, terminals of descending pathways and glial cells. Excitatory amino acids are also involved in the transfer of nociceptive information from the spinothalamic tract to the thalamus, and from the spinomesencephalic tract to the periaqueductal grey (Ericson et al. 1995; Azkue et al. 1997).

Tissue damage, inflammation and nerve injury are associated with local biochemical changes effected by the liberation of intracellular substances into the extracellular fluid surrounding the primary afferent fibres, evoking local pain, tenderness and hyperalgesia (Bonica 1987). These biochemical changes can modify the activity of nociceptors, either by directly activating them, or sensitising them to different types of stimuli. This is of considerable importance as the mechanisms leading to abnormal excitation of peripheral afferent fibres are thought to be a major factor in the development of chronic pain (Rang and Urban 1995). An example of the impact of chemical changes on the activity of nociceptors is the sleeping or silent nociceptor. This class of nociceptor was described during the late-1980s and is resistant to activation under normal conditions, but becomes sensitised under pathological conditions such as inflammation (Schaible and Schmidt 1988;

Handwerker 1991). This class of nociceptor has been described in joint, cutaneous and visceral tissue (Treede et al. 1992; Schaible and Grubb 1993; Schmidt et al. 1995). It is considered to be a chemoreceptor which becomes sensitised to mechanical stimuli due to the activation of second messenger systems by chemical agents such as prostaglandins and bradykinin, which are released in damaged tissue.

There is an extensive and expanding list of the chemicals that are contained and released by primary afferent fibres, and are involved in the transmission and modulation of nociceptive signals. Such chemicals include excitatory amino acids such as glutamate; neuropeptides such as Substance P (SP) and calcitonin gene related peptide (CGRP); the cellular energy source adenosine triphosphate (ATP); nitric oxide (NO); and the phospholipid metabolites, prostaglandins and neurotrophins (growth factors). Furthermore, the release of inflammatory mediators, such as bradykinin, prostaglandins and histamine, activate second messenger systems that also act to sensitise the nociceptors. The chemical composition of primary afferent fibres varies depending on tissue type, the state of the tissue (intact vs. injured or inflamed) and the class of fibre (Millan 1999).

Of the many substances that affect the excitability of the primary afferent fibres, kinins, which are potent algogenic (pain producing) peptides, are of considerable importance (Dray 1997; McHugh and McHugh 2000). These peptides are produced rapidly following injury and initiate a range of chemical interactions impacting on both peripheral and central neurons. Bradykinin, which is released from kininogens in the circulation, and lysyl-bradykinin (kallidin) produce pain and contribute to hyperalgesia (an increased response to a painful stimulus) by the activation of two major kinin receptors: B_1 and B_2. Bradykinin can both directly activate nociceptors, and sensitise nociceptors by the excitation of

postganglionic sympathetic neurones, causing the release of prostanoids such as prostaglandin E2 (Rang and Urban 1995; Besson 1999). Kinins are also produced during acute inflammation (Dray 1997), and bradykinin B_2 receptor antagonists have been demonstrated to be analgesic during these conditions (Steranka et al. 1988; Griesbacher et al. 1994). B_1 receptors have also been shown to have a role during prolonged inflammation, and B_1 receptor antagonists can attenuate the development of the associated hyperalgesia (Perkins et al. 1993).

Countless other neuromodulators and neurotransmitters from a range of sources have also been implicated in modulating nociception at peripheral, spinal and supraspinal stages, and thus altering the subjective qualities of pain. These transmitters and modulators are too numerous to detail herein, but include cytokines, endorphins, enkephalins, gamma-aminobutyric acid, dopamine, acetylcholine, epinephrine and norepinephrine. Figure 1-1 depicts the chemical environment of sensory nerve fibres (Dray 1997). It should be noted that these transmitters and modulators may have pronociceptive or antinociceptive actions. For comprehensive reviews, see Millan (1999) and Dray (1997).

1.4.5. Descending modulation

The experience of pain is also subject to modulation by both descending inhibitory (Fields and Basbaum 1994) and facilitatory pathways, which have been the subject of a recent review (Millan 2002).

A series of studies conducted in the late 1960s and early 1970s revealed an important feature of pain processing and modulation, with the discovery that analgesia could be produced upon stimulation of the periaqueductal grey region of rat brain (Reynolds 1969;

Mayer and Liebeskind 1974). Studies later revealed that stimulation in the same areas of the human brain in patients with intractable pain also produced analgesia (Adams 1976; Hosobuchi et al. 1977). It was also demonstrated that the opioid antagonist naloxone attenuated the analgesia produced by stimulation (Akil et al. 1976), suggesting the involvement of opioid systems.

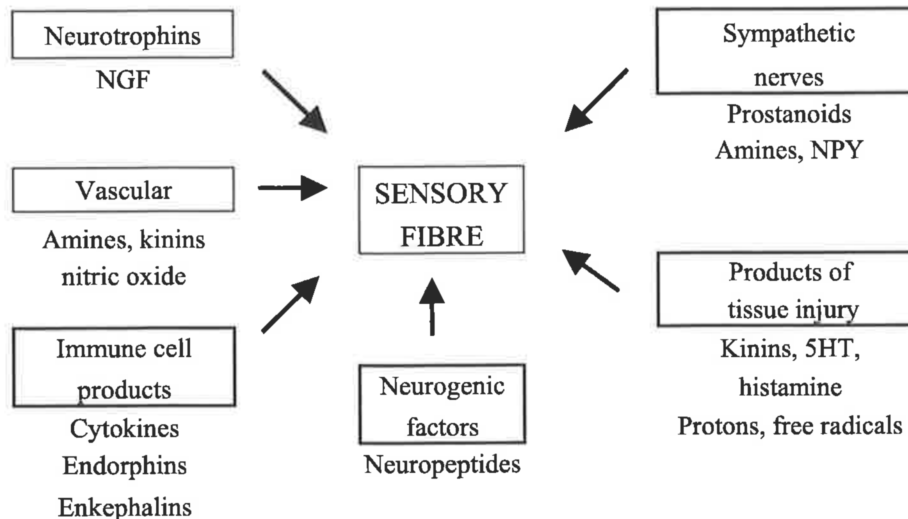

Figure 1-1. The chemical environment of sensory nerve fibres. A large number of mediators produced by several tissues can affect the excitability and phenotype of sensory neurons. NGF nerve growth factor, NPY neuropeptide Y, 5HT 5-hydroxytryptamine (serotonin). (Reproduced from Dray, 1997).

Descending pathways projecting from cerebral structures to the dorsal horn play a significant role in the integration of nociceptive messages in the dorsal horn, modulating the release of neurotransmitters from primary afferent fibres and inhibiting the activity of the projection neurones that transmit nociceptive messages to the higher centres (Millan 1999).

Descending pathways involved in the modulation of nociception do not have a uniquely inhibiting role in the dorsal horn. Individual neurotransmitters can exert either inhibitory or

excitatory effects in the dorsal horn depending, for example, on the receptor activated (Millan 1999).

1.5. Types of pain

The pain experience may be classified into four subgroups or types of pain. These are divided according to the time course and nature of the experience.

1.5.1. Transient pain

Transient pain occurs frequently in everyday life and rarely leads to the need for medical attention. It is the fleeting pain experienced, for example, during venepuncture. Activation of nociceptive transducers in the skin or other tissues elicits transient pain, although tissue damage seldom occurs. The function of this pain pertains to its rapid speed of onset, and the speed with which the pain dissipates once the noxious stimulus has been removed (Loeser and Melzack 1999). This type of pain presumably has a protective role, evoking a reflex response to remove the stimulus that is impinging on the body, thus reducing the likelihood of tissue damage.

1.5.2. Acute pain

Acute pain is associated with significant injury of body tissue, which activates nociceptive transducers at the site of tissue damage (Loeser and Melzack 1999). It is often seen after trauma, surgical procedures, and following some diseases, and will usually result in the individual seeking medical attention. While medical interventions may assist in both reducing the pain and accelerating the healing process, it is often not necessary, and healing can occur without treatment. Acute pain may persist for several days or weeks as the healing process takes place.

1.5.3. Chronic pain

Chronic pain has previously been defined as pain which persists beyond a six-month period (Russo and Brose 1998). It has been argued, however, that duration should not be the distinguishing feature of chronic pain, but rather the ability of the body to restore functioning to normal homeostatic levels (Loeser and Melzack 1999). Indeed recent reports define chronic pain by its character rather than by duration (Schaible and Richter 2004). Chronic pain states include back pain and postherpetic neuralgia. While all types of chronic pain typically lead individuals to seek medical care, often the pain is not treated effectively and due to the unrelenting nature of the pain, stress and environmental factors may further contribute to the problem (Loeser and Melzack 1999). A common form of chronic pain is neuropathic pain, which is associated with damage to the nociceptive pathway, rather than a result of excessive peripheral stimulation as described in transient and acute pain.

1.5.4. Experimental pain

Producing pain in an experimental setting is an important part of research, particularly in the assessment of analgesic drugs. However, it is impossible to produce pain in an experimental setting that is comparable to "real life" pain. For many years the validity of experimental pain, especially in assessing analgesic medication, has been a challenging and contentious issue amongst clinicians and researchers (Keats et al. 1950; Beecher 1953; Moore et al. 1997). Perhaps the most prominent of critics was Henry Beecher, who argued that experimentally induced pain was qualitatively different from the pain produced by injury or disease (Beecher 1962). He asserted that experimentally induced pain is without significance or meaning to the individual, whereas pain that results from injury or disease

has significance to the patient and involves other parameters, particularly psychological factors, such as anxiety.

Notwithstanding, several methods of experimental pain induction have been developed over time that yield reliable and reproducible data (Stacher et al. 1986), and are widely used in the assessment of pain sensitivity and tolerance.

1.5.5. Techniques for experimental pain induction

Techniques that have been developed for the induction of experimental pain in humans include tests of ischemic, heat, cold, pressure and electrical pain. Most frequently, study participants are required either to rate the pain experienced, or are instructed to proceed with the testing as long as the pain can be tolerated. These tests have not been standardised, normative values have not been established, and tests are typically subject to different methods according to the investigative group and the outcome measures of the study. The cold pressor and electrical stimulation tests are two of the most widely used human pain induction techniques. Experimental pain induction is described in greater detail in Chapter 3.

1.5.5.1. Cold pressor

The cold pressor test is a common experimental pain induction technique used in clinical investigation. Originally used in the 1930s as a measure of blood pressure variation (Hines and Brown 1933), it is now used in a variety of experimental settings. It became particularly prevalent in experimental pain research with the pioneering study conducted by Wolff and co-workers (1940), which initially demonstrated that intramuscular morphine produced a dose-dependent increase in cold pressor pain tolerance. Later studies have

consistently confirmed that the test is a highly sensitive assay for opioids, including morphine (Wolff et al. 1966; Jones et al. 1988), dipipanone (Posner et al. 1985), and codeine (Garcia de Jalon et al. 1985), and that this response can be distinguished from both placebo (Posner et al. 1985; Jones et al. 1988) and non-opioid analgesics (Jones et al. 1988). The cold pressor test is of particular value as a model of experimental pain induction as it is produces prolonged, deep sensations that are characteristic of many clinical pain states (Wolff 1984), being likened to dental and back pain (Chen et al. 1989), and has high reproducibility for repeated applications (Walsh et al. 1989; Grach et al. 2004).

There have been many variations on the cold pressor technique, but the test essentially involves the immersion of a limb (usually the forearm) into a bath of very cold water. The participant is typically required to indicate when pain is first experienced (threshold), and when pain can no longer be tolerated (tolerance), both of which are measured in terms of time (seconds) elapsed from initial immersion into the cold water. Alternatively, subjects may be asked to continuously rate pain intensity during cold water immersion.

The cold-water bath may be preceded by the immersion of the limb in a warm water bath for two minutes, and the presence of a water pump in the cold water to keep the water moving, reducing the effect of laminar warming around the limb.

1.5.5.2. Electrical stimulation

Electrical stimulation has been described as one of the most suitable methods of experimental pain induction as it is simple to apply and easy to reproduce (Harris and Blockus 1952). A number of studies have successfully used electrical stimulation to test

the analgesic efficacy of opioids, including morphine (Willer 1985), codeine (Stacher et al. 1986) and methadone (Dyer et al. 1999), and other analgesic compounds, including non-steroidal anti-inflammatory agents (NSAIDs) (Stacher et al. 1979; Stacher et al. 1986; Walker et al. 1993; Walker and Carmody 1998).

Electrical stimulation typically involves attaching electrodes to some area of the body, often the earlobe, hand or finger, and conducting square wave pulses of electricity in increasing volts. Resembling the cold pressor test, the subject indicates the level of pain experienced in terms of both pain sensitivity (when pain is first felt) and tolerance (maximum tolerable pain), however these parameters are measured in volts or amps rather than time.

1.6. Opioids*

1.6.1. The history of opioids

In 1915, the eminent pharmacologist D.I. Macht wrote "If the entire materia medica at our disposal were limited to the choice and use of only one drug, I am sure that a great many, if not the majority, of us would choose opium" (Macht 1915). Opium is the milky sap extracted from the seed pod of the poppy, *Papaver somniferum*, more commonly referred to as the opium poppy. First classified by the botanist Linnaeus in 1753, the species takes its name from the Latin word meaning "sleep-inducing". However, the properties of this plant

* The term opioid denotes all compounds that interact with opioid receptors in the central and peripheral nervous system (Foley, 1993). The term opiate refers only to drugs that are derived from the juice of the

have been known for thousands of years. In 3400 BC, the Sumerians, the world's first civilisation and agriculturalists, were cultivating the opium poppy throughout the Tigris-Euphrates river systems of lower Mesopotamia. The ideograms used by the Sumerians for the poppy translate as "joy plant", suggesting that this ancient civilisation was aware of the psychological and pain-relieving qualities of the plant. It is thought that, just as the Sumerian invention of writing gradually spread to other societies, so too did knowledge of opium. By the end of the second millennium BC, opium use was prevalent in much of the ancient world. Throughout the ages, the use of opium flourished. In its time, it has been hailed as a magical panacea, used for pain relief, as an antidote to sorrow, in the treatment of insomnia, as a cure for stomach ailments, to treat coughs and colds, as a crude anaesthetic during surgery, in religious rites, as a form of recreation, and as a convenient poison in murder and suicide (Booth 1996). However, as early as the fifth century BC, the dangers of this universal remedy were recognized. Erasistratus advocated complete abstinence from opium and later, in the third century BC, the philosopher Diagoras of Melos proclaimed that it is better to suffer pain than to become dependent upon opium.

By the sixteenth century AD, opium was well established in Europe, however its use was predominantly medicinal. Publications of the mid-late 1700s describe aspects of opium addiction and the difficulty of withdrawal; however there lacked any notion of moral denunciation for either medicinal or recreational use. In fact, at this time, there was a widespread movement, particularly in the literary community, away from the restrained ethos of classicism in favour of spontaneity, imagination, a greater awareness of the natural

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opium poppy. Hence, the synthetic compound methadone is classed as an opioid due to its interaction with opioid receptors, however it is not an opiate, as it is not derived from the opium poppy.

world, and a more liberated expression of passion. Opium played a significant role in the growth of this movement, which has become known as the Romantic Revival, and included literary figures such as Samuel Taylor Coleridge, Lord Byron, Elizabeth Barrett Browning, Goethe, and Thomas De Quincey, author of the notorious "Confessions of an English Opium-eater" of 1821.

In 1806 the German pharmacist Friedrich Sertürner isolated a pure alkaline substance from opium. The compound was named morphine, after the Greek god of dreams Morpheus. Coupled with the invention of the hypodermic syringe in 1853, and the synthesis in 1874 of the highly potent opioid, diacetylmorphine, the magnitude of opioid dependence presented a significant public health concern. Diacetylmorphine, considered at the time to be a non-addictive alternative to morphine, was marketed by the Bayer pharmaceutical company in the late 1800s as a cough syrup, and named "Heroin". In response to the mounting problem of opioid dependence, US Congress passed legislation in the early 1900s restricting the medical and recreational use of opioids. This action has since been echoed throughout most of the world.

The quest for potent, non-addictive analgesics led to the synthesis of drugs such as methadone and pethidine in the late-1930s and 1940s; however, these compounds displayed typical morphine-like properties, including the potential to produce dependence. It was also during this time that the first opioid antagonist was synthesised. Pohl (1915, see Gonzalez and Brogden 1988) first noted the pharmacology of opioid antagonists, drugs that block the effects of opioids, in the early 1900s.

1.6.2. Opioid classification

Opioids exert their effects by interacting with specific receptors on nerve cells in the CNS and periphery (see discussion below). Opioids are classified according to their interaction with these receptors and the pharmacological effects they produce. These compounds are classed as agonists, antagonists or partial agonists (Zacny and Walker 1998). Opioid agonists, such as methadone, morphine and heroin, exert a very strong or maximal effect at the receptor, inducing the cellular actions described below (section *1.2.4*). Antagonists have minimal or no intrinsic action themselves, but act to block the effects of opioid agonists. Opioid antagonists include the short-acting naloxone and longer-acting naltrexone. Partial agonists such as buprenorphine have less intrinsic activity at the receptor, and thus may exert agonist activity, but also may exert some degree of antagonist activity by displacing full agonists that have higher intrinsic activity at the receptor.

1.6.3. Opioid receptors

The mounting social problems associated with opiate dependence in the 1940s and 1950s stimulated a surge of research focused on the still unrealised goal of developing a potent, non-addictive analgesic. However, from this research emerged the finding that many of the pharmacological effects of opioids were highly stereospecific. This generated the hypothesis that these drugs exert their effects by binding with specific sites on nerve cells. The first indication of stereospecific opioid binding in mouse brain was published in 1971 (Goldstein et al. 1971). In 1973, three research groups independently produced decisive evidence for specific opioid receptors in animal brain (Pert and Snyder 1973; Simon et al. 1973; Terenius 1973), and soon after in human brain (Hiller et al. 1973). With the observation that morphine and several of its analogues had different pharmacological profiles, and that opioid antagonists blocked various agonists differentially, it was

postulated that, rather than opioid receptors being one homogeneous group as first thought, there existed different types of opioid binding sites. Evidence for multiple receptor types was soon produced, and three receptor types proposed: μ, κ and σ (Gilbert and Martin 1976; Martin et al. 1976). Later research revealed a fourth receptor type, which was labelled δ (Lord et al. 1977). Further investigation revealed that the σ receptor was not opioid, as effects mediated by this receptor were not reversed by opioid antagonists, even at very high doses. Furthermore, findings indicated that the σ receptor was a binding site for non-opioid drugs such as phencyclidine (PCP) (Vincent et al. 1979; Zukin and Zukin 1979). More recent investigation has demonstrated that this class of receptor represents a group of binding sites with high affinity for a range of non-opioid compounds (Henderson and McKnight 1997).

Molecular cloning of first the δ (Evans et al. 1992; Kieffer et al. 1992), and then κ (Yasuda et al. 1993) and μ receptor (Chen et al. 1993) has facilitated the pharmacological characterisation of these binding sites (Reisine and Bell 1993; Raynor et al. 1994; Satoh and Minami 1995). Subtypes of these receptors have been proposed (Dhawan et al. 1996); however pharmacologically defined subclasses of the main receptor types have not been well established (Connor and Christie 1999; Williams et al. 2001).

In 1994, a fourth receptor clone was isolated from a number of species, including mouse (Nishi et al. 1994), rat (Fukuda et al. 1994; Lachowicz et al. 1995) and human (Mollereau et al. 1994). Although this novel receptor shares a high level of sequence homology with traditional opioid receptors, in mammalian cells the receptor demonstrated very little binding affinity with opioids (Henderson and McKnight 1997; Barlocco et al. 2000). Hence, the novel receptor was considered by many to be an orphan receptor (Mogil and

Pasternak 2001), and labelled ORL1 (opioid-receptor-like 1). In 1995 an endogenous ligand for this receptor, orphanin FQ or nociceptin, was identified (see discussion below) (Meunier et al. 1995; Reinscheid et al. 1995). There remains, however, some controversy regarding this receptor type, and whether it should be considered an opioid receptor (Henderson and McKnight 1997).

Extensive investigation has revealed that the three traditional opioid receptor types (μ, κ and δ) demonstrate distinct anatomical distributions and functions in the CNS and periphery (see Table 1-2), and that there is generally a correlation between receptor density in a particular brain region and the functional importance of opioids in that area (Mao 1999). Opioid receptors are distributed widely in the mammalian CNS (Mansour and Watson 1993). Dense μ-receptor binding is found in regions important for nociceptive regulation, basal ganglia, limbic structures and thalamic nuclei, however there is considerable intra-species variation in distribution. Similarly, while κ sites are widely distributed throughout the forebrain, midbrain and brainstem, considerable differences exist between mammalian species. By comparison, the distribution of delta receptors is more restricted, being concentrated in forebrain regions, and is more consistent between species. While the main effects of opioids are mediated by the CNS, receptors distributed throughout the periphery produce hormonal, immunological and some analgesic effects (King et al. 2001).

Receptor type	Primary functions
μ	Nociception (↓) Respiration (↓) GI motility (↓) Feeding (↓) Learning and memory (↓) Locomotor activity (↓)* Hormone secretion (↑) Cardiovascular regulation Immune function Thermoregulation Associated with positive subjective effects, highly reinforcing
δ	Nociception (↓) GI motility (↓) Respiration (↓) Locomotor activity (↑) Cognitive function (↓) Cardiovascular regulation Olfaction Immune function Associated with some reinforcing properties
κ	Nociception (↓) Diuresis (↑ urinary excretion) Feeding (↓) Neuroendocrine secretion Immune function Thermoregulation No positive subjective effects, can produce dysphoria in humans
*effects depend on dose and species ↓ decreased activity/effect, ↑ increased activity/effect	

Table 1-2. Primary functions of opioid receptors (adapted from Dhawan et al., 1996).

1.6.4. Endogenous opioid ligands

With the detection of stereospecific binding sites for opioids in the early 1970s, it was hypothesised that there must exist an endogenous ligand for these receptors. This notion was first supported with evidence of opiate-like activity in brain extract (Kosterlitz and

Waterfield 1975), and soon after the enkephalins were isolated and characterised (Hughes 1975; Hughes et al. 1975). Following these studies, a fragment of pituitary hormone β-lipotropin was found to have high affinity for opioid receptors, and was renamed β-endorphin (from *end*ogenous m*orphine*) (Bradbury et al. 1976). Some years later, a third class of endogenous opioid was discovered, and labelled the dynorphins (Goldstein et al. 1979). These three classes of endogenous ligands each demonstrate greater binding affinity with one of the receptor types: enkephalins with the δ-receptor, endorphins with the μ-receptor, and dynorphins with the κ-receptor (van Ree et al. 1999). However, it should be noted that these ligands also bind with other opioid receptor types, for example, the enkephalins have affinity for the μ-receptor (Mansour et al. 1995), and β-endorphin binds potently with δ-receptors (Reisine 1995).

In recent years, several advances have been made in our understanding of endogenous opioids. Recently discovered in mammalian cells, ligands termed endomorphin 1 and 2 demonstrate potent binding affinity and are highly selective for the μ-receptor (Zadina et al. 1997; Goldberg et al. 1998; Zadina 2002). As mentioned, a novel neuropeptide, orphanin FQ or nociceptin, was identified in 1995, and demonstrated to be the endogenous ligand for the ORL1 receptor (recently renamed NOP_1, or nociceptin/orphanin FQ peptide). There is still controversy regarding whether NOP_1 should be considered a fourth member of the opioid receptor family.

1.6.5. Second messengers and effectors

Cloning has confirmed that opioid receptors belong to the superfamily of G-protein-coupled receptors, which activate and regulate multiple second messenger pathways associated with effector coupling, receptor trafficking and nuclear signalling (Williams et

al. 2001). Signalling via these receptors has been shown to be mediated by interaction with guanine nucleotide-binding proteins, or G-proteins. Opioids have been shown to interact preferentially with pertussis-toxin (PTX)-sensitive G_i/G_o-proteins, although evidence indicates that opioids may also interact with other families of G-proteins, for example the PTX-insensitive G_z and G_{16} (see Connor and Christie 1999 for review). The cellular actions produced by opioid receptor activation are common to all three types of receptor. The most common actions are inhibition of adenylyl cyclase (reducing intracellular cAMP), activation of potassium conductance, inhibition of voltage-gated calcium channels, and inhibition of transmitter release. Recent observations suggest that activation of opioid receptors is also associated with activation of protein kinase C (PKC), release of calcium from extracellular stores, nuclear signalling (e.g. the activation of the mitogen-activated protein kinase (MAPK) cascade) and receptor trafficking (Williams et al. 2001). The overall consequence is a reduction in the excitability of the cell due to hyperpolarization and the inhibition of neurotransmitter release, however, opioids may also produce an excitatory effect by preventing the release of inhibitory neurotransmitters (Williams et al. 2001).

1.7. Opioid effects

Opioids are predominantly used for the euphoria and feelings of well-being they inspire, and for their exceptional analgesic qualities. Most clinically used opioid agonists exert their effects primarily through μ receptors. While the pharmacokinetic profile of these compounds varies widely, many share a similar pharmacodynamic profile, exerting both desirable and undesirable effects (Mather 1990). Opioids affect a wide range of physiological systems, including the respiratory, cardiovascular, genitourinary and gastrointestinal systems. Typical opioid effects include analgesia, euphoria or feelings of

well-being, respiratory depression, reduced gastrointestinal motility, pupillary miosis, pruritus, sedation, nausea, vomiting and cognitive impairment. These effects are typically dose-dependent. While clinical use of opioids is principally for their powerful analgesic actions, they are also prescribed for their anti-tussive and anti-diarrhoeal effects. The discussion below will focus primarily on the effects of morphine, the prototypic μ receptor agonist used in the management of pain.

1.7.1. Analgesia

Opioids are the mainstay of pain management. They are the most effective and frequently used class of analgesic drug for the treatment of moderate to severe pain (Gutstein and Akil 2001). Referred to as "God's own medicine" by Sir William Osler, morphine remains the standard by which drugs with analgesic actions are assessed.

The analgesic effects of opioids are mediated through spinal, supraspinal and peripheral mechanisms (see Figure 1-2). Opioid receptors are prominent in the brain and spinal cord regions involved in the transmission and modulation of pain (see Ossipov et al. 2004). Binding of opioid agonists to these specific receptors inhibits nociceptive activity, resulting in potent analgesia (Fields 1993; Codd et al. 1995; McNally 1999).

Opioids inhibit the release of Substance P (SP) by nociceptive afferent neurons at the dorsal horn, and directly inhibit the pain transmission neuron. Opioids further produce analgesia by activating descending inhibitory pathways. Opioids excite neurons in the periaqueductal grey (PAG) and in the nucleus reticularis paragigantocellularis (NRPG), which in turn project to the rostroventral medulla, including the nucleus raphe magneus (NRM). From the NRM, neurons containing 5HT and enkephalin run through fibres in the dorsolateral

funiculus to the substantia gelatinosa of the dorsal horn, exerting an inhibitory influence on transmission. The locus ceruleus also plays a role in the inhibition of transmission via noradrenergic neurons that project to the dorsal horn.

While previously thought to act only centrally, opioids are now understood to have an analgesic role in the periphery also. Peripherally administered opioids and endogenous ligands that are locally released during inflammation bind with opioid receptors on peripheral nerves (Stein and Yassouridis 1997; Stein et al. 2001; Zajaczkowska et al. 2004).

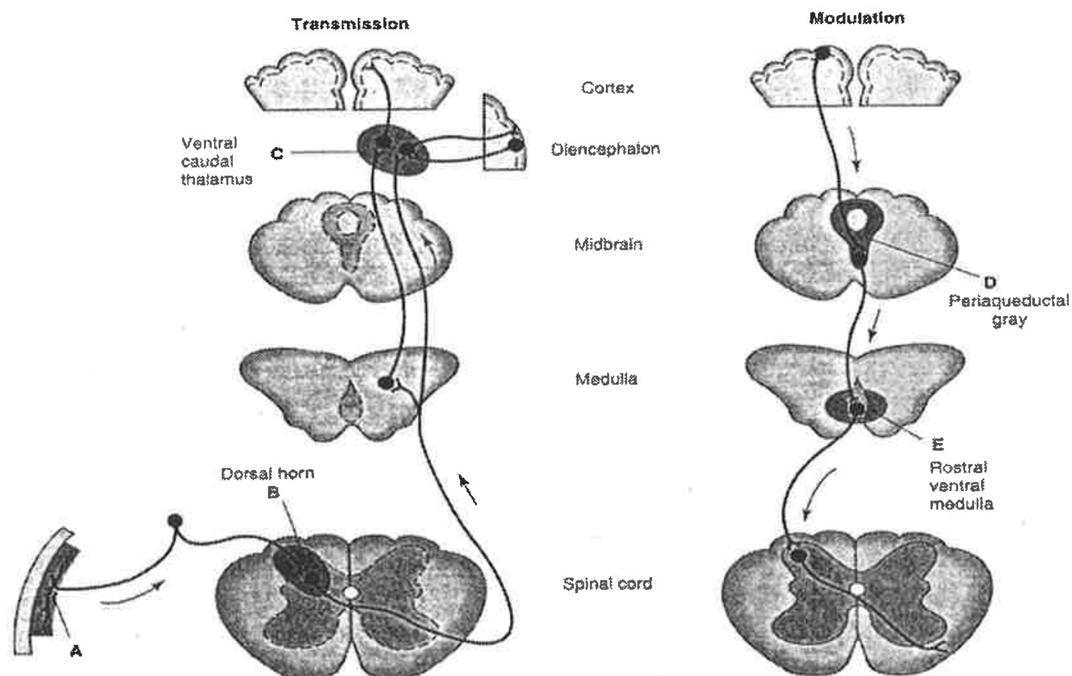

Figure 1-2. Putative sites of action of opioid analgesics. **Left:** ***Sites of action on pain transmission pathway from the periphery to the higher centres.*** **Right:** ***Action on pain modulating neurons secondarily affects pain transmission pathways. A.: Possible direct action of opioids on inflamed peripheral tissues. B: Inhibition occurs in the spinal cord. C: Possible site of action in the thalamus. On the right, actions of pain-modulating neurons in the midbrain (D) and medulla (E) secondarily affect pain transmission pathways. (Way et al. 2000)***

1.7.2. Respiratory depression

Respiratory depression is one of the most serious adverse effects associated with opioid analgesics (Hill 1993). It is one of the principal adverse effects that limits their therapeutic use (Florez and Hurle 1993), and death from morphine toxicity is typically the result of respiratory arrest (Gutstein and Akil 2001). Respiration is controlled primarily through the medullary respiratory centres, namely the dorsal respiratory group and ventral respiratory group of neurones, while chemoreceptors and stretch receptors in the periphery contribute to the control of breathing rate and pattern (White and Irvine 1999). Agonists at μ- or δ-

opioid receptors can result in respiratory depression. Such agents can cause a decrease in respiratory rate, a decrease in tidal volume, irregular respiratory rhythm and obstructive apnea during sleep (Macintyre and Ready 2001). The medullary and pontine centres involved in the regulation of respiration are inhibited by opioid interaction with both μ- and δ-receptors (Martin 1983). Opioids inhibit chemoreceptors in the periphery, reducing response to changes in oxygen and, importantly, increases in carbon dioxide levels. This effect is chiefly mediated by interaction with μ-receptors (White and Irvine 1999).

Following IV administration of morphine, respiratory depression peaks within approximately 10 minutes and depression may persist for up to 5 hours. While therapeutic doses of morphine depress all phases of respiratory activity, rarely do such doses produce clinically significant depression (Gutstein and Akil 2001). A greater risk occurs with the combination of opioids and other CNS depressants, such as sedative-hypnotics, general anaesthetics and alcohol, the use of which may be problematic in both acute and chronic pain patients.

1.7.3. Sedation

Respiratory depression is often preceded by sedation (Macintyre and Ready 2001). Sedation occurs primarily in the early stages of treatment (Foley 1993), and can compromise analgesia by limiting dose escalations that may be required for adequate pain relief (Macintyre and Ready 2001).

1.7.4. Nausea and vomiting

All clinically used opioid analgesics produce nausea and vomiting to some extent, although the magnitude of this effect varies considerably between individuals. These side effects

are caused by direct stimulation of the chemoreceptor trigger zone for emesis. The likelihood of nausea and vomiting is also increased in ambulatory patients compared to recumbent patients. This suggests the involvement of the vestibular system, and indeed there is evidence that opioids may be involved in the modulation of neurotransmission in the peripheral vestibular system (Andrianov and Ryzhova 1999). Opioids can increase vestibular sensitivity, and in some patients even slight movements can provoke nausea and vomiting (Macintyre and Ready 2001).

1.7.5. Effects on mood

Opioid use is associated with euphoria and decreased emotional distress. The mood altering properties of opioids are considered one of the primary reasons for illicit (non-medical) use of this class of drug (van Ree et al. 1999). Opioid abuse and dependence presents a significant public health challenge throughout much of the world, being associated with significant morbidity and mortality. While the prevalence of illicit opioid use is considerably lower than other drugs such as alcohol and cannabis, the economic cost is staggering. The mechanisms by which opioids exert their rewarding effects have not been fully elucidated (O'Brien 2001); however it is thought that the systems involved in mediating mood and reward are distinct from those associated with analgesia and the development of physical dependence (Koob and Bloom 1988). The rewarding effects of drugs of abuse, including opioids, are thought to be mediated by the midbrain dopaminergic system, which is also involved in the rewarding effects of other stimuli such as food (Hyman and Malenka 2001).

1.7.6. Constipation

Opioids can cause delayed gastric emptying, inhibition of bowel motility and constipation. This inhibition of smooth muscle activity is mediated by both central opioid receptors and receptors on the bowel wall (Gutstein and Akil 2001).

1.7.7. Pupillary miosis

Most μ and κ agonists cause pupillary constriction by exerting an excitatory effect on the parasympathetic nerve innervating the pupil (Gutstein and Akil 2001).

1.7.8. Cardiovascular effects

Therapeutic doses of opioid analgesics are associated with peripheral vasodilation, reduced peripheral resistance and inhibition of baroreceptor reflexes. Arterial or venous vasodilation may occur by a direct effect of vascular muscle or by histamine release. While in a supine position, there is no significant effect on blood pressure or cardiac rate, though upon movement to a sitting or standing position, hypotension and fainting may occur.

Opioid agonists such as morphine are also known to have utility in the treatment of angina pectoris and acute myocardial infarction, and have more recently been reported to have a cardioprotective effect (Schultz and Gross 2001).

1.7.9. Cough suppression

The mechanism by which opioids suppress the cough reflex is thought to involve a direct effect on the cough centre in the medulla (Gutstein and Akil 2001). The dose required for cough suppression is lower than that required for analgesia (Foley 1993).

1.7.10. Pruritus

Opioids can trigger histamine release from mast cells, which can create itching and flushing of the skin. Opioid-related pruritus primarily occurs on the face, neck and trunk, or at the injection site (Macintyre and Ready 2001). Pruritus does not occur with all opioids (Duthie and Nimmo 1987), and has been reported to occur more commonly following epidural or spinal opioid administration (Ballantyne et al. 1988).

1.8. Barriers to adequate pain management with opioids

There are a number of barriers to effective pain control with opioids. Several of these are societal barriers, including a stigma associated with the use of opioids that fails to adequately distinguish between legitimate and illegitimate use, and an apprehension amongst clinicians to prescribe opioids for fear of the patient developing a psychological dependence on the drug (Hill 1993; Rupp and Delaney 2004).

Moreover, significant limitations in the use of opioids for pain control are the adverse effects associated with this class of drug, and the adaptations that occur with prolonged use.

1.8.1. Adverse effects

Adverse effects associated with opioids can present significant limitations to pain management. As described above, the most significant adverse effects are respiratory depression and sedation, as these are potentially dangerous and can also limit dose escalation required for adequate pain control. While tolerance develops to the emetic effects of opioids (see 1.8.2), constipation is an ongoing adverse effect that can impact upon comfort and quality of life, especially in cases of prolonged opioid administration for chronic pain.

1.8.2. Tolerance

Chronic opioid administration results in tolerance, which is characterised by a decrease in activity of a drug after a previous exposure to the same or a similar drug (Foley 1993). Opioid tolerance is associated with decreased magnitude and duration of effects such as analgesia and euphoria, as well as several of the adverse effects associated with opioids, including respiratory depression, nausea and sedation (Collett 1998). The decreased effect associated with opioid tolerance can be very pronounced, with morphine doses many times greater than would be administered for analgesia producing only mild effects in chronic opioid users (Gregory et al. 1992; O'Brien 2001). However tolerance to different opioid effects does not develop at the same rate (Ling et al. 1989); for example, tolerance to the euphoric effects of opioids develops much earlier than tolerance to the gastrointestinal effects.

Several different types of tolerance have been described, which may be innate or acquired (O'Brien 2001). Innate tolerance refers to a genetic predisposition towards a lesser (or greater) response to a drug. Acquired tolerance may be classified as pharmacokinetic, pharmacodynamic or learned tolerance. Pharmacokinetic tolerance describes the increase in clearance (CL) of an agent with repeated use, resulting in a lower plasma drug concentration. Learned tolerance refers to the development of behaviours or mechanisms that reduce the magnitude of drug effect. For example, compensatory behaviours may be developed in order to maintain functioning despite intoxication. Pharmacodynamic tolerance describes adaptive changes occurring in systems that are affected by the drug, which result in a reduction in effect.

The majority of investigations of opioid tolerance have focused on the μ receptor, as this is the major site of action of interest. The involvement of κ- and δ-opioid receptors in tolerance has received less attention.

The mechanisms underlying tolerance have not been fully elucidated. While a number of cellular and synaptic adaptations have been demonstrated to occur with chronic administration of an opioid agonist, how these adaptations result in the physiological and behavioural phenomena of tolerance remains unclear. A blunting of opioid receptor response, by receptor de-coupling or internalisation (Borgland 2001; Williams et al. 2001), or an increase in opioid receptor coupling with excitatory G-proteins (Crain and Shen 2000) have been implicated in the development of tolerance. However it is apparent that there are a number of other adaptations that occur with the chronic administration of opioids, and are increasingly understood to play a role in tolerance.

Phosphorylation of occupied receptors is thought to play a significant role in receptor desensitisation and internalisation by uncoupling receptors from their G-proteins. G-protein coupled receptor kinase (GRK)-mediated receptor phosphorylation allows binding of the cellular protein arrestin, which disrupts G-protein binding with receptors. A number of other kinases may mediate receptor desensitisation by phosphorylation, including protein kinase C (PKC) (Inoue and Ueda 2000; Ueda et al. 2001), calcium/calmodulin-dependent protein kinase II (CaM kinase II) and tyrosine kinase (Borgland 2001). Mu-receptor desensitisation is associated with a decrease in binding sites on the plasma membrane (Pak et al. 1996). Receptor phosphorylation by GRKs is considered to play a central role in receptor internalisation (Capeyrou et al. 1997).

These cellular adaptations have been demonstrated to occur rapidly upon administration of an opioid, often within minutes of administration (Williams et al. 2001). The role these initial changes play in the development of chronic tolerance with prolonged dosing is unclear. It has been reported that the degree of change observed at the cellular level is much smaller in magnitude than the effects observed clinically (Harrison et al. 1998; Williams et al. 2001), suggesting the involvement of other adaptational changes in the development of tolerance. Long-term adaptations are thought to involve functional uncoupling of μ receptors from signalling pathways as a consequence of downregulation of opioid receptors on the surface of cell membranes (Williams et al. 2001).

A well-established adaptation occurring with chronic opioid administration of opioids is the upregulation of cAMP pathways (Nestler 2001). Opioids inhibit activity of the cAMP pathway. With chronic administration, activity of cAMP pathways recovers with the induction of adenylyl cyclase and protein kinase A.

The N-methyl-D-asparate (NMDA) receptor has also been implicated in the development of tolerance (Elliott et al. 1994; Mao 1999; Trujillo 2000), and indeed NMDA receptor antagonists have been demonstrated to attenuate the development of tolerance (Trujillo and Akil 1991; Tiseo and Inturrisi 1993). There is also evidence for the involvement of nitric oxide and nitric oxide synthase (Pasternak 1995; Aley and Levine 1997).

1.9. Enhancing analgesia through drug combinations

While opioids are considered the gold standard in the management of moderate to severe pain, their use is limited by the adverse effects described. Due to these limitations, research is increasingly focusing on ways in which to improve the use of opioids in pain

management; that is, to enhance the analgesic effect while minimising the incidence and severity of adverse effects. The notion of combining agents for enhanced analgesic effect and reduced incidence of adverse effects is increasingly being viewed as an important consideration for effective pain treatment. The management of post-operative pain has received particular attention, with the recognition that inadequate post-operative pain relief can adversely influence organ functioning and contribute to morbidity (Kehlet 1994; Kehlet 1997). It has been acknowledged that optimal pain relief is unlikely to result from a single drug or treatment modality (Kehlet and Dahl 1993; Mehlisch 2002). Drug combinations producing either additive or synergistic analgesic effects, which can lower doses of individual drugs and thus moderate the incidence and severity of side effects, may significantly reduce postoperative morbidity, hospital stay and convalescence (Kehlet 1995; Kehlet 1997).

One approach is the addition of a non-opioid analgesic to opioid treatment, the rationale being that a lower dose of opioid would thus be required to achieve antinociception, thereby reducing the incidence and severity of adverse effects.

An increasing body of literature has demonstrated that in some circumstances, the addition of an alternative agent to opioid treatment can *potentiate* the analgesic effect of an opioid. Importantly, it has also been demonstrated that some combinations may attenuate the development of tolerance. Two drug classes that have received considerable attention for the therapeutic advantages observed in concomitant administration with an opioid agonist are NMDA receptor antagonists, and paradoxically, ultra-low doses of opioid receptor antagonists.

1.9.1. Opioid agonist-NMDA antagonist combinations

NMDA receptors, a class of excitatory glutamatergic receptors, have been implicated in the neural plasticity associated with the development of opioid tolerance and physical dependence (Mao 1999; Trujillo 2000). NMDA receptor antagonists are a diverse range of compounds, including the over-the-counter antitussive dextromethorphan and the anaesthetic ketamine. The addition of an NMDA receptor antagonist to opioid treatment has received considerable attention since the pioneering work of Trujillo and Akil reported that the repeated co-administration of the NMDA receptor antagonist MK-801 and morphine attenuated the development of tolerance to the antinociceptive effects of the morphine in the rat (Trujillo and Akil 1991). Subsequent investigation has substantiated this finding in animal models (Tiseo and Inturrisi 1993; Allen and Dykstra 1999; Allen and Dykstra 2000), and demonstrated that attenuation of antinociceptive tolerance by NMDA receptor antagonists may be observed with a variety of other opioids, including the μ-receptor agonists etorphine and dezocine (Allen and Dykstra 2000), as well as some δ- (Bhargava and Zhao 1996; Zhao and Bhargava 1996) and κ-receptor agonists (Bhargava and Thorat 1994).

There have also been conflicting reports suggesting that NMDA receptor antagonists can modulate acute opioid antinociception. It has been demonstrated that the addition of NMDA receptor antagonists can, in some circumstances, potentiate morphine antinociception in animal models (Plesan et al. 1999; Belozertseva et al. 2000; Carlezon et al. 2000; Kozela et al. 2001; Alvarez et al. 2003; Redwine and Trujillo 2003), and in some clinical trials of healthy volunteers and pain patients (Bell et al. 1999; Caruso 2000; Katz 2000; Mercadante et al. 2000; Weinbroum et al. 2002; Sveticic et al. 2003). In contrast, other studies have reported no effect of the addition of an NMDA receptor antagonist on

opioid antinociception (Trujillo and Akil 1991; Trujillo and Akil 1994; Gonzalez et al. 1997; Allen and Dykstra 1999). Indeed, a small number of studies has reported that the addition of an NMDA receptor antagonist *inhibited* the acute antinociceptive effects of morphine (Lutfy et al. 1993; Plesan et al. 1999). There is no identifiable factor distinguishing investigations that demonstrate potentiation and those that do not, and it has been suggested that due to the idiosyncratic nature of the synergism, it is unlikely that the effects are related to NMDA receptor blockade (Redwine and Trujillo 2003).

1.9.2. Opioid agonist-antagonist combinations

An alternative strategy that has received some attention is the co-administration of an opioid agonist and ultra-low doses of an opioid antagonist. It is widely accepted that opioid antagonists such as naltrexone (NTX) and naloxone (NLX) are "pure" opioid antagonists (Blumberg and Dayton 1973); that is, they have no intrinsic agonist action. Given the blockade of opioid binding associated with administration of an opioid antagonist, it would be anticipated that administration of such an agent would either produce no analgesic effect, or conceivably *increase* pain sensitivity due to blockade of endogenous opioid ligands that may mediate nociception. This has been consistently demonstrated in experimental pain models with healthy volunteers showing NLX (0.4-8 mg IV) (El-Sobky et al. 1976; Grevert and Goldstein 1977; Davis et al. 1978; Grevert and Goldstein 1978; McCubbin and Bruehl 1994) and NTX (50-100 mg orally) (Volavka et al. 1979) either increased or had no significant impact on pain response.

However, the role of opioid antagonists in the modulation of pain has been shown to be considerably more complex, and the potential role of these agents in pain management strategies is increasingly compelling (McNicholas and Martin 1984). This section will

review evidence for the role of opioid antagonists in modulating nociception and response to opioids, and enhancing pain management.

1.9.2.1. Enhanced opioid sensitivity following chronic antagonist pre-treatment

There is a wealth of findings from animal studies indicating that chronic exposure to an opioid antagonist results in the upregulation of opioid systems (Pert and Snyder 1976; Zukin et al. 1982; Yoburn et al. 1985; Cote et al. 1993; De Vries et al. 1993; Marley et al. 1995; Daws and White 1999) with resultant supersensitivity to the agonist effects of morphine (Hollt et al. 1978). While several putative mechanisms underlie these changes, increased opioid receptor density has been most intensively studied. The development of a quantitative immunohistochemical assay, used in conjunction with radioligand binding, investigated whether this upregulation of the opioid system is simply a function of an increase in the *total* number of opioid receptors, or if there is also an increase in the number of receptors in an active binding formation (Unterwald et al. 1998). It was revealed that the changes are brain region specific. In some brain regions (for example, hippocampus, amygdala, thalamus) there was found to be an increase of between 35% and 130% in the total number of opioid receptors, as well as a substantial increase in the proportion of receptors in active binding conformation (between 35% and 195%). However, in other areas (for example, hypothalamus, central grey, globus pallidus), an increase in the proportion of binding receptors (between 43% and 200%) was evident, without an increase in the total number of receptors *per se*.

1.9.2.2. Analgesic actions of low-dose opioid antagonists

As described, administration of high doses of opioid antagonists has consistently been shown to either increase or have no impact on sensitivity to noxious stimuli in healthy

volunteers across a range of pain induction techniques (El-Sobky et al. 1976; Grevert and Goldstein 1977; Davis et al. 1978; Grevert and Goldstein 1978; McCubbin and Bruehl 1994). In contrast, numerous authors have reported that, in certain conditions, administration of NLX in low doses itself produces analgesia. Early investigation with healthy volunteers demonstrated analgesia and hyperalgesia to ischemic pain with low (2 mg SC) and high dose (8-10 mg SC) NLX, respectively (Lasagna 1965). Subsequent investigations substantiated this bi-directional dose-dependent effect in studies with pain-free animals (Kokka and Fairhurst 1977; Woolf 1980; Ueda et al. 1986; Taiwo et al. 1989; Miaskowski et al. 1990) and in animals suffering from acute (Kayser et al. 1988; Iwasaki et al. 1991) or chronic (arthritic) (Kayser and Guilbaud 1981; Kayser et al. 1986; Kayser et al. 1987) pain.

A certain degree of analgesia may be attributed to the expectation of pain relief simply by virtue of drug administration (placebo analgesia). It has been consistently demonstrated that clinical pain relief may be achieved by administering a neutral agent that a patient believes has analgesic properties, and that this pain relief can be antagonised by high dose NLX (Levine et al. 1978). The possibility that the analgesia associated with antagonist administration was in fact the result of expectation, rather than a drug effect, is an important consideration. To distinguish antagonist analgesia from placebo analgesia, Levine and colleagues employed the use of a hidden programmable infusion pump that had previously been reported to eliminate placebo analgesia (Levine and Gordon 1986). The findings demonstrated selective analgesia produced by low-dose NLX, but also revealed that the hyperalgesia associated with higher doses was absent, suggesting that the increase in pain normally evident with higher doses is the result of blocked placebo analgesia (Levine and Gordon 1986).

Trials in clinical pain have confirmed the bi-directional analgesic effect of opioid antagonists (Levine et al. 1979). However, it has been noted that the effect of antagonist administration may depend upon the characteristics of the patient sample. Individual differences have been observed in the effect of antagonists on pain response. In most cases, variation in the activity of endogenous opioid systems is implicated. For example, in a study of post-operative dental pain that distinguished placebo responders from placebo non-responders, it was revealed that a NLX bi-directional dose-response was evident only in the cohort of placebo responders (Levine et al. 1979). In this group, the degree of analgesia associated with low-dose NLX (0.4 and 2 mg) was significantly greater than was associated with placebo analgesia. Amongst the placebo non-responders NLX had a minimal effect on pain response though, in comparison with the effect of placebo, NLX demonstrated a trend towards hyperalgesia.

The effects of antagonist administration on pain sensitivity have also been assessed according to variation in baseline pain response. Buchsbaum and colleagues divided a sample of healthy volunteers into two groups on the basis of basal sensitivity to electrical pain. NLX (2 mg) was associated with antinociception, but only in the cohort classified as pain sensitive. Those grouped as pain insensitive (i.e. higher baseline tolerance of pain) demonstrated a greater level of hyperalgesia to electric shock following NLX administration (Buchsbaum et al. 1977). These findings are in accord with a later animal study revealing that acute doses of NLX (0.1-0.2 mg/kg IV) were associated with hyperalgesia only in rats classified as 'good adaptors' to noxious stimuli (Satoh et al. 1979). In contrast, an investigation by Volavka and colleagues reported that among a cohort of healthy male volunteers, comparatively high acute doses of NLX (50 mg and 100

mg) were associated with analgesia in those participants with lower baseline pain threshold (Volavka et al. 1979).

Mechanisms proposed to explain the paradoxical antinociception observed with low concentrations of antagonist have included the selective blockade of a putative endogenous opioid system that is antagonistic to analgesia (Gillman and Lichtigfeld 1985; Gillman and Lichtigfeld 1989), and blockade of an endogenous dynorphin "anti-analgesia" system (Wu et al. 1983; Fujimoto and Rady 1989; Holmes and Fujimoto 1993).

1.9.2.3. Reduction in side effects with the addition of low-dose antagonist

Early reports demonstrated that the co-administration of an opioid antagonist in low doses with morphine could reduce opioid side effects. This was first established in animal models, with reports of the reversal of respiratory depression without a reduction in analgesia (Hensel et al. 1983). These findings were substantiated in subsequent trials with human pain patients. Brookshire and colleagues reported that the IV infusion of low-dose NLX (0.4 mg bolus, then 0.6 mg/hr for 23 hours) significantly decreased the incidence of pruritus, urinary retention and vomiting in 31 women receiving intrathecal morphine for painful labor (Brookshire et al. 1983). It was also demonstrated that intermittent administration (Korbon et al. 1983) or continuous infusion (Rawal et al. 1986) of low-dose NLX could reverse respiratory depression without reducing analgesia from epidural morphine. Gueneron and colleagues investigated whether reduced incidence of opioid side effects associated with NLX infusion could be extended to epidural fentanyl analgesia (Gueneron et al. 1988). While drowsiness, pruritus and nausea were reduced with the administration of a low dose NLX infusion, respiratory depression was not reversed. As anticipated, high dose NLX effectively reversed both opioid induced side effects and

analgesia. The authors proposed that the failure of low-dose NLX to reverse respiratory depression following epidural fentanyl was related to the differing lipid solubility of morphine and fentanyl.

An intriguing finding emerged from a 1997 study of 60 post-hysterectomy patients investigating the addition of a low-dose NLX infusion to morphine in patient controlled analgesia (PCA). It was revealed that the continuous infusion of low-dose NLX (0.25 μg/kg/hr) not only attenuated opioid side effects, but was also associated with reduced opioid requirements (42.3±24.1 mg) compared with morphine alone (59.1±27.4 mg) ($p<0.05$) over the 24-hour study period. Continuous infusion of a higher dose of NLX (1.0 μg/kg/hr) was associated with greater morphine requirements (64.7±33.0 mg); however this was not significantly different from morphine requirement without NLX (Gan et al. 1997). The authors proposed that the reduction in side effects may be due to different concentration-response curves for different opioid effects, that is, a lesser concentration of an opioid antagonist is required to antagonise effects such as nausea, vomiting and pruritus while a higher concentration is required to antagonise analgesia.

A 1999 study investigating the effect of varying doses of the antagonist nalmefene (15 or 20 μg IV) on the incidence of patient controlled morphine-related side effects in the post-operative period revealed a significant reduction in the need for anti-emetic and anti-pruritic medications among those receiving nalmefene as compared to placebo. There was no difference in total morphine consumption between the nalmefene and placebo groups, though patients who received nalmefene retrospectively reported less severe pain (Joshi et al. 1999).

Recent studies have also demonstrated that epidural naloxone can attenuate pruritus ($p<0.05$) and nausea ($p<0.05$) in post-hysterectomy patients (Choi et al. 2000) and intestinal hypomotility (constipation) in post-gastrectomy patients ($p<0.001$) (Lee et al. 2001) without compromising epidural morphine analgesia. There were no significant differences between groups in pain ratings in either of these investigations.

1.9.2.4. Enhanced analgesia and attenuation of opioid tolerance with co-administration of low-dose antagonist

The opioid sparing effect revealed in the study by Gan and colleagues (Gan et al. 1997) and the reduction in pain severity reported by Joshi and colleagues (Joshi et al. 1999) are perhaps not unexpected when considered in the context of, not only the reported analgesic actions of opioid antagonists, but also the large body of *in vitro* and rodent *in vivo* studies investigating the combined effects of opioid agonists and low-dose antagonists. Results from many of these studies have formed the basis of the bimodal opioid receptor model, the principal model postulated to account for observations of analgesic potentiation with co-administration of opioid agonists and ultra-low dose antagonists.

1.9.2.4.1. *In vivo* animal studies

Combining morphine with ultra-low (nanomole) doses of the opioid antagonist naloxone (1ng/kg) in mice has been found to increase the analgesic potency of morphine in a radiant heat tail flick test (Holmes and Fujimoto 1993). Acute, concomitant administration of IP morphine (1-3 mg/kg) and ultra-low dose naltrexone (10-100 ng/kg) in mice revealed significantly enhanced antinociception using the hot-water tail-flick assay, while chronic co-treatment with morphine (30-50 mg/kg) and low dose naltrexone (10 μg/kg) markedly attenuated the development of tolerance (Crain and Shen 1995; Shen and Crain 1997).

In a more recent series of investigations, the effect of the co-administration of ultra-low dose naltrexone on morphine antinociception was investigated following acute dosing, in attenuating the development of tolerance, and in reversing established tolerance (Powell et al. 2002). Responses to thermal (radiant heat tail-flick test) and mechanical (paw pressure test) nociceptive stimuli were assessed in rats, using both systemic and spinal (IT) routes of administration. Acute administration of IT (0.05 and 0.1 ng) or systemic (10 ng/kg IP.) naltrexone enhanced the antinociception associated with an acute submaximal dose of IT (5 μg) or systemic (7.5 mg/kg) morphine in the tail-flick test (results for the mechanical stimuli were not reported). Chronic IT (0.005 and 0.05 ng) or systemic (10 ng/kg) naltrexone combined with IT (15 μg) or systemic (15 mg/kg) morphine over a 7-day period inhibited the decline in morphine analgesia and prevented the loss of morphine potency in both nociceptive tests.

In studying the reversal of established tolerance by naltrexone, tolerance was induced by administration of IT (15 μg) or systemic (15 mg/kg) morphine daily over 5 or 7 days for the IT and systemic groups, respectively. Subsequent IT (0.05 ng) co-administration of naltrexone produced a progressive recovery of antinociception to approximately 70% of baseline in thermal stimuli, and 50% of baseline in mechanical stimuli, by day 10. Systemic co-administration of naltrexone also significantly restored antinociception. However, the 10 ng/kg dose was significantly more effective than the 50 ng/kg. In both attenuating and reversing the development of tolerance, IT administration of morphine and naltrexone was significantly more effective than systemic administration, suggesting that this effect is expressed at a spinal level, or that the peripheral effects of naltrexone may interfere following systemic administration.

1.9.2.4.2. *In vitro* studies: the basis of the bimodal opioid receptor model for enhanced opioid analgesia with ultra-low antagonist

Classically, opioids exert their effects by binding with specific receptors belonging to the superfamily of G-protein coupled receptors, interacting preferentially with receptors linked to pertussis-toxin (PTX)-sensitive G_i/G_o-proteins (see 1.6.5). At the cellular level, opioids inhibit voltage-gated calcium channels, activate potassium channels and inhibit adenylate cyclase (North and Williams 1983; Ikeda et al. 1995). The overall result of this activity is neuronal inhibition and analgesia.

The inhibitory effects of G_i/G_o–coupled receptor activation, such as shortening of the calcium component of the action potential and inhibition of neurotransmitter release, provide a cellular model of opioid analgesia (Mudge et al. 1979; Werz and Macdonald 1983). In nociceptive type dorsal root ganglion (DRG) neurons in culture it has been demonstrated that micromolar and nanomolar concentrations of opioid agonists decrease and increase action potential duration (APD), respectively (Chen et al. 1988; Shen and Crain 1989). The mechanism proposed to explain this dual action has been termed the "bimodal opioid receptor model" (Crain and Shen 2000). Electrophysiological studies of the effects of opioids on nociceptive types of DRG neurons in culture have revealed that a subgroup of opioid receptors is coupled to an excitatory second messenger system. These studies have established that treatment of these DRG neurons with extremely low concentrations (picomolar to nanomolar) of an opioid agonist elicits excitatory effects, such as prolongation of the APD, which are mediated by a cholera-toxin (CTX)-sensitive G_s-coupled opioid-receptor (Crain and Shen 1995) (see Figure 1-3). This G_s-coupled mode of receptor is reported to elicit excitatory effects via an adenylate cyclase/ cAMP/ protein kinase A-mediated transduction system. These excitatory effects may attenuate the

inhibitory effects of concurrent activation of G_i/G_o-coupled opioid receptors on these cells (see Figure 1-3). The excitatory effects are typically overlooked as they are masked by the inhibitory effects of the higher concentrations (micromolar) used therapeutically.

Several opioid alkaloids and peptides have been identified as selective antagonists of excitatory G_s-coupled opioid receptor functions, including clinically used opioid antagonists such as NTX and NLX, as well as opioid agonists such as the potent analgesic etorphine. At therapeutic (millimolar) concentrations, agents such as NTX and NLX antagonise activity at the inhibitory G_i/G_o-coupled opioid receptors, while etorphine is a robust agonist at these receptors. When present in extremely low (picomolar) concentrations, however, these agents have been shown to selectively antagonise excitatory, but not inhibitory, opioid-receptor mediated functions in DRG. It is postulated, then, that the enhanced analgesia associated with low- or ultra-low doses of an opioid antagonist is a result of this selective blockade of excitatory, anti-analgesic opioid effects.

It should be noted that while this model may account for the enhanced antinociception apparent when morphine is co-administered with ultra-low dose antagonist, it does not readily account for all the paradoxical actions observed with low or ultra-low dose antagonists. For example, it does not explain the antinociceptive action of low dose antagonists administered alone. In response to this, it may be argued that the putative endogenous pronociceptive opioid system proposed by earlier authors (Gillman and Lichtigfeld 1985; Gillman and Lichtigfeld 1989) is, in fact, the activation of excitatory G_s-coupled receptors by endogenous opioids, which is selectively antagonised by low concentrations of an antagonist, thus enhancing the antinociceptive action of the endogenous system. The potential clinical benefit of opioid agonist-antagonist combination

depends on the enhanced analgesia occurring without a simultaneous increase in adverse effects. The bimodal opioid receptor model would not predict the selective enhancement of only the antinociceptive effect of opioids without an increase in other opioid effects. While there have been reports of enhanced analgesia without a simultaneous increase in adverse effects (see section 1.9.2.4.4), this approach has not been well studied in humans.

1.9.2.4.3. Putative role of excitatory G_s-coupled opioid receptors in the development of tolerance

It has been suggested that activation of G_s-coupled receptors and the resultant excitatory effects may provide insight into the mechanisms underlying opioid tolerance and hyperalgesia. As described previously, the mechanisms underlying opioid tolerance are not fully understood. One mechanism that has been proposed is that sustained opioid exposure increases excitatory G_s-coupled receptor binding, leading to a reduction in agonist potency (Crain and Shen 1998). GM1 ganglioside is an endogenous glycolipid occurring in abundance on the surface of neuronal cells. Recent studies with cloned opioid receptors indicate that these receptors are readily converted from the inhibitory G_i/G_o-coupled mode to excitatory G_s-coupled mode when the concentration of GM1 ganglioside on the surface of the neuron increases (Wu et al. 1997; Wu et al. 1998). GM1 ganglioside levels are regulated by a cAMP/protein kinase A-dependent glycotransferase (Scheideler and Dawson 1986), which may be activated following G_s-mediated increases in cAMP and protein kinase A (Crain and Shen 1990; Crain and Shen 1992). A positive feedback loop is therefore postulated, whereby G_s-coupled receptor binding increases cAMP and PKA, which can result in an increase in GM1 ganglioside levels, triggering the conversion of additional inhibitory G_i/G_o-coupled receptors to excitatory G_s-coupled mode, and so forth. Thus GM1 ganglioside is considered to play a key role in the regulation of G_s-coupled

excitatory receptor function and, via this positive feedback loop, a critical role in the modulation of opioid analgesia, tolerance and dependence (Crain and Shen 1998; Shen and Crain 2001) (Figure 1-3).

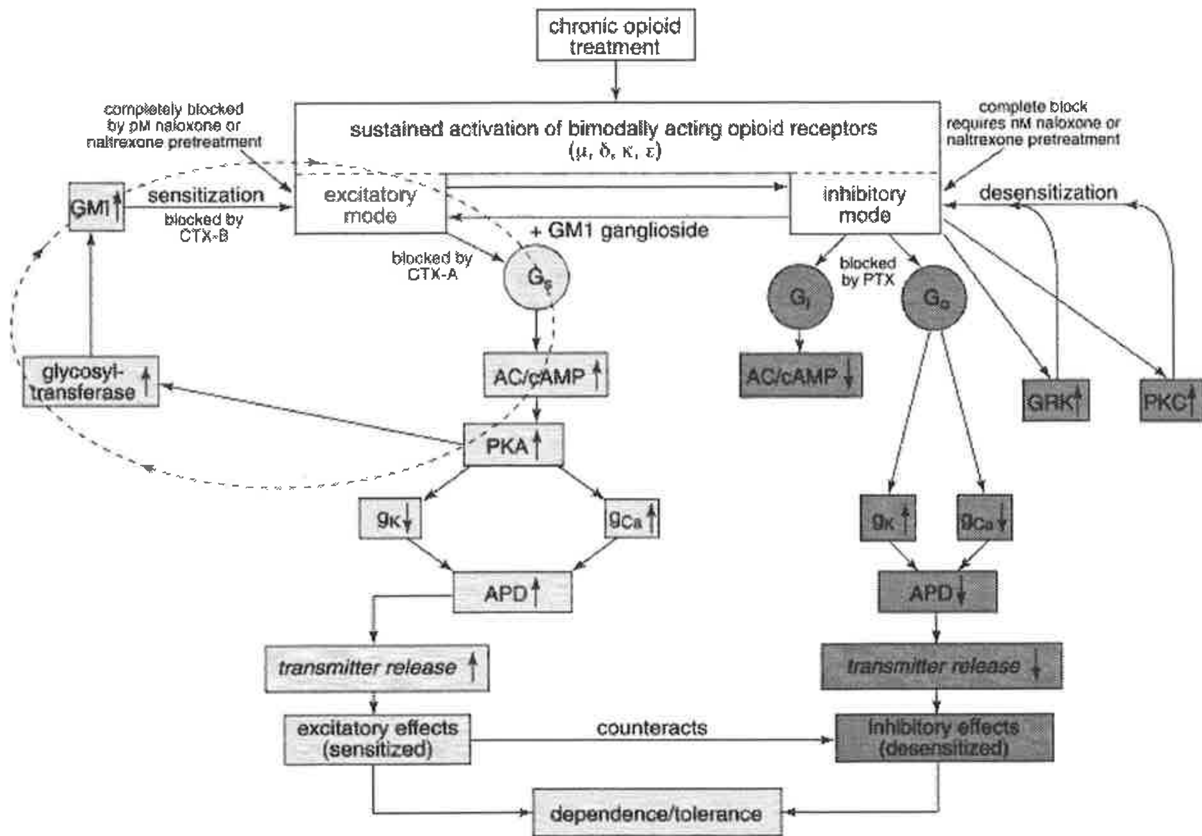

Figure 1-3. Bimodal opioid receptor model, including GM1 ganglioside-induced interconversion of opioid receptors between the G_i-G_o-coupled inhibitory mode (right) and the G_s-coupled excitatory mode (left) in sensory DRG neurons. Note the sharply contrasting linkages of these G_i-G_o-coupled receptors compared with G_s-coupled receptors to K+ and Ca2+ conductances (gK and gCa), which control action potential duration (APD) and transmitter release in pre-synaptic terminals of nociceptive neurones involved in opioid analgesic systems CTX-A, CTX-B: A and B subunits of cholera toxin. (reproduced from Crain and Shen, 1998).

Studies have demonstrated increased sensitivity of DRG neurons to the excitatory effects of opioid agonists following brief treatment with low concentrations of GM1 ganglioside (Shen et al. 1991). This is thought to be due to GM1 ganglioside binding with an allosteric regulatory site on opioid receptors (Shen and Crain 1990), and thereby enhancing the

efficacy of excitatory G_s-coupled opioid receptor functions (Crain and Shen 1998). This is consistent with evidence that injection of low doses of GM1 in mice reduces the analgesic effect of morphine, producing what has been termed "acute tolerance" (Crain and Shen 2000).

Furthermore, unlike most G-protein coupled receptors that become desensitised with chronic exposure to an agonist, G_s-coupled opioid receptors become increasingly sensitised during sustained exposure of DRG neurons to bimodally acting opioid agonists (Crain and Shen 1992). Indeed, G_i/G_o-coupled receptors have been shown to become progressively desensitised by activation of G-protein receptor kinases and arrestins, and by activation of PKC (Harris and Williams 1991; Ueda et al. 1995; Chuang et al. 1996), and it is thought that this may also contribute to the development of tolerance with chronic opioid treatment.

1.9.2.4.4. Human models of analgesic potentiation with low-dose antagonist

Conflicting findings have emerged from human studies investigating the use of opioid antagonists in conjunction with opioid agonists to enhance analgesia (summarised in Table 1-3). In contrast to the opioid-sparing effect reported by Gan and colleagues (Gan et al. 1997) (see 1.9.2.3), Cepeda and colleagues reported no decrease in opioid requirements combining low-dose (Cepeda et al. 2002) or ultra-low dose (Cepeda et al. 2004) NLX with morphine in patient controlled analgesia (PCA) during the post-operative period. Low-dose NLX (an average of 0.5 μg/kg/hour in the first hour, followed by an average of 0.06 μg/kg/hour afterwards) combined with morphine did not produce a decrease in opioid requirements, and pain intensity ratings were in fact higher in the group receiving NLX (Cepeda et al. 2002). However, this study was limited in that only one dose of NLX was

used. Recent findings (see below and Table 1-3) indicate that both the dose of agonist and the agonist:antagonist ratio are crucial in producing potentiation. In a more recent study, the same group trialled the same methodology utilising a lower dose of NLX. In this study patients received an average of 0.05 μg/kg/h NLX in the first 2 hours, and an average of 0.009 μg/kg/h subsequently. At this dose, it was revealed that NLX still failed to reduce opioid requirements, but significantly decreased the incidence of opioid side effects (Cepeda et al. 2004).

A case study of a 61-year old diabetic with painful polyneuropathy revealed that ultra-low dose naltrexone potentiated the analgesia afforded by methadone, and decreased the incidence and severity of side effects (Cruciani et al. 2003).

Several studies have also investigated other opioid agonists in combination with low dose antagonists. There have been reports of NLX potentiating the effects of opioids that mediate antinociception via other opioid receptor types, such as the κ partial agonists nalbuphine and pentazocine. In an early investigation, analgesia produced by morphine (8 or 15 mg) and pentazocine (60 mg) administered individually and in combination with low-dose NLX (0.4 mg) were compared (Levine et al. 1988). Analgesia produced by pentazocine (60 mg) was potentiated by the addition of NLX, while morphine analgesia (8 mg) was attenuated with the addition of the antagonist. The analgesia associated with the combination of pentazocine and NLX was significantly greater than was associated with administration of either NLX, pentazocine or high-dose morphine (15 mg) alone. Patient use of diazepam was considered a potentially confounding effect in these results; therefore a correlative study was conducted with rats. Findings from the animal study substantiated the results of the clinical trial. More recent investigation has focused on another κ agonist,

nalbuphine. It has been reported that the addition of low-dose NLX (itself not associated with analgesia) to low-dose nalbuphine produced marked post-operative analgesia (Gear et al. 2000), and a report of three case studies describes improved relief of neuropathic trigeminal pain with the combination of nalbuphine and low-dose NLX (Schmidt et al. 2003).

Study	Sample	Design	Agonist	Antagonist (dose)	Route	Outcome measure	Outcome
Gan et al., 1997	Post-surgical patients (hysterectomy) *(n=60)*	DB RCT	Morphine (PCA)	Low (0.25 μg/kg/h) or high (1.0 μg/kg/h) dose NLX. Continuous infusion	IV	Cumulative morphine requirement over 24h period (PCA)	35% reduction in nausea and vomiting, 30% reduction in pruritus ($p<0.05$), no difference in respiratory rate Morphine requirement reduced by one third (59.1 mg to 42.3 mg) in low-dose NLX group($p<0.05$) High dose NLX group had higher morphine requirements ($p>0.05$)
Joshi et al., 1999	Post-surgical patients (lower abdominal surgery), females only *(n=120)*	DB RCT	Morphine (PCA)	Nalmefene (15 mg or 25 mg)	IV	Cumulative morphine requirement in 1st 24 h post-surgery Anti-emetic and anti-pruritic drug requirement Retrospective pain intensity	No difference in morphine requirement ($p>0.05$) Reduced retrospective rating of pain intensity ($p<0.05$). Reduced need for antiemetic and antipruritic medication ($p<0.05$)
Cepeda et al., 2002	Post-surgical patients (abdominal, thoracic, orthopedic surgery of < 3 h duration) *(n=166)*	DB RCT	Morphine (PCA)	NLX (average of 0.5 μg/kg/h 1st 2 hours; 0.06 μg/kg/h subsequently) intermittent infusion (administered in combination with morphine)	IV	Cumulative morphine requirement in 1st 24 h post-surgery (PCA) Pain intensity ratings Patient satisfaction with pain treatment Side effects	Morphine requirement *higher* in morphine+NLX group (15.6 mg) compared to morphine group (13.4 mg) ($p=0.009$) More treatement failures due to inadequate analgesia in morphine+NLX group (n=38) compared to morphine group (n=22)($p=0.025$) Higher percentage of patients in naloxone group not satisfied with pain treatment ($p=0.01$) No differences between groups in side effects ($p>0.05$)
Cepeda et al., 2004	Post-surgical patients (abdominal, thoracic, orthopedic, craniofacial surgery) of < 3 h duration) *(n=265)*	DB RCT	Morphine (PCA)	NLX (average of 0.05 μg/kg/hr in 1st 2 hours, 0.009 μg/kg/hr subsequently) Intermittent infusion (in combination with morphine)	IV	Cumulative morphine requirement in 1st 24h post-surgery (PCA) Pain ratings	No difference in morphine requirement between morphine+naloxone (25.4 mg) and morphine (27.0 mg) groups ($p>0.05$) Nausea and pruritus reduced in NLX group ($p=0.01$) Other side effects similar between groups
Cruciani et al., 2003	61-year old diabetic, painful neuropathy *(n=1)*	Case report	Methadone	NTX	Oral	Pain ratings Methadone dose Side effects	Potentiated analgesia Decreased incidence and severity of opioid side effects (NB Case report)
Levine et al. 1988	Post-surgical patients (removal of impacted third molars) *(n=105)*	DB RCT	Pentazocine (60 mg) Morphine (8 mg combined with NLX & 15mg alone)	NLX (0.4 mg)	IV	Pain ratings	Pentazocine + NLX produced greater analgesia than pentazocine ($p<0.01$) and high-dose morphine (15 mg) ($p<0.01$) Significant enhancement maintained at end of study (3 h 10 min post-dosing) ($p<0.01$) Addition of NLX attenuated morphine (8 mg) analgesia ($p<0.05$)
Gear et al. 2000	Post-surgical patients (removal of impacted third molars) *(n=88)*	DB RCT	Nalbuphine (5 mg)	NLX (0.4 mg)	IV	Pain ratings, males vs. females	Nalbuphine+NLX associated with enhanced analgesia in both males and females compared to either nalbuphine or NLX alone ($p=0.0001$)
Schmidt et al., 2003	Trigeminal neuropathic pain *(n=3)*	Case report	Nalbuphine	NLX	IV	Pain ratings	Nalbuphine + NLX reduced pain ratings by 50% compared to prior treatment (varied) (NB case report)

DB Double-blind RCT Randomised controlled trial PCA Patient-controlled analgesia

Table 1-3. Summary of clinical reports and human studies of potentiation of analgesia with opioid agonist:antagonist combinations.

1.9.3. Limitations of the human studies

The inconsistencies associated with the findings from human studies of analgesic potentiation with opioid agonist:antagonist combinations may be related to differences in trial methodology, outcome measures and doses used. For example, Gan and colleagues showed analgesic potentiation with the administration of NLX by continuous infusion (Gan et al. 1997), whereas Cepeda and colleagues administered NLX intermittently in combination with morphine and detected no enhancement of analgesia (Cepeda et al. 2002; Cepeda et al. 2004). There were also differences between studies in the amount of NLX administered. The average NLX dose administered to patients in the first 2 hours of the initial study by Cepeda and colleagues (0.5 μg/kg/h) (Cepeda et al. 2002) was midway between the low (0.25 μg/kg/h) and high (1.0 μg/kg/h) doses used by Gan and colleagues, which were associated with lower and higher morphine requirements, respectively. However, the amount of NLX administered by Cepeda and colleagues was decreased to an average of 0.06 μg/kg/h after the initial 2 hours of observation (Cepeda et al. 2002). It has also been noted that the amount of NLX administered by Cepeda and colleagues was higher than the equipotent dose of nalmefene used by Joshi and colleagues (Joshi et al. 1999), which was associated with enhanced analgesia (Mehlisch 2003). The subsequent study by Cepeda and colleagues aimed to investigate whether the failure to detect enhanced analgesia was related to the comparatively higher doses of NLX administered (Cepeda et al. 2004). In this study, patients received an average of 0.05 μg/kg/h in the first 2 hours, and an average of 0.009 μg/kg/h subsequently (compared to an average of 0.5 and 0.06 μg/kg/h administered in the initial study, as described above). While a decrease in side effects was observed with this combination, there was no evidence of analgesic potentiation.

Significant variation in average cumulative morphine requirement between studies has also been noted (Mehlisch 2003). The average amount of morphine administered in the 24 hours post-surgery in the first study by Cepeda and colleagues (Cepeda et al. 2002) (13.4-15.6 mg) was considerably lower than used over an equivalent time period by post-hysterectomy patients in the studies by both Gan and colleagues (42.3-64.7 mg) (Gan et al. 1997) and Joshi and colleagues (45-56 mg) (Joshi et al. 1999). It has been proposed that the difference in morphine requirement is indicative of fundamental differences between studies in terms of type of surgical procedure, level of pain associated with the procedure, and/or method of drug administration (Mehlisch 2003). Cepeda and colleagues have defended the design of their study, responding that morphine requirements during the first 2 hours post-surgery (10.7-12 mg) were similar to those reported by Joshi and colleagues (8 mg), and that differences in opioid consumption subsequent to this are likely due to the PCA dose used (0.5 mg rather than 1.0 mg) (Carr and Cepeda 2003). Notwithstanding, both human studies that have shown enhanced analgesia with the morphine and NLX combination recruited patients undergoing the same surgical and anaesthetic procedures (Gan et al. 1997; Joshi et al. 1999), whereas both studies that failed to demonstrate analgesic potentiation recruited patients undergoing a wide range of different surgical procedures (Cepeda et al. 2002; Cepeda et al. 2004). The relative advantages and disadvantages of these approaches are discussed below. Further, those studies reporting enhanced analgesia administered the antagonist by continuous infusion (Gan et al. 1997). In contrast, studies reporting no analgesic potentiation administered NLX in combination with morphine in intermittent boluses (Cepeda et al. 2002; Cepeda et al. 2004). The implications of this discrepancy are discussed below.

There are several limitations associated with the human studies of analgesic potentiation using opioid agonist:antagonist combinations. All studies were conducted with clinical

pain rather than experimental pain. As discussed previously, the validity of experimental pain, particularly in assessing analgesic medication, has been a challenging and contentious issue amongst clinicians and researchers (Keats et al. 1950; Beecher 1953; Moore et al. 1997). It has been argued that experimental pain is qualitatively different from pain associated with injury or disease (Beecher 1962). While it is recognised that the effectiveness of an analgesic intervention must be assessed in the circumstances for which it is intended, there is a strong argument that the measurement of dose-response, evaluation of optimal dosage and comparison of relative efficacy with known substances are best conducted with a sample of healthy volunteers who are as homogeneous as possible (Bromm 1985).

There are two broad justifications for the use of an experimental pain paradigm *prior* to clinical evaluation of agonist:antagonist combinations. Firstly, clinical pain involves considerably more variability in both patient characteristics and pain experience. As described in Chapter 3, the experience of pain is subject to considerable inter- and intra-individual variability. Numerous factors have been shown to impact upon the pain experienced and reported in an experimental or clinical setting. In an experimental context, factors such as sex, age, ethnicity, psychological variables, current or past substance dependence, presence of medical conditions, cigarette smoking, previous chronic exposure to opioids and history of chronic pain can be better controlled for in participant recruitment. Importantly, the type and intensity of noxious stimuli can also be standardised. In a clinical context, not only is it considerably more difficult to control for these factors, but even greater variability is introduced. The type of pain frequently investigated in trials with clinical populations is post-surgical pain, and indeed all randomised trials of agonist:antagonist combinations for enhanced analgesia have assessed this type of pain. The use of post-operative pain introduces numerous sources of

variability. These may include fear of surgery and associated stress, differing anaesthetic techniques and recovery from the anaesthetic procedure, prior experience with surgical procedures, concomitant medication (both administered for surgery and for coexisting medical conditions) and the purpose and type of surgical procedure itself, incorporating factors such as time in theatre and differences in baseline pain, pain duration and general discomfort typically associated with a certain procedure. Furthermore, side effects (which have been used as an outcome measure indicative of treatment quality) observed in the post-operative period may be related to the surgery, the anaesthetic technique or concomitant medications rather than the analgesic agent(s) administered. Several of the studies in question attempted to reduce potential sources of variability by recruiting patients who had undergone equivalent surgical and/or anaesthetic procedures (Levine et al. 1988; Gan et al. 1997; Joshi et al. 1999; Gear et al. 2000). An alternative strategy is to recruit patients who have undergone surgical procedures lasting within a certain time range, ostensibly as time in theatre provides a degree of standardisation of the gravity of an operation, anticipated degree of post-surgical pain or recovery process (Cepeda et al. 2002; Cepeda et al. 2004). Despite these approaches to reducing variability, the use of clinical pain significantly reduces the investigator's control over the many sources of variability within a sample.

It has been argued that recruiting patients who have undergone a wide range of surgical procedures enhances the generalisability of the findings and provides a more naturalistic study in terms of the likely clinical utility of the intervention (Carr and Cepeda 2003). However, in the case of agonist:antagonist combinations for enhanced analgesia, where application to humans remains in the preliminary stages and "proof of concept" is yet to be adequately demonstrated, it may be considered that establishing optimal dose and agonist:antagonist ratio using experimental pain would be preferable. Indeed, the findings

from one human study that failed to detect analgesic potentiation with the agonist:antagonist combination have been censured for utilising a flawed methodology (Mehlisch 2003). It has been argued that this study (Cepeda et al. 2002), which aimed to investigate the use of agonist:low-dose antagonist combinations using intermittent administration (described in section 1.9.2.4.4) suffered a number of significant flaws both in terms of departure from the methodology of previous studies and confounding factors that were not adequately controlled (Mehlisch 2003).

The second justification for using an experimental pain paradigm prior to clinical pain trials in the investigation of agonist:antagonist combinations relates to the importance of identifying the optimal agonist dose and agonist:antagonist dose ratio. As discussed in greater detail below (see 1.9.4), animal studies have demonstrated that both the dose of agonist administered and the agonist:antagonist ratio are critical in producing antinociceptive potentiation. While it is recognized that the optimal dose ratio in managing clinical pain may be different from that demonstrated experimentally, in a clinical pain setting it is considerably more difficult to establish dose-response curves for the agonist alone and in combination with different antagonist ratios. The human studies of agonist/antagonist combinations for enhanced analgesia have been limited by a failure to vary the antagonist dose relative to the agonist dose, or have at best administered either "high" or "low" antagonist doses (Gan et al. 1997). Furthermore, the use of PCA introduces additional variability in terms of the amount of each agent administered to each patient. PCA has been used in several studies examining agonist/antagonist combinations in analgesia, offering the advantage of an accurate representation of common clinical procedure, and the useful outcome measure of a reduction in opioid requirements to indicate analgesia. However, in studies that have administered the antagonist in continuous infusion (Gan et al. 1997) irrespective of patients' use of morphine, the ratio

would differ between patients according to their use of the agonist. In other studies, the antagonist has been combined with morphine, such that the antagonist was administered simultaneously with the morphine and only if the morphine was accessed. In these circumstances, while the ratio is consistent between patients, the absolute amount of both agonist and antagonist received varies according to the need for additional doses. Moreover, if the ratio is not optimal, such that the dose of antagonist is too high relative to the agonist, as has been postulated in one study (Cepeda et al. 2002), it is conceivable that the antagonist may attenuate analgesia, resulting in an increase in reported pain intensity and the need for another bolus, thereby further enhancing the hyperalgesia. The intermittent administration of low-dose antagonist according to the methodology of Cepeda and colleagues (Cepeda et al. 2002) has also provoked criticism (Mehlisch 2003) as it has been demonstrated in acutely and chronically morphine treated rodents that intermittent administration of a low-dose antagonist precipitates long-lasting hyperalgesia (Celerier et al. 1999; Shen and Crain 2001). Indeed, the 2002 study by Cepeda and coworkers that used intermittent antagonist dosing reported that patients admininstered morphine plus naloxone experienced less pain relief and used more opioids than those administered morphine plus saline (Cepeda et al. 2002).

The valid assessment of outcome measures is complicated further by the requirement in some protocols of the mandatory administration of a rescue dose when reported pain intensity exceeded a set point, and that the PCA dose be augmented by 20%. These studies also incorporated a mandatory decrease in PCA dose by 20% if side effects attributed to the PCA were experienced (Cepeda et al. 2002; Cepeda et al. 2004). While these measures are reasonable for ethical reasons, they further complicate the valid comparison between groups. In these studies, the requirement for rescue doses and the incidence of side effects were recorded. However, as noted by Mehlisch (2003), side effects may have been related

to other factors (i.e. the surgical procedure, the anaesthetic procedure or concomitant medication). Thus rescue doses may have been administered when they were not, in fact, required.

A further concern is the difference in time course and duration of effect associated with the agonist and antagonist administered, and the lack of control over the timing of drug administration in clinical pain settings.

In summary, the use of agonist:antagonist combinations for enhanced analgesia in humans has produced inconsistent findings. It is proposed that differences in trial methodology, outcome measures and doses used may account for these differences. Moreover, the failure to assess a wide range of dose ratios and the degree of variability and lack of control associated with the clinical pain trials described, reduces the capacity for valid assessment between treatment groups and may prevent the observation of enhanced analgesia. There have been no published studies of opioid agonists combined with ultra-low or low-dose antagonists in a human experimental pain paradigm.

Given the importance of agonist dose and agonist:antagonist ratio in observing analgesic potentiation, it is proposed that the investigation of agonist/antagonist combinations be first assessed in an experimental pain paradigm with healthy volunteers to establish the optimal opioid dose and agonist:antagonist ratio. In an experimental setting many of the sources of variability that complicate assessment in clinical pain studies may be controlled.

1.9.4. Buprenorphine and antagonist combinations

Buprenorphine (BUP) is an opioid receptor partial agonist with potent analgesic effects (see 4.2). Chronic exposure to BUP may be associated with mild physical dependence,

with limited withdrawal signs and symptoms. The adverse effects of BUP are similar to other opioid agonists. Findings suggest that BUP may have a ceiling effect for respiratory depression and other effects in humans. Thus, it may be safer than other opioids (Walsh et al. 1995).

There have been very few investigations of BUP in combination with a low-dose antagonist. An early investigation reported that prior treatment of rats with NLX significantly *reduced* the analgesic action of low-dose BUP in a tail flick assay (Rance et al. 1980). A subsequent study demonstrated that pretreatment with NTX shifted the BUP analgesic dose-response curve to the right (Dum and Herz 1981). Following a report describing potent analgesia resulting from the addition of NLX in two patients recovering from cholecystectomy (Pedersen et al. 1985), Bergman and colleagues conducted a trial investigating this drug combination in a rabbit tooth pulp assay. This study revealed enhanced antinociception with the combination of NLX (0.001 mg/kg IV) and BUP (0.10 mg/kg IV), with a peak % maximum possible effect (MPE) of 78% compared to 48% produced with BUP only ($p<0.05$). NLX at a higher dose (0.1 mg/kg) did not alter antinociception ($p>0.05$).

There have been no published investigations of BUP combined with ultra-low or low-dose antagonists in humans.

While the utility of opioids in the management of neuropathic pain is controversial (Portenoy et al. 1990; McCormack 1999), mounting evidence suggests that BUP may have a special role in this capacity (Benedetti et al. 1998; McCormack et al. 1998; Kouya et al. 2002; Radbruch 2003). Given this potential for BUP in the treatment of neuropathic pain, Cougnon-Aptel and colleagues (Cougnon-Aptel et al., unpublished) recently investigated

the use of BUP combined with low-dose opioid antagonists in rats with peripheral neuropathy induced by the chronic constriction injury model (CCI) (Bennett and Xie 1988). This model involves loose ligation of the sciatic nerve to induce neuropathy. Over the 7-10 days following ligation, animals develop pain behaviours including thermal and mechanical hyperalgesia and allodynia in the hindpaw ipsilateral to the site of nerve ligation. These behaviours can persist for up to 7 weeks following ligation (Bennett and Xie 1988; Attal et al. 1990). Nociceptive testing commenced one week following nerve ligation, and following confirmation of neuropathy. Thermal nociceptive threshold, as determined by hindpaw withdrawal latency, was assessed for increasing doses of BUP, and two BUP:NLX ratios, 15:1 and 20:1.

As described in Chapter 4, one of the most intriguing features of the pharmacological profile of BUP is the bell-shaped dose-response curve observed with many effects, including antinociception in animal models. Antinociception peaked at a dose of 20 μg/kg, decreased to a trough at 40 μg/kg, but then increased at 50 μg/kg, which may be indicative of the biphasic dose-response curve that has previously been associated with BUP (see Cowan 1995). The combination of BUP and naloxone was associated with significantly enhanced antinociception, but this effect was dependent upon the BUP dose (Cougnon-Aptel et al. unpublished). Interestingly, the combination was associated with significant antinociception only when the dose of BUP alone produced minimal or no antinociception. This was evident at both ends of the BUP dose-response curve. When the dose of BUP alone was associated with significant antinociception, the addition of naloxone reduced the magnitude of antinociception, ostensibly having an antagonistic effect. The enhanced antinociception associated with the drug combination was observed with both ratios, though the greatest effect was evident with the 15:1 ratio. In light of the suggestion in previous findings that reduced opioid requirements can occur *without* a simultaneous

increase in adverse effects (Rawal et al. 1986; Gan et al. 1997), the potentiation evident at the lower end of the BUP dose-response curve is of particular clinical relevance. The optimal dose of BUP in the lower range of the dose-response curve associated with enhanced antinociception when combined with NLX was 2.5 μg/kg. This dose was then tested in combination with NLX and the long-acting opioid antagonist naltrexone, as well as morphine and naloxone combinations, in a range of ratios. The BUP:naloxone combination in a 15:1 ratio was the most effective in enhancing antinociception. These data indicate that both the BUP dose and the agonist:antagonist ratio are critical in producing potentiation of BUP antinociception by ultra-low doses of antagonist.

1.10. Summary

The negative consequences of pain management are costly to the community and associated with increased morbidity for the patient. While opioid analgesics are highly effective in the management of pain, they are associated with a number of unpleasant and dangerous adverse effects that limit their use. One approach by which the use of opioids may be improved is the administration of an opioid in combination with another agent that either itself produces analgesia or potentiates the analgesia associated with the opioid. A lower dose of the opioid is therefore required to achieve an adequate level of analgesia, thus reducing the incidence and severity of adverse effects.

The combination of an opioid agonist with ultra-low dose opioid antagonist has been reported to potentiate the pain relieving effect of the opioid in animal studies and in some clinical investigations. Critical to the clinical utility of this drug combination is whether the adverse effects are potentiated in the same manner as has been observed with pain relief. Previous experience with the addition of low doses of opioid antagonists to opioid treatment suggests that adverse effects may be reduced without a simultaneous reduction in

analgesia, or unaffected despite enhanced analgesia. If the combination of an opioid agonist and ultra-low dose antagonist can potentiate analgesia without a simultaneous increase in adverse effects, the negative effects that limit the use of opioids in pain management may potentially be overcome.

BUP is a highly potent opioid analgesic, with low abuse liability and a potentially better safety profile than other opioid analgesics such as morphine. BUP is also considered to have potential in the management of neuropathic pain, for which other opioids have been less effective (Kouya et al. 2002). Recent animal data suggest that BUP antinociception may be potentiated with the addition of ultra-low dose NLX in a model of neuropathic pain (Cougnon-Aptel et al. unpublished). The antinociceptive effect of BUP combined with ultra-low doses of an opioid antagonist has not been investigated in humans.

1.11. The present research

The broad aim of the present research was to determine whether BUP combined with ultra-low doses of NLX enhances antinociception in a human model of experimental pain. A further aim was to determine whether any antinociceptive potentiation observed with the drug combination was associated with a simultaneous increase in the incidence or severity of adverse opioid effects, including respiratory depression, sedation and nausea.

Due to the ratio-dependent nature of the enhanced antinociception observed in the animal study of BUP:NLX combinations (Cougnon-Aptel et al., unpublished), a further aim was to identify the optimal ratio for antinociceptive potentiation, if this occurred.

This thesis will describe four studies, which are organised by chapter:

1. Normative study of the nociceptive tests (Chapter 3)

The nociceptive tests to be used in this series of trials had not been standardised, and normative values had not been established. Furthermore, numerous variables have been reported to contribute towards inter-subject variability in response to experimental noxious stimuli, and it is unclear to what extent these factors should be controlled for in subject recruitment. The purpose of the initial study was to determine normative values for the two nociceptive tests to be used, the cold pressor and electrical stimulation tests, and to assess the contribution of a range of biological and psychosocial variables to inter-individual variability in test performance. The findings from this study guided subject selection and exclusion criteria for the subsequent drug studies.

2. Buprenorphine dose-finding study (Chapter 4)

Unlike many other opioid analgesics, BUP had not been evaluated in a human experimental pain paradigm. A dose-finding study was required to determine whether the nociceptive tests to be used are sensitive assays for BUP antinociception, and to identify a sub-analgesic dose of BUP to be used in the subsequent BUP:NLX ratio studies.

3. Antinociceptive activity of buprenorphine and naloxone combinations (Chapter 5)

The effects of BUP combined with NLX were compared with the effect of BUP combined with saline. The BUP:NLX combination was administered in the following ratios: 15:1, 20:1 and 25:1. These ratios were selected on the basis of findings from the BUP:antagonist animal study (Cougnon-Aptel et al., unpublished).

4. Optimising the BUP:NLX ratio for antinociception (Chapter 6)

Based on the results of the first ratio study, a second BUP:NLX ratio study was conducted to further investigate the effects of this drug combination over a wider range of ratios.

This study investigated the effects of BUP combined with NLX in a 5:1, 10:1 and 12.5:1 ratio, compared to the effects of BUP combined with saline.

1.11.1. Aims

CHAPTER 3:

1. To establish normative values for healthy volunteers on the cold pressor and electrical stimulation tests.
2. To establish an upper and lower limit in baseline pain response to guide subject selection in subsequent studies.
3. To determine the factors that contribute to intra-individual variation in pain response, and may thus be important to control in subject recruitment.

CHAPTER 4:

1. To determine whether the pain induction tests selected for use in these studies are sensitive assays for BUP antinociception.
2. To determine a BUP dose-response curve for doses in the lower range and below therapeutic doses for analgesia
3. To select a sub-antinociceptive dose of BUP for use in the subsequent BUP:NLX ratio studies.

CHAPTER 5

1. To investigate whether the addition of ultra-low doses of NLX significantly enhances antinociception to experimental pain compared to the same dose of BUP alone in healthy, pain-free volunteers

2. To determine whether the addition of ultra-low doses of NLX significantly enhances adverse opioid effects compared to the same of BUP alone in healthy, pain-free volunteers

3. To identify the optimal BUP:NLX ratio for enhanced antinociception to experimental pain.

CHAPTER 6

1. To investigate further the effect of BUP:NLX combinations on antinociception and adverse effects.

2. General Methods

2.1. Introduction

This project incorporated four studies: a normative study of two experimental pain induction techniques, a dose-finding study of intravenous BUP in experimental pain, and two studies investigating the antinociception associated with the combination of intravenous BUP and naloxone in different ratios. The purpose of this chapter is to describe methods that were common to all four studies, namely the nociceptive tests. Methods common to the three drug studies (Chapters 4, 5 and 6) are also described. Methods specific to each individual study are described in the respective chapters.

2.2. Nociceptive testing

2.2.1. Cold pressor (CP) test

The methods have been adapted (Doverty et al. 2001) from Eckhardt and colleagues (Eckhardt et al. 1998). The test utilises two plastic cylindrical containers, one of which is filled with warm water and the other with a combination of water and crushed ice to achieve a "slushy" consistency. The subject immerses the non-dominant forearm and hand into the warm water for exactly 2 minutes. At 1 minute 45 seconds, a blood pressure cuff on the immersed arm is inflated to a pressure 20 mmHg below the diastolic blood pressure. The blood pressure cuff minimises the role of blood flow in determining the reaction to cold. At exactly 2 minutes, the forearm is transferred from the warm water to the cold water bath. The subject's eyes are covered for the entire procedure to minimise distraction and cues for time. Upon immersion of the limb in the cold water bath, subjects are asked to indicate when they first experience pain (pain threshold, CPTHR), then asked to leave their arm submerged until they can no longer tolerate the pain (pain tolerance, CPTOL). Pain

threshold and tolerance times are recorded in seconds from immersion in cold. An undisclosed cut-off of 180 seconds is imposed, after which time pain tolerance can no longer be accurately assessed due to numbness.

2.2.1.1. Materials

- 2 x 20 litre plastic cylindrical containers (38cm in depth; 30cm in diameter)
- Digital Thermometer
- Blindfold
- Sphygmomanometer
- Digital timer (with second display)
- Thermoregulator (Unistat 110, Thermoline Scientific, Sydney, Australia)
- Aquatic water pump (Brolga MV 1500, Brolga Australia Pty. Ltd., Haberfield, NSW, Australia)
- Towel

2.2.1.2. Set-up procedure

One container was filled with warm water (to 5 cm from top of container), and the thermoregulator immersed in container and set at 35°C. The second container was filled with crushed ice (to 10 cm below top of the container). Water was added until the container was filled to 5cm below the top of the container, and stirred to ensure water and ice were mixed evenly and there were no large clumps of ice. The temperature of the water/ice combination was then checked with a digital thermometer, and water or ice added as required to achieve a temperature between 0.5 and 1.0°C. Containers were placed on the trolley 10 cm apart with the warm water container on the left hand side. The water pump was placed at the bottom of the cold water container on the far side of the container (away from where the subject will stand) with the water jet facing upwards.

2.2.1.3. Test administration

The experimenter described to the subject the purpose of the test and the procedure as follows:

This is the cold pressor test. It is a test of your tolerance to cold pain. Here are two water containers, one filled with warm water, one filled with ice and cold water. You will place your non-dominant arm into the warm water container for two minutes, then take it out and put it immediately into the cold water container. When your arm is in the cold water container, there are two things I will ask you to tell me: tell me when you first feel pain, then leave your arm in the cold water as long as you can possibly tolerate the pain. Tell me when you feel you can no longer tolerate the pain, and remove your arm from the water. I will pass you a towel, which you may use to dry your arm. While you are completing the test you will be blindfolded, and I will inflate a blood pressure cuff on your arm just before you transfer your arm to the cold water container. This is to control for other factors that may interfere with the results. There is a water pump in the cold water container to keep the water circulating and stop the ice from clumping together. When you put your arm in each water container, immerse your arm quickly but carefully. As you will be blindfolded, I will help you transfer your arm from the warm water to the cold water. Keep your fingers straight and spread apart. Do not touch the sides or the bottom of the container and try not to move your arm around too much in the water.

I will not speak to you during the test except to give you reminder instructions. You should not speak during the test unless you have an urgent question or concern. The pain you experience from the test disappears quickly after removing your arm from the cold water, and there is no risk of permanent damage.

Every person is different in terms of his or her pain sensitivity. It is very important that we obtain an accurate and honest assessment of your pain tolerance. There is no

reward for setting a record time, but please try to perform the test honestly and leave your arm in the cold water as long as you can tolerate the pain.

The experimenter then ensured that the subject understood the instructions and enquired whether the subject had any questions before commencing the test. The subject was seated in a comfortable chair and his/her blood pressure taken. The subject then stood in front of the containers at an appropriate distance such that the non-dominant arm could be fully immersed in the container (see Figure 2-1). The thermoregulator was then switched off. The temperature of the water in each container was checked with a digital thermometer, and adjusted if necessary to ensure the temperature was within the required range (warm water: 34.5-35.5°C; cold water: 0.5-1°C). A blood pressure cuff was attached to the non-dominant arm, and a blindfold placed over the eyes. With the assistance of the experimenter, the subject rapidly immersed the non-dominant arm into the warm water container. The fingers of the immersed hand were spread apart comfortably, the arm held vertically and immersed such that there was no contact with the sides of the container and the fingertips were just above the bottom of the container. The digital timer was activated as soon as the arm was immersed. At 1 minute 45 seconds, the blood pressure cuff was inflated to 20mmHg below diastolic (obtained when blood pressure was taken just prior to the cold pressor testing) and remained inflated for the subsequent duration of the test. At exactly 2 minutes, the subject was assisted in transferring the immersed arm to the cold water container. The digital timer was started as soon as the arm was immersed in the cold water. The experimenter reminded the subject *"Tell me when you first feel pain"*. The time was recorded (in seconds from the immersion of the arm in cold water) when the subject verbally indicated the onset of pain (CPTHR). The experimenter then instructed the subject *"Now leave your arm in the water as long as you can tolerate the pain"*. The subject verbally indicated when the pain could no longer be tolerated (CPTOL), the time

was recorded, and the subject was assisted in removing the arm from the water. The subject was offered a towel to dry the arm, the blindfold was removed and the blood pressure cuff deflated. If a subject's arm remained in the cold water container beyond 180 seconds from the time of immersion, he/she was asked to withdraw his/her arm and informed that beyond this point the numbness of the arm prevented the test from continuing. In these circumstances, CPTOL was recorded as 180 seconds.

Figure 2-1. Cold pressor test administration.

2.2.2. Electrical stimulation (ES) test

The test involves delivering electrical pulses (frequency 0.7 pulses per second) of 14 milliseconds duration through an electrode attached to the earlobe. The pulses are increased by 2-volt increments (starting at 0V) every 1.42 seconds. Subjects indicate when the sensation becomes painful (pain threshold, ESTHR) and when the pain can no longer

be tolerated (pain tolerance, ESTOL). Pain threshold and pain tolerance are recorded in volts, with a maximum of 100 volts.

2.2.2.1. Materials

- Conductive gel (Livingstone Conductive Gel, Livingstone International Pty Ltd, Sydney, NSW, Australia)
- Grass stimulator (Grass Instrument Co., Model S6C, Quincy, M.A., USA)

2.2.2.2. Set-up procedure

The Grass stimulator is adjusted to the following settings outlined in Table 2-1 (also see Figure 2-2).

Frequency	7 (x.1)
Delay	7 (x10)
Duration	14 ms (x1)
Volts	0 (x10)
Output	Mono
Polarity	Normal
Mode	Repeat
Stimulus	Regular

Table 2-1. Grass stimulator settings for ES test.

2.2.2.3. Test administration

The subject was seated in a comfortable chair and the procedure explained as follows:

This is the electrical stimulation test. It is a test of your tolerance to electrical pain. I will smear some gel on your earlobe and then attach this earclip. The earclip is an electrode connected to this machine [indicate], which delivers electrical current in pulses. When you are ready to commence, I will ask you to close your eyes and will start sending very low voltage current through the electrode. At first you won't feel anything. I will slowly increase the voltage and you will begin to feel a sensation through the clip. It won't hurt at first; it will feel like someone is lightly pinching your

earlobe in pulses. I will continue to slowly increase the voltage and I would like you to tell me when that sensation becomes painful. I will then continue to increase the voltage until you tell me you can no longer tolerate the pain, at which time I will deactivate the machine and remove the earclip.

So, there are two things I'd like you to tell me: when the sensation you feel becomes painful, and when you can no longer tolerate that pain. Aside from indicating these things, do not speak during the test unless you have an urgent question or concern. I will not speak to you during the test except to give you reminder instructions.

Electro-conductive gel was applied to the earlobe and electrode, and the electrode clipped onto the lobe. The subject closed his/her eyes and upon verbally indicating readiness to commence, the experimenter increased the pulses by 2-volt increments (starting at 0v) every 1.42 seconds (as determined by the light indicator) (Figure 2-2). The subject verbally indicated onset of pain (ESTHR). The experimenter then instructed the subject *"Now tell me when you can no longer tolerate the pain"*, and continued to increase the voltage as described. The subject verbally indicated when the pain could no longer be tolerated (ESTOL) and the stimulator was immediately turned off. The electrode was removed from the earlobe and the gel wiped from both the earlobe and the electrode.

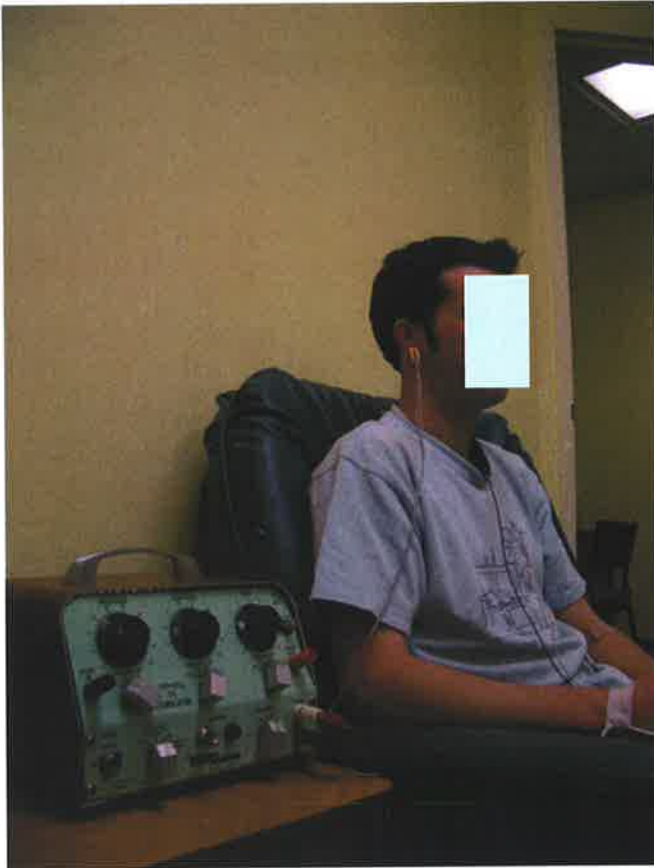

Figure 2-2. Electrical stimulation test administration.

2.2.3. Procedures for repeated testing

In several of the studies described herein, subjects were tested repeatedly over one or several days. In these circumstances the procedure for preparing the test and instructing the subject varied, by necessity, from the procedures described above.

The descriptions provided to the subject prior to the first testing occasion are detailed and lengthy, and not warranted on each occasion a subject repeats the test, particularly considering the studies described herein required the subject to complete the test up to 13 times in one day. In these circumstances, the experimenter described the full details of the test on the first testing occasion, and gave brief reminder instructions prior to each subsequent testing.

The set-up of the tests according to the details above was conducted at the beginning of a testing day, and this apparatus used for testing at multiple time points throughout that day. For the cold pressor, the thermoregulator maintained the warm water at the appropriate temperature between testing occasions, and immediately prior to each testing occasion, the cold water container was assessed by digital thermometer for temperature, and by visual inspection for consistency. Ice was added to achieve the correct temperature and the same consistency as at initial set-up. The electrical stimulation machine was switched off between each testing occasion, and remaining conductive gel wiped from the ear clip.

2.2.4. Testing environment

Nociceptive testing during each study was conducted in the same environment, with minimal background noise, audible voices and no clock with audible ticking. Ambient room temperature and lighting was consistent for each study. At no time did the experimenter discuss with the subject his/her performance on the test, or answer any questions related to the average pain tolerance time or any previous results.

2.3. Methods common to drug studies (Chapters 4, 5 and 6)

Common testing procedures were employed for all drug studies. Upon arrival at the testing centre participants provided a urine sample, which was tested for drugs of abuse (opioids, cannabinoids, benzodiazepines and sympathomimetic amines) by an independent laboratory and, for female subjects, pregnancy. A 22 gauge indwelling venous catheter (Insyte™) was inserted into the best available forearm vein on each arm (above the CP immersion line for the non-dominant arm). A male luer lock adaptor injection site (Interlink♦ Injection site, Baxter Healthcare Corp, Deerfield, IL, USA) was attached to each catheter. One catheter was used for blood sampling throughout the testing day, and the other for the infusions. The participant was then connected to an Agilent A3®

(Phillips) monitor, which was set to continuously monitor physiological parameters for the duration of the testing day.

2.3.1. Drug administration

On each testing day, participants received a 30-minute unblinded intravenous infusion of saline, followed by one or more 30-minute drug (or placebo) infusions. The purpose of the initial saline infusion was two-fold: to establish whether any changes in pain or physiological parameters would occur as a response to the infusion process itself, and to ensure that there was no obstruction to venous access via the catheter and the infusion pump was operating correctly.

Infusions were administered using Graseby Syringe Pump 3100 (SIMS Graseby Ltd., Herts, UK) (see Figure 2-3). Drugs and saline were prepared by the Royal Adelaide Hospital Pharmacy in 30ml BD Plastipak syringes. Infusions were run at a rate of 20ml per hour for 30 minutes. Each syringe was attached to a minimum volume extension set (150cm tubing, female luer lock, male luer lock, 0.5mL/30cm) (Tuta Healthcare, Lane Cove, NSW Australia). The male luer lock was attached to a lever lock cannula (BD Interlink♦, Franklin Lakes, NJ, USA). The extension set was primed with the drug/saline, and inserted into the injection site (Interlink♦ Injection site, Baxter Healthcare Corp, Deerfield, IL, USA). In studies requiring the simultaneous infusion of two drugs (via one cannula), a Y-type catheter extension set (Interlink♦ System Baxter Healthcare Corp, Deerfield, IL, USA) with two injection sites was attached to the catheter, and the lever lock cannulas (connected via the minimum volume extension set to each syringe) were inserted in each of the injection sites.

Figure 2-3. Graseby Syringe Pumps used for drug infusions.

2.3.2. Testing time points

Measurements were taken at numerous time points during each testing day. Each testing time point consisted of the following measures in the order listed: nausea and sedation recorded, blood sample taken, physiological parameters recorded (pulse, oxygen saturation and blood pressure), nociceptive testing completed, and respiration recorded (breaths per minute counted for one full minute during warm water component of CP). The schedule for testing time points is outlined below.

2.3.2.1. Blood sampling

Plasma samples taken during the studies were for use in an investigation unrelated to this thesis. Blood sampling (10 ml on each occasion) was conducted immediately prior to nociceptive testing throughout each testing day. Each sample was obtained via the catheter inserted for blood sampling at the beginning of the testing day. Following each sample, the catheter was flushed with 5ml saline. Where sampling from the catheter was not possible, the blood sample was taken, with the subject's consent, by venepuncture.

2.3.2.2. Monitoring of physiological parameters

Oxygen saturation, blood pressure and pulse were monitored throughout testing days using the Agilent A3♦ (Phillips) monitor. These parameters were recorded immediately prior to each blood sampling occasion throughout the testing days. Respiration was also recorded at these times by observation during the warm water component of the CP test for one full minute.

2.3.2.3. Monitoring of nausea and sedation

Nausea (Del Favero et al. 1992) and sedation (Royal Adelaide Hospital Sedation scale) were also monitored throughout the day, and recorded immediately prior to blood sampling. Nausea was recorded according to the following scale: 0, no nausea; 1, mild nausea; 2, moderate nausea; 3, severe nausea. Sedation was recorded according to the scale outlined Table 2-2.

0	None
1	Mild Occasionally drowsy, Easy to rouse
2	Moderate Constantly drowsy, easy to rouse
3	Severe Somnolent, difficult to rouse

Table 2-2. Sedation scale.

2.3.2.4. Monitoring of other opioid effects

Any other subjective experiences reported by the subject or observations made by the experimenter were recorded throughout each testing day. This included nausea, vomiting or sedation occurring in the periods between assessment time points (rated according to the scales described above), and any other experiences such as euphoria, headache, difficulty concentrating, sweating, light-headedness, or general discomfort.

2.3.2.5. Nociceptive testing

Nociceptive testing was conducted as described above. To reduce the impact of any order effects in studies using both CP and ES tests, participants were randomised to receive either CP followed by ES, or vice versa, for the duration of the study.

2.3.3. Testing schedule

The above measures were taken at set intervals throughout each testing day. These testing time points were as follows: 1. Prior to the commencement of infusions; 2. Twenty minutes after the commencement of the 30 minute saline infusion; 3. Twenty minutes after the commencement of the 30 minute drug infusion[1], and hourly following the cessation of the (last) drug infusion for a period of 6 (Study 2 and 3) or 10 (Study 4) hours. This is referred to as the washout period. The purpose of conducting the testing 20 minutes after commencing each 30 minute infusion was to allow time for testing to be completed before starting the subsequent infusion.

2.4. Methods of statistical inference

Methods of statistical inference used in the current series of experiments are described for each study in the relevant chapter. In several of the experiments, multiple tests of statistical inference have been conducted with a set of data. The practice of adjusting the alpha level used to determine statistical significance is a common practice in cases where multiple comparisons are undertaken within a data set (Tukey 1977). The rationale for adjusting the alpha level is that the chance of Type I error (a false positive, that is, a statistically significant ($p<0.05$) result occurring by chance) increases with the number of

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[1] *The dose finding study described in Chapter 4 involved four consecutive 30-minute drug infusions. The testing battery was conducted 20 minutes after the commencement of each infusion.*

comparisons made. However, the ethical and scientific validity of this practice has been questioned, and is the subject of ongoing debate (Perneger 1998; Aickin 1999; Bender and Lange 1999; Perneger 1999). Several problems with the practice of alpha level adjustment for multiple comparisons have been identified (Perneger 1998). Firstly, it has been proposed that the practice defies common sense, creating a scenario whereby the number of tests performed determines the findings of a study rather than the data. Moreover while an alpha level adjustment can prevent an increase in Type I error rate, this entails an increase in the rate of Type II error (a false negative, that is, *not* finding a significant relationship where one *does* exist), which can lead to practical and ethical dilemmas.

Describing what tests have been used and why, allowing the reader to reach a reasonable conclusion without the use of alpha level adjustment, has been proposed as the best approach to addressing the problem. To avoid the problems described, an alpha level of $p<0.05$ has been used throughout the studies described herein. Where relevant, the potential impact of this approach to the clinical implications of the findings will be considered in the interpretation of results.

3. ESTABLISHING NORMAL VALUES FOR THE COLD PRESSOR TEST AND ELECTRICAL STIMULATION TEST IN HEALTHY VOLUNTEERS

3.1. Introduction

3.1.1. The validity of experimental pain

The use of experimental pain models in the evaluation of analgesic interventions has for many years been a challenging and contentious issue amongst clinicians and researchers (Keats et al. 1950; Beecher 1953; Beecher 1957; Moore et al. 1997). Perhaps the most prominent of critics was Henry Beecher, who argued that experimentally induced pain was qualitatively different from the pain produced by injury or disease (Beecher 1962). He asserted that experimentally induced pain is without significance or meaning to the individual, whereas pain that results from injury or disease has significance to the patient and involves other parameters, most notably psychological factors such as anxiety.

While it is recognised that analgesic interventions must be evaluated in the clinical setting to which they will be applied, experimental pain models capable of evaluating analgesic efficacy under standardised conditions are of considerable utility and importance. As described below, the experience of pain is subject to numerous sources of intra- and inter-individual variability. Experimental pain research with pain-free humans allows a level of control and standardisation not possible in a clinical pain setting (Gracely 1999). It has been argued that investigations of dose-response, optimal dosage or comparison of relative efficacy with known substances are best conducted with a sample of healthy volunteers who are as homogeneous as possible (Bromm 1985). The evaluation of analgesic interventions in an experimental pain paradigm prior to assessment in a clinical pain

population allows far greater control over factors that may contribute to variability and compromise the validity of findings.

Numerous pain induction techniques have been developed for use in experimental pain studies with humans. These techniques can typically be classified according to the type of pain induced, such as mechanical (pressure), chemical, thermal (heat or cold), electrical and ischaemic pain. The electrical stimulation (ES) and cold pressor (CP) tests were selected for use in the series of experiments described herein as they each provoke a different type of pain (tonic and phasic), both tests have consistently been demonstrated to be sensitive to the antinociceptive effects of opioids, have been shown to produce reliable and valid outcomes under controlled conditions, are applied easily, produce a distinct pain sensation, are associated with rapid onset and offset of pain (though in the case of the CP offset is more prolonged), are reproducible and suitable for multiple administration (Gracely 1991). For example, a recent study compared the sensitivity of five pain tests to clinical doses of the opioid alfentanil. Antinociceptive effects were observed with electrical, CP and pressure pain while no significant antinociceptive effect was observed with ischemic or heat pain (Luginbuhl et al. 2001).

3.1.2. Considerations in experimental pain induction

The development of new pharmacological strategies for pain management depends upon techniques for the induction and accurate assessment of experimental pain in healthy, pain-free humans. The valid assessment of experimental pain is limited by several factors. Firstly, the methods used for common pain induction techniques have not been standardised. Consequently, there is a poor understanding of the normal pain response using these techniques and it is difficult to make meaningful comparisons between findings from different investigative groups (Eccleston 1995). Secondly, the sensitivity of a test in

detecting a drug effect can be compromised by inter- and intra-individual variation in pain response. While a substantial body of research has been concerned with the impact of biological and psychosocial factors on experimental pain response, we lack an appreciation of the relative contribution of these variables to test performance and, moreover, to what extent these factors should be taken into consideration in the design of experimental pain studies in order to reduce inter-subject variability. To maximise both the statistical power of a study and the likelihood of observing an analgesic effect, a pain induction technique should be associated with minimal variation, both in terms of variation between individuals, and variation within an individual over time.

Pain induction techniques typically have a maximum time or stimulus limit, after which point the assessment of maximum tolerated pain (MTP) is no longer valid (for example, due to numbness when inducing cold pain) or safe (for electrical stimulation or ischaemic pain). For the evaluation of analgesic interventions an individual's baseline pain response must be sufficiently below this ceiling to allow a significant increase to be measured. There are two approaches by which the magnitude of MTP may be reduced: modifying the pain induction methods and excluding subjects whose baseline MTP is too close to the censor point to allow a significant increase to be measured. Such an approach may induce a bias in subject selection. An alternative is to exclude all potential subjects outside a notional normal range, whether at the extreme high or extreme low ends of the distributions. This approach has previously been employed (Eckhardt et al. 1998). However, there have been no standardised data upon which to justify the inclusion range. In establishing the upper and lower boundaries of MTP for inclusion in an experimental pain trial it is preferable that standardised data be available to assess individual performance relative to the distribution within the population.

3.1.3. Determinants of pain response

Numerous studies have examined the impact of biological (physiological and genetic) and psychosocial variables on pain response, though in many cases findings have been inconsistent. Much of this research has been instigated by clinical observations, such as the apparent difference in pain sensitivity between men and women.

3.1.3.1. Sex

Sex differences in pain sensitivity and tolerance have been a major focus of pain research. While it is often reported that there is minimal sex difference in terms of pain threshold, pain tolerance is consistently higher in males compared to females (Berkley 1997; Riley et al. 1998). Lower pain tolerance or greater pain report in females compared to males has been demonstrated using a range of pain induction techniques including the CP test (Walsh et al. 1989; al'Absi et al. 1999), noxious heat stimuli (Feine et al. 1991; Fillingim et al. 1998), pressure (Jensen et al. 1992; Chesterton et al. 2003) and mechanical pain (Sarlani and Greenspan 2002). These differences may be attributed to a variety of factors, which may include sociocultural influences such as gender role expectation (Robinson et al. 2001; Wise et al. 2002) and participant and experimenter gender (Levine and De Simone 1991; Robinson and Wise 2003; Kallai et al. 2004; Robinson and Wise 2004) as well as biological (physiological and genetic) influences such as hormonal changes (Riley et al. 1999; Hellstrom and Lundberg 2000) and differences in central pain processing (Paulson et al. 1998; France and Suchowiecki 1999; Naliboff et al. 2003; Sarlani et al. 2004).

3.1.3.2. Ethnicity/race

Ethnicity, or race, has also been shown to influence tolerance to experimentally induced pain. Studies of ethnicity and pain have focused predominantly on differences between African-Americans and Caucasians, with early studies concluding that Caucasians

demonstrated greater pain tolerance than African-Americans (Chapman and Jones 1944; Woodrow et al. 1972). The results of more recent studies continue to support the notion of differences in pain tolerance between ethnic groups (Edwards et al. 2001). Studies of experimentally induced pain have generally not focused on Asian populations. In the late 1980s, Zatzick and Dimsdale conducted a review of studies investigating ethnicity differences in pain (Zatzick and Dimsdale 1990). Thirteen English language articles published between 1943 and 1989 were identified. Of these thirteen papers, only four included an Asian group, and findings were inconsistent. Two of the studies reported greater pain tolerance amongst Caucasians than Asians using cold pressor (Knox et al. 1977) and mechanical pressure tests (Woodrow et al. 1972; Knox et al. 1977). One study used small numbers of Nepalese (n=6) and Caucasians (n=5) and revealed a higher pain threshold in the Nepalese group. The fourth study reported a higher pain threshold amongst North American Asians than North American Caucasians, but the latter group evidenced a higher pain threshold than native Asians. These inconsistent results may be attributed in part to different methods of pain induction, differences in subject numbers, and variation in the operational definition of ethnicity as distinct from race. The distinction between race and ethnicity is discussed in further detail below (section 3.6). A recent study investigated differences in heat pain threshold between three East Asian Ethnic groups (Malay, Indian and Chinese), and revealed no significant differences between the groups (Yosipovitch et al. 2004).

3.1.3.3. Age

Investigations of age-related differences in response to noxious stimulation have generally demonstrated that older subjects are less tolerant of experimentally induced pain (Woodrow et al. 1972; Edwards et al. 2003). However, it has been observed that age-related differences are influenced by the method of pain induction and the outcome

measure (for example, whether subjects are required to rate the intensity of the pain elicited by a stimulus, or are asked to report at what point a stimulus becomes intolerable) (Gibson and Helme 2001).

3.1.3.4. CNS stimulants

Studies examining the impact of stimulants such as caffeine and nicotine on pain response have produced variable findings. An early study reported that cigarette smoking did not influence performance on either the CP test or ES test (Sult and Moss 1986), while a 1993 study revealed that, amongst habitual smokers, elevated thermal pain threshold occurred when they were cigarette-deprived (Pauli et al. 1993). A recent study revealed that the administration of 250 mg of caffeine was associated with higher CP pain threshold and tolerance than placebo (Keogh and Witt 2001), and a later study suggested that caffeine-induced hypoalgesia may be related to anxiety sensitivity (Keogh and Chaloner 2002).

3.1.3.5. Menstrual cycle

Several studies have also investigated the impact of menstrual cycle on response to experimental pain stimuli. A meta-analytic review of 16 published studies revealed a relatively consistent pattern of changes in pain sensitivity, but this was dependent upon the modality of stimulus used. Studies using ES found that the luteal phase was associated with higher pain threshold, while studies employing other pain induction techniques found higher pain threshold and tolerance to be evident during the follicular phase (Riley et al. 1999). A recent study of 500 healthy volunteers found no significant effect of menstrual cycle on heat or cold pain (Kim et al. 2004).

3.1.3.6. Body weight/size

The role of body weight in pain response has been examined, with an early investigation demonstrating that "obese" individuals were more sensitive to pressure pain than "non-obese" individuals (McKendall and Haier 1983). The presence of an eating disorder such as anorexia nervosa has also been associated with altered pain sensitivity (de Zwaan et al. 1996; de Zwaan et al. 1996; Raymond et al. 1999), though findings have been inconsistent and it remains unclear whether any effect observed is a physiological correlate of the disorder itself or related to body weight.

3.1.3.7. Psychological/cognitive factors

Psychological factors that have been considered include the role of attention (de Wied and Verbaten 2001; Villemure and Bushnell 2002), mood (Weisenberg et al. 1998) and coping strategies (Baker and Kirsch 1991) in modifying the experience of pain (see Chen et al. 1989; Fields 2000), as well as emotional constructs such as catastrophizing (Sullivan et al. 2001; Edwards et al. 2004), fear of pain (Crombez et al. 1999; Vlaeyen and Linton 2000; Keefe et al. 2004; Roelofs et al. 2004) and anxiety (Janssen and Arntz 1996; Rhudy and Meagher 2000). Taken together, this research provides strong support for the notion that cognitive factors impact considerably on the pain experienced in both clinical and experimental settings, although the exact nature of the effect on pain response, as well as potential interactions with other variables such as gender, are yet to be clearly elucidated.

While numerous reports describe the impact of a variety of factors on pain response, absent from the literature is a comparison of the relative contribution of these variables to experimentally induced pain, and an indication of the degree to which these factors should be controlled for in the design of experimental pain studies.

3.1.4. Cold pressor test

The cold pressor (CP) test was first studied as a pain induction technique in the 1940s (Wolf and Hardy 1941) and established as a method of analgesic evaluation some years later (Wolff et al. 1966; Wolff et al. 1966). The test is a tonic pain model, activating slow-conducting, unmyelinated C-fibres. The pain experienced has been likened to dental or back pain (Chen et al. 1989). There have been many variations on the CP technique, but the test essentially involves the immersion of a limb (usually the hand or forearm) in a bath of very cold water. The participant is generally required to indicate when pain is first experienced (pain threshold), and when pain can no longer be tolerated (tolerance), or is required to continuously rate pain throughout the procedure using a VAS. Threshold and tolerance are measured in terms of latency (seconds) from initial immersion in the cold water.

The CP test has become one of the most widely used methods of experimental pain induction for the evaluation of analgesic interventions, including pharmacological (Berntzen et al. 1985; Garcia de Jalon et al. 1985; Posner et al. 1985; Jones et al. 1988; Compton 1994; Eckhardt et al. 1998; Yuan et al. 1998; Compton et al. 2000; Compton et al. 2001; Doverty et al. 2001; Compton et al. 2003), cognitive (Gilligan et al. 1984; Spanos et al. 1984; Dolce et al. 1986) and other approaches (Hilgard et al. 1974; Ashton et al. 1984). The test has also increasingly been used to characterise pain response in different groups, such as individuals with a current or past history of opioid-dependence (Compton 1994; Compton 1998; Compton et al. 2000; Compton et al. 2001; Doverty et al. 2001; Doverty et al. 2001). The effect of NSAIDs on CP response has been inconsistent. Therapeutic doses of ibuprofen (Jones et al. 1988) and indomethacin (Telekes et al. 1987) have failed to increase VAS ratings of CP pain. However, in a double blind, placebo controlled investigation, Yuan and colleagues demonstrated a significant increase in VAS

pain ratings with acetaminophen (paracetamol, 1000 mg p.o.) in 18 healthy volunteers (Yuan et al. 1998). Compton and colleagues investigated the influence of sex on the effect of ketorolac (10 mg p.o.) on CP pain tolerance in healthy volunteers, and revealed a moderate (though not statistically significant) analgesic effect in women. A considerable increase in CP tolerance was evident in the male sample. However, this could not be distinguished from the comparably large placebo effect (Compton et al. 2003). Studies have consistently confirmed that the test is a highly sensitive assay for opioids, including morphine (Wolff et al. 1966; Jones et al. 1988), dipipanone (Posner et al. 1985), and codeine (Garcia de Jalon et al. 1985), and that this response can be distinguished from both placebo (Posner et al. 1985; Jones et al. 1988) and non-opioid analgesics (Jones et al. 1988). Furthermore, the test is associated with high reproducibility for repeated applications (Walsh et al. 1989) and, in terms of subjective experience, the test is considered comparable to clinical pain (Wolff 1984).

The lack of standardised procedures for the CP test provoked concern regarding the reliability of the technique (Blasco and Bayes 1988). It was observed that there was significant variation in the methods used by different investigative groups, and that these procedural differences hampered replication of results and comparison between studies. This lack of standardisation compromises the reliability of the test as a number of the procedural differences relate to factors that may impact on test performance. These methodological differences include a lack of consistency in the temperature of the cold water, the immersion of the arm in warm water for a period of time prior to cold water immersion, the proportion of the limb immersed (i.e. hand or forearm), the induction of ischemia in the immersed arm by inflation of a blood pressure cuff, the laterality of the arm used (i.e. dominant, non-dominant or unspecified), the instructions given to the subject, subject blinding (i.e. eyes open, closed or blindfolded), the use of a water pump to

circulate the cold water (preventing laminar warming around the immersed limb), and the outcome measures used (i.e. pain threshold and tolerance, or visual analogue scales of pain intensity).

A 1989 normative model of the CP test established by Walsh and colleagues represents the only published study of this kind to date (Walsh et al. 1989). The methods used in this investigation required subjects to immerse the non-dominant hand and arm into cold water (1-2 °C) until the pain could no longer be tolerated. Walsh and colleagues used Cox regression to develop a normative mathematical model for CP pain tolerance according to this simplified technique. This analysis revealed the best model for predicting performance on the test contained the following covariates and interactions: sex, race (Anglo-Saxon vs. non-Anglo-Saxon) by sex, sex by age, and race by age. Findings indicated that, when controlling for age, pain tolerance was greatest in Anglo-Saxon males, followed by non-Anglo-Saxon males, Anglo-Saxon females and non-Anglo-Saxon females. Since that time, however, several features have been incorporated by various experimental groups (for example, Garcia de Jalon et al. 1985) to the current CP methodology with the aim of reducing variability and mean tolerance time. Standardised, normative data according to these methods have not been established.

3.1.5. Electrical stimulation test

The electrical stimulation (ES) test is a phasic pain model, activating fast-conducting Aδ-fibres, and associated with sharp, localised pain. Despite its extensive use in human pain investigation, no normative studies have been published. As with the CP test, a number of methods have been used to induce pain by electrical stimulation in humans, varying both in terms of the apparatus used to deliver electrical current and area of the body to which the stimulus has been applied. Most commonly, ES has been applied cutaneously to the

finger, hand or earlobe, and has been relatively consistent in demonstrating sensitivity to analgesic compounds. An early study by Wolff and colleagues demonstrated a significant increase in tolerance to ES applied cutaneously to two fingers with the administration of 60 mg of codeine as compared to placebo (Wolff et al. 1966). These findings were replicated in a later study demonstrating a significantly greater increase in threshold and tolerance to electrical stimulation of the earlobe with 60 mg codeine than placebo (Stacher et al. 1986). A significant increase in analgesia compared to placebo has also been demonstrated using the same method with a range of other compounds (Stacher et al. 1979; Stacher et al. 1982; Stacher et al. 1983). As described above, Luginbuhl and colleagues also recently reported that clinical doses of alfentanil were associated with significant analgesia to electrical stimulation of the toe (Luginbuhl et al. 2001). Evidence of a significant increase in ES performance compared to placebo with the administration of NSAIDs has been less consistent. Several studies have reported a significant effect (Stacher et al. 1979; Stacher et al. 1986; Walker et al. 1993; Walker and Carmody 1998), while others have failed to detect an increase in ES response (Wolff et al. 1966; Moore et al. 1971; von Graffenried et al. 1978). It has been suggested that this failure to detect a significant effect may be due to insufficient subject numbers, given the substantial variability in response to analgesic drugs (Stacher et al. 1986).

There are several advantages associated with the application of electrical current to the earlobe in ES testing. In comparison with other areas of the body that have previously been used, it has been argued that the use of the earlobe offers advantages in reducing stimulus detection threshold and reducing variability by minimising individual differences and confounding factors (Walker et al. 1993; Walker and Carmody 1998). For example, results from stimulation of sites that overlie muscle (e.g. the hand) may be compromised due to muscle contraction. Variation in skin thickness may also be minimised with use of

the earlobe. For example, with use of the hand, skin thickness may vary considerably according to a number of factors, including the sex of the subject and participation in occupational or recreation activities involving manual activity. The impact of stress-induced sweating may also be reduced with use of the earlobe.

The application of ES to the earlobe has recently been used in a number of studies of pain response in opioid dependent individuals and healthy volunteers. Dyer and colleagues assessed the antinociceptive activity of methadone prescribed as a maintenance pharmacotherapy to opioid dependent individuals. This study reported a decrease in pain sensitivity with increasing plasma methadone concentrations, further substantiating both the capacity of the ES method to evaluate the antinociceptive activity of opioids, and the reproducibility of the technique (Dyer et al. 1999). Subsequent studies have also applied this technique to opioid-dependent populations in comparing pain response to that of healthy volunteers (Dyer et al. 1999; Doverty et al. 2001).

3.2. Purpose and aims of the present study

The aims of the present study were: to demonstrate the distribution of values for the CP and ES tests, which may guide in the selection of subjects in future studies; to establish whether the current methods produce less variation and a lower mean than earlier techniques; to determine the replicability of the tests, and to assess the factors that may be important to control for when using these techniques in order to minimise variability.

3.3. Methods

3.3.1. Participants

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 011119). Subjects were 100 healthy, drug-free volunteers, who met the criteria detailed below. Participation in the study was on a voluntary basis. Participants were financially remunerated $AU15 for participation. Participants who completed a second testing session were remunerated $AU40 for the two sessions (i.e. $AU15 for the first session, $AU25 for the second). The purpose of offering higher remuneration for the second testing session was to enhance the incentive to return, and to reduce a potential bias in the sample by attracting only the subjects who did not find the tests overly aversive. Written informed consent was obtained from all participants prior to commencing the trial.

3.3.1.1. Inclusion criteria

- Aged between 18 and 65 years
- Males and females in equal numbers
- Signed informed consent provided

3.3.1.2. Exclusion criteria

- A history of opioid dependence according to DSM-IV criteria
- History of significant chronic pain
- Prior chronic opioid use (in excess of one week)
- Current regular use of any other drug, including recreational and non-prescription drugs such as allergy medication (with the exception of the contraceptive pill) (NB Regular use considered in excess of once per week)

- Any history of substance dependence
- Alcohol consumption exceeding National Health and Medical Research Council (NHMRC) guidelines
- Prior knowledge of, or exposure to, the CP or ES tests
- Suffering from Raynaud's Syndrome

3.4. Procedures

3.4.1. Recruitment and screening procedures

Participants were recruited using an advertisement flyer posted in key areas (e.g. university and hospital notice boards), and through word of mouth. The experimenter conducted a brief screening interview with each participant prior to organising the testing session.

3.4.2. Experimental procedures

The subject was provided with a study information sheet, and was given the opportunity to read the details and purpose of the study, discuss the procedures with the investigator and ask any questions. All participants attended the testing centre on an individual basis for one testing session of approximately 20 minutes. Participants were instructed to refrain from taking any drug (excluding nicotine, alcohol and the contraceptive pill) in the 24 hours prior to testing. Testing was conducted in a quiet room at controlled temperature (approximately 23°C), with only the investigator present. Participants completed a questionnaire providing demographic information, including age, sex, ethnicity, average daily alcohol and caffeine consumption, cigarette smoking, and phase of menstrual cycle (where appropriate). Height and weight were recorded and body mass index (BMI) calculated (weight (kg)/height (m)^2). Average daily alcohol and caffeine consumption and

cigarette smoking were established by the questions displayed in Table 3-1. Caffeine consumption was calculated as follows: instant coffee 95 mg; brewed/percolated coffee 135 mg; espresso coffee 100 mg ; decaffeinated coffee 3 mg ; tea 50 mg. These values were based on the ranges determined by Bunker and McWilliams (Bunker and McWilliams 1979). Data were collected between March and May 2002.

Do you smoke cigarettes? yes / no

if yes, on average how many do you smoke per day?

(a) less than 10
(b) between 10 and 20
(c) between 20 and 30
(d) between 30 and 40
(e) more than 40

During the past 30 days, on how many days did you have at least one alcoholic drink?

(a) 0 days
(b) 1 or 2 days
(c) 3 to 5 days
(d) 6 to 9 days
(e) 10 to 19 days
(f) 20 to 29 days
(g) every day

On these days, how many drinks did you have on average?

(a) 1 to 2
(b) 2 to 5
(c) 5 to 8
(d) more than eight

During the past 30 days, on how many days did you have 5 or more drinks in a row (that is, within a few hours)?

(a) 0 days
(b) 1 day
(c) 2 days
(d) 3 to 5 days
(e) 6 to 9 days
(f) 10 to 19 days
(g) 20 or more days

Please write in each box the number of cups of each "type" of coffee you would normally drink in a day

	instant
	plunger/percolater
	espresso
	decaffeinated

How many cups of tea would you normally drink in a day?

Table 3-1. Assessment of average alcohol and caffeine consumption and cigarette smoking.

Phase of menstrual cycle was recorded for those female participants who reported a regular menstrual cycle (n=44). Regularity of menstrual cycle was established by self-report, with subjects indicating whether they had four menses in the previous six months, and if they considered their cycle to be regular. For those reporting a regular cycle, phase at testing was determined by self-report, with phases categorised according to the following classification: menstrual (days 1-5), follicular (days 6-12), ovulation (days 13-16) and luteal (days 17-28) (Sherwood 1997).

A skinfold thickness measure was taken around the volar aspect of the right forearm using skinfold calipers (Holtain Ltd., Crymych, UK). This was performed on three consecutive occasions and a mean calculated. This variable was assessed as skinfold thickness around the arm was considered to be a potential source of variability in CP performance. Participants then completed the State Trait Anxiety Questionnaire (STAI) (Spielberger 1983). This inventory independently assesses two constructs of anxiety: state anxiety and trait anxiety, using two 20-item scales. The state anxiety scale assesses situational anxiety, requiring subjects to respond to a series of items according to how they feel "right now at this moment". The trait anxiety scale aims to assess the more enduring, stable personality trait of proneness to anxiety. This scale requires participants to respond to items according to how they "generally feel". Each scale has a possible scoring range of 20 – 80, with a higher score indicative of greater anxiety. This inventory is used widely in research and clinical practice, and has been demonstrated to be psychometrically sound (Spielberger 1983).

Participants then completed the nociceptive tests, the CP and ES, once in a randomised order. These tests were conducted according to the methods described in Chapter 2. Following the nociceptive testing, participants completed the Fear of Pain Questionnaire-III (FPQ-III). The FPQ-III is a 30-item questionnaire assessing the degree to which an

individual fears the pain associated with a range of different experiences. Participants indicate how much they fear the pain associated with a range of situations, such as breaking an arm or receiving an injection, by rating a 5-point Likert-type scale with the categories "not at all", "a little", "a fair amount", "a lot" and "extreme". Scores may be summed to produce a Total Fear of Pain score (with a possible scoring range of 30-150), or may be grouped into three subscales of 10-items each: Fear of Medical Pain, Fear of Severe Pain, and Fear of Minor Pain. The FPQ-III has been shown to be psychometrically sound, demonstrating high reliability and validity (McNeil and Rainwater 1998).

To determine the replicability of the techniques, a cohort of thirty subjects completed the pain tests a second time, between 14 and 21 days after the initial session.

3.4.3. Statistical analyses

Statistical analyses were conducted using SPSS® for Windows version 11.5 (SPSS Inc., Chicago, United States of America). To assess the intra-subject variability between two sessions, Wilcoxon signed rank tests for related samples were conducted to identify differences in nociceptive test performance between first and second testing for the cohort of 30 subjects who completed two sessions. This non-parametric test was used due to deviations from the normal distribution and the presence of censored data as a result of the test upper limits. A number of subjects did not report reaching MTP before the upper limit of the tests (180 seconds for CP, 100 volts for ES), and are therefore censored data. The percent change between first and second testing for both CP tolerance and ES tolerance were also calculated for each of the 30 returning subjects. Survival analyses (Cox proportional hazards regression) were conducted for CP tolerance and ES tolerance to assess the relative contribution to test performance of the biological and psychosocial variables assessed. The presence of censored data necessitated the use of survival analysis

to determine the relative contribution of each variable to test performance. Cox regression assesses the effect of each variable on the likelihood of a subject "surviving" until the maximum limit of the test, that is, *not* reaching pain tolerance during the observation period. To determine whether there were any interactive effects between the variables, Cox regression analysis was performed with the main effects and interaction terms for each possible pair of variables. Where significant interactions were detected, the interaction term was also entered into the full Cox regression.

Among the female participants who reported a regular menstrual cycle, no data were censored due to reaching the maximum limit. To analyse the effect of menstrual phase on test performance among this cohort, simple linear regression with self-reported phase of menstrual cycle at time of testing was conducted. Unless otherwise indicated, data are described as mean (±SEM, interquartile range).

3.5. Results

3.5.1. Sample characteristics

The sample comprised 100 healthy unrelated volunteers (50:50 sex ratio), who ranged in age from 18 to 56 (26.1±0.88) years, with a BMI between 16 and 42 (23±0.44), and body weight between 40 and 108 kg (66.8±1.5). Ethnicity was established by self-identification, with 63 identifying as Caucasian and 37 identifying as Asian. Subject demographics are detailed in Table 3-2. The proportion of female subjects at each phase of the menstrual cycle at first testing is detailed in Table 3-3.

	Mean	±SEM	Median	IQR
Age (years)	26.11	0.88	24.00	8.00
BMI	23.15	0.44	22.50	5.18
Skinfold thick. (mm)	6.84	0.26	6.37	3.19
Daily caffeine (mg)	148.55	14.8	135.00	211.25
FPQ-III (total)	88.30	1.63	88.00	21.75
STAI Trait	35.85	0.81	35.50	11.75
STAI State	32.47	0.81	32.00	10.75
Ethnicity	Caucasian 63% Asian 37%			
Cigarette smoker	Yes 10% No 90%			
If yes, how many/day?	< 10: 9% 10-20: 1%			
Alcohol		%		%
During the past 30 days, on how many days did you have at least one alcoholic drink? (n=100)	0 days	34	10 to 19 days	9
	1 or 2 days	16	20 to 29 days	2
	3 to 5 days	19	every day	0
	6 to 9 days	20		
On these days, how many drinks did you have on average? (n=66)	1 to 2	37.8	5 to 8	4.5
	2 to 5	56.1	> 8	1.5
During the past 30 days, on how many days did you have 5 or more drinks in a row (n=100)	0 days	61	6 to 9 days	3
	1 day	11	10 to 19 days	0
	2 days	6	20 or > days	0
	3 to 5 days	19		

IQR Interquartile range; BMI Body mass Index (kg)/height (m)^2; FPQ-III Fear of Pain Questionnaire-III, scoring range 30-150; STAI State Trait Anxiety Inventory, Trait and State subscale scores, scoring range 20-80 on each scale. See section 3.4.2 for description of measures.

Table 3-2. Demographic parameters of normative study sample (n=100). IQR, Interquartile range.

	N	%
Menstrual	5	10
Follicular	14	28
Ovulation	5	10
Luteal	20	40
Irregular (or absent due to contraception)	4	8
Absent due to hysterectomy	2	4

Table 3-3. Phase of menstrual cycle reported by female subjects (n=50) at initial testing.

As described, a cohort of 30 participants completed a second testing between 14 and 21 days later. Prior to testing, participants were offered the opportunity to enrol for one or two testing occasions. The offer of a second testing occasion was made to all participants until 15 males and 15 females had been recruited for two testing sessions. All subjects who initially agreed to participate in a second testing session did so. The cohort of 30 who were tested on two occasions ranged in age from 18 to 56 (25.3±1.69), with a BMI between 17.3 and 42 (23.28±0.91). There were no significant differences between those who participated in one or two testing sessions in terms of age, BMI, pain parameters, average alcohol or caffeine consumption, fear of pain or cigarette smoking. There was, however, a significant difference between those who participated in one or two testing sessions in terms of state anxiety as measured by the STAI, such that those who agreed to return for a second testing reported lower state anxiety than those who enrolled for one testing occasion only (see Table 3-4). However, a comparison of only the subjects who were *offered* the opportunity of returning for a second session (i.e. until 15 males and 15 females had been recruited for two sessions) revealed no significant differences between those who did and did not enrol for two sessions (see Table 3-5). This suggests that the difference in state anxiety between the sample returning for a second testing (n=30) and those participating in only one testing session (n=70) does not reflect a sampling bias.

	Subjects attending 1 session *(n=70)*		**Subjects attending 2 sessions** *(n=30)*	
	Mean	(±SEM)	**Mean**	(±SEM)
Age (years)	26.46	1.04	25.30	1.69
BMI	23.09	0.50	23.28	0.91
Alcohol intake^	2.53	0.18	2.73	0.27
Caffeine intake (mg)	160.61	18.66	120.42	23.08
FPQ-III (total)	90.14	1.93	84.00	2.94
STAI Trait	36.74	0.96	33.77	1.45
STAI State*	33.59	1.01	29.87	1.21
CP Threshold (seconds)	9.69	0.54	9.27	0.59
CP Tolerance (seconds)	50.86	5.22	56.63	8.34
ES Threshold (volts)	31.54	0.99	36.87	1.94
ES Tolerance (volts)	55.03	1.79	58.27	3.09
	%		%	
Ethnicity	Caucasian 60% Asian 40%		Caucasian 70% Asian 30%	
Cigarette smoker	Yes 6% No 94%		Yes 20% No 80%	

FPQ-III Fear of pain questionnaire-III; STAI State Trait Anxiety Inventory trait and state anxiety scales. See section 3.4.2 for details of measures. ^How many days in previous month consumed at least one drink. *Significant mean difference, $p<0.05$ (Independent samples t-test).

Table 3-4. Comparison of subjects according to number of testing sessions completed: all subjects completing one session vs. subjects completing two sessions.

	Subjects offered 2nd session, performed 1 only *(n=26)*		Subjects performing 2 sessions *(n=30)*	
	Mean	(±SEM)	**Mean**	(±SEM)
Age (years)	24.77	1.48	25.30	1.69
BMI	23.34	0.91	23.28	0.91
Alcohol intake^	2.50	0.29	2.73	0.27
Caffeine intake (mg)	142.88	26.02	120.42	23.08
FPQ-III (total)	88.08	2.97	84.00	2.94
STAI Trait	36.31	1.56	33.77	1.45
STAI State	32.00	1.53	29.87	1.21
CP Threshold (seconds)	10.77	1.22	9.27	0.59
CP Tolerance (seconds)	60.04	9.74	56.63	8.34
ES Threshold (volts)	33.08	1.18	36.87	1.94
ES Tolerance (volts)	53.62	2.69	58.27	3.09
	%		%	
Ethnicity	Caucasian 53% Asian 47%		Caucasian 70% Asian 30%	
Cigarette smoker	Yes 8% No 92%		Yes 20% No 80%	

FPQ-III Fear of pain questionnaire-III; STAI State Trait Anxiety Inventory trait and state anxiety scales. See section 3.4.2 for details of measures. ^How many days in previous month consumed at least one drink. No significant mean differences (Independent samples t-test).

Table 3-5. Comparison of subjects according to number of testing sessions completed: subjects completing one session but given option of 2nd vs. subjects completing two sessions.

3.5.2. Normative data

Normative data and frequency distributions for the pain tests in all 100 subjects are displayed in Table 3-6 and Figure 3-1. Distributions for ES threshold and tolerance approximated a normal distribution, with both skewness (0.8 and 0.9, respectively) and kurtosis (0.8 and 0.5) near zero. CP threshold and tolerance distributions demonstrated a moderate degree of positive skew (1.6 and 1.8, respectively) and kurtosis (4.5 and 2.3, respectively).

	Mean	SD	95% CI Upper Lower	Median	IQR	CV (%)	S	K
Cold Pressor								
Threshold (seconds)	9.6	4.1	8.7 10.4	9.0	4.0	42.7	1.6	4.5
Tolerance (seconds)	52.6	44.1	43.8 61.3	35.5	31.8	83.8	1.8	2.3
Electrical Stimulation								
Threshold (volts)	35.9	9.0	34.1 37.3	35.0	12.0	25.1	0.8	0.8
Tolerance (volts)	56.0	15.6	52.9 59.9	53.0	21.5	27.9	0.9	0.5
SD Standard deviation; CI Confidence Intervals; IQR Interquartile range; CV Coefficient of variation; S Skewness; K Kurtosis.								

Table 3-6. Descriptive data of cold pressor and electrical stimulation test parameters (n=100). Note that due to the potential for censoring on CP and ES tolerance, median and IQR more accurately represent the distribution for these parameters than mean and SD.

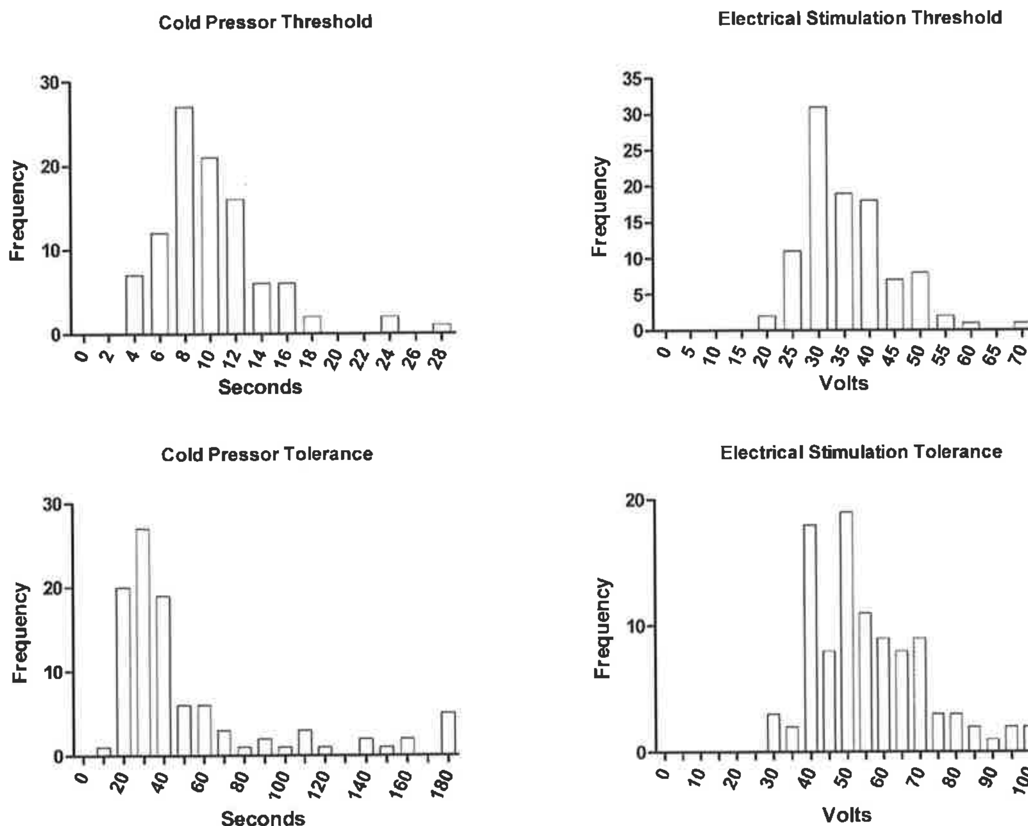

Figure 3-1. Frequency distributions for cold pressor and electrical stimulation test parameters in 100 healthy volunteers. Note that the values of 180 seconds for CP tolerance and 100 volts for ES tolerance represent censored data (maximum limits).

3.5.3. Intra-subject variability

There were no statistically significant differences between testing sessions for CPTHR (1st: 9.3 (±0.6, 4.3) seconds; 2nd: 8.7 (±0.7, 5.0) seconds; z=-0.51, p=0.61); CPTOL (1st: 56.6 (±8.3, 44.0) seconds; 2nd: 52.6 (±7.7, 39.3) seconds; z=-1.034, p=0.30); ESTHR (1st: 36.9 (±1.9, 15.0) volts; 2nd: 35.7 (±1.5, 13.5) volts; z=-0.822, p=0.41) or ESTOL (1st: 58.3 (±3.1, 25.0) volts; 2nd: 58.1 (±2.9, 23.0) volts; z=-0.239, p=0.81). The mean percent change between first and second testing was 15.1% (±SEM 2.37) and 11.6% (±SEM 1.72) for CPTOL and ESTOL, respectively.

3.5.4. Factors impacting upon test performance

3.5.4.1. Cold pressor tolerance (CPTOL)

There were no significant interactions between variables in CPTOL. Eleven variables were entered in the Cox regression model for CPTOL: sex, age, BMI, skin-fold thickness, ethnicity, cigarette smoking, average alcohol and caffeine consumption, fear of pain, state anxiety and trait anxiety. Two of these variables were significant predictors of survival on the CP test after adjustment for other variables in the model: fear of pain and sex (see Figure 3-2). The likelihood of an individual reaching pain tolerance within the observation period (i.e. not being censored) increased by 1.027 times for every unit increase in total FPQ score (exp(0.026)=1.027, p=0.001). For the predictor variable sex, females were approximately half as likely to reach the pain tolerance cut-off point, relative to males (exp(-0.789)=0.454, p=0.005).

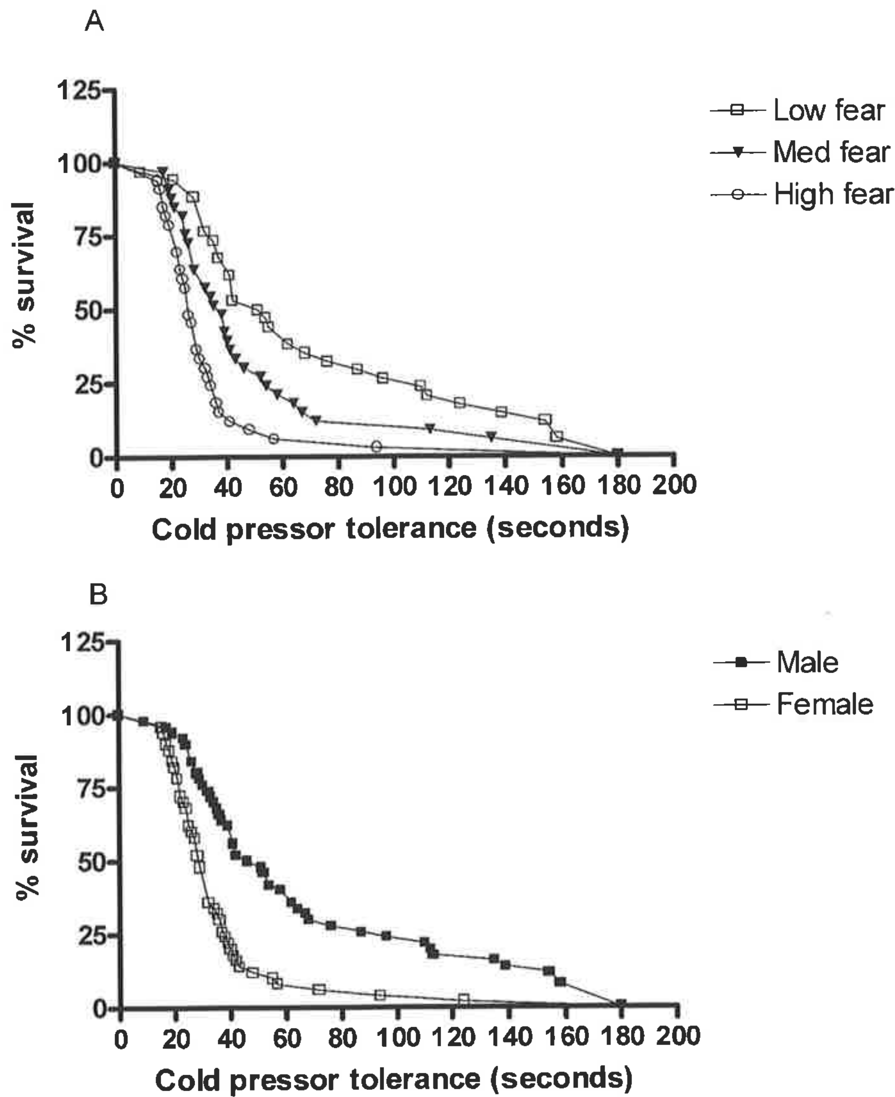

Figure 3-2. Percent survival on the CP test by (A) fear of pain (FPQ-III) and (B) sex in 100 healthy volunteers. Note that, for the purpose of clarity, the FPQ-III variable has been converted to a categorical variable. This was performed by the SPSS® categorize variable function, producing three variables: low (n=34), medium (n=33) and high (n=33) fear of pain. Survival analyses were conducted with the raw data (continuous variable).

3.5.4.2. Electrical stimulation tolerance (ESTOL)

A significant interaction effect for ESTOL was detected between ethnicity and sex ($F_{1,96}$=5.87, p=0.017). Ten[•] individual variables (sex, age, BMI, ethnicity, cigarette smoking, alcohol and caffeine consumption, fear of pain, state anxiety and trait anxiety) and one interaction term (sex*ethnicity) were entered into the Cox regression for ESTOL. Fear of pain and the sex*ethnicity interaction were found to significantly predict survival on the ES test (see Figure 3-3). The likelihood of reaching pain tolerance prior to the cut-off point increasing by 1.021 times for every unit increase in FPQ score, after adjustment for the effects of the other variables in the model (exp(0.021)=1.021, p=0.006). The interaction between ethnicity and sex also contributed significantly to ESTOL (exp(-1.53)=0.216, p=0.004). This interaction indicated that while Caucasian females were less tolerant of ES pain than Caucasian males, Asian females were more tolerant of ES pain than Asian males.

[•] *Skinfold thickness was excluded from the electrical stimulation tolerance regression as this variable was taken around the forearm and considered only to be a potential source of variance in cold pressor performance.*

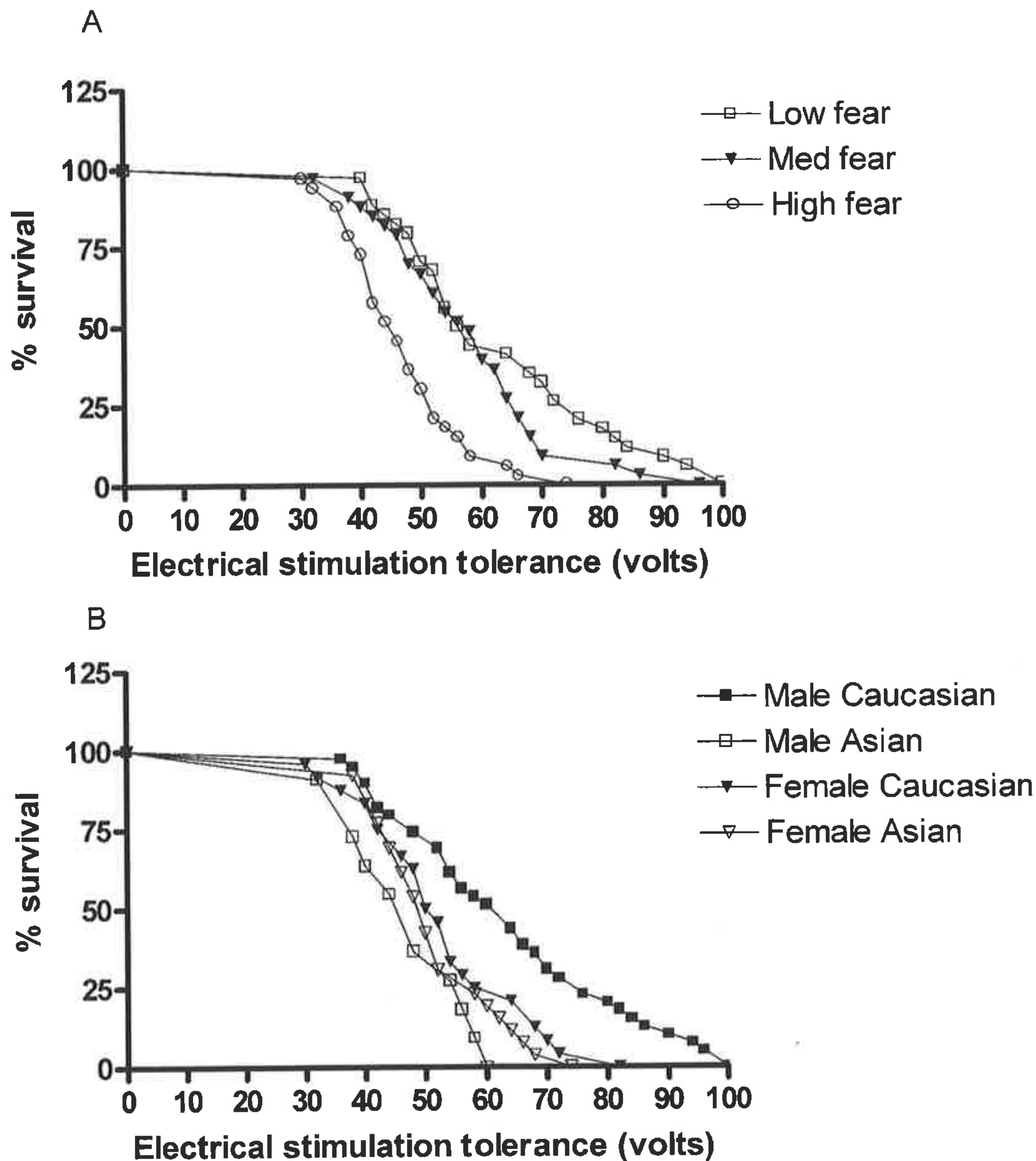

Figure 3-3. Percent survival on the ES test by (A) fear of pain (FPQ-III) and (B) sex ethnicity interaction in 100 healthy volunteers. Note that, for the purpose of clarity, the FPQ-III variable has been converted to a categorical variable. This was performed by the SPSS® categorize variable function, producing three variables: Low (n=34), medium (n=33) and high (n=33) fear of pain. Survival analyses were conducted with the raw data (continuous variable).

3.5.4.3. Impact of menstrual phase

Menstrual phase was not a significant predictor of CPTOL or ESTOL in the 43 normally menstruating females subjects. Simple linear regression analysis revealed that menstrual phase described 3.5% of variance in CPTOL (R^2_{adj}=1.2) ($F_{1,41}$=1.50, p=0.228) and 1.9% of variance in ESTOL (R^2_{adj}=0.5) ($F_{1,41}$=0.78, p=0.382).

3.5.4.4. Test order effects

There were no significant differences on any nociceptive parameter according to the order in which the two tests were performed. Independent samples t-tests of CPTHR and ESTHR revealed no significant differences in response between those who completed the CP followed by the ES test, and those who completed the tests in the reverse order (CP: t(98)=0.75, p=0.45; ES: t(98)=-0.19, p=0.84). Mann-Whitney U tests revealed no test order effects for CPTOL (Z=-1.55, p=0.12) or ESTOL (Z=-0.27, p=0.79).

3.6. Discussion

The central aims of this study were to establish normal values for two human pain induction techniques, and to determine which factors should be controlled for in the selection of subjects for clinical trials of healthy, pain-free humans using these techniques. The CP and ES tests are commonly used techniques, but the methods used for each vary considerably between investigative groups. Standardisation enables comparison between studies, and provides an index of normal values by which to gauge performance. Moreover, due to the maximum limit imposed on pain induction tests, it is imperative when investigating an analgesic intervention that a subject's baseline MTP is sufficiently below the maximum limit to allow a significant increase to be measured. In this respect, the establishment of a normal response range for these tests provides a basis from which to impose inclusion criteria for the upper and lower boundaries of pain response. On the

basis of these normative data, a baseline MTP cut-off in subject selection of one standard deviation above and below the log mean for each test was decided upon for use in subsequent studies. This produces a range of 21-85 seconds and 42-68 volts for subject inclusion on the CP and ES tests, respectively. This range was selected as the upper limit permits an increase of 50% in ESTOL and over 100% in CPTOL before the censor point on each test. In a previous study of healthy volunteers, a steady state plasma morphine concentration of 23 ng/ml was associated with mean increase in CPTOL of 52% and in ESTOL of 15% (Athanasos et al. 2002). The exclusion of subjects according to baseline response to experimental pain induction has previously been reported in a study of the CP test. However, the inclusion range in that study was not based on standardised data (Eckhardt et al. 1998).

Modification of the pain induction technique is another approach by which mean MTP may be reduced. The CP method used in the present study was adapted from Eckhardt and colleagues (Eckhardt et al. 1998), and was developed with the aim of reducing intra-subject variability and reducing the mean MTP. The results suggest that these measures have been effective in reducing CP MTP, with the mean tolerance time associated with the current method (52.6 seconds) being considerably lower than the mean withdrawal latency reported in healthy volunteers using the simplified method, which has been as high as 138 seconds in healthy controls (Compton et al. 2001).

The current technique differs from the model evaluated by Walsh and colleagues (Walsh et al. 1989) in the addition of several features. The inflation of a sphygmomanometer cuff prior to cold water immersion and for the subsequent duration of the test reduces the role of blood flow in determining reaction to cold. It has been reported that restricting blood flow to the immersed limb does not impact upon response to cold (Wolf and Hardy 1941).

However, Garcia de Jalon and colleagues proposed that better control over the haemodynamic condition of the immersed arm may decrease the inter-subject variability that compromises the applicability of the technique to small sample sizes (Garcia de Jalon et al. 1985). In their investigation, a sphygmomanometer cuff attached to the subject's arm was inflated to 20 mm Hg below diastolic 10 seconds prior to immersion in cold and for the subsequent duration of the test. Using a sample of 6 healthy volunteers, a significant difference in pain response was observed between codeine (60 mg) and placebo. An earlier report failed to detect a significant difference between the same dose of codeine, placebo and aspirin (1 g) with a much larger sample size (n=56) (Wolff et al. 1969). The restriction of venous return may also contribute to reducing the mean pain tolerance time. Indeed, the investigation by Garcia de Jalon reported a mean baseline pain tolerance of approximately 30 seconds, which is considerably lower than has been associated with studies that do not induce ischemia (for example Compton et al. 2001).

The immersion of the arm in warm water for two minutes prior to cold water immersion is an adaptation procedure considered to modify the response to the cold water (Blasco and Bayes 1988). The methodology used by Garcia de Jalon and colleagues also involved a brief warm water immersion prior to cold and this may have contributed to the lower mean pain tolerance. The blindfold covering the subject's eyes during the test prevents visual distraction and time cues, and the water pump in the cold water container circulates the ice and cold water to prevent laminar warming around the immersed limb. Consistent with the procedures described in the normative study by Walsh and colleagues, subjects in the current study immersed the forearm and hand in the cold water. This differs from several other studies requiring only the immersion of the hand. While an early investigation reported that immersion of a finger in the cold water induces an equivalent degree of pain

to the immersion of the entire hand (Wolf and Hardy 1941), subsequent findings support the notion of spatial summation in CP pain (Westcott et al. 1977; Martikainen et al. 2004). As discussed, there have been no normative studies of the ES test. Comparison of ES results from the current study with different methods is more complicated as many earlier studies have used current (amps) as the outcome measure (Gracely 1999), whereas voltage was used in the present study. However, ESTOL in the current study is consistent with other studies of healthy volunteers that have assessed response to electrical stimulation of the earlobe in volts (Dyer et al. 1999; Doverty et al. 2001; Athanasos et al. 2002). Walker and Carmody reported a lower mean pain tolerance to electrical stimulation of the earlobe (24±0.4 and 21±0.4 volts in males and females, respectively) compared to the findings of the current study (60±2.6 and 52±1.6 volts in males and females, respectively), though this study assessed a relatively small sample size (n=10 males, n=10 females) (Walker and Carmody 1998).

Findings from the present study indicate that both the CP and ES tests are associated with low intra-subject variability, with no significant differences detected between testing occasions 14 to 21 days apart, for either test. While it has been reported that there is a novelty factor associated with the pain tests used, such that the first test will produce a different result from subsequent tests, the mean intra-subject variation between pain tolerance at first and second testing was minimal (15.1% for the CP, 11.6% for the ES). It may be argued that this is perhaps due to the length of time between testing. To the author's knowledge, the capacity for restoration of the novelty response associated with first testing, given sufficient time between testing occasions, has not been investigated.

Variation in pain response between individuals has been identified as a limitation of pain induction techniques testing MTP. Comparison with other studies is limited, as many

publications do not report mean MTP and standard deviation (SD) or SEM from which to calculate coefficient of variation (CV). Comparison is further complicated by the many variations in methodology, outcome measure, sample size and characteristics. Notwithstanding, the CP tolerance CV associated with the present method (83.8%) is lower than has been observed with the method established by Walsh and colleagues (Walsh et al. 1989), which has produced a CV as high as 124% (Compton 1994). Another report describes a substantially lower CV (23.5%), though this method used a continuous visual analogue scale (VAS) of pain intensity, and the area under the curve of the VAS-time curve was used as the outcome measure (Luginbuhl et al. 2001). The CV for ES tolerance in the current study was 27.9%. The CV associated with the ES tolerance in earlier studies has varied considerably. Luginbuhl and colleagues reported a CV of 32.2% (Luginbuhl et al. 2001) from ES of the toe, while a study by Walker and Carmody found a CV of 5.25% and 6% from ES of the earlobe for males and females, respectively (Walker and Carmody 1998). This may be considered to support the assertion that the use of the earlobe in electrical stimulation is preferable to the use of other stimulation sites (Walker et al. 1993; Walker and Carmody 1998). It must be noted that in the distribution of all parameters, most notably the CP parameters, there is some deviation from the normal distribution, having a moderate degree of positive skew and kurtosis. Moreover, due to censoring of data at the maximum limit of the tests, mean and SD (from which CV is calculated) are less reliable indices of tolerance distribution than median and IQR. In this respect, CV for these parameters should be considered with due appreciation of these limitations.

Despite any decrease in inter-subject variability that may be associated with the current methods, the level of inter-subject variability observed remains high. As described previously, variables implicated in determining pain sensitivity include sex (Riley et al.

1998; Fillingim 2000), age (Edwards et al. 2003; Gibson and Farrell 2004), ethnicity (Zatzick and Dimsdale 1990; Edwards et al. 2001), caffeine consumption (Keogh and Chaloner 2002), cigarette smoking (Pauli et al. 1993), menstrual cycle (Riley et al. 1999), psychological parameters (Chen et al. 1989; Baker and Kirsch 1991; McGrath 1994; Turk 1999) and body size (McKendall and Haier 1983; Lautenbacher and Strian 1993). However, evidence is inconsistent and often contradictory, and there is little indication of the extent to which each should be considered in the design of experimental pain studies with healthy, pain-free subjects. A recent study investigated the involvement and interaction of sex, ethnicity, "psychological temperament" and genetic variation on response to experimental noxious stimuli among 500 healthy volunteers. Sex, ethnicity and "psychological temperament" (as measured by the Temperament and Character Inventory) were found to contribute significantly to response to noxious cold and heat stimuli by interactions with polymorphisms of molecules involved in pain perception (Kim et al. 2004).

Of the variables measured in the present study, only sex and fear of pain contributed significantly to CPTOL. Fear of pain and the interaction between ethnicity and sex together contributed significantly to ESTOL. The findings of the current study, that sex, ethnicity and fear of pain are significant determinants of response to experimentally induced pain, are in line with the recent report that sex, ethnicity and psychological factors contribute significantly to individual variation in response (Kim et al. 2004). Our finding that phase of menstrual cycle did not impact significantly on CP or ES response is also consistent with this investigation, which reported no statistically significant effect of menstrual phase on heat or cold pain (Kim et al. 2004).

It is widely accepted that psychological factors play a significant role in determining pain response (McGrath 1994). Pain-related fear has been shown to contribute to morbidity among chronic pain patients, and in particular in the adjustment to persistent pain (Vlaeyen and Linton 2000; Keefe et al. 2004). In studies of selective attention, chronic pain patients have demonstrated a bias for pain-related material (Pincus and Morley 2001), though it has been suggested that this attentional bias may be mediated by emotional state, in particular pain-related anxiety or fear (Asmundson et al. 1997). Important in the context of this investigation, selective attention bias for pain-related material has also been demonstrated among healthy, pain-free volunteers with high fear of pain (Keogh et al. 2001). It has been suggested that this preferential processing of pain-related material among high-fear, pain-free individuals may be indicative of a greater disposition towards negative responses to pain (Keogh et al. 2001), analogous to the attentional biases that are observed in cases of non-clinical anxiety and considered a vulnerability factor in the development of emotional disorders (Eysenck 1992; Williams et al. 1997). It must be noted that evidence for the existence of selective attentional bias for pain-related material among high-fear, pain-free individuals has been inconsistent (Keogh et al. 2003; Roelofs et al. 2003) and further research is required. Nonetheless, if there were such a cognitive bias in the processing of pain-related information, it may interfere with performance not only on pain induction tests, but also on other tasks performed within experimental pain studies. Thus, while controlling for fear of pain in experimental pain trials may reduce inter-individual variability on pain tests, it may also address the potentially confounding effects of cognitive processing bias on other tasks. Indeed, a recent study reported that the effect of distraction or sensory focusing in modifying subject rating of CP pain among pain-free females was moderated by fear of pain (Roelofs et al. 2004).

Despite the role of pain-related fear in tolerance to nociceptive stimuli, neither state- nor trait-anxiety contributed significantly to test performance. The role of anxiety in experimental pain has not been fully elucidated and findings have been inconsistent (James and Hardardottir 2002). Further, it has been suggested that fear and anxiety represent qualitatively different emotional constructs (Rhudy and Meagher 2000). The current findings support a recent report that fear of pain correlated significantly with ratings of CP pain intensity, while no correlation existed between fear of pain and trait anxiety (Sullivan et al. 2004). It should also be noted that attentional bias for pain-related material among pain-free individuals with high fear of pain was not related to anxiety sensitivity, defined as the fear of anxiety-related sensations (Keogh and Cochrane 2002).

The significant contribution of sex to CPTOL, with males being more tolerant of CP pain than females, is consistent with extensive clinical and experimental reports of sex differences in pain. As described, greater pain sensitivity in females compared to males has been demonstrated in cold (Walsh et al. 1989), heat (Feine et al. 1991; Fillingim et al. 1999), pressure (Jensen et al. 1992; Chesterton et al. 2003), electrical (Lautenbacher and Rollman 1993) and mechanical pain (Sarlani and Greenspan 2002).

The influence of ethnicity on test performance in the current study raises several issues. Firstly, the interaction between sex and ethnicity in ESTOL was such that males were more tolerant of ES pain than females, but only among Caucasians. Asian females were more tolerant of ES pain than Asian males. While the greater tolerance of ES among Asian females compared to Asian males is not consistent with the majority of literature on differences in pain between the sexes, sex differences within specific ethnic groups have not been well defined. Secondly, the lack of significant effect of ethnicity on CPTOL in the current study, either individually or as an interaction with sex, is not consistent with

several reports of the impact of ethnicity on CP performance (Knox et al. 1977; Walsh et al. 1989; Kim et al. 2004). However, the role of ethnicity in the current study must be considered in the context of limitations associated with this investigation as well as those inherent to the study of ethnicity and pain. Many investigations have examined ethnicity and pain, yet there remains a lack of consensus regarding the role of ethnicity in response to noxious stimuli. In experimental pain research, findings often vary according to the type of stimulus employed (Lautenbacher and Rollman 1993). A further difference is the ethnic groups investigated. In a 1990 review of studies examining pain and ethnicity, it was noted that, of the thirteen studies reviewed, no two investigations studied the same combination of cultural groups (Zatzick and Dimsdale 1990). It is therefore difficult to make meaningful comparisons with other investigations. It should also be considered that the current study included only Caucasians and Asians, and thus the generalisability of findings to other ethnic or racial groups is uncertain.

Several additional problems are encountered in the study of ethnicity and pain that complicate the interpretation of findings. Edwards and colleagues have described the important distinction between ethnicity and race (Edwards et al. 2001), though defining and classifying the ethnicity of a sample itself is complicated (Aspinall 1997). In the current study, ethnicity was established by self-identification. More correctly, this classification determined race, as it failed to take into consideration the numerous social, linguistic and cultural factors that characterise ethnicity. Cultural beliefs and practices related to the experience of pain and the individual's response to pain can vary considerably (Melzack and Wall 1996). It would, therefore, have been preferable to distinguish between the cultural identity or heritage of the participants, in addition to the biological and physical characteristics associated with race. It would have been beneficial to differentiate, for example, between Asians who had been born in Australia and those

who had immigrated at a later age. Indeed, it is probable that subjects in the current study who identified as Caucasian also derived from different cultural backgrounds and influence. The lack of recognition of the heterogeneity within ethnic groups is a common limitation (Morris 2001). It is unlikely that such broad classifications as Caucasian, Asian and African will elicit a true representation of the impact of ethnicity on test performance, given not only the broad genetic variation within these groups, but also the role of cultural experiences and upbringing (Melzack and Wall 1996). It becomes apparent, then, that the classification of participants according to race or ethnicity is a complex and multifaceted undertaking, and this classification is inadequately addressed in the majority of experimental pain studies. Indeed the difficulties involved in defining and classifying the ethnicity of a sample have previously been recognised (Aspinall 1997). In this respect, the findings from the current study regarding the impact of ethnicity should be interpreted with due appreciation of these limitations.

The method by which nociception is evaluated in experimental pain trials has been the subject of significant debate. There is a significant limitation inherent to the assessment of nociception in humans. Pain is a subjective and private experience, which may be modulated by many variables, and for which there is no reliable, objective marker (Kumar et al. 2002). Determining the level of pain experienced by an experimental subject or by a clinical pain patient is, for the most part, dependent on the report of the individual in pain. While the noxious input and testing environment may be standardised, the method by which the degree of nociception or antinociception produced is quantified remains a complicated and contentious issue. Physiological correlates such as autonomic indices (e.g. skin conductance and resistance, pulse rate, and skin temperature) (Bromm and Scharein 1982; Dowling 1983), direct recording from peripheral nerves (Hallin and Torebjork 1974; Fors et al. 1984), and evoked potentials (Bennett and Jannetta 1980;

Coger et al. 1980; Stohr et al. 1981) have been investigated as indices of pain. Such measures in isolation are typically not considered to be reliable indices of the pain experience. Neuroimaging has increasingly received attention as a measure of pain (Bradley et al. 2000; Bornhovd et al. 2002). However, these methods are costly and are typically not readily accessed or used. Notwithstanding, the field of pain measurement has experienced considerable development in recent years and several promising approaches are emerging (Gracely 1999). The use of rating scales, such as category judgements and visual analogue scales (VAS) requiring subjects to report on the intensity and unpleasantness of the stimulus is a common approach as it is economical and straightforward, both to administer and for the subject to understand. The validity and reliability of these techniques have been the subject of several investigations. These assessments have, for the most part, focused on the repeatability of VAS measurements of pain. Early reports concluded that the use of VAS in pain measurement exhibited very high test-retest correlations (Gracely et al. 1978; Price et al. 1983). There has been considerable debate regarding the validity of the correlational method of establishing repeatability, which has recently been outlined by Rosier and colleagues (Rosier et al. 2002). This investigation sought to determine methods by which reproducibility of VAS pain assessment may be maximised, concluding that session-to-session variability in VAS may be minimised by incorporating several features into the structure of the assessment procedure.

The use of threshold and tolerance, as employed in the present study, is another widespread approach, though it has received some criticism. It has been argued that such methods are highly subjective, that responses vary widely according to the instructions given, and that subjects can too easily be biased in their response (Gracely 1999). However, under carefully controlled conditions, the use of threshold and tolerance can

produce valid outcomes (see Chapman et al. 1985). It has been observed that opioid analgesia is less evident in pain threshold than pain tolerance, and that tolerance more reliably detects analgesic effects (Luginbuhl et al. 2001). Indeed, tolerance has been demonstrated to be reliably altered by morphine and other opioids as discussed earlier. However, tolerance has been criticised as it represents the level of pain a subject is willing to endure, and this may alter as the subject becomes more comfortable with the technique, or more familiar with the experimenter and experimental environment (Chapman et al. 1985). It has also been suggested that testing paradigms requiring subjects to endure a stimulus until the pain can no longer be tolerated does not measure pain tolerance but rather an alternative construct related to endurance (Wolff 1971; Cleeland et al. 1996). Notwithstanding, experimental pain paradigms using tolerance have been reported to approximate clinical pain more than other experimental pain techniques, as there is typically a level of anxiety or arousal involved in testing (Chapman et al. 1985). Overall, it is apparent that there is a lack of an objective, reliable index of the pain experience. The current study demonstrates the reproducibility of pain threshold and tolerance on the CP and ES tests, supporting evidence that these indices can be associated with valid, reliable outcomes in a carefully controlled study.

Excluding subjects according to the baseline pain response criteria suggested (i.e. 21-85 seconds and 42-68 volts for CP and ES tolerance, respectively) would have precluded 29% and 32% of those tested from participation in future trials, based on MTP for the CP and ES, respectively (see Table 3-7). Interestingly, there was very little consistency between those who were outside the normal range (determined to be the standard deviation above and below the log mean) on the CP and the ES. That is, those who fall into the CP normal range are typically not the same individuals who fall into the normal range on the ES. Only five of the subjects tested (5%) were outside the normal range on both CP and ES (4

above normal range on both tests, 1 above normal range on CP and below normal range on ES). This finding has several implications. Firstly, it suggests that a high or low level of sensitivity to a particular nociceptive stimulus cannot be generalised to other stimulus modalities. The finding supports the recommendation that studies using experimental pain paradigms include more than one type of test (Wolff et al. 1976). Moreover, it intimates that different factors underlie sensitivity to different types of pain.

	% excluded due to tolerance *below* normal range	% excluded due to tolerance *above* normal range	Total excluded (%)
CP	12	17	29
ES	14	18	32

Table 3-7. Proportion of sample (n=100) who would be excluded from future trials on the basis of baseline nociceptive tolerance outside the determined normal range (of 21-85 seconds for CP, and 42-68 volts for ES tolerance).

This introduces the issue, then, that if more than one stimulus modality is to be used in an investigation, whether subjects be enrolled only if within the normal range on all pain tests. There is a strong justification for having only subjects participating in a study who are within the normal range on all tests used, given the requirement that baseline pain response be sufficiently below cut-off to allow a significant increase to be observed, and for the purpose of reducing inter-subject variability in response to each test. There are also practical implications for subject recruitment based on these results. If subjects were to be excluded from participation in a study if their baseline MTP on either test was outside of the normal range, of the cohort of 100 subjects enrolled in the current study, 61% would have been excluded (24% based on their CP response, 27% based on their ES response, and only 5% based on both CP and ES). By excluding such a large proportion of potential subjects, not only is the task of recruitment made considerably more complicated, but raises significant concerns regarding the representativeness of the sample. It must be emphasised that the fact that only 39% of the *normal* population would be included does

not indicate that only this proportion fall into the *normal* range. Rather, it is that only 39% fall into the normal range for both tests. It may be argued that the "normal" range determined by the present study (the standard deviation above and below the log mean) is too stringent given the relatively large proportion of individuals who fall outside this range. However, previous studies have demonstrated an increase in CP tolerance from 52% (Athanasos et al. 2002) up to 100% (Doverty et al. 2001) following administration of morphine to healthy controls. Including subjects whose baseline pain tolerance is above the inclusion cut-off of 85 seconds (CP) or 68 volts (ES) may preclude the valid assessment of the magnitude of the analgesic effect associated with an intervention. An alternative approach is to identify the primary outcome test and recruit subjects on the basis of performance on this test. For example, as described, the CP test has previously been associated with a greater magnitude of opioid effect than ES (Luginbuhl et al. 2001). Thus this test may be selected as the primary outcome measure and subjects screened for baseline performance on this test only. This approach has been taken in subsequent studies described herein (see Chapters 4, 5 and 6).

In summary, the present study has established normative data for the CP and ES tests and provided the basis for upper and lower boundaries of baseline MTP for subject selection in studies with healthy, pain-free volunteers. Fear of pain contributed significantly to performance, with greater fear associated with lower MTP. Consistent with previous findings, sex was a significant contributor to CPTOL. The interaction between sex and ethnicity contributed significantly to ESTOL.

An increasing number of physiological and genetic factors has been implicated in determining or altering response to pain, including the involvement of the cardiovascular (Edwards et al. 2001) and immune systems (Hutchinson et al. 2004), and chronic exposure

to opioids (Vanderah et al. 2001). In recent years the role of genetic determinants of pain has increasingly become a focus of research. Initially animal studies and, more recently human studies, have investigated the extent to which genotype is involved in pain sensitivity, concluding that the genotype of an individual can significantly impact upon pain response. A recent study investigated the involvement and interaction of sex, ethnicity, "psychological temperament" and genetic variation on response to experimental noxious stimuli among 500 healthy volunteers. Sex, ethnicity and "psychological temperament" (as measured by the Temperament and Character Inventory) were found to contribute significantly to response to noxious cold and heat stimuli by interactions with polymorphisms of molecules involved in pain perception (Kim et al., 2004).

It is likely that our increasing understanding of the role of genetics in pain response will elucidate a greater understanding of inter-individual variability in response to noxious stimuli. While there are limitations inherent to the induction and valid assessment of pain, when performed in carefully controlled circumstances, experimental pain testing can produce reliable, valid results, and can be of considerable utility in the development of novel analgesic strategies.

4. ANTINOCICEPTIVE ACTIVITY OF BUPRENORPHINE IN EXPERIMENTAL PAIN: DOSE FINDING STUDY

4.1. Overview

The unique and complex pharmacological profile of buprenorphine (BUP) has attracted substantial interest and investigation since it was first synthesised in 1966. Derived from thebaine, BUP is a μ-opioid receptor partial agonist that has consistently demonstrated its utility as an analgesic agent in animals and in clinical populations (Cowan 2003). With moderate intrinsic activity, high binding affinity and slow receptor kinetics at the μ receptor, and an apparent respiratory depression ceiling at high doses (Walsh et al. 1994; Walsh et al. 1995), BUP is associated with a long duration of action, lower dependence and abuse liability, relatively mild withdrawal following chronic dosing, and a good safety profile compared to other μ agonists (Jasinski et al. 1978). In recent years, the role of BUP in the management of opioid dependence has been the subject of extensive investigation (Mattick et al. 2004) and its use for this indication has grown significantly. BUP has also classically been recognised as an antagonist at the κ-opioid receptor; however as described below, recent investigation suggests the nature of BUP's interaction with both κ- and δ-opioid receptors may be more complex (Reisine and Bell 1993; Zhu et al. 1997; Huang et al. 2001). The analgesic effect of the compound is generally thought to result from its interaction with the μ-opioid receptor, though recent reports propose that this effect may be compromised by interaction with the ORL1 receptor (Lutfy et al. 2003). Some evidence suggests that BUP produces its effects by mechanisms that differ from those of classic μ-opioid receptor agonists, and this may underlie several reports that BUP might be useful in the treatment neuropathic pain (Kouya et al. 2002).

The antinociceptive effects of BUP have been well characterised in animal models, and its efficacy in treating both acute and chronic pain has been consistently demonstrated. Unlike many other opioid and non-opioid analgesics, BUP has not been evaluated in human experimental pain paradigms. Hence, we do not know whether BUP produces measurable antinociception to experimental noxious stimuli in healthy volunteers, the steepness of the dose response curve, nor how this response may vary according to different types of stimuli. Importantly, given the lack of evidence for BUP in human experimental pain, a dose-response curve for BUP antinociception in a human experimental pain paradigm has not been established. As described, the broad aim of this research project is to investigate the combined effects of BUP and the opioid antagonist naloxone (NLX) on experimentally induced pain. Animal data have indicated that the addition of nanomole doses of naloxone to sub-analgesic doses of BUP results in enhanced antinociception in a rodent model of neuropathic pain (Cougnon-Aptel et al. unpublished). To determine whether this effect occurs in humans, an investigation in an experimental pain paradigm will be conducted. The advantages of evaluating this concept in an experimental pain paradigm prior to investigation in a clinical pain population have been described (see 1.9.3).

Given the lack of evidence for the effect of BUP on human experimental nociceptive tests, a dose-finding study in healthy volunteers was required. This study served two purposes: to establish whether the selected pain induction techniques, the cold pressor (CP) and electrical stimulation (ES) tests, are sensitive assays for BUP antinociception, and if so, to allow a sub-optimal dose of BUP to be selected for use in the subsequent BUP:NLX ratio studies. The current chapter will review the pharmacological profile of BUP and evidence for its analgesic efficacy, and describe the methodology and results from the dose-finding study.

4.2. Pharmacology of buprenorphine

BUP [21-cyclopropyl-7α-[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydrooripavine] has a number of characteristics that make it well suited for use as an analgesic and, as has increasingly occurred in recent years, for use as a maintenance pharmacotherapy for opioid dependence. With moderate intrinsic activity, high binding affinity and slow receptor kinetics at the μ-receptor, and an apparent respiratory depression ceiling at high doses (Walsh et al. 1994), BUP is associated with lengthy duration of action, lower dependence and abuse liability, relatively mild withdrawal following chronic dosing, and a good safety profile compared to other μ agonists (Jasinski et al. 1978). Recommended doses for pain relief in adults are 300-600 μg IM or by slow IV injection every 6-8 hours, or 200-400 μg sublingually every 6-8 hours. The effective dose range for opioid substitution varies but has been reported as 2-32 mg (Johnson et al. 2000; Mattick et al. 2003).

BUP is a partial agonist at the μ-opioid receptor (Rothman et al. 1995; Lee et al. 1999; Huang et al. 2001). First proposed in 1956, the classification of "partial" agonism relates to the intrinsic activity of a compound (Stephenson 1956): a partial agonist has less intrinsic activity, that is, will exert a lesser maximal effect, than a full agonist. Furthermore, by displacing a full agonist, a partial agonist may exert antagonistic effects. Thus a partial agonist may behave as an agonist or an antagonist in different circumstances (Ariens 1983). Accordingly, as a μ-opioid receptor partial agonist, BUP has lower intrinsic activity at the μ-receptor than a full agonist such as morphine and methadone. While BUP may act as an agonist at the μ-receptor, it may also antagonise μ-receptor-mediated effects of full (high intrinsic activity) agonists and can thus precipitate withdrawal in opioid-dependent animals and humans. Indeed, preclinical studies have demonstrated that acute pretreatment with BUP blocks the behavioural effects of μ-receptor agonists (Cowan et al. 1977; Leander

1983), and chronic administration of BUP in clinical studies can attenuate or block the effects of opioid agonists, including morphine and heroin (Jasinski et al. 1978; Mello et al. 1982; Bickel et al. 1988; Teoh et al. 1994).

BUP also acts as a κ- and δ-opioid receptor antagonist, and has thus been described as a mixed agonist-antagonist with partial agonist activity at the μ-receptor (Bickel and Amass 1995; Cowan and Lewis 1995). While BUP is classically considered to act as an antagonist at the κ receptor (see review by Cowan 1995), BUP's interaction with this receptor type has also been controversial, with some reports of low-efficacy partial agonist activity (Zhu et al. 1997; Huang et al. 2001). Contributing to the complex pharmacological profile of BUP, norbuprenorphine (norBUP), a major dealkylated human metabolite of BUP, has been reported to be a full agonist at the δ-opioid receptor, while the parent drug acts as a competitive antagonist against the effects of norBUP at this receptor (Huang et al. 2001). Thus the drug may exert varying actions, either primarily agonistic or antagonistic, at the δ receptor according to the relative concentrations of BUP and norBUP (Huang et al. 2001). Furthermore, differential effects of chronic dosing with BUP on binding sites for δ_2 receptors but not δ_1 receptors have led to the suggestion that the drug exerts diverse effects on the two subtypes *in vivo* (Huang et al. 2001).

Traditionally BUP has been reported to have a high affinity for both μ and κ receptors, and a low affinity for δ receptors (Lewis 1985), though BUP has been shown to have a high affinity for the cloned δ receptor (Reisine and Bell 1993). Villiger reported K_i values of 0.31 nM and 0.38 nM for BUP binding with μ and κ receptors, respectively (Villiger 1984). More recent reports describe a high affinity for all three classic opioid receptor types for both BUP (Miller et al. 2001; Negus et al. 2002) and norBUP (Huang et al. 2001). Miller and colleagues have reported K_i values in the subnanomolar to nanomolar ranges for

BUP binding with all opioid receptor types, including the ORL1 receptor (Miller et al. 2001). Rank order of binding affinity was $\mu > \kappa > \delta >$ ORL1. Huang and colleagues recently reported comparable high affinity of BUP and norBUP for μ, κ and δ receptors, with K_i values in the subnanomolar to nanomolar range, and low binding affinity for the ORL1 receptor (Huang et al. 2001)(see Table 4-1).

	μ	δ	κ	ORL1
BUP	0.08 (±0.02)	0.42 (±0.04)	0.11 (±0.05)	285 (±30.0)
norBUP	0.07 (±0.01)	3.14 (±0.30)	0.91 (±0.14)	7330 (±2884)
K_i values calculated from the equation $K_i = IC_{50}/(1+[L]/k_d)$				

Table 4-1. Apparent K_i values (nM) of BUP and norBUP for the opioid receptors and the ORL1 receptor. Reproduced with permission from Huang et al.,2001.

As described in section 4.2.2.3, peak BUP and norBUP plasma concentrations (C_{max}) following a single IV BUP dose of 1.2 mg were 37.52 ng/ml and 0.57 ng/ml, respectively. These concentrations equate to 80.2 nM and 1.38 nM for BUP and norBUP, respectively. Based on the K_i values above, a 1.2 mg IV BUP dose would be associated with BUP binding with μ, δ and κ receptors, and norBUP binding with μ and κ receptors. However, a much higher concentration would be required for either BUP or norBUP to interact with the ORL1 receptor. As a 1.2 mg IV BUP dose is greater than normally administered for pain management (0.3 – 0.6 mg IM or IV), it may reasonably be concluded that BUP or norBUP interaction with the ORL1 receptor would not occur at analgesic doses.

BUP has a longer duration of analgesic action than many other opioid agonists (Cowan et al. 1977). Another distinctive feature of the pharmacological profile of BUP is the compound's slow receptor kinetics. While BUP is highly lipophilic and thus reaches the brain quickly, it dissociates very slowly from the μ receptor (Hambrook and Rance 1976; Schultz and Herz 1976; Wuster and Herz 1976; Tallarida and Cowan 1982). BUP's slow receptor kinetics and high binding affinity for the μ receptor are thought to contribute to its

long duration of action (Kuhlman et al. 1996) and the relatively mild nature of the withdrawal experienced upon cessation of chronic use (Hambrook and Rance 1976; Dum and Herz 1981).

In humans, BUP produces typical μ-opioid agonist effects (Jasinski et al. 1978), and as an analgesic for acute pain, has been estimated to be between 25 and 50 times more potent than morphine (Cowan et al. 1977; Jasinski et al. 1978). As an antagonist, BUP has been shown to be as potent as the opioid antagonist NLX, with a longer duration of action (Cowan et al. 1977; Dum and Herz 1981).

Unlike full agonists such as morphine, BUP is resistant to displacement by μ-receptor antagonists (Rothman et al. 1995). While pre-administration of NLX has been shown to symmetrically shift the bell-shaped dose-response curve to the right (Lewis 1974; Cowan et al. 1977; Rance 1979; Dum and Herz 1981), when NLX is administered *after* BUP, it fails to reverse BUP's agonist effects (Cowan et al. 1977; Orwin 1977; Gibbs et al. 1982). However, there is evidence that NLX can reverse BUP-related respiratory depression. A placebo-controlled, single-blind study evaluated the ability of three (1.0, 5.0, 10 mg) doses of NLX to antagonise the established respiratory depressant effects of a standard analgesic BUP dose (0.3 mg/70kg IV). While 1 mg of NLX had little effect on respiratory depression, both 5 mg and 10 mg doses produced consistent reversal of respiratory depression (Gal 1989).

One of the most intriguing aspects of BUP's pharmacological profile is its dose-response curve (DRC). When tested over a range of doses, BUP has been shown to produce a plateau effect at high doses (Walsh et al. 1994; Liguori et al. 1996) or a bell-shaped dose-response curve (Cowan et al. 1977; Dum and Herz 1981). In laboratory tests of

antinociception, the bell-shaped dose-response curve has been demonstrated in response to medium-high intensity antinociceptive tests (Cowan et al. 1977; Rance 1979; Dum and Herz 1981; McCarthy and Howlett 1984; Hawkinson et al. 2000). This dose-response has been demonstrated across a range of other effects, including respiratory depression, gastrointestinal motility and catalepsy, with either reduced or no greater effects evident with increasing BUP doses (Cowan et al. 1977; Cowan et al. 1977). However, in contrast to the laboratory studies, a submaximal ceiling on the analgesic effect of the drug in clinical use has not been demonstrated (Cowan 1995), though a response plateau at high BUP doses has been demonstrated with some physiological and subjective effects in humans. An increase in the IV BUP dose from 0.3 mg to 0.6 mg was reported to produce a dose-related increase in analgesia and neuroendocrine effects (Watson et al. 1982), though the same doses administered over a 20-minute infusion period produced no such dose response for respiratory depression (de Klerk et al. 1981). More recently, Walsh and co-workers reported that a 32 mg sublingual dose of BUP was associated with no more respiratory depression than a 16 mg dose (Walsh et al. 1994)(see Figure 4-1). This is of considerable clinical importance as respiratory depression contributes significantly to cases of opioid-related mortality (White and Irvine 1999). A bell-shaped dose response curve or flattening of the dose response at higher doses was also reported for other subjective and physiological effects of BUP, including pupillary miosis and scores for subjective measures of positive mood. These findings were substantiated by a subsequent study, which demonstrated that the effects of BUP did not "increase appreciably" at doses above 8 mg (sublingual), even up to to the highest dose administered, 32 mg (Walsh et al. 1995). Such doses far exceed those administered for pain management (0.3 – 0.6 mg IM/IV, or 0.2 - 0.4 mg sublingual).

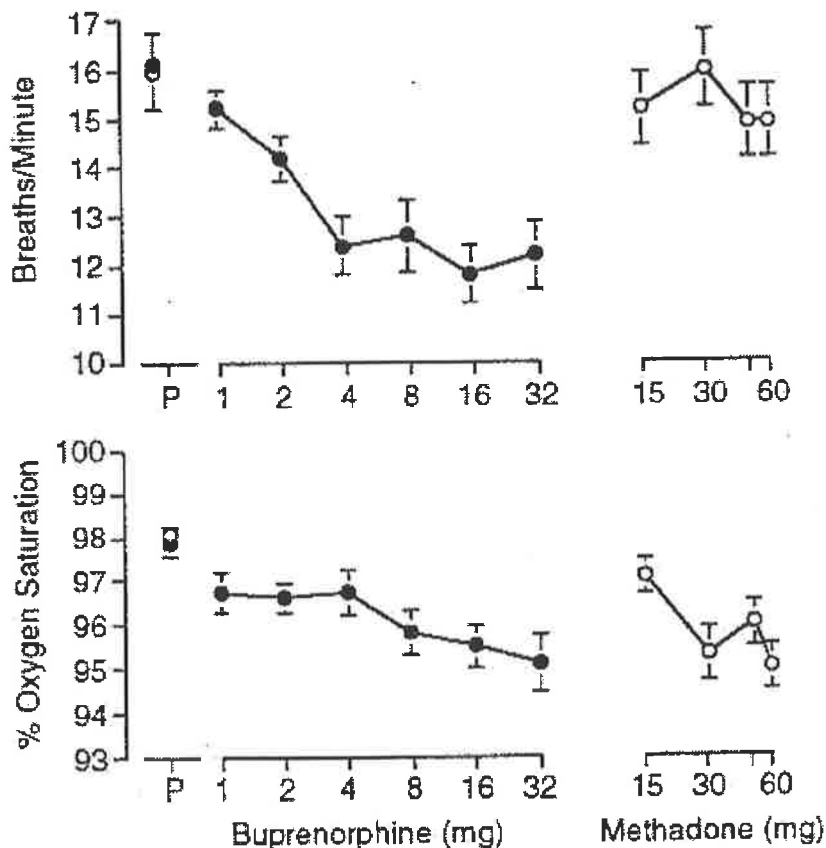

Figure 4-1. The effects of buprenorphine (closed circles) and methadone (open circles) are shown for respiratory rate (upper panel) and arterial oxygen saturation (lower panel). Each vertical bar represents ±1 SEM. Reproduced with permission from Walsh et al., 1994.

The distinctive dose-response is typically explained by BUP's partial agonist effects or by other receptor mechanisms, such as antagonism at the κ receptor (Leander 1987) or activity at the δ-receptor (Sadee et al. 1983). As described, recent findings also indicate that BUP has agonist activity at an additional receptor type, the ORL1 receptor (Wnendt et al. 1999; Bloms-Funke et al. 2000; Hawkinson et al. 2000; Huang et al. 2001). Activation of the ORL1 receptor is increasingly recognised to modify nociception, although the nature of this influence remains contentious (Mogil and Pasternak 2001).

4.2.1. Interaction with the ORL1 receptor

As described previously (see 1.6.3), the ORL1 receptor shares a high level of sequence homology with traditional opioid receptors, but in mammalian cells typically demonstrates very little binding affinity with opioids (Henderson and McKnight 1997; Barlocco et al. 2000). In 1995 an endogenous ligand for this receptor was identified and independently given two names: nociceptin and orphanin FQ (N/OFQ). Initially thought to cause hyperalgesia, Meunier and colleagues named the ligand nociceptin (Meunier et al. 1995). Subsequent findings suggested that the hyperalgesic effect was due to the blockade of stress-induced antinociception, and proposed that the ligand was an anti-opioid peptide (Reinscheid et al. 1995). Despite an increasing focus on the role of N/OFQ, its effect on pain has not been clearly elucidated (Mogil and Pasternak 2001).

Supraspinal activation of ORL1 receptors by N/OFQ has been shown to block the antinociceptive effects of μ-, δ- and κ -opioid receptor agonists (Mogil et al. 1996). In contrast, activation of spinal ORL1 receptors by N/OFQ has been associated with antinociception in the tail-flick assay (see Mogil and Pasternak 2001). Furthermore, N/OFQ has demonstrated additional antinociceptive effects, including attenuating wind-up (Stanfa et al. 1996), inhibiting the release of calcitonin gene related peptide (Helyes et al. 1997), attenuating thermal hyperalgesia in animal models of neuropathic pain, and antinociception on the rat formalin test after intrathecal application (Yamamoto and Nozaki-Taguchi 1997).

BUP has been reported to be a full (Wnendt et al. 1999; Hashimoto et al. 2000) or partial (Bloms-Funke et al. 2000; Hawkinson et al. 2000) agonist at the ORL1 receptor, seemingly depending upon the assay or endpoint used (Bloms-Funke et al. 2000; Huang et al. 2001), while norBUP has been described as a full ORL1 agonist (Huang et al. 2001).

It has been postulated that BUP's antagonism of opioid effects (for example Walker et al. 1995) results from BUP's agonist activity at the ORL1 receptor, given N/OFQ has previously been reported to attenuate the effects of opioids (Mogil et al. 1996). As described previously (section 4.2), both BUP and norBUP have a low affinity for the ORL1 receptor (micromolar range), with K_i values exceeding concentrations found clinically (Huang et al. 2001). Notwithstanding, several recent studies have sought to understand how binding with ORL1 receptors may impact upon BUP's antinociceptive effects. It was hypothesised that the agonist effect of BUP at the ORL1 receptor might contribute to BUP's bell-shaped dose-response curve (Wnendt et al. 1999; Bloms-Funke et al. 2000). Given the inconsistent reports of both anti- and pronociceptive effects with nociceptin binding at the ORL1 receptor, it is difficult to speculate whether BUP agonism of the ORL1 receptor would have anti- or pronociceptive effects. It was proposed that interaction with the ORL1 receptor may have a pronociceptive effect, contributing to the reduction in effect at higher doses (Bloms-Funke et al. 2000). However, while a peak antinociceptive effect in rodents has been identified at a BUP dose of 0.5 mg/kg (s.c.), above which the effect plateaus or diminishes (Dum and Herz 1981), Pick and coworkers reported that at very high doses (in excess of 10 mg/kg), there was a second increasing arm in dose response to the hot water tail flick test in mice (Pick et al. 1997). Thus, interaction with the ORL1 receptor may alternatively be antinociceptive, potentially explaining the increasing dose response arms (Bloms-Funke et al. 2000).

The potential involvement of ORL1 receptor activation in the distinctive dose-response curve observed with BUP has recently been investigated following the development of an ORL1 receptor antagonist. Using the tail flick assay, Lutfy and coworkers (Lutfy et al. 2003) reported that in ORL1 receptor knockout mice, the antinociceptive effect of BUP, but not morphine, was enhanced. This is further supported by the finding that wild-type mice,

but *not* ORL1 knockout mice, co-administered BUP and the ORL1 antagonist J-113397 (Kawamoto et al. 1999) showed significantly enhanced antinociception (Lutfy et al. 2003). Furthermore, the bell-shaped dose-response curve that was observed in the wild-type mice following administration of BUP only was eliminated when BUP was co-administered with the ORL1 antagonist J-113397. In addition, BUP-induced antinociception was absent in μ receptor knockout mice, consistent with previous reports that BUP antinociception is μ receptor mediated (see Cowan 1995). Together these data suggest that BUP antinociception in mice is mediated by the μ-receptor but compromised by activation of ORL1 receptors. There is no published evidence of the effect of ORL1 receptor activation on BUP antinociception in humans.

4.2.2. Human pharmacokinetics

The human kinetics of BUP have been determined by several routes of administration in both analgesic doses and the higher doses administered for the management of opioid dependence. Human pharmacokinetic studies have focused primarily on four routes of administration: intravenous (IV), intramuscular (IM), oral (PO) and sublingual (SL). Low oral bioavailability has drawn increased attention to the enhanced safety and convenience of sublingual administration compared to administration by injection, particularly for outpatient dosing of opioid-dependent patients.

Much of the early pharmacokinetic data were obtained by radioimmunoassay (Bartlett et al. 1980), which is reported to have compromised the reliability of these data due to cross-reactivity with norBUP and the glucuronide conjugate of BUP (Kuhlman et al. 1996). More recent studies have used more specific gas or liquid chromatographic/mass spectrometry assays (Kuhlman et al. 1996; Everhart et al. 1997; McAleer et al. 2003), though sensitivity at the lower doses used for analgesia (300-600 μg) is still a limiting factor. With the recent

focus on the sublingual administration of BUP in its expanding role as a pharmacotherapy for opioid dependence, much of the recent pharmacokinetic work has investigated this route of administration.

The recent development of a transdermal BUP delivery system (Budd 2003) for the management of pain has also demonstrated promising results and is likely to be the subject of increased pharmacokinetic analysis.

This section will outline BUP disposition in humans by different routes of administration.

4.2.2.1. Oral administration

Low oral bioavailability, largely due to substantial first pass metabolism (Brewster et al. 1981; McQuay and Moore 1995), is a significant limiting factor in the oral administration of BUP. Bioavailability following oral administration has been estimated to be 14% (Mendelson et al. 1997).

4.2.2.2. Sublingual administration

Sublingual administration of BUP provides an effective and safer alternative to injectable formulations, which is of particular advantage in the use of BUP as a pharmacotherapy for opioid dependence and in the management of chronic pain. BUP demonstrated clinical efficacy in analgesia by the sublingual route in the late-1970s (Edge et al. 1979; Fry 1979). Early studies evaluated the pharmacokinetic profile of sublingual BUP in the analgesic dose range (Bullingham et al. 1981; Bullingham et al. 1982), while more recent studies have investigated the disposition of sublingual BUP in the higher doses prescribed for opioid dependence (Walsh et al. 1994; Kuhlman et al. 1996; Nath et al. 1999; Schuh and Johanson 1999; McAleer et al. 2003). Kinetics of sublingual BUP in the analgesic dose

range estimated bioavailability at approximately 55% (Bullingham et al. 1982), though significant inter-individual variation in plasma BUP profiles were observed compared to that observed in an earlier study of parenteral administration (Bullingham et al. 1980). Time to highest plasma concentration (T_{max}) was 210±40 minutes and 192±49 minutes following administration of 0.4 mg and 0.8 mg sublingual BUP, respectively (Bullingham et al. 1982). It should be noted, however, that these studies employed the radioimmunoassay developed by Bartlett and coworkers (Bartlett et al. 1980), the limitations of which have already been discussed (section 4.2.2). Nothwithstanding, more recent mean sublingual bioavailability estimates (51.4%) following administration of a substantially greater dose (4.0 mg) are consistent with these early data, as is the large degree of inter-individual variability (12.8–92.2%) (Kuhlman et al. 1996). Mean T_{max} following 4 mg (0.71h) was substantially lower than the means reported following 0.4 mg (3.5h) and 0.8 mg (3.2h) BUP. These shorter T_{max} values reported by Kuhlman and coworkers are in line with another study of high-dose sublingual BUP kinetics reporting T_{max} between 0.5-1.0h following administration of 2-32 mg sublingual BUP (Walsh et al. 1994). Some studies have reported that absorption is dependent on saliva pH (Weinberg et al. 1988; Mendelson et al. 1997), but this finding has been inconsistent (Nath et al. 1999). Terminal half-life ($t_{1/2}$) following sublingual administration has been estimated at 26 hours, but reports have varied considerably (9-69 hours) (McAleer et al. 2003).

The recent focus of sublingual BUP kinetics has been on the comparison between tablet and liquid formulations (Nath et al. 1999; Schuh and Johanson 1999) for use in the treatment of opioid dependence.

4.2.2.3. Intravenous administration

BUP kinetics in acute IV administration have been investigated in postoperative patients at 0.3 mg and 0.6 mg (Bullingham et al. 1980; Watson et al. 1982). These studies used the non-specific radioimmunoassay developed by Bartlett and colleagues to assess BUP in plasma (Bartlett et al. 1980). More recently, IV kinetics have been assessed in healthy volunteers at a dose of 1 mg using a specific gas chromatographic electron-capture detector assay, and in healthy males with a history of opioid dependence at a dose of 1.2 mg using a specific gas chromatographic-tandem mass spectrometric assay (Kuhlman et al. 1996). Single dose IV kinetics have also been studied in anaesthetized patients (n=24) (Bullingham et al. 1980) and anaesthetized patients with renal failure (n=5) (Summerfield et al. 1985) at a dose of 0.3 mg.

Maximum plasma concentration (C_{max}) following IV administration occurs very quickly, though the onset of analgesic action is approximately 15 minutes. Terminal half-life ($t_{1/2}$) following IV administration is considerably shorter than observed following sublingual administration (Kuhlman et al. 1996). Bullingham and colleagues reported a rapid distribution phase with a $t_{1/2}$ of approximately 2 minutes, followed by a slow terminal phase with a $t_{1/2}$ of 2-3 hours (Bullingham et al. 1980). However, when the study duration was increased in a subsequent investigation (from 3 to 13 hours), the estimated terminal $t_{1/2}$ increased to approximately 5 hours (Bullingham et al. 1982). Kuhlman and colleagues reported a C_{max} of 37.52 ng/ml (range, 24.4-55.9) occurring at just over two minutes after a 1.2 mg IV dose, and a t1/2 of 3.2 hours (±1.25, range, 1.62-8.18) (Kuhlman et al. 1996). Mendelson and colleagues reported a comparatively longer T_{max} (mean 26.4±5.4 minutes) and lower C_{max} (14.3±3.0 ng/ml) following a 1.0 mg dose (Mendelson et al. 1997).

Early IV studies have reported high plasma clearance, with a mean in excess of 60 L/hour (McQuay and Moore 1995). This is consistent with the clearance estimate of 62.5 (±21.8) L/hour reported by Medelson and colleagues (Mendelson et al. 1997). Clearance has been shown to vary considerably between anaesthetized and awake patients. In a study assessing IV kinetics in a cohort of patients both during anaesthesia and postoperatively, clearance while anaesthetized was up to 30% lower than during the postoperative period (Bullingham et al. 1980). BUP is almost completely cleared by the liver, thus the difference was attributed to the reduction in hepatic blood flow that has previously been associated with halothane anaesthesia (Juhl and Einer-Jensen 1974). A similar effect of anaesthesia on meperidine kinetics has also been reported (Mather et al. 1975). A second difference was also observed between BUP kinetics during anaesthesia and postoperatively: initial plasma levels of BUP were significantly higher during the anaesthetized phase compared to the awake, postoperative phase. This was thought to reflect lower initial volumes of distribution under anaesthesia, which may have been a function of lowered cardiac output as previously reported during anaesthesia (Prys-Roberts and Kelman 1967). BUP is associated with a large and variable volume of distribution (Vd), with early estimates at 90-190 l (Bullingham et al. 1980; Bullingham et al. 1983), and a more recent estimate of 335 (±116) l (Kuhlman et al. 1996).

4.2.2.4. Intramuscular administration

The only published kinetics of BUP following IM administration are those reported by early work using the radioimmunoassay of Bartlett and coworkers, the potential limitations of which have already been described (see section 4.2.2). The most notable feature of BUP kinetics following IM administration is very rapid absorption, with a T_{max} of approximately 5 minutes after dosing. In a study of 11 patients administered 0.3 mg IM BUP in the postoperative period, a C_{max} of approximately 3.6 ng/ml occurred 2-5 minutes after drug

administration (Bullingham et al. 1980). Systemic availability for the majority (7) of patients was greater than 90%, and between 40% and 60% for the remainder (4) of the sample.

4.2.3. Metabolism and excretion

BUP is metabolised in humans by phase I (oxidative) and phase II (conjugation) reactions to norBUP, and conjugated BUP and norBUP, respectively. BUP is metabolised by N-dealkylation to norBUP by the cytochrome P450 CYP isozyme 3A4. Both BUP and norBUP undergo glucuronidation (Walter and Inturrisi 1995), which is catalysed by numerous isoforms of UGT, primarily UGT1A1 (King et al. 1996) and, to a lesser extent, UGT1A3 (Green et al. 1998).

BUP is primarily excreted in the faeces. Determination of urinary excretion of BUP and its metabolites following subcutaneous, sublingual and oral administration revealed no BUP in urine at any dose or route of administration (Cone et al. 1974). Recovery of metabolites in urine was low, ranging from 1.9 to 14.3% of the dose. Free BUP and norBUP, and to a lesser extent conjugated BUP and norBUP, were present in faeces.

Brewster and coworkers reported enterohepatic circulation of BUP in rats (Brewster et al. 1981). The findings by Cone and coworkers suggest that this occurs in humans also, and this may contribute towards the long duration of pharmacological effect associated with BUP (Cone et al. 1974).

4.2.4. Safety and toxicity

Findings consistently demonstrate that BUP is associated with low abuse and dependence liability (Negus and Woods 1995). An early investigation of the human abuse potential of

BUP reported that the agent was associated with "little, if any" clinically significant physical dependence in a cohort of healthy male prisoner volunteers with prior history of opioid dependence (though not physically dependent on opioids at the time of the study) (Jasinski et al. 1978). There is some evidence that BUP has reinforcing properties in humans, with several cases of BUP abuse being reported among opioid-dependent individuals (Quigley et al. 1984; O'Connor et al. 1988; Chowdhury and Chowdhury 1990; Hammersley et al. 1990; San et al. 1992; Singh et al. 1992; Vidal-Trecan et al. 2003). A recent review of the abuse and dependence liability of BUP describes evidence from preclinical studies (Tzschentke 2002), concluding that BUP is associated with lower abuse and dependence liability than other potent opioids in established models of reward and addiction, including drug discrimination, place preference conditioning and self administration. In addition, following prolonged treatment with BUP, withdrawal from the drug is also reported to be less severe compared to withdrawal from other opioids administered in a similar schedule (Fudala et al. 1990; Bickel and Amass 1995; Cowan and Lewis 1995).

A single dose up to 70-times the recommended analgesic dose was well-tolerated by non-opioid-dependent individuals (Walsh et al. 1995), indicating that the risk of overdose is limited, even in non-dependent opioid users. In terms of overdose mortality among individuals receiving high-dose BUP for opioid dependence, BUP has been associated with a lower proportion of deaths than methadone (Pirnay et al. 2004), and the large majority of reported deaths that have occurred with BUP treatment have involved the concomitant use of additional drugs, typically benzodiazepines (Reynaud et al. 1998; Pirnay et al. 2004).

Some evidence suggests the potential for BUP interaction with human immunodeficiency virus-1 protease inhibitors ritonavir and indinavir, which are extensively metabolized by

CYP3A4 (Iribarne et al. 1998). There has also been a report of metabolic interaction between BUP and the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and fluvoxamine. Iribarne and colleagues reported that fluvoxamine uncompetitively inhibits *in vitro* BUP dealkylation (K_i 260 mM). While fluoxetine did not interact with BUP metabolism, its major active metabolite norfluoxetine was found to inhibit BUP metabolism with a K_i value of 100 nM (Iribarne et al. 1998). BUP has been demonstrated to be a potent *in vitro* inhibitor of CYP3A4 and CYP2D6 (Umehara et al. 2002; Zhang et al. 2003). However, at therapeutic concentrations, neither BUP or norBUP would be expected to have any clinically significant interactions with drugs that are oxidised by these enzymes (Zhang et al. 2003).

The concomitant use of BUP and the benzodiazepine flunitrazepam resulted in unexpected deaths (Reynaud et al. 1998). Both agents undergo *N*-dealkylation by CYP3A4. However, *in vitro* data do not support the hypothesis that a metabolic interaction occurs between the drugs resulting in higher than anticipated plasma concentrations (Kilicarslan and Sellers 2000). Rather, the respiratory depressive effect of both drugs has been proposed as a likely contributor to mortality.

4.2.5. Subjective and physiological effects of BUP

The effects of BUP have been well-characterised in subjects with past or current opioid-dependence (Jasinski et al. 1978; Walsh et al. 1994; Walsh et al. 1995) and in healthy, non-drug using volunteers (Blom et al. 1987; Saarialho-Kere et al. 1987; MacDonald et al. 1989; Zacny et al. 1997). BUP produces similar effects to other μ-receptor agonists. These include analgesia, sedation, nausea and vomiting, positive mood, pruritus, respiratory depression, pupillary miosis, decreased gastrointestinal motility and light-headedness.

Studies of BUP at analgesic doses in healthy volunteers have reported drowsiness, nausea and vomiting, as have most patient studies with BUP (see, for example, Heel et al. 1979). Many studies have described significant psychomotor impairment associated with BUP as measured by tests such as hand-eye coordination and reaction time (Saarialho-Kere et al. 1987; MacDonald et al. 1989; Zacny et al. 1997).

In a comparison of the subjective, physiological and psychomotor effects of BUP and morphine, healthy volunteers received IV BUP (0, 0.075, 0.15 or 0.3 mg/70 kg) or morphine (10 mg/70 kg) on separate occasions in a randomised, double-blind crossover design (Zacny et al. 1997). BUP produced long-lasting, dose-dependent effects on mood, psychomotor performance and physiological effects. In terms of subjective effects, BUP was associated with sedation, difficulty concentrating and dizziness, and there was a high incidence of nausea and vomiting. Physiological effects included a decrease in respiration rate and pupil size. The 0.3 mg/70 kg dose of BUP was associated with a greater magnitude of effect across these parameters than the equianalgesic dose of morphine (10 mg/70 kg). However, it was recognised by the authors that one shortcoming of the study was selecting only one morphine dose for comparison, rather than assessing a range of doses. Furthermore, the dose of morphine selected as equianalgesic to 0.3 mg/70 kg BUP may have under-estimated the potency of BUP, which has been reported at up to 50 times greater than morphine (Jasinski et al. 1978), rather than the 33-fold difference used. In prolonged opioid use for pain management, BUP has been reported to be less constipating than morphine (Bach et al. 1991).

Walsh and co-workers compared subjective and physiological effects of higher dose BUP (2-32 mg SL) with methadone (3.75-50 mg p.o.) in a cohort of healthy volunteers with prior opioid experience but no current dependence (Walsh et al. 1995). Comparable decreases in

respiratory depression, oxygen saturation and pupil size were observed with BUP and methadone, and neither drug was associated with significant changes in heart rate, blood pressure or skin temperature. As described previously, there was a reduction or flattening of the effects of BUP at higher doses.

4.3. Buprenorphine as an analgesic

Identification of BUP as a potent, long-lasting analgesic agent emerged following several early pain induction trials with animals (for example, Skingle and Tyers 1980). Ensuing animal studies evaluated the antinociceptive activity of BUP in response to acute, high-intensity stimuli and to protracted pain states with the use of a model of post-injury pain, the formalin test (McLaughlin and Dewey 1994; Wang et al. 1995).

An early study reported that BUP induced analgesia by agonist activity at the κ receptor, suggesting that BUP resembles nalorphine rather than morphine in analgesic action (Tyers 1980). A later study reported analgesic action via the κ1 and κ3 receptor subtypes (Pick et al. 1997). Notwithstanding, the analgesic effects of BUP are generally considered to be mediated by the μ-opioid receptor (Cowan 1995; Kamei et al. 1995; Lutfy et al. 2003), however the involvement of the κ and, to a lesser extent, the δ receptor have not been definitively elucidated. The potential role of the ORL1 receptor in modulating the antinociceptive activity of BUP has already been described (Lutfy et al. 2003) (see section 4.2.1).

BUP is available in parenteral and sublingual formulations in Australia, and more recently a transdermal delivery system has been developed and registered in some countries. BUP is well-suited to this type of application due to its high analgesic potency, low molecular

weight and high lipophilicity. An oral formulation has not been developed due to its low oral bioavailability.

The intrinsic antinociceptive activity of norBUP was approximately a quarter of that of BUP on the rat tail-flick test (Ohtani et al. 1995). However, it has recently been reported that norBUP is a more efficacious partial agonist at the μ-receptor than the parent drug (Huang et al. 2001).

4.4. Clinical trials with pain patients

Many trials have evaluated the analgesic actions of BUP in a range of clinical settings. These studies have included the use of BUP in the management of both acute and chronic pain. The following section will summarise findings from a selection of these studies.

4.4.1. Acute pain

Several hundred published papers have assessed the use of BUP for pain relief in the post-operative period. A 1980 study of almost 7500 general surgery patients who received BUP in the immediate post-operative period reported good or adequate analgesia of at least 4 hours duration in almost 90% of patients, with few adverse effects, no clinically significant side effects, and less than 1% of patients experiencing opioid-induced respiratory depression (Harcus et al. 1980).

Numerous studies have evaluated the analgesic efficacy and side effect profile of BUP against a range of analgesic agents commonly used in the post-operative period, including morphine (Kay 1978; Ellis et al. 1982; Cuschieri et al. 1984; Ouellette 1984; Green et al. 1985), tramadol (Alon et al. 1981; Fassolt 1981; Alon et al. 1982), sufentanil (Donadoni and Rolly 1987), pethidine (Hovell 1977; Fassolt 1981; Harmer et al. 1983; Khan and

Kamal 1990), pentazocine (Piepenbrock and Zenz 1984), metamizole (Torres et al. 1993), methadone (Carta et al. 1987), meperidine (Carl et al. 1987), ketorolac (Canadell-Carafi et al. 1991), meptazinol (Harmer et al. 1983), dihydrocodeine (Masson 1981) and nalbuphine (Pugh et al. 1987). In the majority of these studies, BUP has been comparable or superior in terms of analgesic efficacy, duration of action and/or incidence of side effects. For example, Kay reported greater and longer duration of analgesia from 0.3 mg IV BUP than 10 mg IV morphine in a double-blind study of 51 abdominal surgery patients (Kay 1978). Hovell and Ward reported similar side effects from 0.3 mg IM BUP and 10 mg IM morphine in post-operative patients, although BUP was associated with greater, and longer lasting analgesia (Hovell and Ward 1977). Similarly, greater magnitude and duration of pain relief has been associated with BUP (0.15 mg IM) compared to meptazinol (50 mg IM) in a double-blind trial with 40 post-cholecystectomy patients (Harmer et al. 1983), and in a double-blind randomised trial comparing BUP (0.3 mg IV) and pentazocine (0.3 mg IV) in 61 patients following abdominal surgery (Piepenbrock and Zenz 1984). Greater pain relief has also been reported with BUP (0.15 mg/ml) compared to nalbuphine (IV infusion, 10 mg/ml) in a continuous IV infusion (0.2 ml/kg/24h) in a double-blind randomised study of 100 abdominal surgery patients (Pugh et al. 1987), and with BUP (0.3 mg IM) compared to tramadol (50 mg IM) in a double-blind randomised trial of 60 post-surgical patients (Alon et al. 1981).

4.4.2. Chronic pain

Opioids are increasingly used in the long-term management of chronic pain (Schug et al. 1991; Zenz et al. 1992; Portenoy 1996; McQuay 1999). BUP has been evaluated in the management of cancer pain, neuropathic pain and other chronic pain states, and reported to produce potent and long lasting analgesia.

In a double-blind, randomised study of 75 cancer patients, Yajnik reported significantly greater and longer lasting analgesia from 0.2 mg SL BUP every 8 hours compared to pethidine (50 mg) and pentazocine (25 mg, both 8 hourly) (Yajnik 1988). In a large open-label trial with 483 pain patients (189 cancer pain, 147 ischaemic pain, 147 other chronic pain), sublingual BUP (0.6-1.2 mg/day) demonstrated comparable analgesic efficacy but significantly fewer side effects than sustained release morphine (60-80 mg/day) (Eriksen et al. 1989). Recent studies also support the analgesic efficacy of the BUP transdermal delivery system, reporting potent analgesia of long duration (Bohme 2002; Sittl et al. 2003; Sorge and Sittl 2004). Side effects associated with BUP in treating chronic pain have been equivalent (Sittl et al. 2003) or lower (Nasar et al. 1986; Bach et al. 1991) compared to other opioids.

4.5. Buprenorphine in the treatment of neuropathic pain

The use of opioids in neuropathic pain is controversial (Portenoy et al. 1990; McCormack 1999). While there has been evidence that opioids are less effective in the management of neuropathic pain (Arner and Meyerson 1988) other reports assert that opioids can provide satisfactory analgesia in such cases (Portenoy et al. 1990). However, this is also considered to depend to a large extent on the type of neuropathic pain (Sindrup and Jensen 1999). Animal studies of opioids in neuropathic pain are numerous but are not clinically informative (Dellemijn 1999). Human studies have been limited by confounding factors associated with neuropathic pain, such as the large variation in aetiology of neuropathic pain among research participants, as well as differences and shortcomings in trial methodology (Dellemijn 1999; Wallace 2001).

Some evidence suggests that BUP may have a special role in the management of neuropathic pain (McCormack et al. 1998; McCormack 1999; Kouya et al. 2002). BUP has

exhibited strong efficacy in animal models of neuropathic pain, including the neonatal formalin test (McLaughlin and Dewey 1994), and in rats with either partial sciatic nerve injury or spinal cord injury (Kouya et al. 2002), the latter of which is particularly resistant to systemic morphine (Xu et al. 1992; Yu et al. 1997). The intrathecal (IT) administration of pertussis toxin (PTX) has been shown to produce hyperalgesia and allodynia (Womer et al. 1997), and provides a further useful model of neuropathic pain (see McCormack et al. 1998). Consistent with the evidence that morphine exerts its effects via PTX-sensitive inhibitory systems, administration of PTX has been shown to dose-dependently attenuate morphine's antinociceptive effect (Wheeler-Aceto and Cowan 1991; Wheeler-Aceto and Cowan 1992; Womer et al. 1997). Reduced opioid effect with the administration of PTX has also been reported with etorphine and fentanyl (Shah et al. 1994), and the μ-receptor agonist PL017 (Wong et al. 1992; Wong et al. 1992). This may also be considered to account for evidence that morphine and other classic μ-receptor agonists are less effective in the management of neuropathic pain. In contrast, BUP has been shown to produce antinociception by agonist-activation of a PTX-insensitive pathway (Wheeler-Aceto and Cowan 1991), and indeed is considered to preferentially activate such pathways, with PTX-sensitive pathways only activated progressively with increasing doses (McCormack 1999). Such findings, however, should be interpreted with due appreciation of the limitations associated with the study of opioids in neuropathic pain described above.

4.6. Buprenorphine in human experimental pain

There have been no published studies investigating the antinociceptive effect of BUP in an experimental pain model in humans. Opioids have consistently been demonstrated to produce significant antinociception to noxious stimuli in experimental studies with healthy subjects. This evidence has been detailed in previous chapters with particular reference to the two tests used in the current studies, the cold pressor (CP) and electrical stimulation

(ES) tests. As a potent opioid analgesic, it may be expected that BUP would be associated with significant antinociception to experimental noxious stimuli. However this must be confirmed and an effective dose range determined.

4.7. The current study

Due to the lack of evidence for the effect of BUP in experimental tests of nociceptive stimuli in humans, a dose-ranging study in healthy volunteers is required to determine whether BUP is associated with significant antinociception on the selected nociceptive tests, and to establish a dose associated with minimal antinociception for use in subsequent BUP:NLX ratio studies. This study will also serve as a dose-ranging study of BUP side effects.

The nociceptive tests to be used are the CP and ES tests as described in previous chapters. As the CP test has previously been shown to be a more sensitive assay for opioids (Athanasos et al. 2002), this test will serve as the primary outcome measure for the BUP:NLX studies. Therefore, the dose selected from the current study will be based on CP response. The ES test will serve as a comparison. The benefits of using two types of nociceptive stimuli have previously been described (Wolff et al. 1976). Tolerance rather than threshold will be used to select the dose, as tolerance more reliably detects antinociceptive effects (Luginbuhl et al. 2001).

4.7.1. Hypothesis

That BUP will be associated with a significant increase in tolerance to nociceptive stimuli.

4.7.2. Aims

The aims of the current study were to assess the antinociceptive activity of BUP in the CP and ES tests, to establish a dose-response relationship to the CP and ES tests and other physiological measures, and to determine a sub-analgesic BUP dose for use in subsequent studies.

4.8. Methods

4.8.1. Participants

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 020820). Subjects were healthy, pain-free volunteers, who met the criteria detailed below. Participation in the study was on a voluntary basis. Participants were financially remunerated $AU250 upon completion of the study. Those who completed the screening process but did not meet the criteria for enrolment were remunerated $AU25. Written informed consent was obtained from all participants prior to commencing the trial.

4.8.1.1. Subject inclusion criteria

- Age range 18-45 years
- Caucasian
- Agreeable to and capable of signing informed consent
- Response to the cold pressor test within the normal range (as determined by prior study, see Chapter 3): 21 – 85 seconds
- Body mass index between 23 and 27
- Completion of pre-study medical screening to the satisfaction of the principal investigators. This included:

- medical history
- physical examination
- laboratory tests for liver (parameters within normal reference ranges), kidney (calculated creatinine clearance greater than 80 ml/minute), bone marrow functions (parameters within normal reference ranges)
- normal ECG

4.8.1.2. Subject exclusion criteria

- Considered unable, unwilling or unlikely to comply with study protocol
- Pregnant or lactating
- Participation in another clinical research project (current or in previous 3 months)
- Taking any medication (oral contraceptive pill allowed)
- Currently taking any analgesic medication
- Alcohol intake exceeding NHMRC guidelines (an average of more than 4 (40 gm alcohol) standard drinks per day in males, and an average of more than 2 (20 gm alcohol) standard drinks per day in females)
- Tobacco smokers
- Current or past history of substance abuse
- Red cross blood bank donation in the previous 3 months
- Laboratory tests outside the ranges listed above
- Any current medical condition, especially heart disease, hypertension, peptic ulcers, any other gastrointestinal disorder, psychiatric disorders, asthma, any other lung disease, any neurological disorder, abnormalities of the blood-forming organs, liver function abnormalities; abnormalities of the blood biochemistry
- Positive urine drug screen for illicit drugs at screening
- Blood pressure lower than 100/60 or higher than 130/80

4.8.2. Study design

This was a randomised, open-label, unblinded trial. The principal aim of the study was to identify a sub-analgesic dose of BUP for use in the subsequent drug combination studies. Subjects were randomised to either the active (BUP) or control (saline) condition. The design of the subsequent drug combination studies would be such that subjects were aware that on each testing day they would be administered BUP (in combination with either NLX or saline). Due to this, the current study was conducted in an open-label design so that subjects in the active phase of the current study were also aware they would be administered BUP.

The purpose of the saline group was to control for the anticipatory effects of pain stimuli, in order to determine whether any significant changes in response may be attributed to practice or order effects. As described, a central aim of the study was to identify a minimally antinociceptive dose; therefore, a dose that was associated with a greater increase in CP tolerance than attributed to practice or anticipatory effects, but not associated with significant antinociception, would be selected. The saline group, then, would also serve as a guide in this selection. Participants randomised to the saline group were aware that they were administered saline.

Due to the lack of data on the antinociceptive activity of BUP in human experimental pain models, an initial pilot study was conducted. The purpose of the pilot study was to determine the antinociceptive activity of BUP associated with the initial dosing schedule, and allow for any dosing adjustment required in order to identify a sub-analgesic dose in the principal study. Each participant in the pilot and principal studies attended for one testing day, during which measures were taken on numerous occasions (see below, section 4.9.2.2.2). Only one subject was tested on each day. The investigator and research nurse

were present for the duration of each testing day. A medical officer inserted the cannulae for drug infusion and blood taking, and was present for the duration of the infusions.

4.9. Pilot study

4.9.1. Sample characteristics

The pilot phase was conducted with two 19-year-old Caucasian male subjects, with cold pressor tolerance (CPTOL) values at screening of 81 seconds and 66 seconds, and body weights of 80.8 kg and 85.9 kg.

4.9.2. Procedures

4.9.2.1. Screening procedures

Subjects attended the testing centre for a screening interview and examination prior to enrolment in the study. The subject was provided with a study information sheet, and was given the opportunity to read the details and purpose of the study, discuss the procedures with the investigator and ask questions. The subject's height, weight, date of birth, ethnicity and sex were recorded. The subject then completed the CP test in order to become familiar with the procedure, and to determine whether baseline pain tolerance lay within the normal range (as determined by the study described in Chapter 3). The screening was continued if baseline pain tolerance was within the acceptable range (see section 4.8.1.1). A medical officer conducted a routine examination with the subject, including a physical examination, medical history, drug use history, and electrocardiogram. A urine sample was taken and tested for drugs of abuse (opioids, cannabinoids, benzodiazepines and sympathomimetic amines). A blood sample was taken and analysed for liver, kidney and bone marrow function, and serology (HIV and Hepatitis B and C). All

blood and urine analyses were conducted by an independent laboratory. If screening was completed successfully according to the criteria listed above, the subject was enrolled in the study.

4.9.2.2. Experimental procedures

Experimental procedures were as described in Chapter 2.

4.9.2.2.1. Drug administration

Four doses of BUP were administered using an escalating and cumulative dosing schedule. Doses were administered by IV infusion, commencing with saline (10 ml over 30 minutes) and followed by 0.5, 1.0, 2.5 and 5.0 μg/kg (i.e. for a 70 kg adult: 35, 70, 175 and 350 μg) each administered over 30 minutes. These doses were selected as they are in the lower range and below the recommended therapeutic dose for pain relief (300-600 μg), in line with the aim of selecting a dose just below that which produces significant antinociception. The saline infusion was administered prior to the BUP to ensure that the parameters measured were not significantly affected by the insertion of the cannulae or the experience of receiving an infusion itself, and also to ensure that the infusion lines were running properly. The total duration of infusion was 2.5 hours, over which time nociceptive testing was performed and physiological parameters recorded according to the schedule of testing time points below. Further details of infusion set-up and procedure are outlined in Chapter 2 (2.3.1).

4.9.2.2.2. Testing protocol and schedule

Subjects were delivered from their homes to the testing centre by taxi in the morning. Subjects had been instructed to refrain from taking any drugs or medication in the 24-hours prior to testing (excluding the contraceptive pill) and to eat a light breakfast on the morning

of testing. A urine sample was taken and tested by an independent laboratory for drugs of abuse (opioids, cannabinoids, benzodiazepines and sympathomimetic amines) and, for female subjects, pregnancy.

Assessments were made at 12 time points throughout the testing day. At each time point a blood sample was taken, nausea, sedation and physiological/subjective parameters were assessed, and the nociceptive tests were completed. The methods used for these procedures are described in Chapter 2. Testing time points occurred prior to the infusions, twenty minutes after the commencement of each infusion (thus measurements were taken 5 times over the infusion period) and then hourly upon completion of the infusions over a 6-hour washout period. Each testing time point started twenty minutes after commencement of each infusion to allow time for all measures to be taken prior to starting the subsequent infusion. Nociceptive testing was the final component of each assessment, thus these measures were taken between 25 and 30 minutes into each infusion and immediately prior to the commencement of the subsequent infusion. Subsequent references to pilot study testing time points are made according to the description in Table 4-2.

Testing time point reference	Description
Pre-saline	Prior to starting the infusion period
Baseline	20 minutes after starting the 30-minute saline infusion
0.5 (μg/kg)	20 minutes after starting the 30-minute 0.5 μg/kg BUP infusion
1.0 (μg/kg)	20 minutes after starting the 30-minute 1.0 μg/kg BUP infusion
2.5 (μg/kg)	20 minutes after starting the 30-minute 2.5 μg/kg BUP infusion
5.0 (μg/kg)	20 minutes after starting the 30-minute 5.0 μg/kg BUP infusion
Washout 1 (hr)	1 hour following cessation of the infusions
Washout 2 (hr)	2 hours following cessation of the infusions
Washout 3 (hr)	3 hours following cessation of the infusions
Washout 4 (hr)	4 hours following cessation of the infusions
Washout 5 (hr)	5 hours following cessation of the infusions
Washout 6 (hr)	6 hours following cessation of the infusions

Table 4-2. Description of pilot study testing time points. At each time point the following was performed: 1) blood sample taken, 2) nausea, sedation, subjective and physiological parameters assessed, 3) nociceptive testing completed.

4.9.2.3. Statistical analysis

No statistical analyses were conducted with the pilot data, due to the small subject number (n=2), and that the purpose of the pilot study was only to provide an indication of the magnitude of antinociceptive effect produced by the BUP dosing schedule, and whether an adjustment would be required for the principal study.

4.9.3. Results

Results for the pilot study are presented for each subject individually. While CP antinociception was the main outcome of interest, ES antinociception, physiological

parameters and adverse effects are also presented. In interpreting these results it should be considered that these are pilot data, and intended only to serve as a guide for the dosing schedule to be used in the principal study.

4.9.3.1. Antinociception

Cold pressor threshold (CPTHR) and CPTOL for the two subjects at each time point are presented in Figure 4-2, and for electrical stimulation threshold (ESTHR) and tolerance (ESTOL) in Figure 4-3.

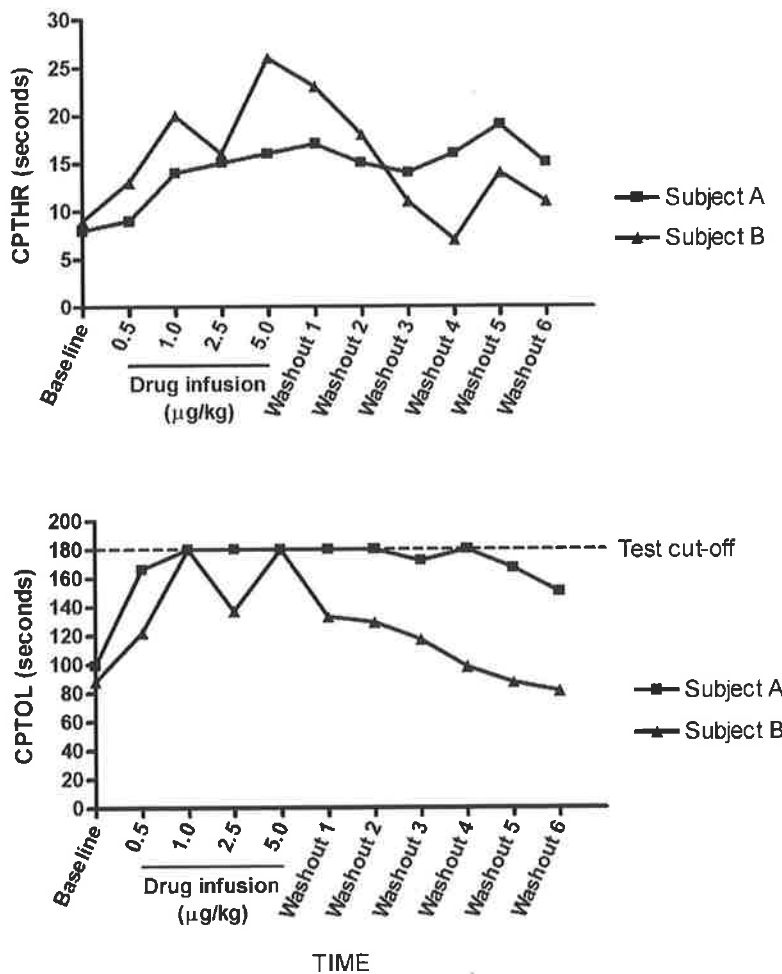

Figure 4-2. CPTHR (seconds) and CPTOL (seconds) for each pilot subject (n=2) at each time point over one day, starting at baseline (pre-drug), at the end of each of 4 cumulative IV BUP infusions (0.5, 1.0, 2.5, 5.0 μg/kg), and hourly following drug administration until 6-hours post-infusion (Washout 6). Maximum time limit allowed on CP test was 180 seconds (test cut-off).

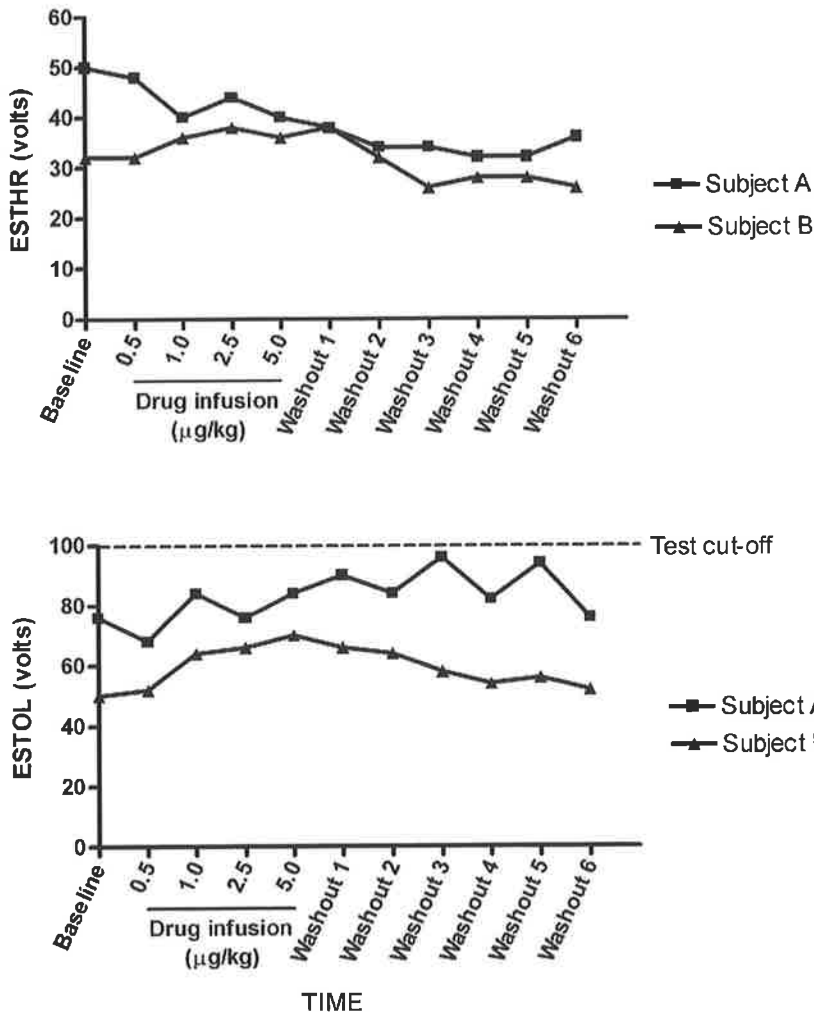

Figure 4-3. ESTHR (volts) and ESTOL (volts) for each pilot subject (n=2) at each time point over one day, starting at baseline (pre-drug), at the end of each of 4 IV BUP infusions (0.5, 1.0, 2.5, 5.0 µg/kg), and hourly following drug administraion until 6-hours post-infusion (Washout 6). Maximum voltage allowed on ES test was 100 volts (test cut-off).

CPTOL and ESTOL data for the two pilot subjects expressed as percent change from baseline are presented in Figure 4-4. Subject A achieved a maximum percent increase in CPTOL of 81.8 %; however this increase was censored due to the maximum time limit imposed on the CP test. This maximum increase occurred at the BUP 1.0 µg/kg time point, was sustained at this level until the Washout 2hr time point, and peaked again at the

Washout 4 hr time point. The maximum increase in CPTOL observed for Subject B also represents censored data, with a peak increase of 105.5% to reach the 180-second cut-off. In line with Subject A, this peak occurred as early as the BUP 1.0 µg/kg time point, returning again at BUP 5.0 µg/kg and gradually declining during the washout period.

Peak increase in ESTOL was 26.3% for subject A, occurring at the washout 3hr time point, and 40% for subject B, occurring at the 5.0 µg/kg time point.

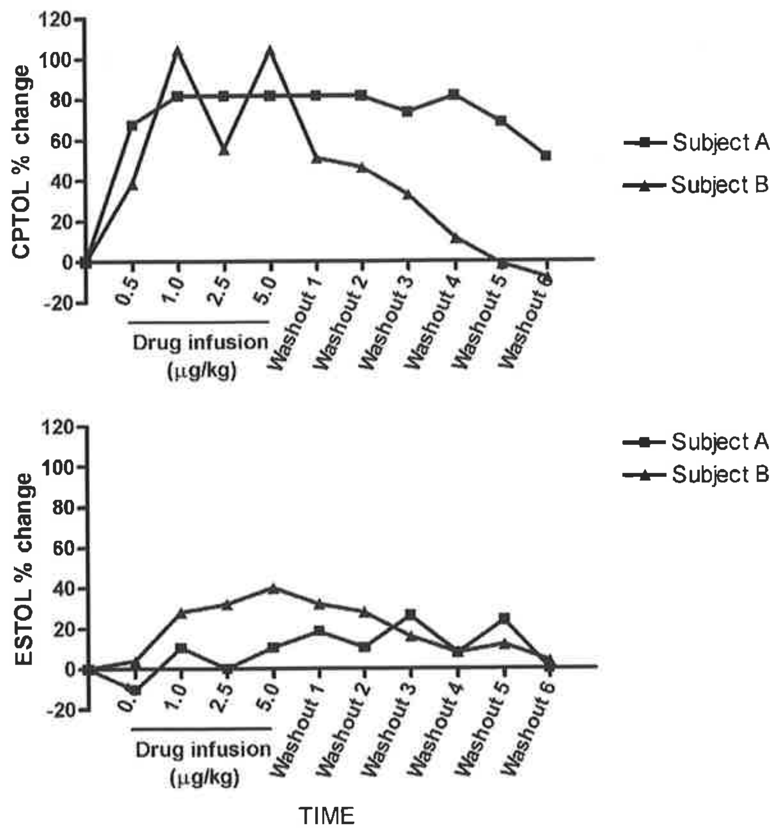

Figure 4-4. CPTOL (seconds) and ESTOL (volts) expressed as percent change from baseline for pilot subjects (n=2) at each time point (baseline, at the end of each of 4 IV BUP infusions {0.5, 1.0, 2.5, 5.0 µg/kg}, and hourly following drug administration until 6-hours post-infusion {Washout 6}) over one day.

4.9.3.2. Physiological parameters

Respiration (as measured by breaths per minute), arterial oxygen saturation, heart rate and blood pressure for each subject at each time point are presented in Figure 4-5. Subject A experience a marked reduction in respiratory depression (52%) from 17 to 8 breaths per

minute, observed at both the washout 1 and washout 3 time points. By contrast, Subject B experienced a maximum decrease in breaths per minute of 17% (12 to 10 breaths per minute) occurring at several time points over the testing day. Despite the marked decrease in breaths per minute observed for Subject A, this subject's arterial oxygen saturation was maintained at 100% throughout the testing day. There was greater fluctuation in arterial oxygen saturation for Subject B, with a peak decrease of 3% (100 to 97%) occurring at the 2.5 μg/kg time point. Heart rate varied during the testing day for each subject, but in alternate directions, with Subject A experiencing an overall increase across the day while Subject B's heart rate increased initially followed by a mild decrease. Systolic and diastolic blood pressure fluctuated mildy over the testing day for both subjects.

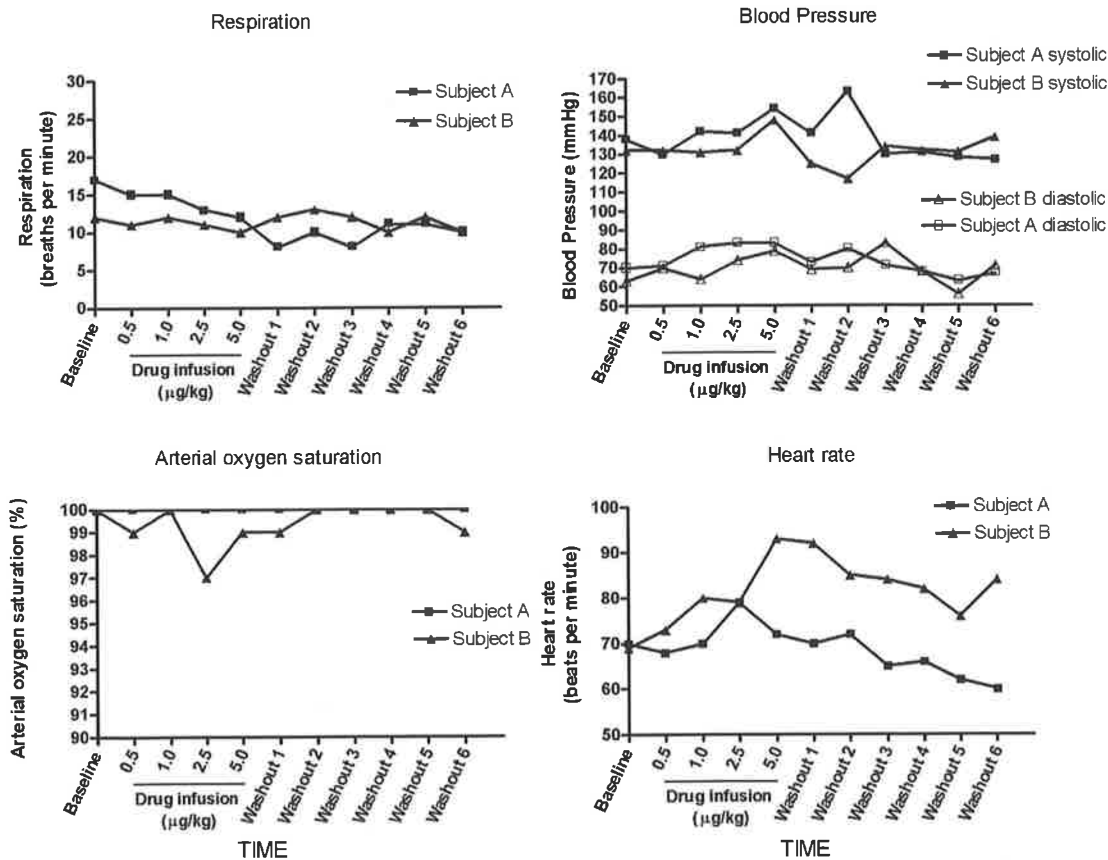

Figure 4-5. Physiological parameters (respiration, arterial oxygen saturation, blood pressure and heart rate) for each pilot subject (n=2) at each time point (baseline {pre-drug}, at the end of each of 4 IV BUP infusions {0.5, 1.0, 2.5, 5.0 μg/kg}, and hourly following drug administration until 6-hours post-infusion {Washout 6}) over one day.

4.9.3.3. Adverse and other drug effects

Nausea was recorded at each time point according to the following scale: 0, no nausea; 1, mild nausea; 2, moderate nausea; 3, severe nausea. Sedation was recorded according to the scale described in Chapter 2. Nausea and sedation occurring during the inter-testing period was also rated and recorded according to these scales.

Adverse effects for each subject are summarised in Table 4-3. Subject A experienced mild nausea with no episodes of vomiting. This subject also experienced mild to moderate sedation from the second BUP infusion, which was still rated as moderate at the final testing time point 6 hours post-drug administration. Subject B did not suffer from nausea, and sedation was less pronounced in this subject. Both subjects experienced light-headedness throughout the day, but again this was less pronounced in Subject B.

Testing time point	Subject A	Subject B
Pre-saline	Nil	Nil
Baseline	Nil	Nil
BUP 0.125 μg/kg	Mild pruritus (neck)	
BUP 0.25 μg/kg	Nausea 1	
BUP 0.5 μg/kg	Nausea 1 Light-headed	Sedation 1
BUP 1.0 μg/kg	Nausea 1 Sedation 1 Light-headed	Sedation 1 Light-headed Dry mouth
Washout 1 hr	Nausea 1 Sedation 2 Mild pruritus (neck) Light-headed Dry mouth	Sedation 1 Light-headed Dry mouth
Washout 2 hr	Nausea 1 Sedation 1 Light-headed Dry mouth	Light-headed
Washout 3 hr	Nausea 1 Sedation 1	Sedation 1 Dry mouth
Washout 4 hr	Nausea 1 Sedation 2 Light-headed (mild)	
Washout 5 hr	Nausea 1 Sedation 2 Light-headed (mild)	
Washout 6 hr	Nausea 1 Sedation 2	
Nausea and sedation reported if experienced at testing time or at any time during period between time points. No episodes of vomiting. See 2.3.2.3 for description of nausea and sedation rating.		

Table 4-3. Incidence and severity of adverse effects experienced by subjects in the pilot study (n=2) at each time point (baseline {pre-drug}, following each of 4 IV BUP infusions {0.5, 1.0, 2.5, 5.0 μg/kg}, and hourly post-drug administration for 6-hours {Washout 6}) over the testing day.

In addition to the adverse effects noted above, both subjects in the pilot study reported difficulty in concentrating and a pleasant drug effect. These effects were described by Subject A as follows:

"When I move my head it takes a while for the rest of the room to catch up." (BUP 0.5 μg/kg)

"I feel like I've had a few beers. It feels good." (BUP 1.0 μg/kg)

"It's hard to concentrate, but it's a good feeling." (BUP 5.0 μg/kg)

Subject B described the subjective drug effects as follows:

"I'm beginning to feel stoned. It's a pleasant feeling." (BUP 1.0 μg/kg)

"I feel trippy" (Washout 1 hr)

4.9.4. Discussion

The primary outcome measure from the pilot study was CPTOL. Even the lowest dose of BUP (0.5 μg/kg) was associated with an increase in CPTOL of 67.7 % and 38.6 % for Subject A and B, respectively. After the second BUP infusion (1.0 μg/kg) both subjects had reached the CPTOL cut-off of 180 seconds. These initial data suggest that the CP test is a sensitive biomarker for BUP, as has been demonstrated for a range of other opioids (see 3.1.4). However, it was apparent that the BUP doses must be reduced for the principal study in order to identify a dose associated with minimal CP antinociception for the subsequent studies.

As anticipated, the magnitude of antinociception to ES was not as substantial as observed on the CP test. Peak increase in ESTOL observed for Subject A was 26.3% at Washout 3hr, and 40% for Subject B at BUP 5.0 μg/kg. This is in line with previous reports showing CP to be a more sensitive biomarker for opioids than ES (Doverty et al. 2001; Athanasos et al. 2002).

Subjective and physiological effects of BUP observed in the pilot study are in line with previous reports of BUP in analgesic doses. Sedation, light-headedness and nausea were observed, although these effects were more pronounced in Subject A. This is in agreement with the widespread observation that some individuals are more responsive to the adverse

effects of opioids (Macintyre and Ready 2001). Subject A also experienced a greater decrease in respiratory depression as measured by breaths per minute. Changes in other physiological parameters (arterial oxygen saturation, heart rate and blood pressure) over the testing day were mild. Dry mouth, mild pruritus and subjective (good) drug effect were also observed.

4.10. Principal study

As described, the results of the pilot study indicated that the BUP dose regimen required modification for the principal study in order to identify a dose appropriate for the subsequent BUP:NLX trials. The following section will describe the principal study, which was conducted according to the same methods as the pilot study but employed markedly reduced doses.

4.10.1. Sample characteristics

Nine Caucasian participants ranging in age from 21 to 41 (mean±SEM, 28.22±2.23) were recruited and randomly assigned to the active (BUP; n=6, 3 male, 3 female) or control (saline; n=3, 1 male, 2 female) arms of the study. There were no significant differences between participants in the active and control conditions in age or CP nociceptive parameters at screening (see Table 4-4), nor was there any significant difference in the mean age of female (28.4±3.57 years) and male (26.8±2.56 years) participants ($p=0.725$). All participants met selection criteria as outlined in sections 4.8.1.1 and 4.8.1.2.

	Active Mean (±SEM)	**Control Mean (±SEM)**	**p**
Age (years)	28.8 (±8.09)	27.0 (±2.00)	0.725
CPTHR at screening (seconds)	8.33 (±0.80)	7.67 (±1.20)	0.154
CPTOL at screening (seconds)	26.67 (±1.43)	35.00 (±5.86)	0.351

Table 4-4. Age (years) and CP parameters (seconds) at screening for the active (BUP, n=6) and control (saline, n=3) groups; no significant differences between groups, p>0.05, independent samples t-tests.

4.10.2. Procedures

4.10.2.1. Screening procedures

The screening procedure for this phase was conducted according to the methods described for the pilot phase above. Phase of menstrual cycle was recorded for female participants who reported a regular menstrual cycle (n=3). Phase was determined by self-report, with phases classified according to the following classification: menstrual (days 1-5), follicular (days 6-12), ovulation (days 13-16) and luteal (days 17-28) (Sherwood 1997). Urine was also taken for pregnancy testing of female subjects.

4.10.2.2. Experimental procedures

Experimental procedures were conducted as described for the pilot study (see 4.9.2.2). Subjects were studied on one testing day only. The infusion doses administered in the active phase were reduced to 0.125, 0.25, 0.5 and 1.0 μg/kg (i.e. for a 70 kg adult: 8.75, 17.5, 35 and 70 μg). Subsequent reference to testing time points are made according to the descriptions in Table 4-5. Control subjects received five 30-minute infusions of saline to control for anticipatory effects of nociceptive stimuli and practice effects.

Testing time point reference	Description
Pre-saline	Prior to starting the infusion period
Baseline / Post-saline	20 minutes after starting the 30-minute saline infusion
0.125 (μg/kg)	20 minutes after starting the 30-minute 0.125 μg/kg BUP infusion
0.25 (μg/kg)	20 minutes after starting the 30-minute 0.25 μg/kg BUP infusion
0.5 (μg/kg)	20 minutes after starting the 30-minute 0.5 μg/kg BUP infusion
1.0 (μg/kg)	20 minutes after starting the 30-minute 1.0 μg/kg BUP infusion
Washout 1 (hr)	1 hour following cessation of the infusions
Washout 2 (hr)	2 hours following cessation of the infusions
Washout 3 (hr)	3 hours following cessation of the infusions
Washout 4 (hr)	4 hours following cessation of the infusions
Washout 5 (hr)	5 hours following cessation of the infusions
Washout 6 (hr)	6 hours following cessation of the infusions

Table 4-5. Description of principal study testing time point references. At each time point the following was performed:1) blood sample taken, 2) nausea, sedation, subjective and physiological parameters assessed, 3) nociceptive testing completed.

4.10.2.3. Statistical analyses

Differences in nociceptive and physiological parameters before and after the 30-minute saline infusion were assessed using paired-samples t-tests. The purpose of this was to determine whether any change in response may be attributed to the infusion process itself, and also provided an indication of the reliability of the baseline from which changes in parameters were measured.

For each antinociceptive and physiological parameter assessed, paired samples t-tests were used to compare scores at baseline with scores at each time point throughout the day for the BUP group. Paired samples t-tests were selected for several reasons. A repeated measures

ANOVA with data from the duration of the testing day may have produced a spurious result, as this analysis would have included time points throughout the washout period, where a significant effect may not be anticipated. Moreover, as the key purpose of this study was to select a BUP dose for the subsequent BUP:NLX ratio studies, the primary interest was in the magnitude of change in each parameter associated with each dose increment. Paired samples t-tests also provide a clear indication of the duration of effect associated with the drug administered.

While these analyses were performed on raw data, results for several parameters are also graphically represented as mean percent change from baseline for each subject to aid visual inspection of the data. This was calculated for each subject's results at each time point according to the equation below. Mean percent change was then calculated from these data for each time point.

$$\text{Percent change from baseline} = \left(\frac{\text{post-drug latency} - \text{baseline latency}}{\text{baseline latency}} \right) * 100$$

Paired samples t-tests were conducted to compare baseline pain tolerance with subsequent results for the saline group in order to determine whether any significant change in response may be attributed to practice or anticipatory effects.

As described in Chapter 3, non-parametric methods of statistical inference are appropriate for the nociceptive tolerance parameters (CPTOL and ESTOL) when data have been censored due to the cut-off associated with the tests (180 seconds for CP, 100 volts for ES). None of the data in the present study included such censored cases, and therefore parametric methods have been applied.

4.10.3. Results

4.10.3.1. Measures pre- and post-saline

There were no significant differences between pre- and post-saline (baseline) measures among the BUP group (see Table 4-6). For all subsequent analyses, then, baseline is taken as the post-saline measure.

	Mean (±SEM) Pre-saline	Mean (±SEM) Post-saline	p
CPTHR (seconds)	7.33 (±0.21)	6.83 (±0.31)	0.076
CPTOL (seconds)	21.50 (±0.92)	21.33 (±1.48)	0.883
ESTHR (volts)	32.67 (±3.57)	29.33 (±1.33)	0.388
ESTOL (volts)	45.00 (±3.96)	42.00 (±2.48)	0.328
Respiration (breaths per minute)	17.33 (±0.42)	17.17 (±0.60)	0.695
Oxygen saturation (%)	99.83 (±0.17)	99.67 (±0.21)	0.363
Heart rate (beats per minute)	76.33 (±3.19)	74.83 (±2.47)	0.632
Systolic blood pressure (mmHg)	117.83 (±3.63)	116.83 (±6.07)	0.832
Diastolic blood pressure (mmHg)	71.33 (±1.86)	69.83 (±3.73)	0.688

Table 4-6. Mean (±SEM) nociceptive and physiological parameters for the BUP group (n=6) at pre- and post-saline infusion (10 ml over 30-minutes) time points. No significant differences in any parameter between time points (p>0.05), paired samples t-tests.

4.10.3.2. Practice/order effects

There were no significant changes in nociceptive tolerance (see Figure 4-6) or any physiological measure (see Figure 4-7) over the testing day for those in the saline group ($p>0.05$). This indicates that responses were not subject to significant practice effects, or to the influence of environmental or physiological effects such as boredom or tiredness. Changes in nociceptive tolerance or physiological parameters observed in the BUP group may therefore be considered not to represent such effects.

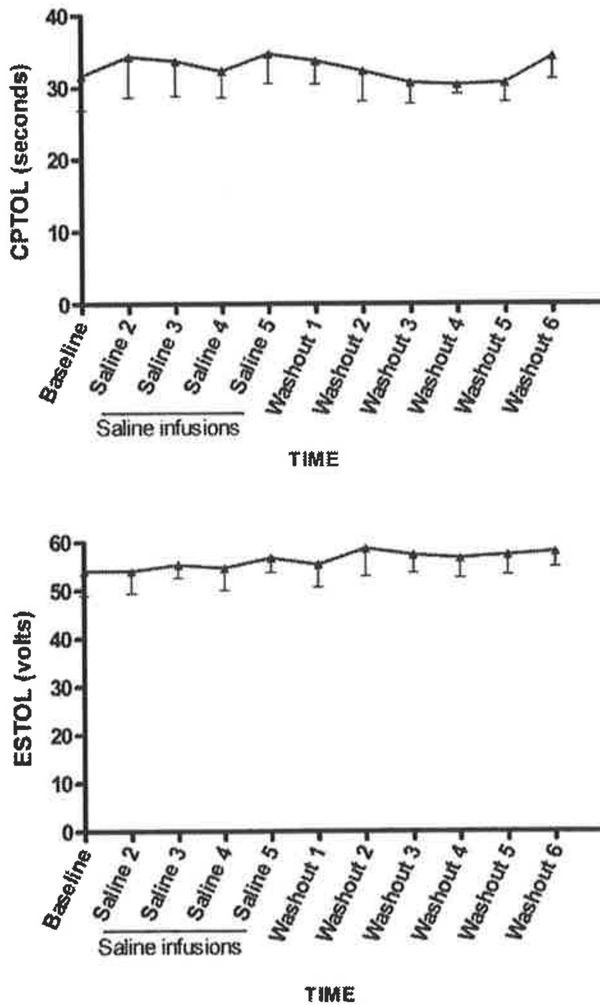

Figure 4-6. Mean(±SEM) CPTOL (seconds) and ESTOL (volts) at each time point (baseline, following each of 4 saline infusions [each 10 ml over 30 minutes], and hourly for 6 hrs) over one day for subjects receiving saline (n=3). p>0.05, no significant differences from baseline (paired samples t-test). Baseline represents measures taken following initial saline infusion (see section 4.10.3.1 for details).

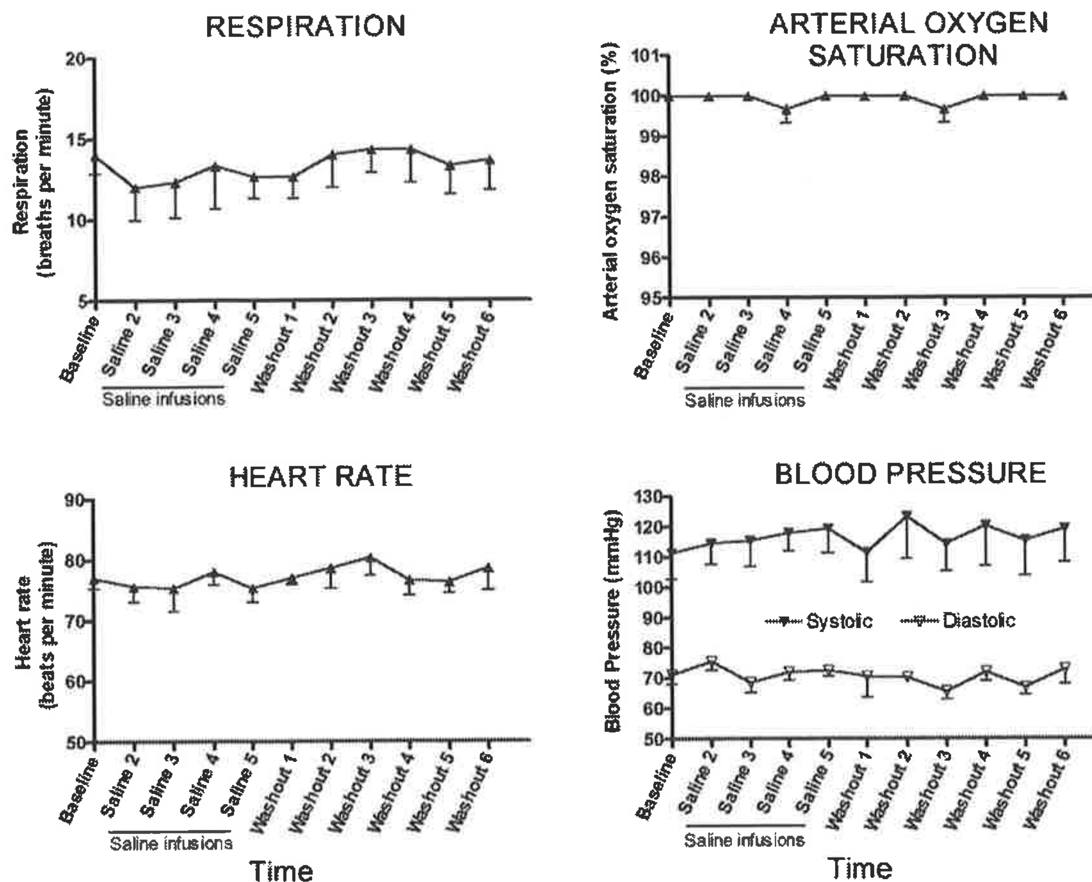

Figure 4-7. Mean(±SEM) respiration, arterial oxygen saturation, heart rate and blood pressure at each time point (baseline, following each of 4 saline infusions [each 10 ml over 30 minutes], and hourly for 6 hrs) over one day for subjects receiving saline (n=3). p>0.05, no significant differences from baseline (paired samples t-test). Baseline represents measures taken following initial saline infusion (see section 4.10.3.1 for details).

4.10.3.3. Antinociception

Mean CPTHR and CPTOL, and ESTHR and ESTOL at each time point for the BUP (active) group are displayed in Figure 4-8 and Figure 4-9, respectively.

4.10.3.3.1. Cold pressor threshold

Mean CPTHR in the BUP group was significantly greater than baseline (6.83±0.31) at the end of the final BUP infusion (1.0 μg/kg) (8.00±0.52 seconds; t(5)=-2.91, p=0.034) and at one hour after the last infusion (Washout 1) (7.83±0.31 seconds; t(5)=-2.74, p=0.041).

Mean CPTHR at the end of the 0.5 μg/kg infusion (8.00±0.63 seconds) was approaching a statistically significant difference compared to baseline (p=0.058) (Figure 4-8).

4.10.3.3.2. Cold pressor tolerance

CPTOL increased significantly from baseline (21.33±1.48 seconds) in the BUP group at the end of the 0.5 μg/kg (26.00±2.60 seconds; t(5)=-2.70, p=0.043) and 1.0 μg/kg (29.33±3.05 seconds; t(5)=-3.55, p=0.016) infusions. One BUP subject did not perform the CP test at the 1-hour washout testing due to nausea and dizziness upon standing and thus the comparison between baseline and Washout 1 included only 5 subjects. This difference (baseline vs. washout 1) was approaching statistical significance (p=0.068). Mean values at each time point are displayed in Table 4-7.

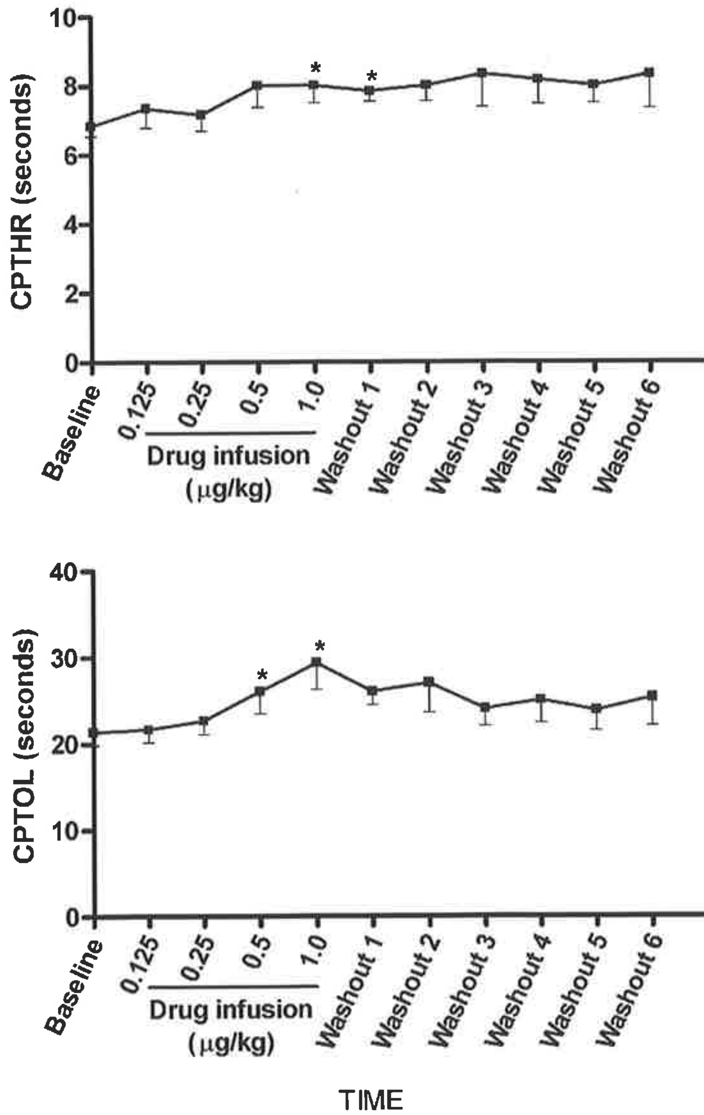

Figure 4-8. Mean(±SEM) CPTHR (seconds) and CPTOL (seconds) at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 μg/kg}, and hourly throughout BUP washout to 6 hrs) over one day for subjects receiving BUP (n=6). *p<0.05, significant difference from baseline (paired samples t-test).

Testing time point	Mean (secs)	±SEM	p (vs. baseline)	% change (±SEM) (range)
Baseline	21.33	1.48	-	-
0.125 µg/kg	21.67	1.52	0.709	1.93 (±4.31) (-11.11-17.65)
0.25 µg/kg	22.67	1.58	0.249	6.84 (±4.52) (-7.69-20.00)
0.5 µg/kg	26.00	2.59	0.043	21.68 (±7.26) (-4.76-48.00)
1.0 µg/kg	29.33	3.05	0.016	37.62 (±10.28) (4.76-64.71)
Washout 1^	26.00	1.51	0.068	16.63 (±7.66) (-3.85-47.06)
Washout 2	27.00	3.44	0.176	29.20 (±17.04) (-23.08-88.24)
Washout 3	24.00	2.00	0.286	14.77 (±12.19) (-11.54-70.59)
Washout 4	25.00	2.57	0.218	19.01 (±12.54) (-19.23-64.71)
Washout 5	23.83	2.29	0.336	13.32 (±10.79) (-26.92-47.06)
Washout 6	25.33	3.29	0.325	22.08 (±19.35) (-26.92-105.88)

Table 4-7. Mean (±SEM) CPTOL (seconds) at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 µg/kg}, and hourly throughout BUP washout to 6 hrs) over one day for subjects receiving BUP (n=6), with corresponding mean (±SEM, range) % change from baseline. Alpha values from paired samples t-tests comparing CPTOL raw data at each time point with baseline. (^n=5, test not completed by one subject due to nausea)

4.10.3.3.3. Electrical stimulation threshold

A comparison of ESTHR at each time point with baseline (29.33±1.33 volts) revealed a significant increase in ESTHR in the BUP group at 4 hours following the cessation of drug infusion (Washout 4) (32.67±1.98 volts; $t(5)=-2.99$, $p=0.031$) (Figure 4-9).

4.10.3.3.4. Electrical stimulation tolerance

A significant increase in mean ESTOL in the BUP group was observed at all time points with the exception of the testing at the end of the 0.25 µg/kg BUP infusion, which was approaching significance ($p=0.058$) (Figure 4-9). Mean values at each time point are displayed in Table 4-8.

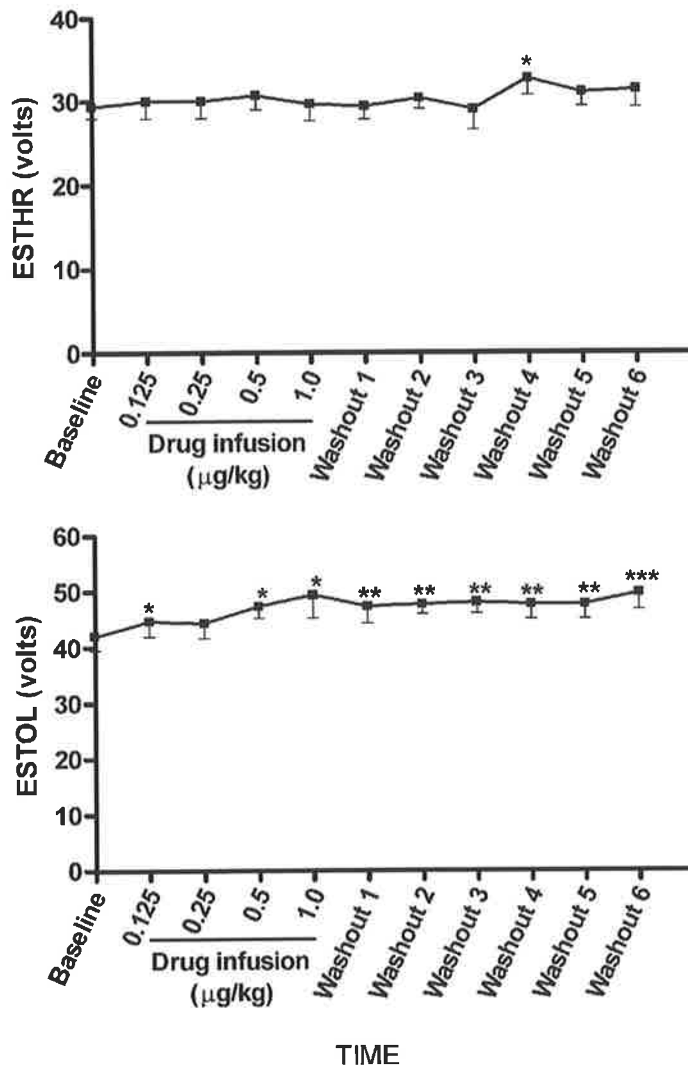

Figure 4-9. Mean(±SEM) ESTHR (volts) and ESTOL (volts) at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 μg/kg}, and hourly throughout BUP washout to 6 hrs) for subjects receiving BUP (n=6). *p<0.05 **p<0.01 ***p<0.001, significant difference from baseline (paired samples t-test).

Testing time	Mean (volts)	±SEM	p (vs. baseline)	% change (±SEM) (range)
Baseline	42.00	2.48	-	-
0.125 μg/kg	44.67	2.72	0.025	6.37 (±1.97) (0-13.64)
0.25 μg/kg	44.33	2.60	0.058	5.62 (±2.41) (0-15.0)
0.5 μg/kg	47.33	2.04	0.025	13.59 (±4.25) (-4.17-25.0)
1.0 μg/kg	49.33	4.09	0.012	16.72 (±3.89) (6.25-29.17)
Washout 1	47.33	3.00	0.010	12.85 (±3.04) (0-20.83)
Washout 2	47.67	1.75	0.005	14.43 (±3.63) (4.17-25.0)
Washout 3	48.00	1.93	0.007	15.19 (±3.80) (0-25.0)
Washout 4	47.67	2.60	0.010	13.95 (±3.30) (0-20.83)
Washout 5	47.67	2.65	0.007	13.87 (±3.31) (4.17-25.0)
Washout 6	49.67	2.94	0.000	18.30 (±1.37) (10.0-22.73)

Table 4-8. Mean (±SEM) ESTOL (volts) at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 μg/kg}, and hourly throughout BUP washout to 6 hrs) for the BUP group (n=6), with corresponding mean (±SEM, range) % change from baseline. Alpha values from paired samples t-tests comparing ESTOL raw data at each time point with baseline.

To aid visual inspection of changes in CPTOL and ESTOL throughout the testing day, these parameters have also been expressed as percent change from baseline (see Figure 4-10). As the antinociceptive response of the saline group will aid in the selection of the BUP dose to be used in the subsequent drug combination studies, mean (±SEM) percent change in tolerance from baseline for the saline group are also displayed.

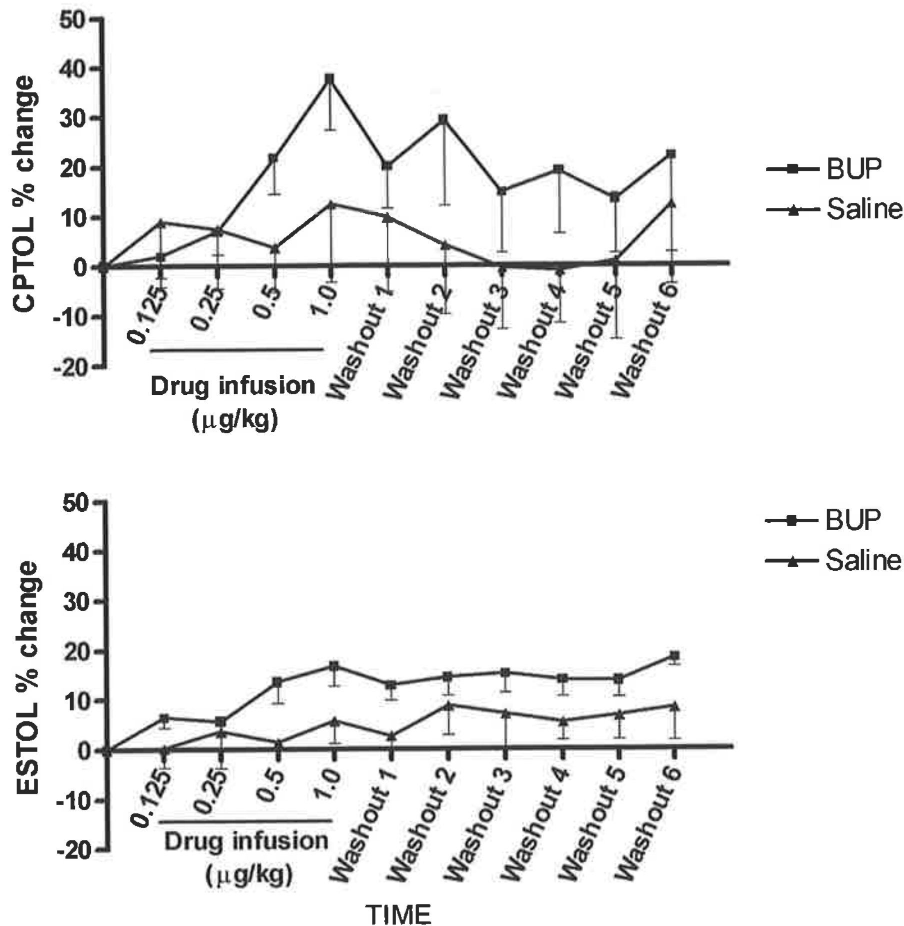

Figure 4-10. Mean (±SEM) percent change from baseline CPTOL and ESTOL for the BUP (n=6) and saline (n=3) groups across all time points (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 µg/kg}, and hourly throughout BUP washout to 6 hrs).

4.10.3.4. Physiological parameters and adverse effects

Physiological parameters for the BUP group at each time point are displayed in Figure 4-11.

4.10.3.4.1. Respiration

A significant decrease in breaths per minute was observed in the BUP group following the 0.5 µg/kg infusion and 1.0 µg/kg infusion, and at 1 hr, 2 hrs and 4 hrs following the cessation of the infusions. Mean breaths per minute at each time point are listed in Table

4-9, with alpha values from paired samples t tests comparing each testing time with baseline.

Testing time point	Mean (volts)	±SEM	p (vs. baseline)
Baseline	17.17	0.60	-
0.125 μg/kg	16.67	0.96	0.688
0.25 μg/kg	15.00	0.96	0.130
0.5 μg/kg *	14.50	0.81	0.014*
1.0 μg/kg *	14.17	0.83	0.017*
Washout 1**	13.83	0.48	0.004**
Washout 2*	15.17	0.75	0.041*
Washout 3	15.83	0.60	0.191
Washout 4*	15.67	0.56	0.045*
Washout 5	15.67	0.42	0.107
Washout 6	16.00	0.45	0.220

Table 4-9. Mean (±SEM) breaths per minute at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 μg/kg}, and hourly throughout BUP washout to 6 hrs) for the BUP group (n=6). Alpha values from paired samples t-tests comparing mean breaths per minute at each time point with baseline. *p<0.05, **p<0.01

4.10.3.4.2. Arterial oxygen saturation

A significant decrease from baseline in arterial oxygen saturation (99.67±0.21%) was found for the BUP group at 1 hour (98.33±0.33%; t(5)=3.162, p=0.025) and 3 hours (98.67±0.33%; t(5)=3.873, p=0.012) following cessation of the infusions.

4.10.3.4.3. Heart rate

Heart rate did not change significantly from baseline throughout the testing day among those receiving BUP (p>0.15).

4.10.3.4.4. Blood pressure

No significant differences from baseline systolic (p>0.20) or diastolic (p>0.15) blood pressure were identified in the BUP group.

4.10.3.4.5. Nausea

Nausea was recorded at each time point according to the following scale: 0, no nausea; 1, mild nausea; 2, moderate nausea; 3, severe nausea. Nausea occurring during the inter-testing period was also rated and recorded according to this scale. Incidence of nausea among subjects receiving BUP are summarised in Table 4-10. Three subjects in the BUP group experienced nausea with vomiting. Bodily movement, such as sitting up or standing, preceded all cases of nausea. Nausea occurred between one and four hours following cessation of the infusions, and ranged from mild to severe. One subject was unable to perform the CP test at the time point one-hour after the final BUP infusion due to severe nausea and vomiting and has been treated as missing data in all analyses. Another subject experienced three episodes of nausea with vomiting, and in all cases a moderate level of nausea developed rapidly and vomiting occurred within two minutes of nausea onset. On all three occasions this subject reported that the nausea was resolved immediately following the episode of vomiting.

4.10.3.4.6. Sedation

All six subjects who received BUP experienced sedation. Sedation was graded according to the sedation scale described in Chapter 2 (2.3.2.3). Comparable to the recording of nausea, while level of sedation was assessed at each time point, sedation occurring during the inter-testing period was also rated and recorded (Table 4-10). In five of the six subjects, sedation was rated as mild. The sedation observed in the BUP group was long lasting, with several subjects experiencing mild sedation up to five hours following the infusion period. One subject experienced severe sedation, at times being difficult to rouse. This subject's level of sedation was rated as moderate to severe up to 6 hours following the end of the infusion period. This was the same subject who experienced the most severe nausea (see Table 4-10).

4.10.3.4.7. Other adverse effects

The most frequent other adverse effect observed was light-headedness (Table 4-10). All six BUP subjects experienced this effect, which was generally mild in nature, preceded by movement, and resolved rapidly with sitting or lying down. Subjects were reclined in an armchair during the inter-testing period, thus light-headedness was often provoked when subjects were roused in preparation for a testing time point. Movement preceded all episodes of light-headedness.

Pruritus was observed in one subject, and this was concentrated in the neck region and mild in nature. This subject also experienced a "hot flush" during a severe episode of nausea with vomiting. One subject complained of a headache at one hour following the infusion period, although it is unclear whether this was a drug-related effect (Table 4-10).

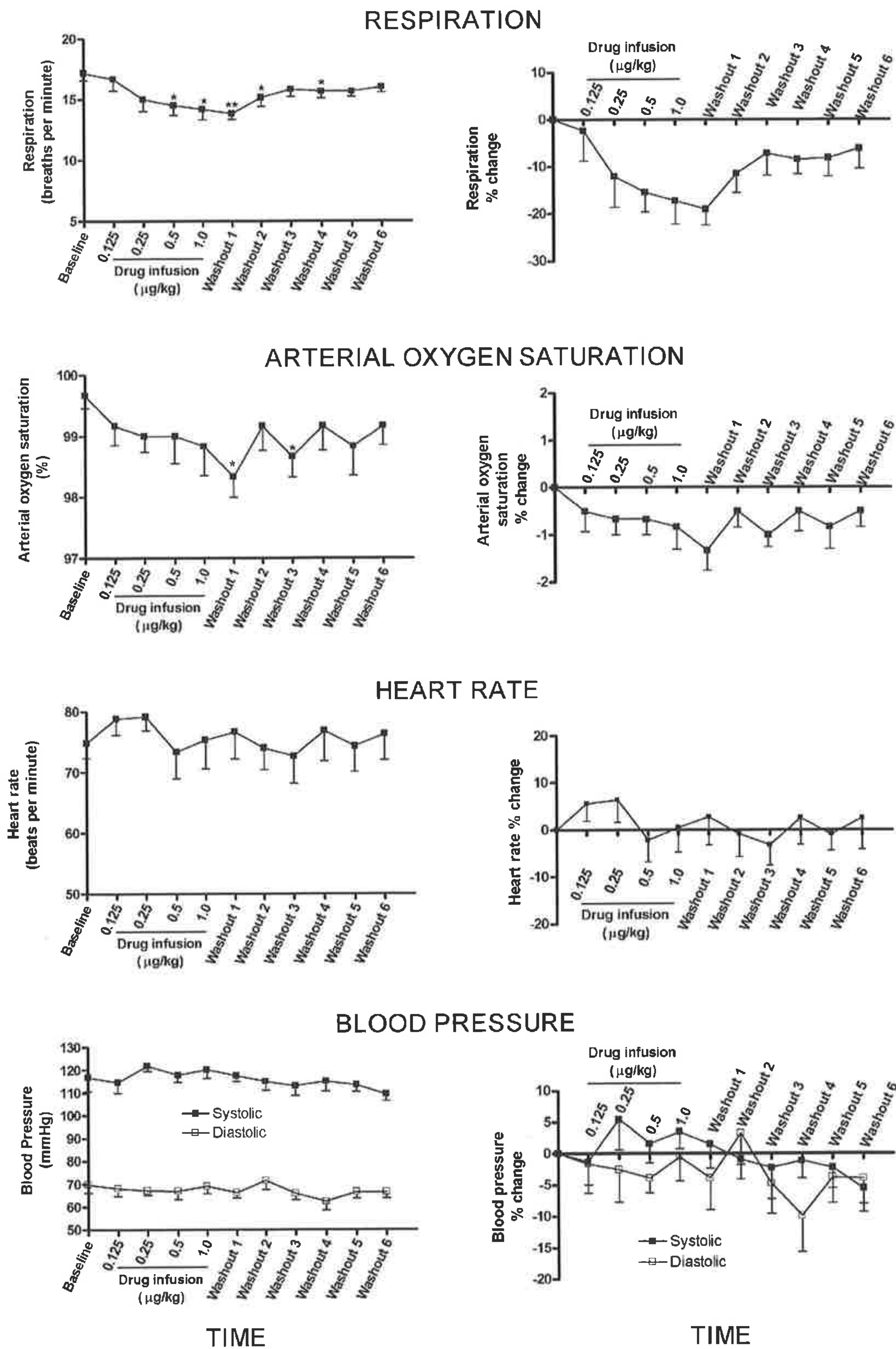

Figure 4-11. Physiological parameters (respiration, arterial oxygen saturation, heart rate and blood pressure) for subjects in the BUP condition (n=6) at each time point (baseline, following each of 4 cumulative 30-minute IV BUP infusions {0.125, 0.25 0.5 and 1.0 μg/kg}, and hourly throughout BUP washout to 6 hrs). Left panel: raw data (mean±SEM), Right panel: data expressed as percent change from baseline (mean±SEM). *p<0.05 **p<0.01, paired samples t-tests.

	Nausea		Sedation		Lightheadedness/ Dizziness	Sweating/ "hot flush"	Pruritus	Headache
	Score	Testing time	Score	Testing time				
Subject A			1 1	Washout 1hr Washout 3hr	Dizziness after BUP 1.0 μg/kg infusion testing Dizziness at Washout 1hr testing.			
Subject B	2	Washout 2hr[+]	1 1	Washout 1hr Washout 2hr	Dizziness upon standing at BUP infusion 1.0 μg/kg testing.			Mild headache during Washout 1hr
Subject C			1 1	Washout 2hr Washout 5hr	Dizziness after BUP 1.0 μg/kg infusion testing			
Subject D	3	Washout 1hr^	1 2 3 2 3 3 2	BUP 1.0 μg/kg Washout 1hr Washout 2hr Washout 3hr Washout 4hr Washout 5hr Washout 6hr	Dizziness upon standing at BUP infusion 1.0 μg/kg testing. Dizziness upon standing at Washout 1hr testing.	Hot flush occurred with nausea and dizziness at Washout 1hr.	Mild, neck region, at Washout 1hr	
Subject E	2 1 2	Washout 1hr* Washout 2hr* Washout 4hr*	1 1 1 1	Washout 2hr Washout 3hr Washout 4hr Washout 5hr	Dizziness upon standing between 1.0 μg/kg and Washout 1hr testing. Resolved once seated.			
Subject F			1 1 1	BUP 0.5 μg/kg BUP 1.0 μg/kg Washout 4hr	Dizziness after BUP 0.5 μg/kg infusion testing			

Note nausea and sedation reported if experienced at testing time or at any time during period between assessment time points.
^Nausea and vomiting upon standing. Metoclopramide (10 mg IV) administered, nausea resolved. CP results from this testing time have been excluded from analyses as test could not be performed properly due to nausea.
*Nausea and vomiting upon standing. Vomiting occurred on each occasion within 2 minutes of onset of nausea. Nausea was immediately resolved by vomiting. Following vomiting at Washout 4, metoclopramide (10 mg IV) was administered. No further episodes of nausea.
[+]Mild nausea upon standing. No vomiting.

Table 4-10. Incidence and severity of adverse effects among subjects in the BUP condition (n=6). Adverse effects are described by subject, time point (see Table 4-5 for description) during which the effect was experienced and, where appropriate, severity of effect.

4.10.4. Discussion

The purpose of the current study was to determine the BUP dose response for CP and ES antinociception. BUP has consistently demonstrated its potent analgesic effects in acute (for example, Harcus et al. 1980; Harmer et al. 1983) and chronic (for example, Sittl et al. 2003; Sorge and Sittl 2004) pain. The activity of many clinically used analgesic agents has been assessed in experimental pain paradigms with healthy volunteers. The capacity for opioid analgesics to produce significant antinociception to experimental pain has been established in a number of experimental pain induction techniques, including tests of cold (Posner et al. 1985; Jones et al. 1988; Doverty et al. 2001) and electrical (Wolff et al. 1966; Stacher et al. 1986) stimulation.

There have been no published reports of the effect of BUP in human models of nociceptive stimulation. In this respect, while BUP is a well-characterised analgesic agent, nothing was reported of its effect in an experimental setting with healthy volunteers. It may be surmised that BUP would produce significant antinociception to experimental stimuli given the potent analgesia observed clinically and the existing evidence for other opioid analgesics with experimental techniques. However, as described previously, the pharmacological profile of BUP differs markedly from opioid agonists such as morphine. Moreover, the BUP dose range associated with significant antinociception to experimental stimuli had not been determined.

The current study assessed the antinociceptive effects of IV BUP in a cumulative dosing schedule in a cohort of healthy volunteers using two common nociceptive tests, the CP and ES tests. Findings demonstrated that BUP is associated with significant antinociception on both the CP and ES tests, with CP showing the largest response.

In a previous study with healthy volunteers, a steady state plasma morphine concentration of 23 (±1) ng/ml was associated with a mean increase in CPTOL of 52% and in ESTOL of 15% (Athanasos et al. 2002). A plasma morphine concentration of 11 ng/ml increased mean CPTOL by approximately 31%, while a plasma morphine concentration of 33 ng/ml increased mean CPTOL by approximately 97% (Doverty et al. 2001). A plasma morphine concentration of 15 ng/ml is required for minimum effective post-operative pain relief (Dahlstrom et al. 1982; Gourlay et al. 1986), while a concentration in the order of 50 ng/ml is sufficient to provide relief from moderate to severe post-operative pain (Berkowitz et al. 1975). By comparison, subjects in the current study received BUP doses lower than would normally be administered for pain management (300-600 μg by slow IV injection every 6-8 hours). Subjects received a total of 1.875 μg/kg over a 2-hour period, equating to a total dose in a 70 kg subject of 131.25 μg. This dosing schedule produced a significant peak mean increase in CPTOL of 37.6% (±10.3) ($p=0.016$).

ESTOL increased significantly at almost all post-BUP time points. In line with previous reports, the mean magnitude of the antinociception was not as great as CPTOL; however these differences were statistically significant due to the small degree of inter-subject variability associated with ESTOL compared to CPTOL. The maximum mean increase in ESTOL was 18.3(±1.6)%, occurring at the Washout 6 hr time point ($p<0.001$). Notwithstanding, CPTOL will remain the major outcome measure of the subsequent studies as it has been reported that the CP test is the human nociceptive test that most closely resembles clinical pain (Wolff 1984), and the findings of the current study have revealed a greater effect with this test than with the ES test.

Consistent with typical opioid effects, a significant decrease in respiration (as measured by breaths per minute) was observed, with a mean trough of 13.8 breaths per minute compared to 17.2 breaths per minute at baseline.

Sedation and light-headedness were the most prevalent adverse-effects, with all subjects experiencing these effects at least once during the testing day. Nausea was experienced by 50% of the BUP subjects. In line with previous reports, nausea and light-headedness occurred upon ambulation or with significant body movement (Macintyre and Ready 2001), supporting the involvement of the vestibular system in these effects (Andrianov and Ryzhova 1999). Sedation was observed to be of particularly long duration, with several subjects being moderately sedated at the final time point 6-hours post-infusion.

The plateau or reduction in effect with increasing dose that is often associated with BUP was not observed for any effect (including nociception and respiration) in the current study. A plateau in effects such as respiration and pupillary miosis has previously been reported to occur at doses exceeding 8 mg (SL) (Walsh et al., 1995), which is greater than doses typically administered for pain management (0.2-0.4 mg SL, 0.3-0.6 mg IM/IV) and far exceeds the comparatively lower doses administered in the present investigation (in a 70 kg adult, 0.131 mg IV over 2 hours). That increasing doses of BUP were not associated with a plateau or reduced effect in the current study is therefore not unexpected, given the comparatively lower doses administered in this study. It may be considered then, that at the doses administered in the current study, BUP produced effects typical of a full opioid agonist.

The open-blind design of this study was necessary due to the fact that all subjects in the subsequent drug combination studies will be aware that they are receiving BUP on each

testing day (the design of these studies is described in more detail in Chapter 5). As the response to BUP in the current study is the basis for anticipated response in subsequent studies, it was necessary to maintain the same subject conditions (i.e. aware that they are receiving BUP). In addition, it is highly unlikely that blinding in this study would have been successful given the obvious subjective and objective effects associated with BUP. Due to the lack of blinding, the influence of subject and experimenter bias, and importantly the impact of placebo analgesia, may have contributed to response.

As described, the principal objective for conducting this dose finding study was to identify a sub-analgesic dose of BUP for use in the subsequent BUP:NLX ratio studies. A dose that was associated with greater CP antinociception than saline but not the maximal effect would be selected. Based on the data presented here, the 0.5 μg/kg dose was selected for the subsequent studies. As can be seen in Figure 4-10 (upper panel), testing at the 0.5 μg/kg infusion was the first to produce an effect above that observed in the saline group. While the time point at the 0.5 μg/kg dose was associated with a statistically significant increase from baseline (21.68%(±7.26), range -4.76-48.00, p=0.043), this infusion had been preceded by a 0.125 and 0.25 μg/kg infusion. Thus the effect of a single 0.5 μg/kg 30-minute infusion would be anticipated to be lower than observed with the cumulative dosing schedule employed in the current study, and is therefore considered suitable for use in the subsequent studies.

It may be argued that determining BUP plasma concentrations following each infusion and selecting a target concentration would have produced a more precise indication of BUP effect than basing the selection on dose. However, there are several practical limitations associated with this approach. Firstly, BUP is difficult to quantify at the very low doses such as those used in the current study, and the equipment required to quantify BUP

concentrations with the required specificity was not available at the time. Moreover, such an approach would require quantification of both BUP and its potent, active metabolite, norBUP. This would entail the selection of BUP and norBUP target concentrations for subsequent studies and that would be extremely difficult to achieve.

In conclusion, this study has demonstrated that both the CP and ES are sensitive tests for BUP antinociception, with doses below those that are used therapeutically for pain relief producing significant antinociception. A dose of 0.5 μg/kg has been selected for use in the subsequent BUP:NLX ratio studies.

5. ANTINOCICEPTIVE ACTIVITY OF BUPRENORPHINE AND NALOXONE COMBINATIONS IN HUMAN EXPERIMENTAL PAIN: RATIO STUDY 1

The management of pain is one of the principal tasks of health care practitioners, and has been described as one of the greatest challenges facing medicine. Despite the substantial advances that have been made in our understanding of the complex mechanisms of pain, it is acknowledged that inadequate treatment of pain remains a significant problem (NHMRC 1999; Kamming et al. 2004; Primm et al. 2004; Viscusi 2004). The consequences of poor pain management are costly to both the individual and the community (Phillips 2000).

Opioids are considered the "gold standard" in moderate to severe pain management, and are the most widely used class of drug in clinical practice for the treatment of moderate to severe pain (Gutstein and Akil 2001). The use of opioids, however, is limited by a number of factors, including the development of tolerance, concerns regarding abuse liability, and unpleasant and potentially dangerous side effects such as respiratory depression, gastro-intestinal problems and pruritus (see section 1.8).

Research is increasingly focusing on ways in which to improve the use of opioids, investigating approaches to enhance the analgesic actions of the drug, while attenuating the development of tolerance and minimising adverse side effects.

A promising drug combination for improved pain management is the co-administration of opioid agonists and ultra-low doses of opioid antagonists. While in millimolar plasma concentrations opioid antagonists have either no effect or enhance pain sensitivity (El-Sobky et al. 1976; Grevert and Goldstein 1977; Davis et al. 1978; Grevert and Goldstein

1978; McCubbin and Bruehl 1994), findings increasingly suggest that opioid antagonists have a more complex role in the modulation of pain.

Firstly, chronic exposure to an opioid antagonist enhances sensitivity to the analgesic effects of subsequent agonist administration (Daws and White 1999). This has been reported to result from the upregulation of opioid systems, and has the obvious clinical advantage of lower opioid requirements, and as a consequence, potentially fewer adverse effects. Secondly, the paradoxical finding has emerged that opioid antagonists in low doses can themselves produce analgesia (Buchsbaum et al. 1977; Levine et al. 1979; Woolf 1980; Levine and Gordon 1986; Ueda et al. 1986; Taiwo et al. 1989; Miaskowski et al. 1990). A bi-directional dose response has been identified, whereby opioid antagonists can be analgesic when administered in low doses and hyperalgesic in higher doses. Thirdly, small (μg) doses of an opioid antagonist co-administered with an agonist can significantly reduce adverse opioid side-effects without reducing analgesia (Brookshire et al. 1983; Korbon et al. 1983; Rawal et al. 1986; Gueneron et al. 1988; Gan et al. 1997; Joshi et al. 1999; Choi et al. 2000; Lee et al. 2001). Moreover, evidence from animal studies has demonstrated that this drug combination can *enhance* analgesia (Lasagna 1965; Bergman et al. 1988; Levine et al. 1988; Vaccarino et al. 1989). Particular emphasis has more recently been drawn to the use of antagonists in "ultra-low" doses (ca. 10 ng/kg) (rather than the "low" doses (ca. 10 μg/kg) employed in other studies) and the reported benefit in terms of enhanced analgesia (Holmes and Fujimoto 1993) as well as attenuation of the development of opioid tolerance (Shen and Crain 1997; Crain and Shen 2001; Powell et al. 2002).

Enhancing the analgesic effect of a given dose of an opioid by the co-administration of an antagonist has the obvious clinical utility of lower dose requirements, and thus fewer

adverse effects. However, critical to this rationale is whether the opioid-related adverse effects would, akin to the analgesia, also be potentiated. As described, several clinical studies have reported the agonist/antagonist combination to be associated with a reduction in opioid side effects without a reduction in analgesia (Rawal et al. 1986; Joshi et al. 1999; Cepeda et al. 2004). There have also been a small number of reports of *enhanced* analgesia in clinical pain patients, with either equivalent (Levine et al. 1988) or a reduced (Gan et al. 1997) incidence of adverse effects, with the addition of low or ultra-low dose antagonists to opioid agonist treatment. These data suggest that the potentiation may not be general to all opioid effects. However, the clinical studies of this drug combination have been few in number, findings have been inconsistent (Levine et al. 1988; Cepeda et al. 2002; Cepeda et al. 2004), and studies have typically been associated with significant limitations (see discussion in 1.9.3). The potential for agonist/antagonist combinations in pain management has thus not been adequately evaluated. A drug combination that could enhance the analgesia afforded by a given dose without a simultaneous escalation in adverse effects could potentially overcome some of the limitations that compromise pain management with opioids.

5.1. Proposed mechanisms of enhanced analgesia with low dose antagonists

Of particular relevance in the context of this investigation are the reports of paradoxical analgesia associated with ultra-low dose opioid antagonist administration, and the enhanced effect of agonist and ultra-low dose antagonist co-administration. A number of mechanisms have been proposed to explain this paradoxical analgesia. Animal studies suggest that low dose NLX selectively blocks a putative endogenous opioid system that is antagonistic to analgesia (Gillman and Lichtigfeld 1985; Gillman and Lichtigfeld 1989), or an endogenous dynorphin "anti-analgesia" system (Wu et al. 1983; Fujimoto and Rady 1989; Holmes and Fujimoto 1993). Alternatively, it has been postulated that low dose

opioid antagonist blockade of presynaptic opioid receptors involved in autoinhibition of enkephalin release may augment release of endogenous opioid peptides (Ueda et al. 1986). However, there have been reports of analgesia produced by doses of NLX (Vaccarino et al. 1988) and NTX (Vaccarino et al. 1989) that were also considered to have postsynaptic actions.

As described in section 1.9.2.4, the principal hypothesis that has emerged to explain the paradoxical actions of antagonists postulates that enhanced analgesia and attenuated tolerance with the addition of a low dose opioid antagonist results from selective antagonism of excitatory opioid-receptor functions (Crain and Shen 2000), the effect of which is reported to be most profound with *ultra*-low doses (pM - nM) of an antagonist (Shen and Crain 1997). This has been termed the bimodal opioid receptor model (see Figure 1-3). It is proposed that in low doses, opioid antagonists and indeed some opioid agonists, selectively antagonise ligand binding with these excitatory, anti-analgesic G_s-coupled receptors, without affecting inhibitory G_i/G_o-coupled receptor binding. Enhanced antinociception is thus observed as the anti-analgesic effects of G_s-coupled receptor binding are blocked. Furthermore, opioid receptors can be readily converted between inhibitory G_i/G_o-coupled mode and excitatory G_s-coupled mode, and this conversion is initiated by increases in the concentration of the glycolipid GM1 ganglioside. It has been postulated that the interaction between GM1 ganglioside and excitatory G_s-coupled receptors may underlie the mechanisms involved in tolerance and hyperalgesia (Crain and Shen 1998).

5.2. Buprenorphine/antagonist combinations in animal models of nociception

BUP is a potent opioid analgesic that is reported to be safer and have less abuse liability than many other opioid analgesics (Jasinski et al. 1978). Mounting reports indicate that

BUP may be particularly useful in the treatment of neuropathic pain (Kouya et al. 2002). In a rat model of neuropathic pain, it has recently been demonstrated that the co-administration of BUP and ultra-low doses of NLX significantly enhanced thermal pain threshold, but this effect was dependent on the BUP dose (Cougnon-Aptel et al. unpublished). The drug combination enhanced antinociception only when the dose of BUP alone was not antinociceptive. As described previously, one of the most intriguing features of the pharmacological profile of BUP is the bell-shaped dose-response curve observed with many effects, including antinociception in animal models. The enhanced antinociception observed by Cougnon-Aptel and co-workers occurred only at the low- and high-dose BUP troughs of the bell-shaped dose-response. When BUP alone produced significant antinociception, the addition of naloxone reduced the magnitude of the antinociception, effectively having an antagonistic effect. In light of previous findings suggesting that enhanced analgesia can occur without a simultaneous increase in adverse effects (Gan et al. 1997; Cruciani et al. 2003; Gear et al. 2003; Schmidt et al. 2003), the potentiation at the lower, sub-antinociceptive BUP doses is of particular potential clinical relevance. Antinociception was assessed at BUP:NLX ratios of 15:1 and 20:1, and potentiation found to be most profound at the 15:1 ratio, with a mean increase in paw withdrawal latency approaching 200% compared to the effect of the BUP alone ($p<0.005$). These data indicate that the agonist:antagonist dose ratio are critical in producing potentiation of antinociception. This may provide some basis for the failure of other studies to detect analgesic potentiation (Cepeda et al. 2002; Cepeda et al. 2004), though it is also possible that the dose-dependence of the effect is unique to BUP. Most studies to date have investigated morphine and, more recently, nalbuphine. As described (see section 4.2), the pharmacological profile, mechanism of action and dose-response relationship associated with BUP are increasingly recognised to differ substantially from those of other opioid μ-receptor agonists.

5.3. Summary

Inadequate pain relief can be associated with increased morbidity and significant cost to the individual and the community. Multimodal approaches to pain relief involving the combination of different drugs are increasingly being investigated for the advantages they offer in terms of enhanced analgesia and reduced incidence of side effects. Opioids remain the optimal treatment for the management of moderate to severe pain. There have been several promising findings from *in vivo* and *in vitro* animal and human studies demonstrating that the addition of an alternative agent to opioid agonist analgesia can improve treatment and attenuate the development of tolerance. Numerous reports have documented a paradoxical analgesic effect with the administration of low- or ultra-low-dose opioid antagonists, and enhanced analgesia with the combination of an agonist and ultra-low dose antagonist. Human studies have produced inconsistent findings, likely due to methodological differences and limitations associated with those investigations.

BUP is a partial μ-opioid receptor agonist with potent analgesic effects (Kay 1978). While the use of opioids in the treatment of neuropathic pain is controversial (Portenoy et al. 1990; McCormack 1999), evidence suggests that BUP may have potential in the management of this complex pain condition.

A compelling finding recently emerged from an investigation of BUP combined with NLX in a neuropathic pain model in rats (Cougnon-Aptel et al. unpublished). These findings demonstrate that NLX can significantly enhance antinociception, but that this effect was evident only when BUP was administered in sub-analgesic doses. These data indicate that the BUP:NLX dose ratio is critical to producing antinociceptive potentiation. The effects of BUP combined with a low- or ultra-low-dose antagonist have not previously been

investigated in humans, and the agonist:antagonist combination has not previously been evaluated in an experimental pain paradigm.

Based on the findings of Cougnon-Aptel and colleagues, that BUP combined with the short acting opioid antagonist NLX produced greater antinociceptive potentiation than the BUP:NTX combination (Cougnon-Aptel et al. unpublished), NLX will be used in the current ratio studies.

5.4. Naloxone

Like other opioid antagonists, the effects of NLX vary depending upon the presence of exogenous opioid agonists or the activation of endogenous opioid systems. Small doses of NLX (0.4-0.8 mg) can rapidly and effectively reverse the effects of opioids, and thus have considerable clinical utility in cases of overdose, and in diagnosing opioid physical dependence (Gutstein and Akil 2001)[2]. NLX is administered for acute opioid toxicity, and in such circumstances has been associated with adverse effects, including pulmonary oedema (Flacke et al. 1977; Prough et al. 1984; Partridge and Ward 1986; Johnson et al. 1995), hypertension (Tanaka 1974; Azar and Turndorf 1979; Levin et al. 1985) nausea, vomiting, hypotension, ventricular tachycardia and fibrillation, bradycardia (MIMS 2004) and cardiac arrest (Cuss et al. 1984). NLX dose can be titrated to reverse respiratory depression without precipitating a full withdrawal syndrome, and as outlined previously, NLX in low doses can attenuate opioid adverse effects without attenuating analgesia in pain patients.

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[2] The small doses of NLX that reverse the effects of opioids (0.4-0.8 mg) exceed those described as "ultra-low". In the current study, the ultra-low NLX doses administered to a 70kg adult would be 2.33, 1.75 and

In a non-dependent person and in the absence of opioid agonists (or activation of endogenous opioid systems) NLX is understood to have few effects. In the absence of opioid agonists, doses of up to 12 mg of subcutaneous (SC) NLX have produced no evident effects in non-dependent humans (Gutstein and Akil 2001).

As described previously (see 1.9.2.2), opioid antagonists including NLX have been reported to produce a dose-dependent biphasic effect, being associated with analgesia in low doses and either producing no effect or reducing pain tolerance at higher doses (Buchsbaum et al. 1977; Levine et al. 1979; Woolf 1980; Levine and Gordon 1986; Ueda et al. 1986; Taiwo et al. 1989; Miaskowski et al. 1990). Opioid antagonists such as NLX can attenuate placebo analgesia and produce effects in other circumstances where the endogenous opioid system has been activated, such as in stress (Gutstein and Akil 2001).

5.4.1. Pharmacology of naloxone

NLX is a "pure" opioid antagonist that binds non-selectively with all three classic opioid receptor types (Goldstein and Naidu 1989). Reported K_i values for NLX binding to μ-opioid sites have ranged from 10.2 nM in avian brain tissue (Magnan et al. 1982), to 1.78 in guinea pig (Magnan et al. 1982) and 1.9 nM in mouse brain homogenates (Lewanowitsch and Irvine 2003). Binding with δ sites is less potent, with K_i values reported between 17.7 nM and 27.0 nM (Magnan et al. 1982; Deviche 1997).

NLX is readily absorbed from the gastrointestinal tract, but is almost completely metabolised by the liver before reaching the systemic circulation and must therefore must

1.4 μg for the BUP:NLX 15:1, 20:1 and 25:1 ratios, respectively and, as such, are more than two orders of magnitude lower than doses administered for antagonism of opioid effects.

be administered parenterally. NLX is metabolised rapidly, primarily by glucuronide conjugation. NLX has a $t_{1/2}$ of approximately an hour (Evans et al. 1974; Gutstein and Akil 2001), and a similarly short duration of action, although this is dependent upon dose and route of administration. Longnecker and colleagues reported reversal of morphine-induced respiratory depression up to 79 minutes following an IV dose of 5 μg/kg and up to 99 minutes following 10 μg/kg (Longnecker et al. 1973). A similar duration of effect has been reported by other investigators (Hasbrouck 1971; Evans et al. 1974). Onset of action following IV administration is rapid (1-2 minutes) (Ngai et al. 1976), and time to peak effect between 5 and 15 minutes.

5.5. Purpose and aims of the present research

The purpose of the present study was to investigate whether the addition of ultra-low doses of NLX significantly enhances antinociception to experimental pain compared to the same dose of BUP alone in healthy, pain-free volunteers. A further aim was to determine whether adverse opioid effects are enhanced by the drug combination. This study also sought to determine whether enhanced antinociception with the BUP:NLX combination is ratio dependent.

This drug combination has not previously been studied in pain-free humans. As described, animal data indicate that there is a narrow BUP dose and agonist:antagonist ratio range over which enhanced antinociception is produced in a neuropathic pain model. It is therefore proposed that a sub-analgesic dose of BUP combined with NLX in a range of ratios be assessed using an experimental pain paradigm in pain-free humans. This investigation will demonstrate whether the enhanced antinociception observed in the study by Cougnon-Aptel and colleagues can be achieved in humans. By using an experimental pain model with pain-free volunteers the many potential sources of variability associated

with a clinical pain paradigm are reduced, allowing effective dose ratios to be identified prior to investigation in a clinical pain population.

5.5.1. Hypothesis

That there will be a significant increase in antinociception, but not adverse effects, with the combination of BUP and NLX compared to BUP alone.

5.5.2. Aims

The aims of the present study were to determine whether the addition of ultra-low dose NLX can potentiate BUP antinociception in two human experimental pain models, and to determine the effect of the addition of NLX on the incidence of adverse effects.

5.6. Methods

5.6.1. Study design

This study was a double-blind, randomised controlled trial. Each participant was studied on four occasions approximately two weeks apart. Participants received, in a randomised manner, each of the following combinations over the four testing days: (i) BUP:saline; (ii) BUP:NLX in a 15:1 dose ratio; (iii) BUP:NLX in a 20:1 dose ratio; (iv) BUP:NLX in a 25:1 dose ratio. These ratios were selected on the basis of the animal data that demonstrated optimal antinociception at a BUP:NLX ratio of 15:1 (Cougnon-Aptel et al. unpublished). BUP was administered at a dose of 0.5 μg/kg body weight based on the results of the dose-finding study described in the previous chapter (see 4.10.3.3). Only one subject was tested on each day, with the investigator and research nurse present for the duration of each testing day. A medical officer inserted the cannulae and was present for the duration of the infusions (see 1.4.4.2.1).

While the inclusion of a saline only condition in the protocol would have controlled for any placebo effect, it was considered that such a control would have been futile given the subjective opioid effects associated with the BUP. Furthermore, the central aim of the investigation was not to assess the effect of the BUP, but rather to investigate the magnitude of difference in response with the drug combination compared to the BUP alone.

5.6.2. Participants

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 020820). Participants were healthy, drug-free volunteers recruited through word of mouth. Participants from the dose-finding study (Chapter 4) were precluded from participating in the current study. Participation in the study was on a voluntary basis. Participants were financially remunerated $AU1000 upon completion of the study. If a participant withdrew from the study for personal reasons prior to completion of the 4 testing days, he/she was remunerated $AU100. If a participant withdrew due to adverse effects related to the study drug or procedures, or the investigator considered it unethical to continue with testing due to adverse effects, the participant was remunerated on a pro-rata basis (i.e. $AU250 per testing day). Those who completed the screening process but did not meet the criteria for enrolment were remunerated $AU25. Written informed consent was obtained from all participants prior to commencing the trial.

5.6.2.1. Inclusion and exclusion criteria

Inclusion and exclusion criteria for this study were as described in Chapter 4 (see 4.8.1.1 and 4.8.1.2).

5.6.3. Sample characteristics

The sample comprised 6 healthy Caucasian volunteers. One male subject withdrew from the study following the first testing day (see details in section 5.7.1.1) and an additional subject recruited in his place. The sample characteristics described represent the final cohort of 6 who completed the trial. Demographic characteristics are displayed in Table 5-1. There were no significant differences between male and female participants in terms of age or CP performance at screening.

	Complete sample (N=6)		
	Mean (±SEM)	Range	CV
Age (years)	30.33 (±3.95)	19 - 45	
CPTHR at screening (seconds)	8.17 (±0.47)	7 - 10	14.31
CPTOL at screening (seconds)	51.67 (±10.77)	22 - 83	51.07

	Sample divided by sex[a] (Mean±SEM)	
	Male (n=3)	Female (n=3)
Age	29.33 (±4.49)	31.33 (±7.54)
CPTHR at screening	8.67 (±0.88)	7.67 (±0.33)
CPTOL at screening	66.67 (±13.91)	36.67 (±12.72)

[a] No significant differences, p>0.05, independent samples t-tests. CV: Coefficient of variation

Table 5-1. Age (years) and CP parameters (seconds) at screening among the entire group (n=6), and the group classified according to sex (3 males, 3 females). No significant differences between males and females in age or CP parameters at screening (p>0.05).

5.6.4. Procedures

5.6.4.1. Screening procedures

Screening procedures for the current study were as described for the dose-finding study (Chapter 4).

5.6.4.2. Experimental procedures

5.6.4.2.1. Drug administration

The infusion period commenced with an IV infusion of 10 ml saline over 30 minutes, followed immediately by the infusion of either BUP and saline, or BUP and NLX in a 15:1, 20:1 or 25:1 ratio over 30 minutes. Participants were randomised to receive each of these conditions over 4 separate testing days (see randomisation schedule, Table 5-3). BUP was administered at a dose of 0.5 μg/kg body weight. BUP and NLX (or saline on the BUP:saline condition) were administered simultaneously from different infusion pumps into the same vein. An example of BUP and NLX doses administered for each condition based on a subject weighing 70 kg is displayed in Table 5-2. The total duration of infusion was 1 hour (30 minutes saline, 30 minutes BUP:saline or BUP:NLX) over which time nociceptive testing was performed and physiological parameters recorded. Details of infusion set-up and procedure are outlined in Chapter 2 (section 2.3.1).

Condition	Pump A	Pump B
BUP:saline	BUP 35 μg	Saline 10ml
BUP:NLX 15:1	BUP 35 μg	NLX 2.3 μg
BUP:NLX 20:1	BUP 35 μg	NLX 1.75 μg
BUP:NLX 25:1	BUP 35 μg	NLX 1.4 μg
Conditions administered in a double-blind, randomised order. Pump A and pump B infused simultaneously for a 30-minute period.		

Table 5-2. Doses administered for each condition based on a 70 kg subject.

	Session 1	Session 2	Session 3	Session 4
Subject A	BUP only	15:1	25:1	20:1
Subject B	20:1	15:1	BUP only	25:1
Subject C	20:1	BUP only	15:1	25:1
Subject D	BUP only	15:1	20:1	25:1
Subject E	15:1	25:1	20:1	BUP only
Subject F	25:1	BUP only	15:1	20:1

Table 5-3. Randomisation schedule for 7 healthy volunteers. Drug conditions were IV BUP:saline (BUP only) 0.5 µg/kg; IV BUP(0.5 µg/kg):NLX in a 15:1, 20:1 and 25:1 ratio. BUP and saline/NLX infused simultaneously into the same vein over 30-minutes. Subjects randomised to receive each condition across 4 individual testing days. Randomisation schedule was created and administered by hospital pharmacy responsible for drug preparation.

5.6.4.2.2. Testing protocol and schedule

Subjects were delivered from their homes to the testing centre by taxi in the morning. Subjects had been instructed to refrain from taking any drugs or medication in the 24-hours prior to testing (excluding the contraceptive pill) and to eat a light breakfast on the morning of testing. A urine sample was taken and tested by an independent laboratory for drugs of abuse (opioids, cannabinoids, benzodiazepines and sympathomimetic amines) and, for female subjects, pregnancy.

Assessments were made at nine time points throughout each testing day according to the procedures described in section 2.3.2. Each assessment time point involved taking a blood sample, recording nausea, sedation and physiological parameters, and completion of the nociceptive tests. This testing procedure took place prior to infusion, twenty minutes after the commencement of each infusion (saline and then BUP with saline or BUP with NLX), and then hourly upon completion of the infusions over a 6-hour washout period. Subjective effects were also recorded throughout the testing day. Subsequent reference to testing time points are made according to the description in Table 5-4.

Testing time point reference	Description
Pre-saline	Prior to starting the infusion period
Baseline	20 minutes after starting the 30-minute saline infusion
Post-bup/ Post-drug	20 minutes after starting the 30-minute 0.5 μg/kg BUP infusion
Washout 1 (hr)	1 hour following cessation of the BUP infusion
Washout 2 (hr)	2 hours following cessation of the BUP infusion
Washout 3 (hr)	3 hours following cessation of the BUP infusion
Washout 4 (hr)	4 hours following cessation of the BUP infusion
Washout 5 (hr)	5 hours following cessation of the BUP infusion
Washout 6 (hr)	6 hours following cessation of the BUP infusion

Table 5-4. Description of testing time point references. At each time point the following was performed:1) blood sample taken, 2) nausea, sedation, subjective and physiological parameters assessed, 3) nociceptive testing completed.

5.6.5. Statistical analyses

Statistical analyses were conducted using SPSS® for Windows (SPSS Inc, Chicago, Illinois, USA) and GraphPad Prism® Version 4. An alpha level of 0.05 was used in all analyses. The analyses conducted are described below.

5.6.5.1. Justification for use of parametric or non-parametric methods

As mentioned previously (see 3.4.3), parametric tests of statistical inference are not appropriate when cases of censored data are included in the data set due to assumptions about the distribution of the data. In the current study, CPTOL was censored due to the maximum time limit (180 seconds) at one or more time points for 3 of the 6 subjects. While these subjects reached the maximum limit at only a few time points throughout the study (at two or less time points for each of the 3 subjects for the duration of the study), the conservative approach has been taken to conduct all CPTOL analyses with non-parametric

techniques. Other parameters met the assumptions for parametric methods (Tabachnick and Fidell 2000) and have therefore been analysed with these methods.

5.6.5.2. Establishing baseline response

Differences in each parameter before and after the 30-minute saline infusion were assessed using paired samples t-tests. The purpose of this was to determine whether any change in nociceptive response or physiological parameters may be attributed to the infusion process itself, and also provided an indication of the reliability of the baseline from which changes in nociception were measured.

5.6.5.3. Assessing the effect associated with each drug condition

To assess the effect associated with each drug condition (BUP only, BUP:NLX 15:1, 20:1 and 25:1), changes from baseline were assessed for each parameter using one-way repeated measures analysis of variance (ANOVA) for each condition (BUP only, and each BUP:NLX ratio). These analyses included results from assessment time points at Baseline, Post-drug and Washout 1 hour. Results from subsequent time points were not included as drug effect may reasonably be expected to be most distinct at the assessments immediately following drug administration (Post-bup and Washout 1hr). Including all time points in these analyses (to 6 hours following cessation of drug administration, by which time the drug effect may be minimal) may have masked a significant effect early in the post-drug period. Furthermore, it has previously been reported that ANOVA using all time points is not appropriate when a biphasic response is anticipated due to a potential overestimation of variance (Angst et al. 2003), such as in cases where effects during both administration and washout of a drug are assessed. Tukey's Multiple Comparison Test was used for post-hoc analyses to identify significant relationships between individual time points when a significant effect had been observed with ANOVA.

As described, non-parametric tests of statistical inference were used to assess changes in CPTOL due to the potential for censored data included in these results (i.e. cases where the subject has not reached maximum tolerable pain before the forced cut-off point on the tests). Friedman's analysis of ranks (a non-parametric equivalent to the one-way repeated measures analysis of variance) was used to analyse these data. Consistent with the other analyses, only Baseline, Post-bup and Washout 1hr were included in these analyses. When significant differences were found, a series of Dunn's Multiple Comparison tests were carried out to provide post-hoc comparisons of the Mean ranks.

5.6.5.4. Comparing the effect of each BUP:NLX ratio with BUP alone

As baseline values were different between conditions, data were expressed as percent change from baseline in order to compare the effect associated with each ratio with the effect of BUP alone. Each participant's nociceptive and physiological response at each time point for each condition was expressed as a percent change from baseline response according to the equation below. Data are expressed as the mean (±SEM) of these values at each post-drug time point for each condition.

$$\frac{\text{Post-drug latency} - \text{baseline latency}}{\text{baseline latency}} * 100$$

As mentioned, ANOVA including all time points is considered inappropriate due to the nature of the data. Two-way repeated measures ANOVA comparing BUP alone with each BUP:NLX ratio using only the 3 time points described above would assess the effect of the addition of the NLX at each ratio early in the post-drug period. However, the magnitude of effect during the latter phase of the washout period was also of interest. Furthermore, no non-parametric equivalent (to analyse the CPTOL data) of the two-way repeated measures ANOVA was available with either of the statistical programs employed.

Therefore, to assess the effect of the addition of NLX at all time points, and for the sake of consistency in analysis of nociceptive and physiological parameters, paired samples t-tests (and the non-parametric equivalent, Wilcoxon signed ranks, for the CPTOL data) were used to compare parameters at each time point for BUP alone and each BUP:NLX ratio.

5.6.5.5. Comparing the effect of each ratio

In addition to comparing the effect of each ratio compared to BUP alone significant differences between the antinociceptive effects of each BUP:NLX ratio were investigated across all time points. As tolerance to the nociceptive stimuli was the primary outcome measure, these further analyses were only conducted with CPTOL and ESTOL.

To investigate differences in antinociception between the BUP:NLX ratios, the percent change from baseline associated with BUP alone at each time point was subtracted from the percent change in nociceptive tolerance associated with each BUP:NLX ratio at the corresponding time point. While this illustrates the time course of antinociception associated with each ratio, the area under the percent change BUP:NLX minus percent change BUP only curve (AUC) from baseline to the end of the monitored washout period was calculated for each ratio to demonstrate the magnitude of change associated with the addition of NLX over the entire dosing and washout period[3]. AUC was calculated by the trapezoidal method. As the statistical software package used to calculate AUC did not not assign a negative value to peaks below the *x* axis, negative and positive peaks were calculated separately, and negative peak area subtracted from positive peak area for each subject. Wilcoxon signed rank tests and paired samples t-tests were used to assess mean differences in AUC between the ratios for CPTOL and ESTOL, respectively.

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[3] AUC($\%change_{BUP:NLX}$ - $\%change_{BUP\ only}$) for each subject in each ratio

5.6.5.6. Subjective and other effects

These effects are reported in tabular form according to effect, subject and time point during which the effect was experienced. Where appropriate the severity of the effect is also reported. No tests of statistical inference have been applied to these data.

5.7. Results

This section is divided into five parts: participant withdrawal and missing data, analysis of pre- and post-saline results to establish baseline, results from nociceptive testing, physiological measurements, and the incidence of adverse effects. The antinociceptive parameters are outlined according to each test, in the order CPTHR, CPTOL, ESTHR, and ESTOL.

5.7.1. Missing data and participant withdrawal/exclusion post-recruitment

5.7.1.1. Participant withdrawal

A 33-year old male subject attended for the first testing day, but was subsequently excluded due to nausea (without vomiting) and light-headedness. The participant received BUP:NLX in a 15:1 ratio. The nausea and light-headedness were of short duration (approximately 5 minutes) but the participant reported "not feeling too good" at several subsequent time points, stating on each of these occasions that he felt too ill to undergo the pain testing. Throughout the intervals between nociceptive testing (50-55 minutes) the participant did not complain of any significant adverse effects. It is considered that this participant's failure to continue with the testing is due in part to an unwillingness to undergo testing procedures for the three subsequent scheduled testing days. The subject stated that he was disinclined to continue with the study due to the adverse effects he had

experienced. The subject was remunerated $AU250 for his participation. An additional male participant was recruited in his place.

5.7.1.2. Missing data

One participant (45-year old female) returned an opioid positive urine on the 20:1 testing day. Data from this participant for the 20:1 testing day have been excluded. Where comparison is made with the BUP only condition (such as in mean difference between performance during a BUP:NLX condition and BUP only condition), the results from this participant for the BUP only condition have also been omitted to avoid confounding results.

5.7.2. Pre- and post-saline infusion

Paired samples t-tests were conducted to detect any significant differences between nociceptive and physiological parameters before and after the 30-minute saline infusion. No significant differences were detected on any parameter at pre-saline testing and post-saline testing ($p>0.05$). Thus it is considered that the infusion process itself had no significant impact on nociceptive or physiological parameters. In all subsequent analyses, then, post-saline values have been used as baseline response.

5.7.3. Cold pressor threshold

5.7.3.1. Effect of each condition

Mean (±SEM) CPTHR values for each condition across all time points are presented in Figure 5-1. One-way ANOVA conducted for each condition over three time points (Baseline, Post-bup and Washout 1hr) revealed a significant increase in the BUP only condition ($F_{2,10}=4.52$, $p=0.039$) (see Table 5-5). Tukey's multiple comparison test

revealed a significant difference between Baseline and Post-bup ($p<0.05$). No significant differences were observed in any of the BUP:NLX ratios. While there was a considerable increase in CPTHR in the 25:1 ratio condition, there was a high degree of inter-individual variability in response. This variability may be attributed to the results of one subject (30 year old female) who experienced a peak increase in CPTHR approaching 300 %. This subject demonstrated a marked increase in CPTHR in the 15:1 condition also, although this was of a lesser magnitude (approximately 50%).

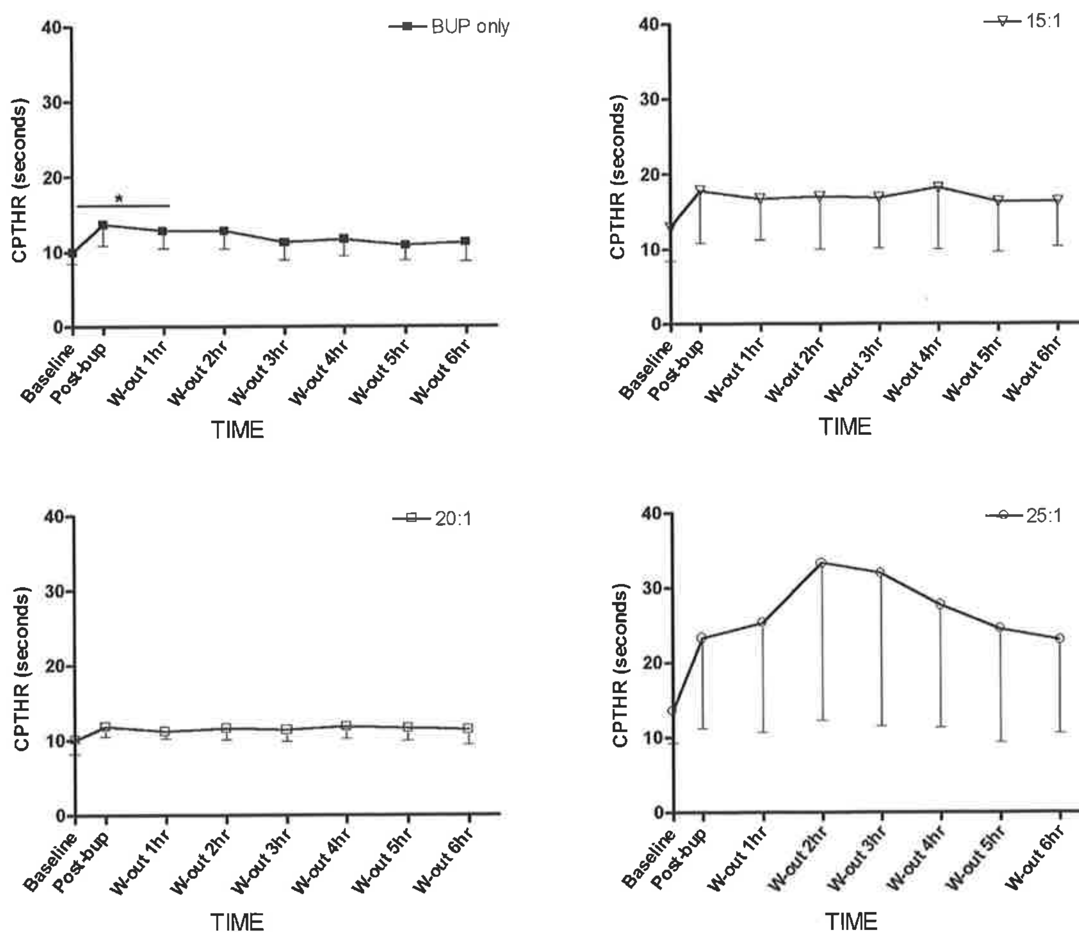

Figure 5-1. Mean (±SEM) CPTHR (seconds) for each condition (BUP only {n=6}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6} ratio) over all time points (baseline, following an IV BUP infusion {0.5 μg/kg over 30 minutes}, and hourly throughout BUP washout to 6 hrs). *p<0.05, significant change over Baseline, Post-bup and Washout 1hr, one-way repeated-measures analysis of variance.

	Mean (±SEM)			Df	F	p
	Baseline	Post-bup	Washout 1hr			
BUP:NLX 15:1 (n=6)	11.00 (±2.72)	13.00 (±4.57)	17.83 (±7.06)	2, 10	2.04	0.181
BUP:NLX 20:1(n=5)	9.20 (±1.59)	10.00 (±1.79)	11.80 (±1.36)	2, 8	0.87	0.287
BUP:NLX 25:1(n=6)	12.83 (±4.35)	13.67 (±4.40)	23.33 (±12.16)	2, 10	1.35	0.305
BUP only(n=6)*	9.67 (±1.71)	10.00 (±1.57)	13.67 (±2.87)	2, 10	4.52	0.039

Table 5-5. One-way repeated measures analysis of variance (ANOVA) of CPTHR (seconds) for each drug condition (BUP only {0.5 µg/kg}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over Baseline, Post-bup and Washout 1hr time points.

5.7.3.2. Effect of BUP:NLX ratio compared to BUP alone

Values for CPTHR mean (±SEM) percent change from Baseline at each time point for each condition are presented in Figure 5-2. Paired samples t-tests between BUP only and each ratio at each time point revealed no significant differences.

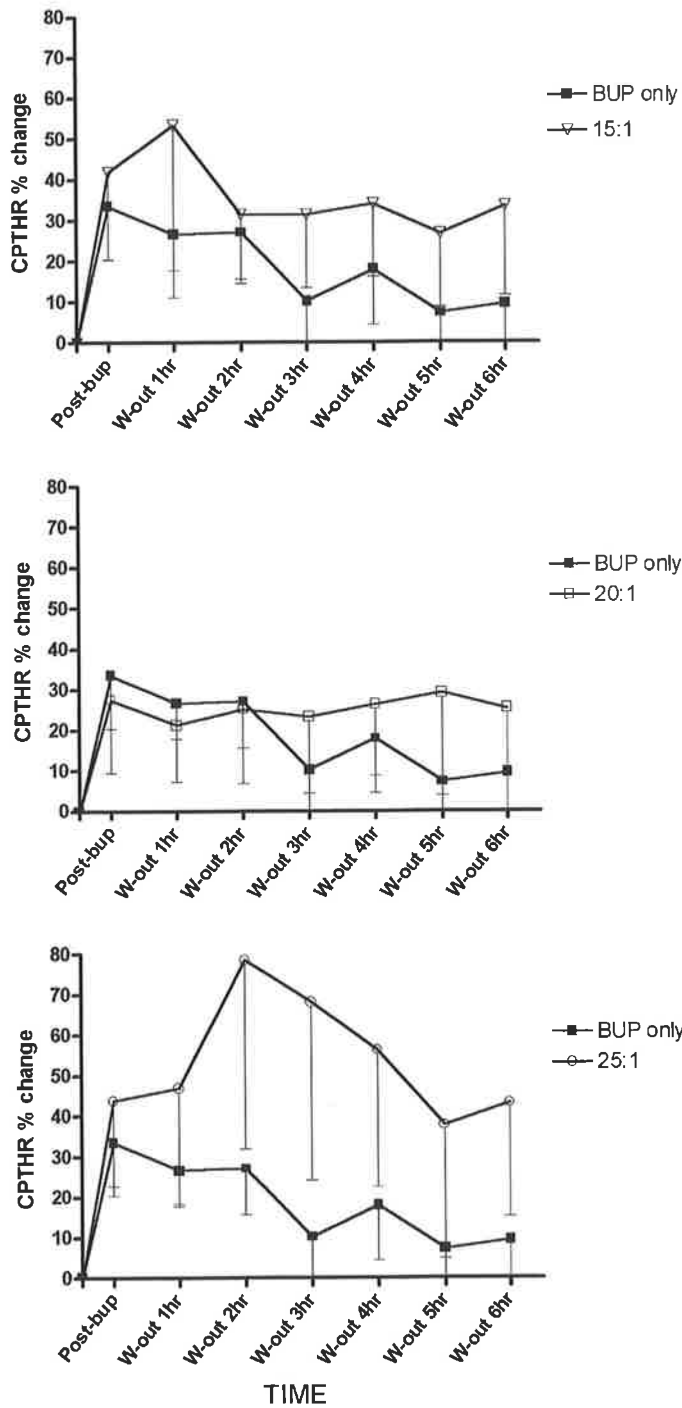

Figure 5-2. Mean (±SEM) CPTHR (seconds) percent change from Baseline for BUP:NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only {n=6} across all time points (baseline, following an IV BUP infusion {0.5 μg/kg over 30 minutes}, and hourly throughout BUP washout to 6 hrs). p>0.05, no significant difference from BUP only, paired samples t-tests. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.4. Cold pressor tolerance

5.7.4.1. Effect of each condition

Mean (±SEM) CPTOL for each condition is presented in Figure 5-3. Friedman's two-way analysis by ranks for each condition (BUP only, 15:1, 20:1 and 25:1 BUP:NLX ratios) over three time points (Baseline, Post-drug and Washout 1hr) revealed a significant increase in CPTOL in the 15:1 condition only ($\chi^2(3)=9.33$, $p=0.005$) (see Table 5-6). Dunn's multiple comparison tests revealed significant differences in Baseline vs. Washout 1hr ($p<0.05$). There were no significant differences in CPTOL in the 20:1 ($p=0.367$) or 25:1 ($p=0.142$) conditions. The difference in CPTOL over these three time points in the BUP only condition was approaching significance ($p= 0.072$).

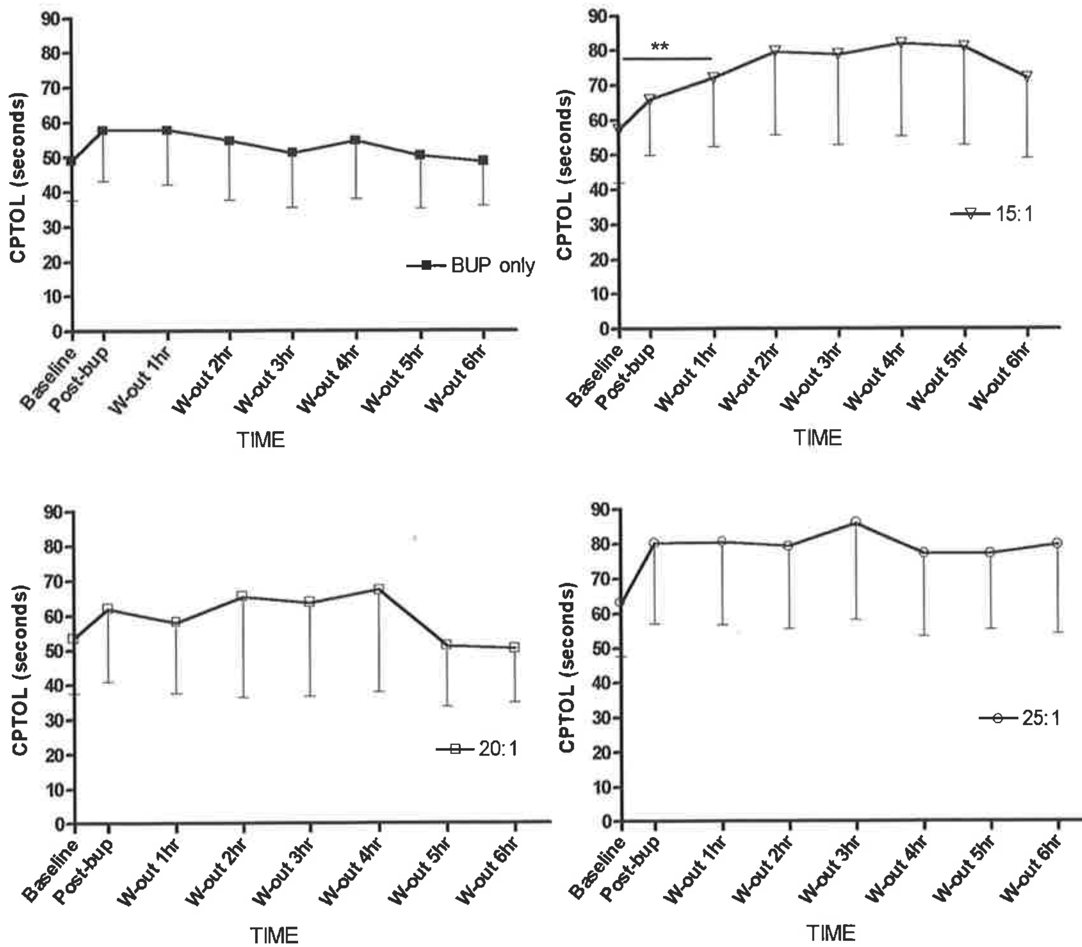

Figure 5-3. Mean (±SEM) CPTOL (seconds) for each condition (BUP only {0.5 μg/kg, n=6}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (baseline, post-BUP infusion, and hourly throughout BUP washout to 6 hrs). **p<0.001, significant difference over Baseline, Post-bup and Washout 1hr, Friedman's test.

	Mean rank			χ^2
	Baseline	Post-drug	Washout 1hr	
15:1 (n=6)	1.00	2.83	3.00	9.33**
20:1(n=5)	1.90	2.70	2.90	0.37
25:1(n=6)	1.75	3.25	2.58	0.14
BUP only(n=6)	1.83	3.17	2.83	4.73

Table 5-6. Mean rank for each condition (BUP only 0.5 μg/kg {n=6}, and BUP{0.5 μg/kg}:NLX in a 15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6} ratio) at Baseline, Post-Bup and Washout 1 hr among healthy volunteers. χ^2 statistic of Friedman's test of CPTOL over Baseline, Post-bup and Washout 1hr for each condition, **p<0.01.

5.7.4.2. Effect of BUP:NLX ratio compared to BUP alone

Figure 5-4 displays mean (±SEM) percent change from baseline CPTOL at each time point for each ratio compared to BUP only. The increase in CPTOL observed in the 15:1 ratio condition was significantly greater than observed with the BUP only condition at Washout 2hr (z=-2.20, p=0.028), Washout 3hr (z=-1.99, p=0.046) and Washout 5hr (z=-2.20, p=0.028). A significantly greater increase in CPTOL was observed in the 20:1 ratio condition compared to BUP only at the Washout 3hr time point (z=-2.02, p=0.043).

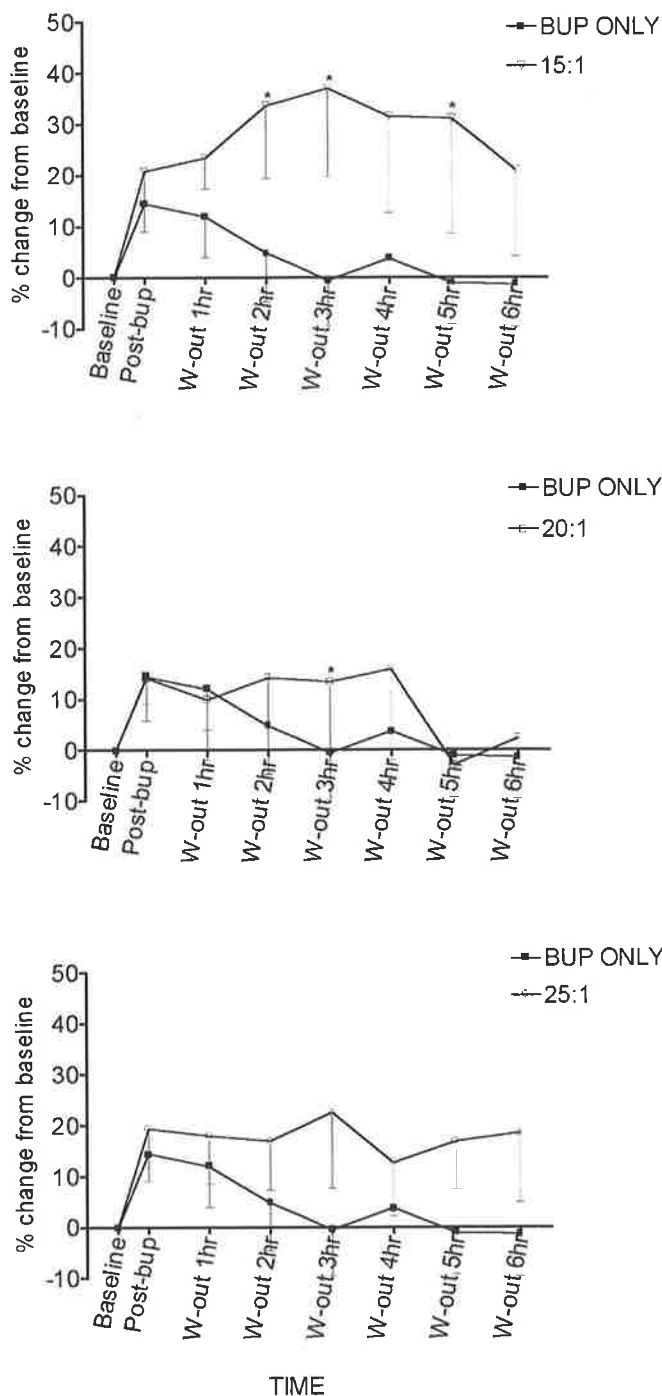

Figure 5-4. Mean (±SEM) CPTOL expressed as percent change from Baseline for BUP(0.5 µg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5}, 25:1 {n=6}) compared to BUP only (0.5 µg/kg, n=6) IV infusion over 30 minutes across all time points (Baseline, Post-bup, and hourly throughout BUP washout to 6 hours). *p<0.05, significant difference from BUP only, Wilcoxon signed ranks. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.4.3. Difference between % change$_{RATIO}$ and % change$_{BUP\ ONLY}$

The difference between the mean percent change from baseline CPTOL for each ratio and for BUP only is presented in Figure 5-5. Wilcoxon signed rank tests revealed no significant differences between the AUC of the difference for each ratio (Figure 5-5, lower panel).

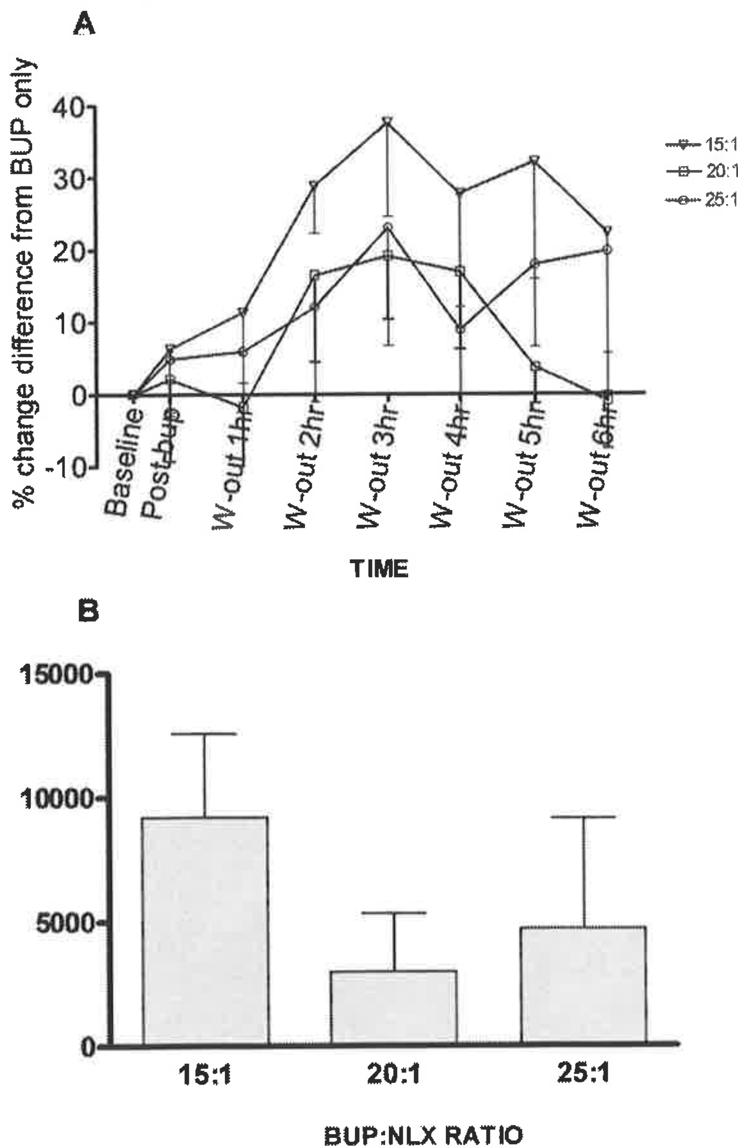

Figure 5-5. A. Mean (±SEM) CPTOL percent change difference from BUP only (0.5 μg/kg, n=6) for each BUP(0.5 μg/kg):NLX ratio (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) over all time points (Baseline, Post-bup, and hourly throughout BUP washout to 6 hours). B. Mean (±SEM) CPTOL AUC percent change difference from BUP only (0.5 μg/kg) for each BUP:NLX ratio. p>0.05, no significant differences between ratios, Wilcoxon signed ranks.

5.7.5. Electrical stimulation threshold

5.7.5.1. Effect of each condition

Mean (±SEM) ESTHR values for each condition are presented in Figure 5-6. One-way analysis of variance (ANOVA) was conducted for each condition (BUP only, 15:1, 20:1 and 25:1 BUP:NLX ratios) over three time points (Baseline, Post-bup and Washout 1hr) to determine whether a significant change in ESTHR was associated with any condition post-drug administration (see Table 5-7). These analyses revealed no significant differences in any condition over the three time points assessed.

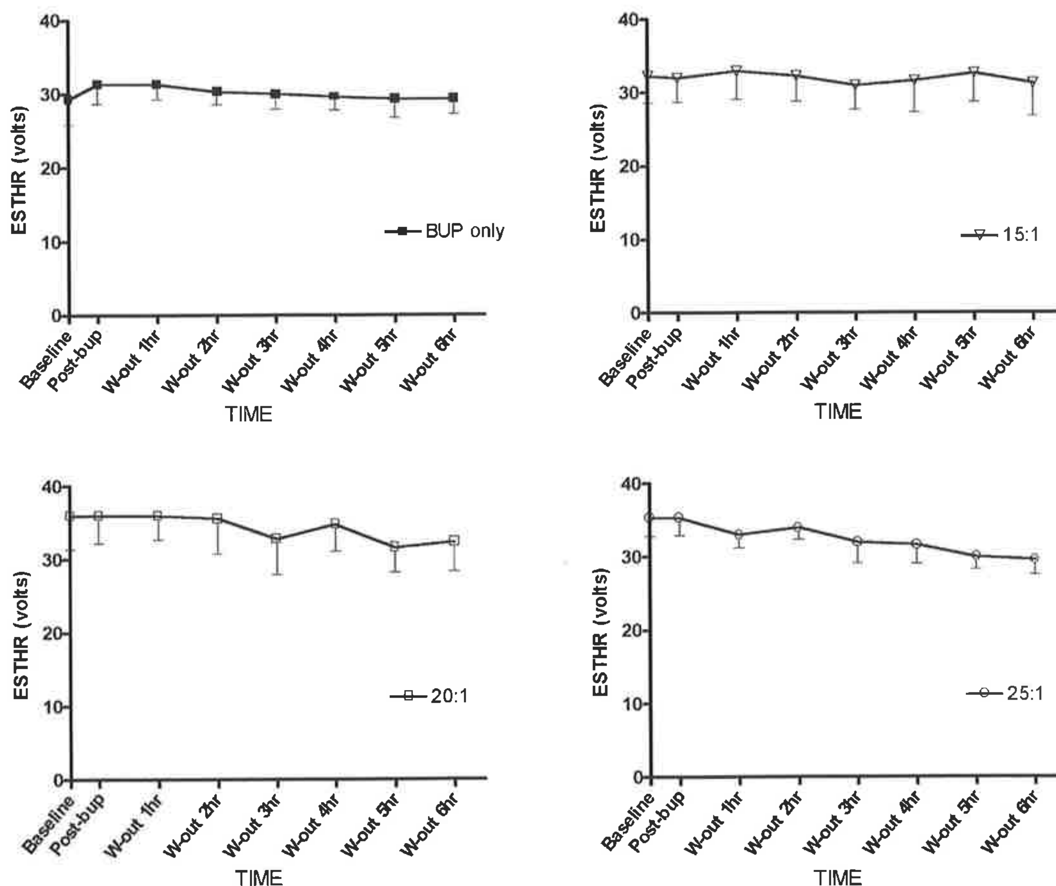

Figure 5-6. Mean (±SEM) ESTHR (volts) for each condition (BUP only{0.5 μg/kg, n=6}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly throughout BUP washout to 6 hours) among healthy volunteers. p>0.05, no significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

	Mean (±SEM)			Df	F	p
	Baseline	Post-drug	Washout 1hr			
15:1 (n=6)	32.33 (±3.70)	32.00 (±3.23)	33.00 (±3.86)	5, 15	0.393	0.685
20:1(n=5)	36.00 (±4.69)	36.00 (±3.79)	36.00 (±3.35)	4, 12	0.000	1.000
25:1(n=6)	35.33 (±2.51)	35.33 (±2.40)	33.00 (±1.77)	5, 15	0.683	0.527
BUP only(n=6)	29.33 (±3.49)	31.33 (±2.61)	31.33 (±2.11)	5, 15	0.750	0.497

Table 5-7. One-way repeated measures analysis of variance (ANOVA) of ESTHR (volts) for each condition (BUP only {0.5 μg/kg, n=6}, and BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over Baseline, Post-bup and Washout 1hr time points among healthy volunteers, p>0.05, no significant differences.

5.7.5.2. Effect of BUP:NLX ratio compared to BUP alone

ESTHR mean (±SEM) percent change from baseline at each time point for each BUP:NLX ratio compared to BUP only are presented in Figure 5-7. Paired samples t-tests revealed no significant differences between BUP only and any of the BUP:NLX ratios.

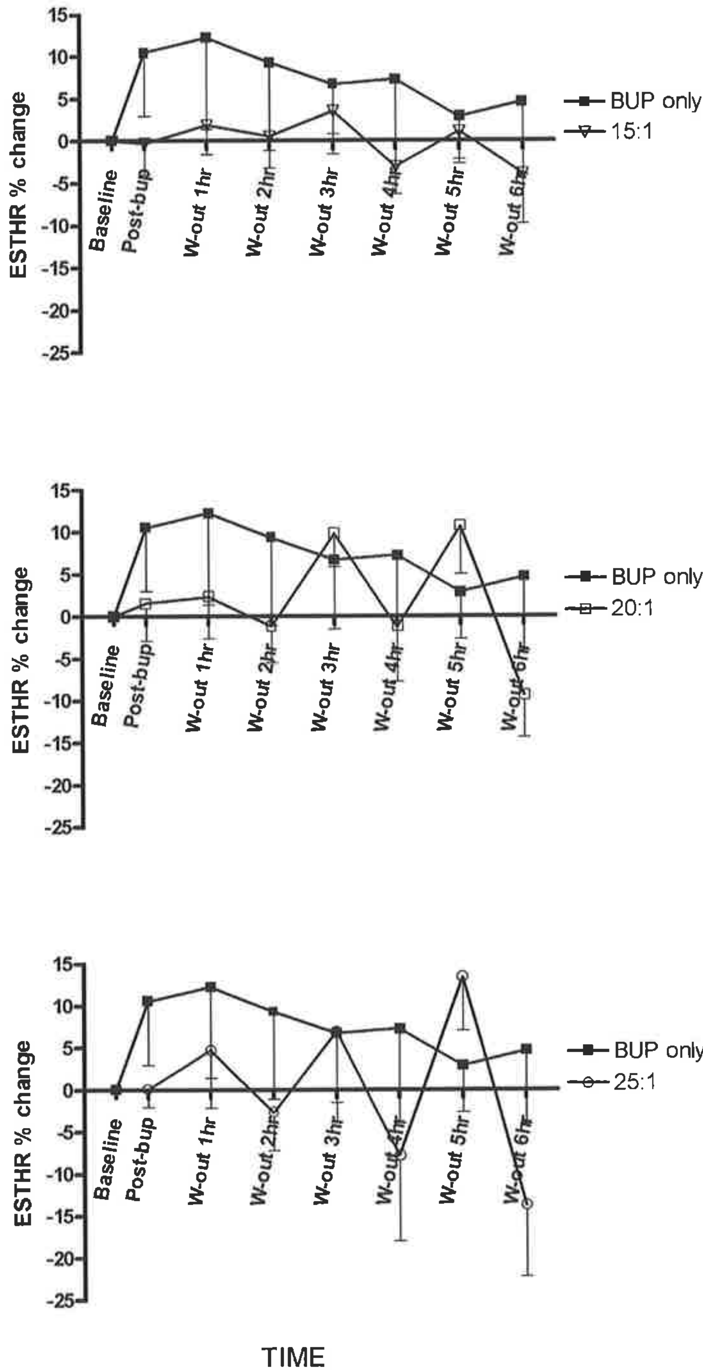

Figure 5-7. Mean (±SEM) ESTHR percent change from baseline for BUP(0.5 μg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 μg/kg, n=6) over all time points (Baseline, Post-bup, and hourly over the BUP washout period to 6 hrs) among healthy volunteers. p>0.05, no significant differences from BUP only, paired samples t-tests. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.6. Electrical stimulation tolerance

5.7.6.1. Effect of each condition

Mean (SEM) ESTOL for each condition is presented in Figure 5-8. One-way ANOVA for each condition over three time points (Baseline, Post-bup and Washout 1hr) revealed no significant differences in mean ESTOL in any condition (see Table 5-8).

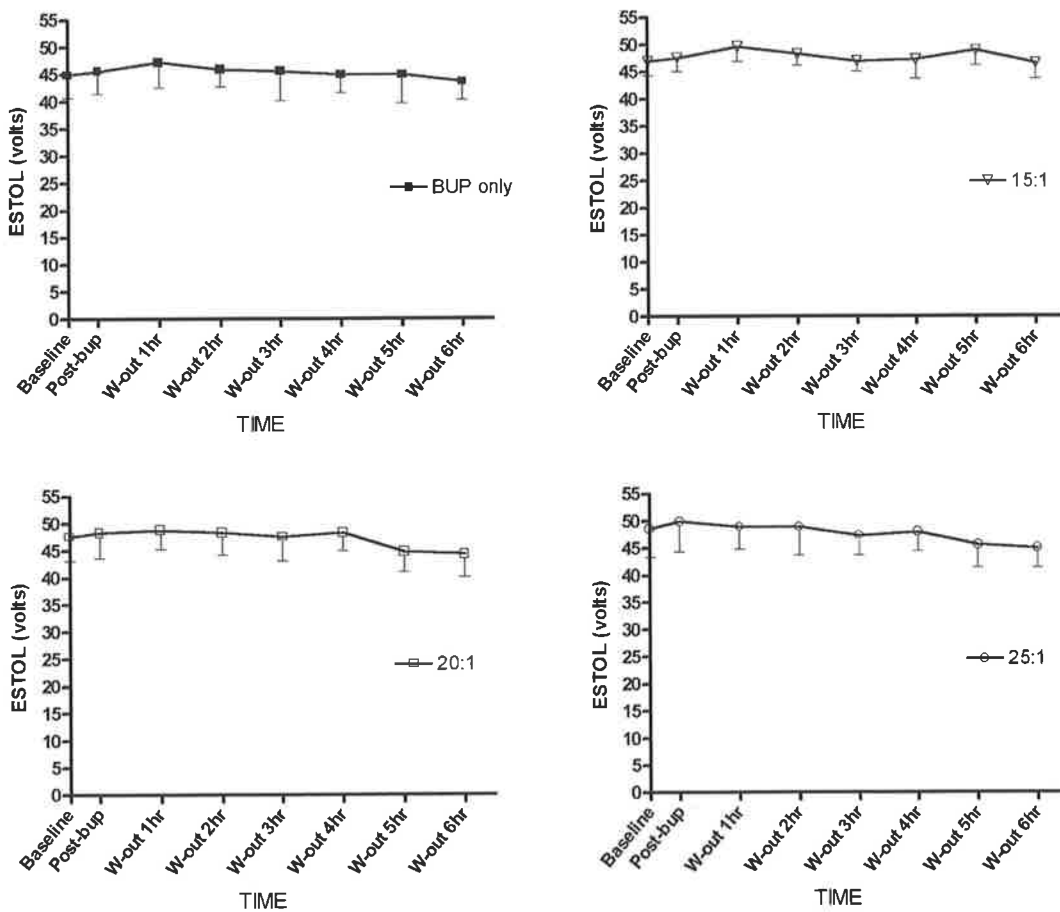

Figure 5-8. Mean (±SEM) ESTOL (volts) raw data for each condition (BUP only {0.5µg/kg, n=6}, BUP{0.5µg/kg}:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly during the BUP washout period to 6 hrs) among healthy volunteers. p>0.05, no significant differences over Baseline, Post-bup and Washout 1hr, one-way ANOVA.

	Mean (±SEM)			Df	F	p
	Baseline	Post-drug	Washout 1hr			
15:1 (n=6)	47.0 (±2.67)	47.67 (±2.60)	49.67 (±2.75)	2, 10	1.429	0.285
20:1(n=5)	47.60 (±4.49)	48.40 (±4.79)	48.80 (±3.49)	2, 8	0.528	0.609
25:1(n=6)	48.67 (±5.26)	50.0 (±5.68)	49.0 (±4.19)	2, 10	0.389	0.687
BUP only(n=6)	45.0 (±4.34)	45.67 (±4.27)	47.33 (±4.72)	2, 10	1.032	0.391

Table 5-8. Mean (±SEM) ESTOL for each condition (BUP only {0.5μg/kg, n=6}, BUP{0.5μg/kg}:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) at Baseline, Post-bup and Washout 1hr time points among healthy volunteers. p>0.05, no significant difference across time points in any condition.

5.7.6.2. Effect of BUP:NLX ratio compared to BUP alone

Paired samples t-tests revealed no significant differences in percent change from baseline for ESTOL between BUP only and any BUP:NLX ratio (see Figure 5-9).

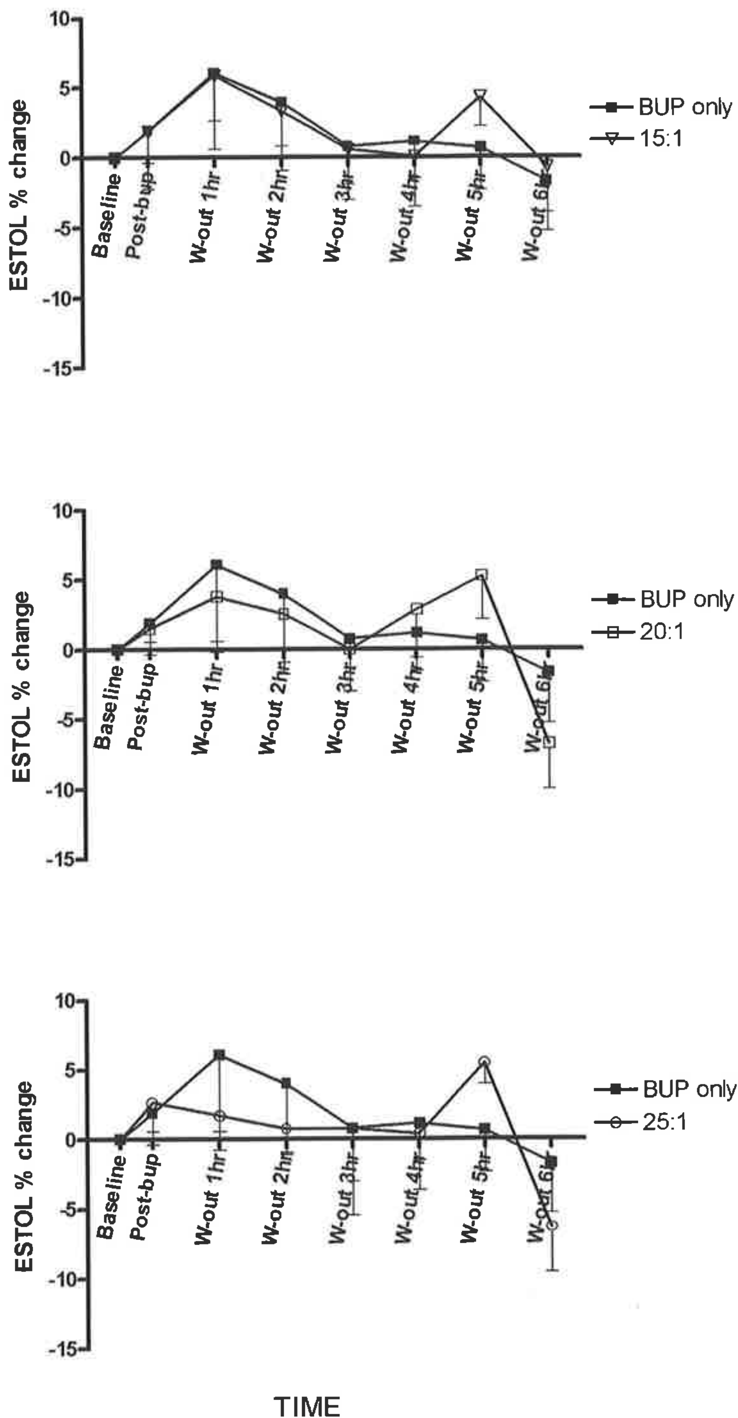

Figure 5-9. Mean (±SEM) ESTOL percent change from baseline for BUP (0.5 µg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 µg/kg, n=6) across all time points (Baseline, Post-bup, and hourly over BUP washout period to 6 hrs) among healthy volunteers. p>0.05, no significant differences from BUP only, paired samples t-tests. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.6.3. Difference between % change$_{RATIO}$ and % change$_{BUP\ ONLY}$

The difference between the mean percent change from baseline CPTOL for each ratio and for BUP only is presented in Figure 5-10. Paired samples t-tests revealed no significant differences between the AUC of the difference for each ratio (p<0.05).

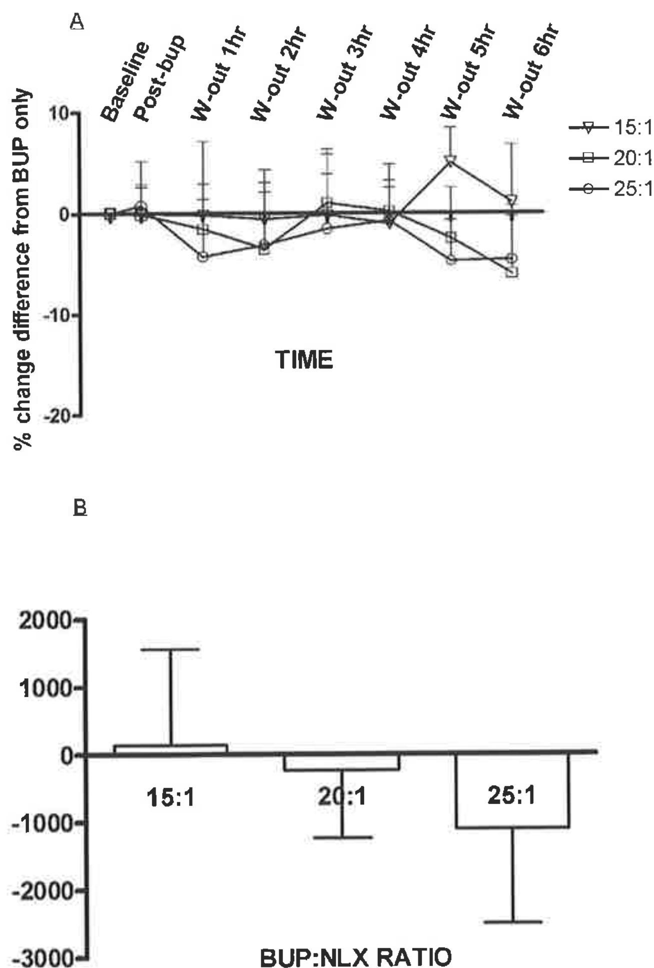

Figure 5-10. A. Mean (±SEM) ESTOL percent change difference from BUP only for each BUP(0.5 µg/kg):NLX ratio (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) over all time points (Baseline, Post-bup, and hourly during BUP washout for 6 hrs) among healthy volunteers. B. Mean (±SEM) ESTOL AUC percent change difference from BUP only for each BUP:NLX ratio. p>0.05, no significant differences between ratios, paired samples t-tests.

5.7.7. Respiration

5.7.7.1. Effect of each condition

One-way repeated-measures ANOVA for each condition over Baseline, Post-bup and Washout 1hr revealed a significant reduction in breaths per minute in the BUP only ($F_{2,10}$=5.71, p=0.022) condition and in the 20:1 BUP:NLX ratio ($F_{2,8}$=5.09, p=0.038) (see Figure 5-11). Post-hoc analyses (Tukey's Multiple Comparison Test) revealed a significant difference in the BUP only condition between Baseline and Post-drug (p<0.05) and in the 20:1 ratio between Baseline and Washout 1hr (p<0.05).

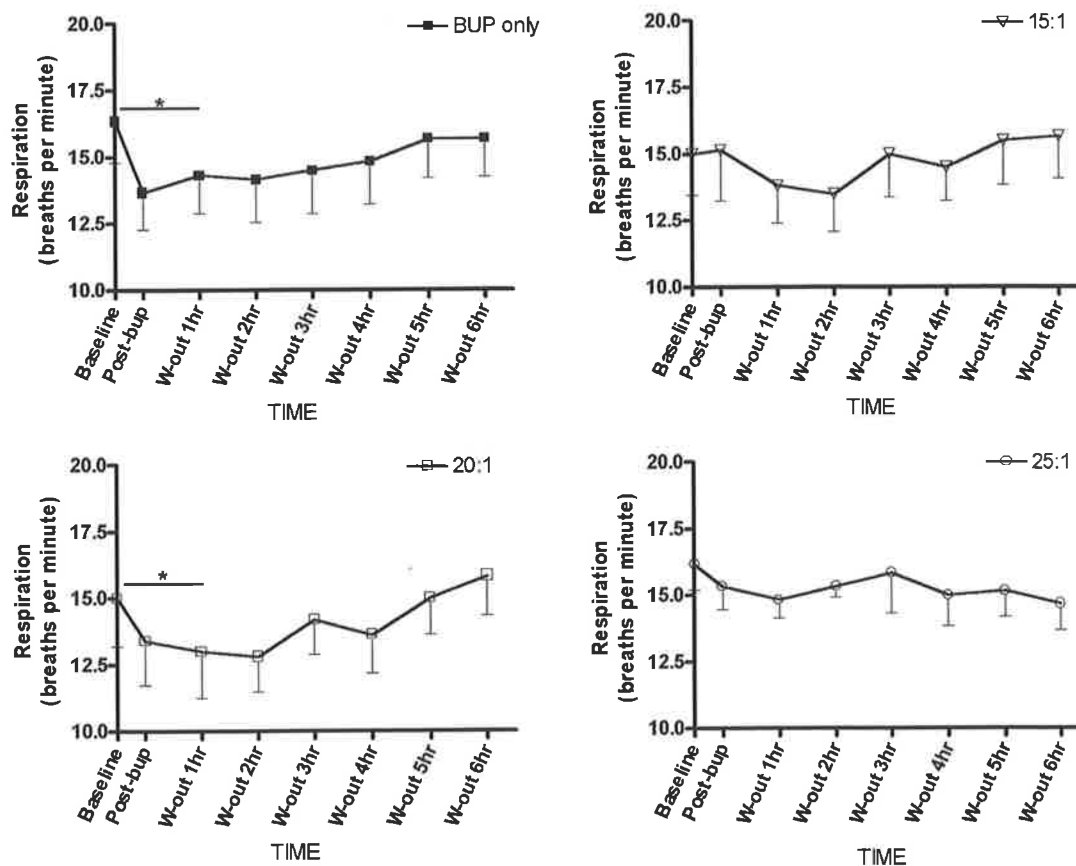

Figure 5-11. Mean (±SEM) breaths per minute for each condition (BUP only 0.5 µg/kg {n=6}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratios) over all time points (Baseline, Post-bup and hourly over the BUP washout period to 6 hrs) among healthy volunteers. *p<0.05, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

5.7.7.2. Effect of BUP:NLX ratio compared to BUP alone

A significantly greater reduction in breaths per minute was observed in the BUP only condition compared to the 15:1 ratio at the Post-bup time point (-15.83±4.37% vs. -0.30±5.94%) ($t(5)=-2.67$, $p=0.044$) (see Figure 5-12). No significant differences were detected between BUP only and the other ratios at any time point.

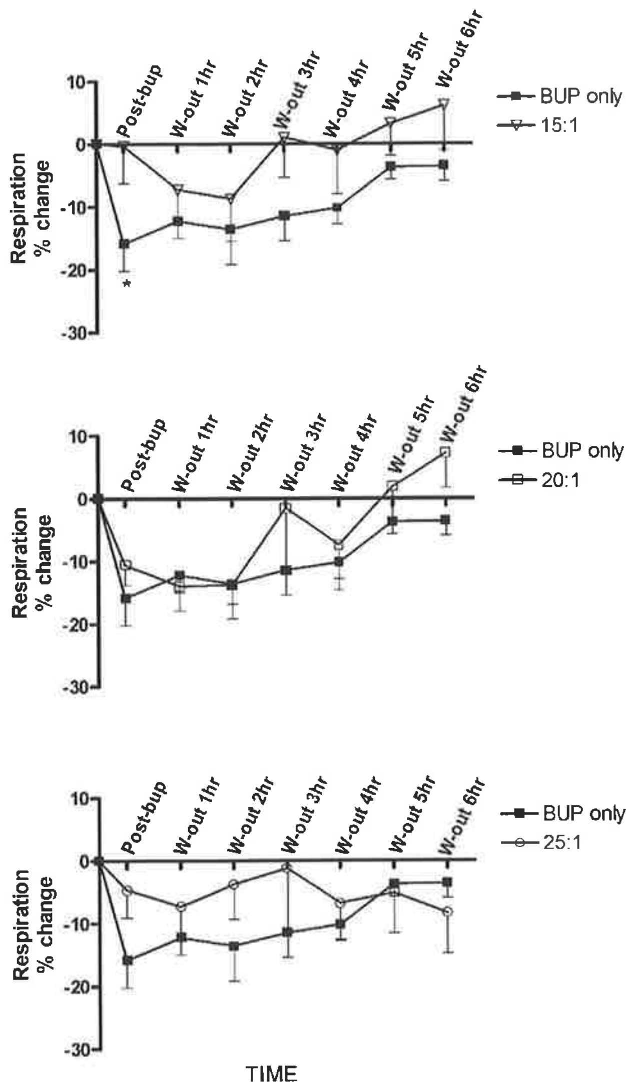

Figure 5-12. Mean (±SEM) breaths per minute percent change from Baseline for BUP (0.5 µg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 µg/kg, n=6) across all time points (Baseline, Post-bup, and hourly over BUP washout to 6 hrs) among healthy volunteers. *p<0.05, significant difference from BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.8. Heart rate

5.7.8.1. Effect of each condition

One-way repeated-measures ANOVA for each condition over the Baseline, Post-bup and Washout 1hr time points revealed a significant change in heart rate in the BUP only condition (F2,10=6.94, p=0.013) (see Figure 5-13). Post-hoc tests revealed a significant difference between Baseline and Post-bup ($p<0.05$, Tukeys Multiple Comparison Test). No significant differences in heart rate were detected over these time points in the 15:1 ($F_{2,10}$=2.63, p=0.121), 20:1 ($F_{2,8}$=2.59, p=0.136) or 25:1 ($F_{2,10}$=0.730, p=0.506) ratios.

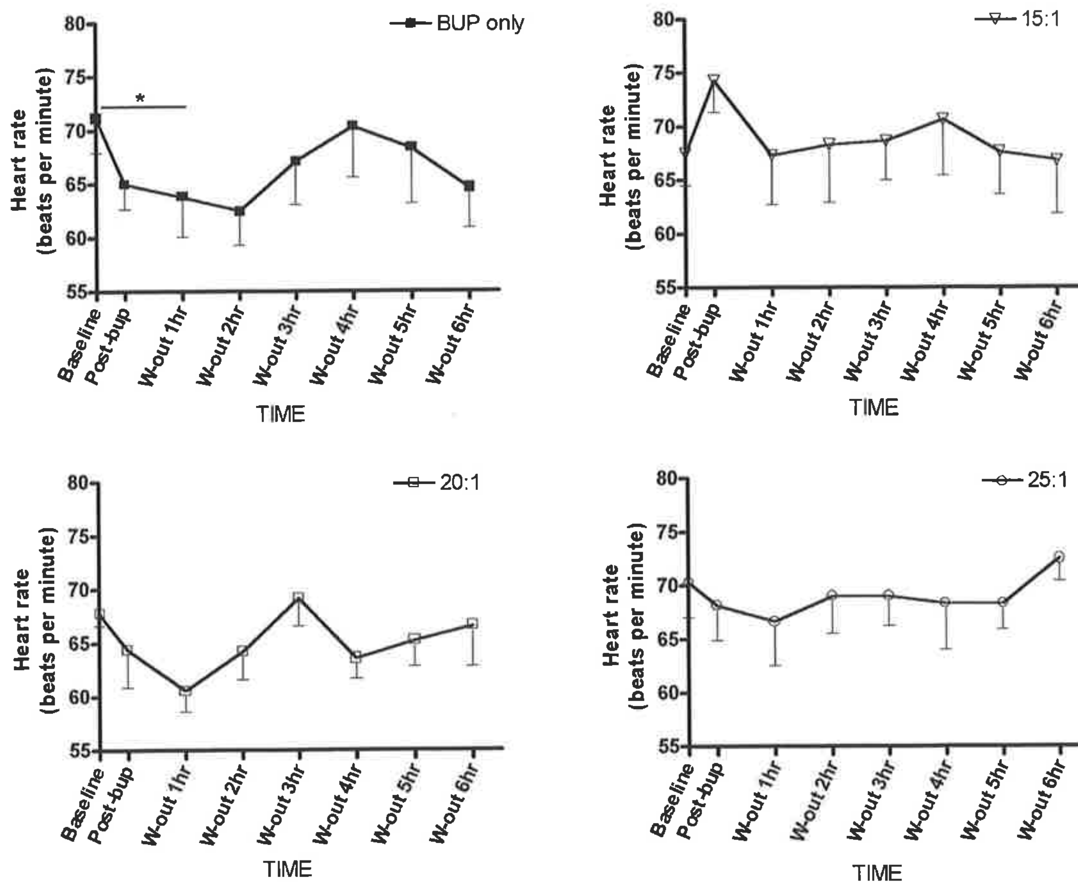

Figure 5-13. Mean (±SEM) heart rate (beats per minute) for each condition (BUP only 0.5 µg/kg {n=6}, BUP 0.5 µg/kg:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly over the BUP washout period to 6 hrs) among healthy volunteers. *p<0.05, significant difference over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

5.7.8.2. Effect of BUP:NLX ratio compared to BUP alone

Significant differences in percent change from baseline in heart rate between the BUP only and ratio conditions were detected between BUP only and the 15:1 ratio at Post-bup (t(5)=-5.15, p=0.004) and Washout 1hr (t(5)=-3.395, p=0.019) (see Figure 5-14). A significant difference from BUP only was also detected in the 25:1 ratio at Washout 2hr (t(5)=-2.80, p=0.038).

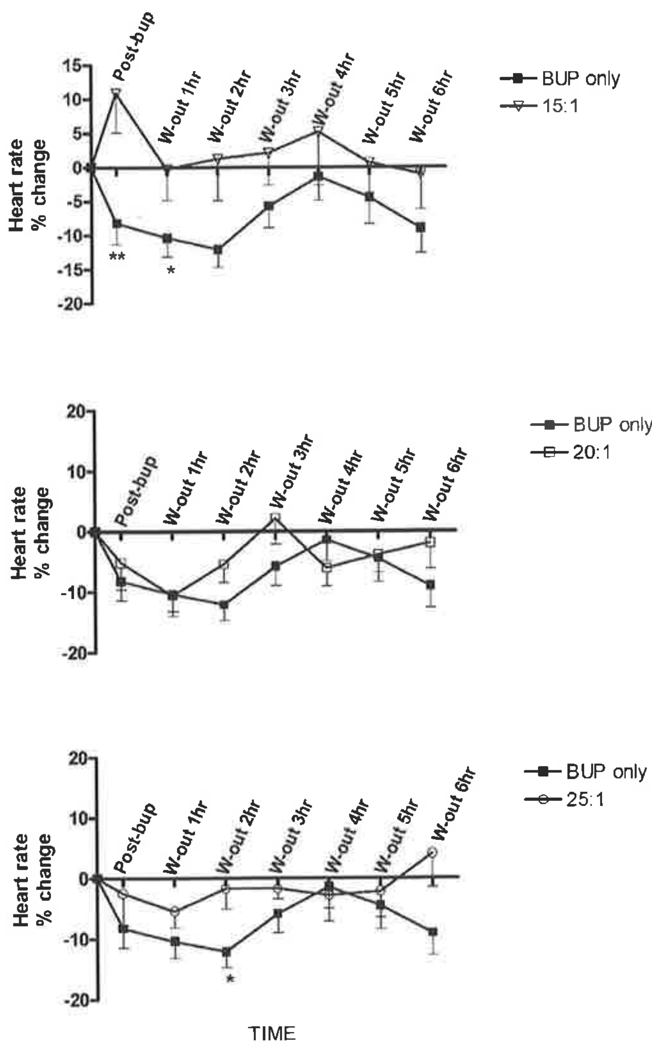

Figure 5-14. Mean (±SEM) percent change from Baseline heart rate for BUP(0.5 µg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 µg/kg, n=6) across all time points (Baseline, Post-bup, and hourly during BUP washout to 6 hrs) among healthy volunteers. *p<0.05 **p<0.01, significant difference from BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.9. Oxygen saturation

5.7.9.1. Effect of each condition

Mean arterial oxygen saturation over all time points for each condition is displayed in Figure 5-15. One-way repeated measures ANOVA over the three initial time points (Baseline, Post-bup and Washout 1hr) revealed no significant effect for BUP only ($F_{2,10}$=2.83, p=0.106), 15:1 ($F_{2,10}$=0.66, p=0.538), 20:1 ($F_{2,8}$=1.46, p=0.289) or 25:1 ($F_{2,10}$=1.15, p=0.354).

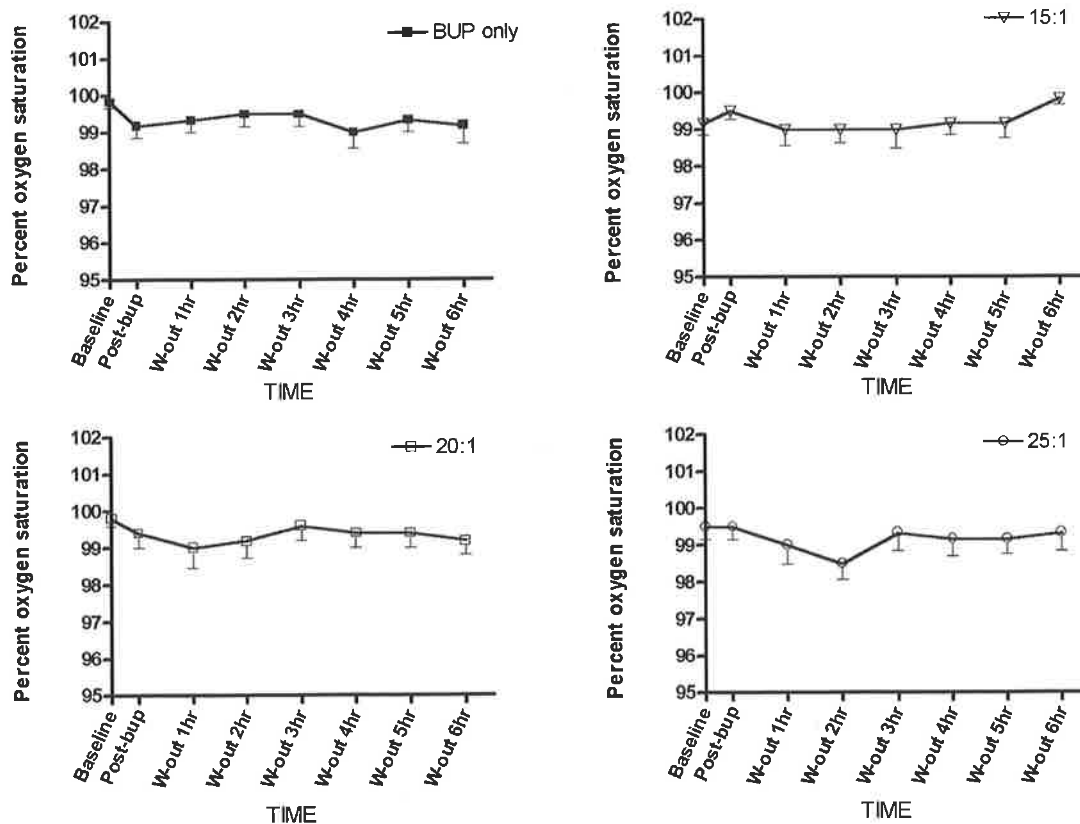

Figure 5-15. Mean (±SEM) arterial oxygen saturation (%) for each condition (BUP only 0.5 µg/kg {n=6}, BUP:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly during BUP washout period to 6 hrs) among healthy volunteers. p>0.05, no significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

5.7.9.2. Effect of BUP:NLX ratio compared to BUP alone

Paired-samples t-tests revealed a significantly greater reduction in arterial oxygen saturation in the BUP only condition compared to the 15:1 ratio condition at Post-bup (-0.67±0.33 vs. 0.34±0.34, $t(5)=-3.89$, $p=0.011$), Washout 4hr (-0.84±0.31 vs. 0.00±0.26, $t(5)=-2.70$, $p=0.04$), and Washout 6hr (-0.67±0.34 vs. 0.68±0.21, $t(5)=-3.15$, $p=0.025$). There were no significant differences in oxygen saturation change from baseline between BUP only and any other ratio (see Figure 5-16).

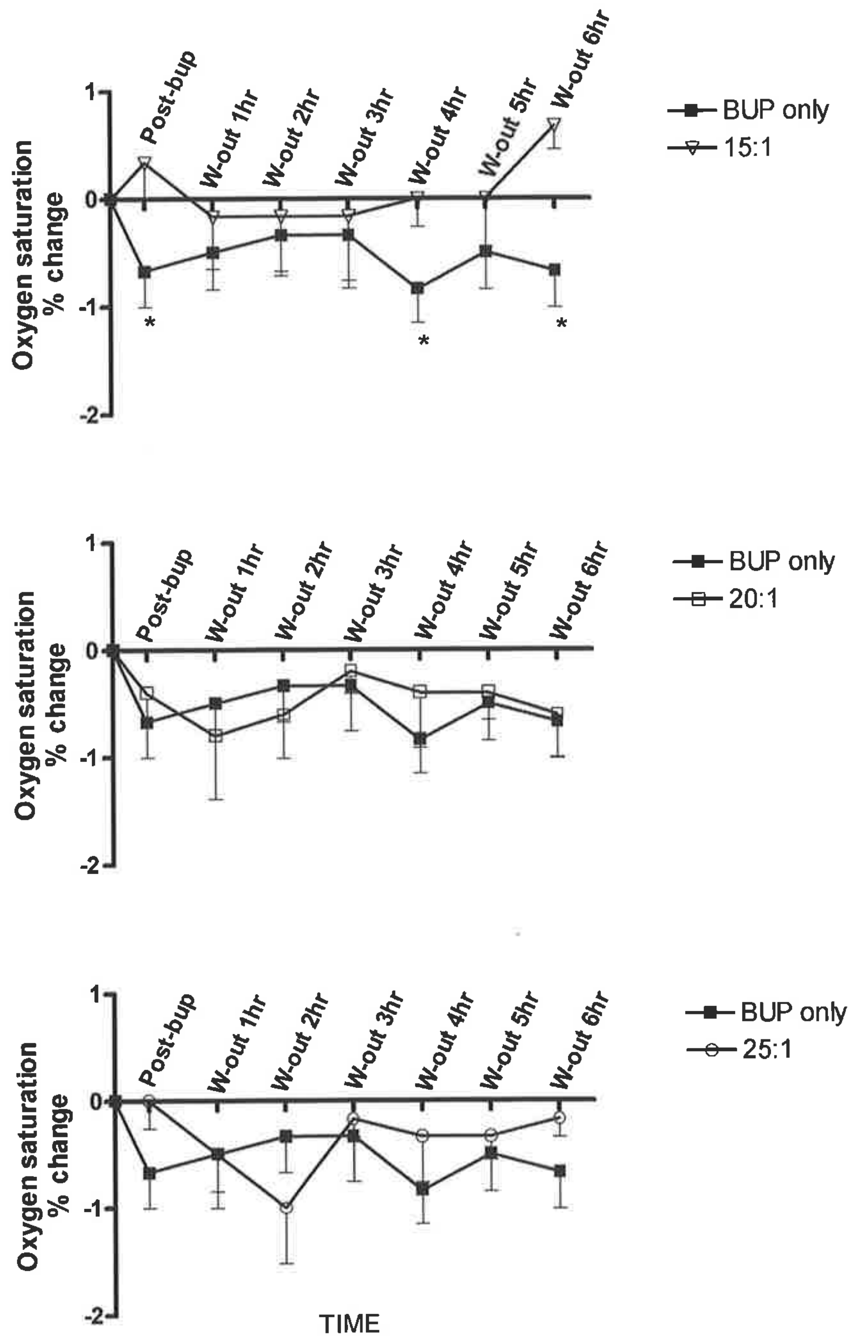

Figure 5-16. Mean (±SEM) arterial oxygen saturation percent change from baseline for BUP(0.5 µg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 µg/kg, n=6) over all time points (Baseline, Post-bup, and hourly during BUP washout period to 6 hrs) among healthy volunteers. *p<0.05, significant difference from BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

5.7.10. Blood pressure

5.7.10.1. Effect of each condition

Mean (±SEM) systolic and diastolic blood pressure for each condition at each time point are displayed in Figure 5-17. One-way repeated measures analysis of variance for each condition over Baseline, Post-bup and Washout 1hr revealed a significant effect on systolic blood pressure in the BUP only condition ($F_{2,10}=5.07$, $p=0.03$). Post-hoc analyses revealed a significant difference between Post-bup and Washout 1hr (Tukey's Multiple Comparison Test, $p<0.05$). There were no other significant differences in systolic blood pressure over these time points for any other condition. A significant difference in diastolic blood pressure over the first three time points was found in the 25:1 ratio ($F_{2,10}=8.49$, $p=0.007$). Tukey's Multiple Comparison Test revealed a significant difference between Baseline and Post-bup ($p<0.001$).

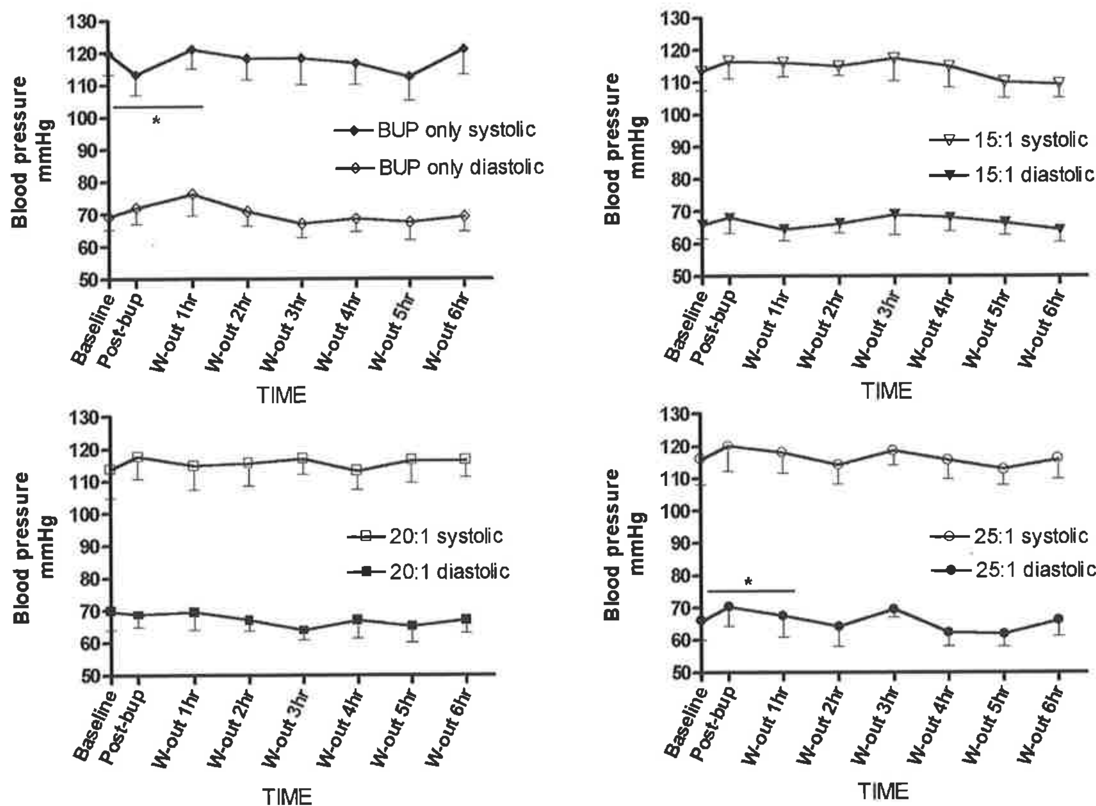

Figure 5-17. Mean (±SEM) blood pressure (mmHg) for each condition (BUP only {0.5 µg/kg, n=6}, BUP{0.5 µg/kg}:NLX in a 15:1 {n=6}, 20:1 {n=5} or 25:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly during the BUP washout period to 6 hrs) among healthy volunteers. *p<0.05, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

5.7.10.2. Effect of BUP:NLX ratio compared to BUP alone

The only significant difference between BUP only and the BUP:NLX ratios in percent change in blood pressure from Baseline was between BUP only and the 25:1 ratio at Post-bup. The mean percent change in systolic blood pressure at Post-bup in the BUP only condition (-5.44±0.83) was significantly different from that observed in the 25:1 ratio (3.88±2.73) ($t(5)=-3.076$, $p=0.028$) (see Figure 5-18).

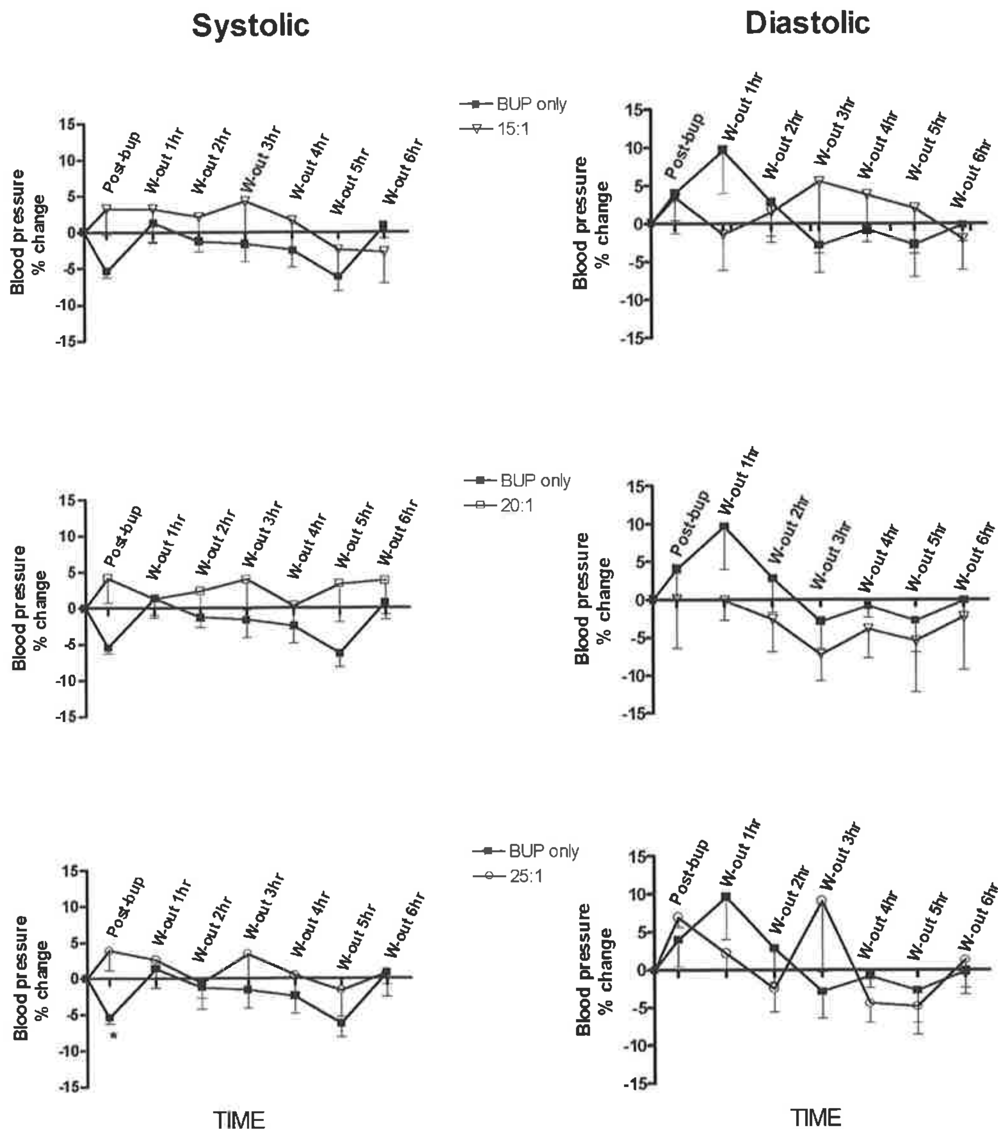

Figure 5-18. Mean (±SEM) percent change from baseline blood pressure for BUP(0.5 μg/kg):NLX ratios (15:1 {n=6}, 20:1 {n=5} and 25:1 {n=6}) compared to BUP only (0.5 μg/kg, n=6) across all time points (Baseline, Post-bup, and hourly during BUP washout to 6 hrs) among healthy volunteers. *$p<0.05$, significant difference from BUP only, paired samples t-test. Note that systolic and diastolic data for the BUP only condition are presented in all graphs on the left and right panel, respectively.

5.7.11. Subjective effects

Subjective effects experienced by each subject in each condition are outlined in Table 6-7. Overall, subjects experienced a similar subjective effect profile in all conditions, with no greater prevalence of subjective effects during the BUP:NLX ratio conditions than during the BUP only condition. Subjective effects observed in the current study are consistent with the earlier dose-finding study (Chapter 4) and previous reports (Macintyre and Ready 2001) that some individuals experience more side effects than others. For example, subject 1 experienced a greater number of effects than other subjects, and this was consistent throughout all drug conditions.

BUP only	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**% of subjects**
Sedation^	W-out 3hr W-out 4hr W-out 5hr	W-out 4hr W-out 5hr					33
Nausea^	W-out 5hr						17
Pleasant feeling	Post-bup W-out 1hr			W-out 1hr			33
Dizziness	W-out 1hr		W-out 1hr		Post-bup W-out 1hr		50
Difficulty concentrating							0
Pruritus							0
"Hot sweats"	W-out 5hr		W-out 5hr				33
BUP:NLX 15:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**% of subjects**
Sedation^	W-out 1hr W-out 2hr W-out 4hr W-out 6hr				Post-bup W-out 1hr W-out 3hr	W-out 5hr	50
Nausea^	W-out 2hr				W-out 1hr		33
Pleasant feeling	Post-bup						17
Dizziness	W-out 2hr				W-out 1hr W-out 2hr		33
Difficulty concentrating	W-out 2hr					W-out 1hr	33
Pruritus	W-out 2hr *						17
"Hot sweats"	W-out 2hr						17
BUP:NLX 20:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**% of subjects**
Sedation^	W-out1hr W-out 5hr						20
Nausea^	W-out 4hr						20
Pleasant feeling							0
Dizziness	W-out 3hr				W-out 1hr		40
Difficulty concentrating							0
Pruritus							0
"Hot sweats"	W-out 3hr						20
BUP:NLX 25:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**% of subjects**
Sedation^	Post-bup W-out 1hr W-out 2hr W-out 5hr W-out 6hr	W-out 4hr W-out 5hr			W-out 5hr		50
Nausea^	Post-bup						17
Pleasant feeling					W-out 1hr		17
Dizziness	W-out 1hr			W-out 1hr	W-out 1hr W-out 5hr W-out 6hr		50
Difficulty concentrating							0
Pruritus							0
"Hot sweats"							0

^All nausea and sedation mild (1). No episodes of vomiting; nausea resolved without anti-emetic medication. *Mild, neck region. Shaded area represents missing data due to opioid positive urine on this testing day.

Table 5-9. Incidence of subjective effects among healthy volunteers following IV infusion of BUP only (0.5 μg/kg), or BUP (0.5 μg/kg):NLX in a 15:1, 20:1 or 25:1 ratio. See Table 5-4 for time point descriptions.

5.8. Discussion

The principal aim of this study was to determine whether the co-administration of BUP and ultra-low dose NLX would be associated with significantly greater antinociception than the same dose of BUP alone in healthy, pain-free volunteers. Greater analgesia, typically demonstrated by reduced opioid requirement and/or pain ratings, has been reported in pain patients with the combination of an opioid agonist and ultra-low dose antagonist compared to the agonist alone (Levine et al. 1988; Gear et al. 2000; Cruciani et al. 2003; Gear et al. 2003; Schmidt et al. 2003); however findings have been inconsistent (see discussion in 1.9.2.4.4). BUP combined with ultra-low dose NLX demonstrated significantly increased antinociception in a rodent model of neuropathic pain (Cougnon-Aptel et al. unpublished). The combination of BUP and an antagonist had not previously been investigated in humans.

The second key aim of the current study was to determine whether the addition of ultra-low dose NLX to BUP would impact upon the prevalence of adverse effects such as respiratory depression, sedation and nausea. Increasing the pain relief associated with a given dose of an analgesic has the obvious clinical advantage of lower dose requirements to achieve a given effect. However, this is based on the supposition that the enhancement is selective for the analgesic effect of the agent only, and does not extend to unwanted effects. Previous reports from clinical pain patients have described a reduced incidence of side effects with the administration of low- or ultra-low dose antagonist without compromising analgesia (Korbon et al. 1983; Rawal et al. 1986; Gueneron et al. 1988; Cepeda et al. 2004) or enhanced analgesia with either no increase or a reduction in side effects (Gan et al. 1997; Gear et al. 2000; Cruciani et al. 2003; Schmidt et al. 2003).

The current study is the first known study to investigate BUP combined with ultra-low dose NLX in humans, and the first investigation of effects associated with opioid agonist:antagonist combinations in an experimental pain paradigm.

Findings from the current study suggest that antinociception may be significantly enhanced with the combination of BUP and NLX compared to BUP alone. The co-administration of BUP and NLX in a 15:1 ratio was associated with a significantly greater increase in CPTOL compared to BUP alone. Indeed, while comparison between studies is limited, the 15:1 BUP:NLX ratio was associated with an equivalent mean percent increase in antinociception (see Figure 5-4) as the higher doses of BUP administered in the dose-ranging study (cumulative dose of 1.875 μg/kg, see Figure 4-10), but with a marked reduction in the incidence of adverse effects (see Table 4-10 and Table 5-9).

In the present study, BUP alone initially demonstrated a moderate degree of CP antinociception with a mean increase from baseline of approximately 15% in CPTOL, a difference that was approaching statistical significance (p=0.072), before decreasing to reach baseline levels by the Washout 3hr time point. In contrast, CPTOL associated with the 15:1 BUP:NLX ratio increased to reach a peak mean increase from baseline of 37% at the Washout 3 hr time point, at which point the BUP only condition had returned to baseline CPTOL (p=0.046). Significantly greater CP antinociception compared to BUP only was also observed at the 2 hr (p=0.028) and 5 hr (p= 0.028) washout time points.

It is particularly interesting to note that the significant enhancement of antinociception observed in the 15:1 BUP:NLX ratio was observed up to 5 hours following drug administration, suggesting not only a significant enhancement in antinociception but also an increased duration of effect. Levine and colleagues reported that patients receiving

pentazocine combined with low-dose NLX (see Table 1-3) experienced significantly greater and more prolonged analgesia at the final observation time (3 h 10 min post-dosing) than was associated with pentazocine or even high-dose (15 mg) morphine alone (Levine et al. 1988). Gear and colleagues have also reported a significant prolongation of nalbuphine analgesia with the administration of NLX (0.2 mg) compared to the nalbuphine alone up to the end of the experiment 3 h post-dosing (Gear et al. 2003). In their report of enhanced analgesia among 3 patients with neuropathic trigeminal pain treated with low-dose nalbuphine and naloxone, Schmidt and colleagues reported that the magnitude of analgesia associated with the combination remained maximal at 3 h in 2 out of 3 patients (Schmidt et al. 2003). To my knowledge, the current study is the first to have monitored response to a single dose of agonist combined with a single dose of ultra-low-dose antagonist for as long as 6 hours. Gan and colleagues reported prolonged analgesia among patients receiving the combination of morphine and the lower dose of naloxone (see Table 1-3) as evidenced by reduced cumulative opioid requirement in PCA over a 24-hour study period. However, the Gan study administered a continuous infusion of NLX throughout the study period. Thus, a comparison cannot be made with the extended duration observed in the present study following a 30-minute infusion of NLX. Given the extended duration of effect observed in the current study, the washout monitoring period will be extended for the subsequent BUP:NLX ratio study.

No significant effect was observed in CPTHR, which is in line with earlier reports that opioid antinociception is less evident in pain threshold than pain tolerance, and that tolerance more reliably detects analgesic effects (Luginbuhl et al. 2001). There were also no significant differences associated with ES parameters. While this is consistent with previous reports indicating that this technique is a less sensitive test for opioid effects than the CP test (Athanasos et al. 2002), the dose-finding study (Chapter 4) revealed that the ES

test is a sensitive assay for BUP, with statistically significant changes in ESTOL following BUP administration. Notwithstanding, the mean magnitude of increase in ESTOL in the dose-finding study was considerably smaller than was associated with CPTOL. Differences between the CP and ES tests in the antinociceptive effect of opioids have previously been described, with methadone maintained patients demonstrating hyperalgesia to cold but not electrical stimulation (Doverty et al. 2001). The sensations associated with phasic stimuli (such as electrical stimulation) are qualitatively different from those associated with the deep, prolonged sensations produced by tonic stimuli (such as the cold pressor test) that are often associated with clinical pain (Chen et al. 1989). Indeed Chen and colleagues have postulated that these experiences involve different neurophysiological pathways and may be differentially affected by opioids (Chen et al. 1989).

Importantly, findings from the present study indicate that the enhanced antinociception associated with the BUP:NLX combination does not extend to the other opioid effects measured. Both BUP alone and BUP:NLX 25:1 ratio were associated with a significant decrease in respiratory depression over the first 3 time points (Baseline, Post-bup and Washout 1hr), while no such decrease occurred with the other BUP:NLX ratios. Moreover, the BUP:NLX 15:1 ratio was associated with significantly less respiratory depression (as measured by breaths per minute) than BUP only at the initial Post-bup time point (16% decrease in breaths per minute with BUP only vs. no decrease with BUP:NLX 15:1). Furthermore, the decrease in breaths per minute associated with the 15:1 ratio peaked at Washout 2hr with a decrease of 8%, and returned to baseline levels by Washout 3hr. By comparison, the BUP only condition was associated with a decrease of more than 10% at every time point until Washout 5hr. These respiratory data are supported by the

arterial oxygen saturation findings, which demonstrate a significant decrease in saturation in the BUP only condition compared to the BUP:NLX 15:1 ratio at several time points.
In terms of other physiological parameters, there was a significant decrease in heart rate in the BUP only condition over the first three time points (Baseline, Post-bup and Washout 1hr), and a significantly greater decrease in heart rate in the BUP only condition compared to the 15:1 and 25:1 BUP:NLX ratios for at least one time point. There were no significant differences between BUP only and any BUP:NLX ratio in change in blood pressure throughout all time points.

Other adverse effects, including sedation, nausea and light-headedness, were mild and did not require treatment (i.e. anti-emetic medication) in any drug condition. Overall, these adverse effects were no more prevalent or severe in the BUP:NLX ratio conditions than in the BUP alone condition. The incidence of subjective effects is largely consistent with previous observation (Macintyre and Ready 2001) that some individuals are more responsive to adverse opioid effects than others, and will respond in such a manner each time opioids are administered. One subject experienced an episode of mild nausea following drug administration in all conditions. A second subject experienced a mild episode of nausea in the 15:1 ratio only. Dizziness/lightheadedness occurred in all conditions, with the lowest proportion of subjects experiencing this effect during the 15:1 ratio condition (33% vs. 50% {BUP only}, 40%[4] {BUP:NLX 20:1}, and 50% {BUP:NLX 25:1}). Three of the 6 subjects experienced sedation in the 15:1 and 25:1 ratio conditions,

[4] Note that data are missing for one subject for the BUP:NLX 20:1 ratio due to an opioid positive urine on this day. It is considered unlikely that this subjet would have experienced dizziness on this day as she had not experienced this effect with any other condition. It is likely, then, that the 20:1 and 15:1 ratios would have been associated with an equally low incidence of dizziness.

while 2 of 6 subjects, and 1 of 5 subjects experienced sedation in the BUP only and 20:1 conditions, respectively.

Overall, the adverse opioid effect data from the current study, in particular the rates of respiratory depression, are consistent with findings suggesting that ultra-low or low-dose opioid antagonist can reduce the incidence and severity of adverse opioid effects (Korbon et al. 1983; Rawal et al. 1986; Gueneron et al. 1988; Cepeda et al. 2004), or that enhanced analgesia can occur without a simultaneous increase in adverse effects (Gan et al. 1997; Gear et al. 2000; Cruciani et al. 2003; Schmidt et al. 2003).

There were no significant differences between the BUP:NLX ratios in magnitude of antinociceptive effect compared to BUP alone. However, the finding that BUP:NLX in a 15:1 ratio produced significantly greater antinociception than BUP alone, while ratios either side did not, further supports the finding by Cougnon-Aptel and colleagues (Cougnon-Aptel et al. unpublished) and Gear and colleagues (Gear et al. 2003) that dose ratio is critical for enhanced antinociception with combinations of BUP:NLX and nalbuphine:NLX, respectively. It is noted, however, that a statistically significant increase in antinociception relative to the same dose of BUP alone was observed with small subject numbers (n=6), contributing to the large variability in cold pressor response. It is possible that investigation with a larger sample size may produce a similar effect with other BUP:NLX ratios.

As the mechanism for enhanced antinociception has not been elucidated it is difficult to speculate why dose ratio appears so critical to enhanced antinociception. While it is also unclear whether the mechanisms underlying BUP:NLX potentiation are the same mechanisms that underlie morphine or other agonist:antagonist potentiation, it may be that

the failure of other studies to observe enhanced analgesia with an agonist:antagonist combination in pain patients relates to the ratio. This underscores the advantage of assessing this drug combination in an experimental pain paradigm, in order to establish "proof of concept" and identify the effective dose ratio range prior to assessment in a clinical setting.

6. OPTIMISING THE BUPRENORPHINE:NALOXONE DOSE RATIO IN HUMAN EXPERIMENTAL PAIN

6.1. Introduction

As described in the previous chapter, the addition of NLX to BUP in a 15:1 (BUP:NLX) ratio was associated with significantly enhanced tolerance to cold pain as measured by the cold pressor (CP) test, with less respiratory depression compared to BUP alone.

The animal study of antinociception with BUP:NLX combinations revealed that BUP:NLX dose ratio was critical in observing enhanced antinociception (Cougnon-Aptel et al. unpublished). The previous study assessed the antinociceptive and physiological effects of three BUP:NLX combinations (15:1, 20:1 and 25:1) compared to BUP alone. In line with the animal data, only the 15:1 ratio was associated with significant antinociception.

These findings lead to the suggestion that a lower ratio may produce a similar or possibly greater effect than the 15:1 ratio. In order to define the range of BUP:NLX ratios that are associated with enhanced antinociception, the present study replicates the first ratio study described in Chapter 5 with lower BUP:NLX ratios. The ratios selected for use in the current study are 12.5:1, 10:1 and 5:1.

6.1.1. Hypothesis

That there will be a significant increase in antinociception, but not adverse effects, with the combination of BUP and NLX compared to BUP alone.

6.1.2. Aim

To investigate further the effect of BUP:NLX combinations on antinociception and adverse effects over a lower dose ratio range and for an extended duration post-drug administration than previously investigated.

6.1.3. Study design

This study was a double-blind, randomised controlled trial. The protocol replicated that of the first ratio study (Chapter 5). Subjects were administered 3 BUP:NLX combinations and BUP:saline by IV infusion over 4 separate days. As opioid effects were evident in the previous ratio study even at the final assessment time point 6 hours after drug administration, the washout monitoring period was extended to 10 hours for the current study.

6.1.4. Participants

This study was conducted with the approval of the Research Ethics Committee of the Royal Adelaide Hospital, South Australia (RAH Protocol 030923). Written informed consent was obtained prior to commencing the trial. Subject recruitment and remuneration were as described for the first ratio study (see 5.6.2).

6.1.4.1. Inclusion and exclusion criteria

Inclusion and exclusion criteria for this study were as described in Chapter 4 (see 4.8.1.1 and 4.8.1.2).

6.1.4.2. Sample characteristics

The sample comprised 7[5] healthy Caucasian volunteers, ranging in age from 21 to 37 years (see Table 6-1). The mean age of female and male participants was 27.25(±3.25) and 22.33(±0.88) years, respectively (p=0.265). Participants undertook a screening interview and medical examination prior to commencing the trial to ensure that they were in good health. Subjects also completed the cold pressor (CP) test at the screening session to ensure that pain response was within the normal range as determined by La Vincente and colleagues (La Vincente et al. 2003). Participants had no prior exposure to the pain tests, had no history of chronic pain or drug use, and were not taking any medication on a regular basis (excluding the contraceptive pill). For the purpose of this study, regular use of a medication constituted more than once per week and included recreational use of drugs. Participants did not use any medication or drug (excluding the contraceptive pill in 3 of the 4 females) in the 24 hours prior to each testing day.

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[5] Eight subjects completed the study (4F:4M), however data from one male subject were excluded due to an opioid positive urine on the BUP only testing day (see 6.2.1.2). The sample demographics detailed here describe the final sample of 7.

	Complete sample (N=7)		
	Mean	±SEM	Range
Age	25.15	±2.03	21 - 37
CPTHR at screening (seconds)	14.43	±3.54	6 - 33
CPTOL at screening (seconds)	43.00	±6.73	29 - 80

	Sample divided by sex[a] Mean(±SEM)	
	Male (n=3)	Female (n=4)
Age	22.33 (±0.88)	27.25(±3.25)
CPTHR at screening	11.67(±2.85)	16.5(±6.03)
CPTOL at screening	40.33(±5.78)	45.00(±11.76)

[a] No significant differences, p>0.05, independent samples t-tests.

Table 6-1. Age (years) and CP parameters (seconds) at screening among the entire group (n=7), and the group classified according to sex (3 males, 4 females). No significant differences between males and females in age or CP parameters at screening (p>0.05).

6.1.5. Procedures

6.1.5.1. Screening procedures

Screening procedures for the current study were as described for the dose-finding study (see 4.9.2.1).

6.1.5.2. Experimental procedures

6.1.5.2.1. Drug administration

The infusion period commenced with a 30-minute infusion of saline (10 ml) followed immediately by the infusion of either BUP and saline, or BUP and NLX in a 5:1, 10:1 or 12.5:1 ratio over 30 minutes. Participants were randomised to receive each of these conditions over 4 separate testing days each one week apart (see randomisation schedule,

Table 6-3). BUP was administered at a dose of 0.5 μg/kg body weight. BUP and NLX (or saline on the BUP:saline {BUP only} condition) were administered simultaneously from different infusion pumps into the same vein. An example of BUP and NLX doses administered for each condition based on a subject weighing 70 kg is displayed in Table 6-2. The total duration of infusion was 1 hour over which time nociceptive testing was performed and physiological parameters recorded. Details of infusion set-up and procedure are outlined in Chapter 2 (section 2.3.1).

Condition	Pump A	Pump B
BUP:saline ("BUP only)	BUP 35 μg	Saline 10ml
BUP:NLX 5:1	BUP 35 μg	NLX 7 μg
BUP:NLX 10:1	BUP 35 μg	NLX 3.5 μg
BUP:NLX 12.5:1	BUP 35 μg	NLX 2.8 μg
Conditions administered in a double-blind, randomised order. Pump A and pump B infused simultaneously for a 30-minute period.		

Table 6-2. Doses of BUP and NLX administered based on a 70 kg subject.

	Session 1	Session 2	Session 3	Session 4
Subject A	5:1	10:1	12.5:1	BUP only
Subject B	10:1	12.5:1	BUP only	5:1
Subject C	5:1	10:1	BUP only	12.5:1
Subject D	5:1	10:1	12.5:1	BUP only
Subject E	10:1	5:1	BUP only	12.5:1
Subject F	12.5:1	BUP only	5:1	10:1
Subject G	10:1	5:1	BUP only	12.5:1
Subject H	12.5:1	BUP only	5:1	10:1
Subject I	5:1	10:1	BUP only	12.5:1
Subject J	5:1	BUP only	12.5:1	10:1

Table 6-3. Randomisation schedule for 7 healthy volunteers. Drug conditions were IV BUP with saline (BUP only) 0.5 µg/kg; IV BUP(0.5 µg/kg):NLX in a 5:1, 10:1 and 12.5:1 ratio. BUP and saline/NLX infused simultaneously into the same vein over 30-minutes. Subjects were randomised to receive each condition across 4 individual testing days. Randomisation schedule was created and administered by hospital pharmacy responsible for drug preparation. Shaded subjects excluded: Subject B and E after first testing day due to adverse effects (see 6.2.1.1), Subject G due to opioid positive urine on BUP only testing day (see 6.2.1.2).

6.1.6. Testing procedure and schedule

Subjects were delivered from their homes to the testing centre by taxi in the morning. Subjects had been instructed to refrain from taking any drugs or medication in the 24-hours prior to testing (excluding the contraceptive pill) and to eat a light breakfast on the morning of testing. A urine sample was taken and tested by an independent laboratory for drugs of abuse (opioids, cannabinoids, benzodiazepines and sympathomimetic amines) and, for female subjects, pregnancy. The testing procedures are outlined in Chapter 2. Each assessment time point involved taking a blood sample, recording nausea, sedation and physiological parameters, and completion of the nociceptive tests. This testing procedure took place prior to infusion, twenty minutes after the commencement of each infusion (saline and then BUP with saline or BUP with NLX), and then hourly upon completion of the infusions over a 10-hour washout period. Subjective effects were also recorded throughout the testing day. Subsequent reference to time points is made according to the references in Table 6-4.

Due to the lack of significant effect observed with the ES test in any condition in the previous study this technique was not performed in the current investigation. Antinociception was assessed by the CP test as described (section 2.2.1).

Testing time point reference	Description
Pre-saline	Prior to starting the infusion period
Baseline	20 minutes after starting the 30-minute saline infusion
Post-bup/ Post-drug	20 minutes after starting the 30-minute 0.5 μg/kg BUP infusion
Washout 1 (hr)	1 hour following cessation of the BUP infusion
Washout 2 (hr)	2 hours following cessation of the BUP infusion
Washout 3 (hr)	3 hours following cessation of the BUP infusion
Washout 4 (hr)	4 hours following cessation of the BUP infusion
Washout 5 (hr)	5 hours following cessation of the BUP infusion
Washout 6 (hr)	6 hours following cessation of the BUP infusion
Washout 7 (hr)	7 hours following cessation of the BUP infusion
Washout 8 (hr)	8 hours following cessation of the BUP infusion
Washout 9 (hr)	9 hours following cessation of the BUP infusion
Washout 10 (hr)	10 hours following cessation of the BUP infusion

Table 6-4. Description of testing time point references. At each time point the following was performed:1) blood sample taken, 2) nausea, sedation, subjective and physiological parameters assessed, 3) nociceptive testing completed.

6.1.6.1. Statistical analyses

Methods of statistical inference were as described for the previous BUP:NLX ratio study (section 5.6.5). Consistent with the first BUP:NLX ratio study described in the previous chapter, CPTOL included censored data due to the maximum time limit associated with the

test (180 seconds). As described, such censored cases occur when subjects failed to declare maximum tolerated cold pressor pain before the 180 second time limit had lapsed. Two of the 7 subjects reached this maximum limit over the course of the 4 testing days. As described previously (see 3.4.3 and 5.6.5.1), nonparametric methods of statistical inference are appropriate when such cases are included in the data set. Therefore, consistent with the previous ratio study (Chapter 5), all analyses of CPTOL in the current study have employed nonparametric tests, while other parameters have been analysed with parametric tests.

6.2. Results

6.2.1. Participant withdrawal and missing data

6.2.1.1. Participant withdrawal

Two female subjects withdrew from the study following the first testing day. One 22-year old experienced moderate nausea (without vomiting) at the Post-bup time point, requiring administration of metaclopramide (10 mg IV). An episode of nausea occured each time the subject was required to move from a supine position in preparation for testing. Nausea compromised the performance of the nociceptive tests and was unpleasant for the subject. The subject also experienced a mild level of sedation. It was agreed that it was not appropriate to continue with testing. The subject remained in the testing centre for safety monitoring for 10 hours after drug administration, at which time adverse effects had been resolved. All subjective effects had diminished by this time. The subject had received BUP:NLX in a 10:1 ratio on this day.

There was significant difficulty with cannula insertion with a 23-year old female subject. Numerous attempts were made to achieve adequate venous access; however, the cannulae were repeatedly failing after only one blood sampling. It was considered inappropriate to continue due to the significant discomfort associated with repeated cannula insertions. The subject had been randomised to the BUP:NLX 10:1 condition on this day.

Both subjects were remunerated $AU250 for this testing day and two additional female subjects recruited.

6.2.1.2. Missing data

One 37-year old male subject returned an opioid positive urine on the BUP only testing day. The exclusion of data from this testing day necessitated the exclusion of all results from this subject, as the BUP only session serves as a comparison for the BUP:NLX ratio data.

A 24-year old female subject returned an opioid positive urine on the BUP:NLX 12.5:1 testing day. Data from this testing day have been excluded from all analyses. Where statistical comparisons are made between BUP only and BUP:NLX ratio data, this subject's BUP only data have also been excluded.

6.2.2. Pre- and post-saline infusion

Paired samples t-tests were conducted to detect any significant differences between nociceptive and physiological parameters before and after the 30-minute saline infusion. There were no significant differences between pre- and post-saline response for any measure ($p>0.05$). It is considered, then, that the infusion process itself had no significant

impact on nociceptive or physiological parameters. In all subsequent analyses, then, post-saline values have been used as baseline response.

6.2.3. Cold pressor threshold

6.2.3.1. Effect of each condition

Mean (±SEM) CPTHR for each condition across all time points is presented in Figure 6-1. One-way ANOVA conducted for each condition over three time points (Baseline, Post-bup and Washout 1hr) revealed a significant change in CPTHR in the BUP only condition (see Table 6-5). Post-hoc analyses (Tukey's Multiple Comparison Test) revealed a significant difference between Baseline and Washout 1hr ($p<0.05$). While there were no significant differences over these time points for any of the BUP:NLX ratios, the difference over these time points in the 12.5:1 ratio condition was approaching significance ($p=0.079$).

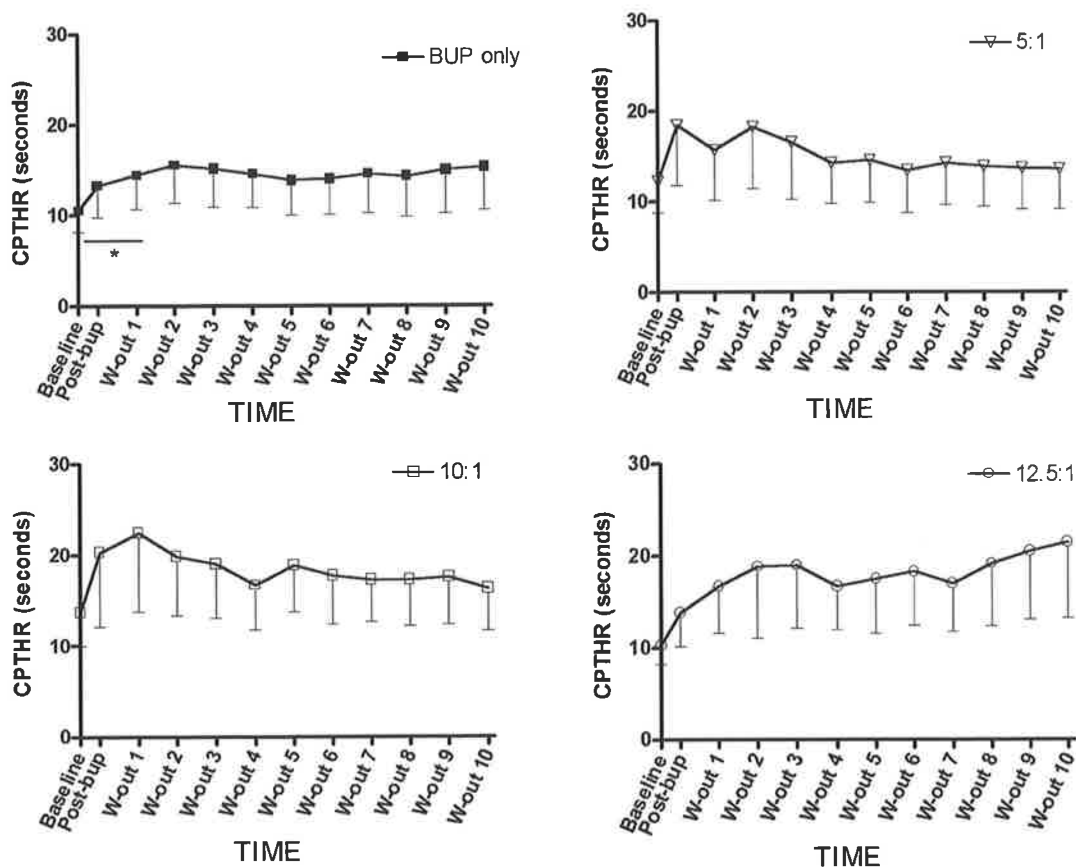

Figure 6-1. Mean (±SEM) CPTHR (seconds) for each condition (BUP only 0.5 µg/kg {n=7}, BUP{0.5 µg/kg}:NLX in a 5:1 {n=7}, 10:1 {n=7} or 12.5:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly post-infusion to 10 hrs) among healthy volunteers. *p<0.05, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated-measures analysis of variance.

	Mean (±SEM) (seconds)			Df	F	p
	Baseline	Post-bup	Washout 1hr			
5:1 (n=7)	12.29 (±3.53)	18.43 (±6.62)	15.71 (±5.63)	2,12	2.253	0.148
10:1(n=7)	13.71 (±3.75)	20.29 (±8.17)	22.43 (±8.72)	2,12	2.305	0.142
12.5:1(n=6)	10.33 (±2.16)	13.83 (±3.71)	16.67 (±5.09)	2,10	3.296	0.079
BUP only(n=7)*	10.37 (±2.42)	13.29 (±3.53)	14.43 (±3.85)	2,12	5.584	0.019*

Table 6-5. Mean(±SEM) CPTHR (seconds) at Baseline, Post-bup and Washout 1hr time points for each condition (BUP only {n=7}, BUP:NLX in a 5:1 {n=7}, 10:1 {n=7} or 12.5:1 {n=6} ratio). F statistic, degrees of freedom (Df) and alpha value (p) for one-way repeated measures analysis of variance (ANOVA) of CPTHR including Baseline, Post-bup and Washout 1hr for each condition. *p<0.05, significant difference in CPTHR over these 3 time points.

6.2.3.2. Effect of BUP:NLX ratio compared to BUP alone

CPTHR mean (±SEM) percent change from baseline at each time point for each condition is presented in Figure 6-2. There were no significant differences in percent change in CPTHR from baseline between BUP only and any BUP:NLX ratio (p>0.05).

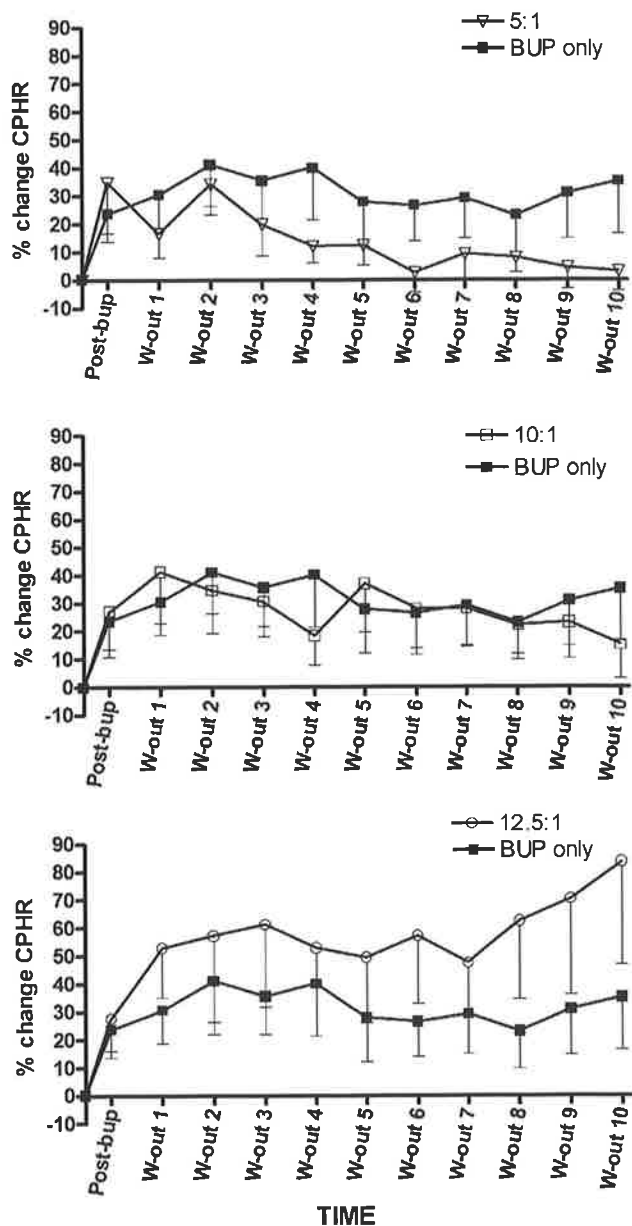

Figure 6-2. Mean (±SEM) CPTHR percent change from Baseline for BUP(0.5µg/kg):NLX ratios (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5 µg/kg, n=7) across all time points (Baseline, Post-bup, and hourly over BUP washout to 10 hrs) among healthy volunteers. p>0.05, no significant differences between BUP:NLX conditions and BUP only, paired samples t-tests. Note that data for the BUP only condition are presented in all 3 graphs.

6.2.4. Cold pressor tolerance

6.2.4.1. Effect of each condition

Mean (±SEM) CPTOL for each condition is presented in Figure 6-3 (see Table 6-6). Friedman's test including data from Baseline, Post-bup and Washout 1hr time points revealed a significant effect in the BUP only condition (χ^2=7.185, p=0.021) and the 12.5:1 BUP:NLX condition (χ^2=11.00, p=0.0001). Dunn's multiple comparison tests revealed significant effects between Baseline and Washout 1hr for both the BUP only (p<0.05) and 12.5:1 (p<0.01) conditions.

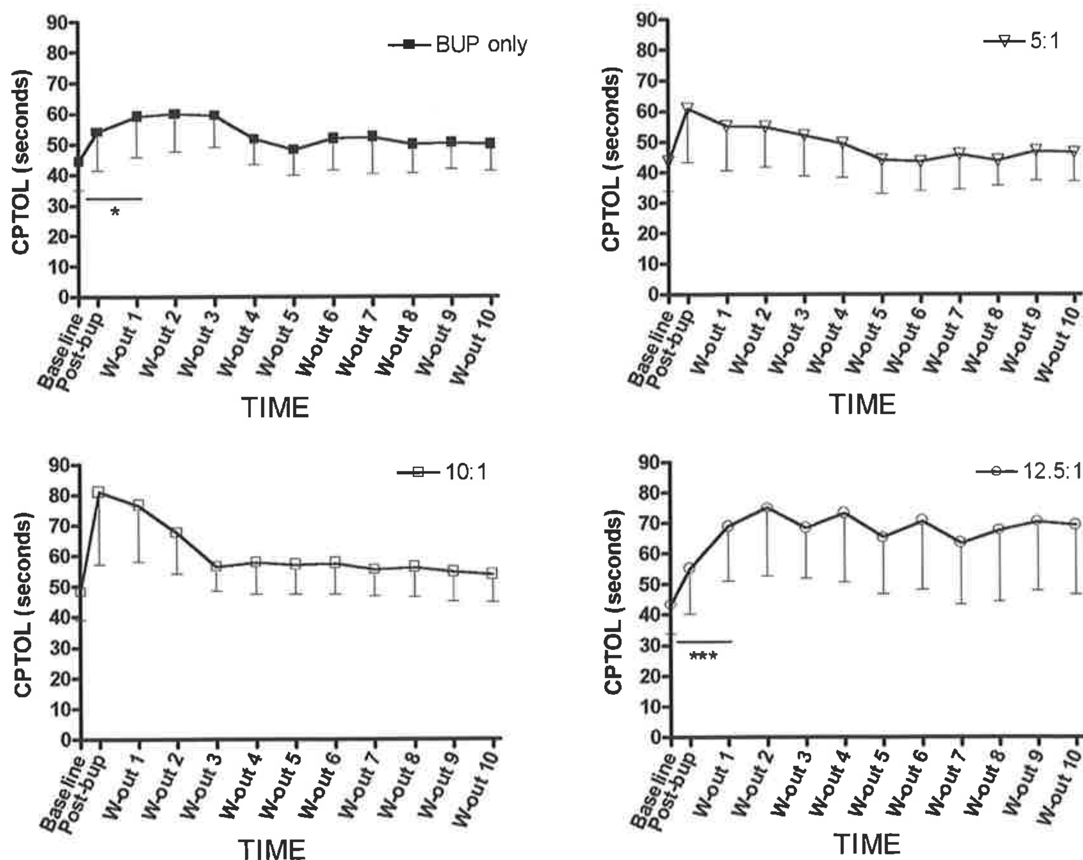

Figure 6-3. Mean (±SEM) CPTOL (seconds) for each condition (BUP only {0.5 μg/kg, n=7}, BUP{0.5 μg/kg}:NLX in a 5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6} ratio) over all time points (Baseline, Post-bup, and hourly during BUP washout to 10 hrs) among healthy volunteers. *p<0.05, ***p<0.001, significant difference over Baseline, Post-bup and Washout 1hr, Friedman's test.

	Mean rank			χ^2
	Baseline	Post-bup	Washout 1hr	
5:1 (n=7)	1.50	2.21	1.86	4.963
10:1(n=7)	1.43	2.00	2.57	4.571
12.5:1(n=6)	1.21	2.00	2.79	11.00***
BUP only(n=7)	1.21	2.21	2.57	7.185*

Table 6-6. Mean rank for each condition (BUP only 0.5 µg/kg {n=7}, and BUP{0.5 µg/kg}:NLX in a 5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6} ratio) at Baseline, Post-Bup and Washout 1 hr among healthy volunteers. χ^2 statistic of Friedman's test of CPTOL over Baseline, Post-bup and Washout 1hr for each condition, *p<0.05 ***p<0.001.

6.2.4.2. Effect of BUP:NLX ratio compared to BUP alone

Figure 6-4 displays mean (±SEM) percent change from baseline CPTOL at each time point for each ratio compared to BUP only. The 12.5:1 ratio was associated with significantly greater increase in CPTOL compared to BUP only at Washout 1hr (z=-1.992, p=0.046), Washout 4hr (z=-1.992, p=0.046), Washout 5hr (z=-2.201, p=0.028), Washout 9hr (z=-2.201, p=0.028) and Washout 10hr (z=-1.992, p=0.046). There were no significant differences in change from baseline CPTOL between BUP only and the 5:1 or 10:1 ratios at any time point.

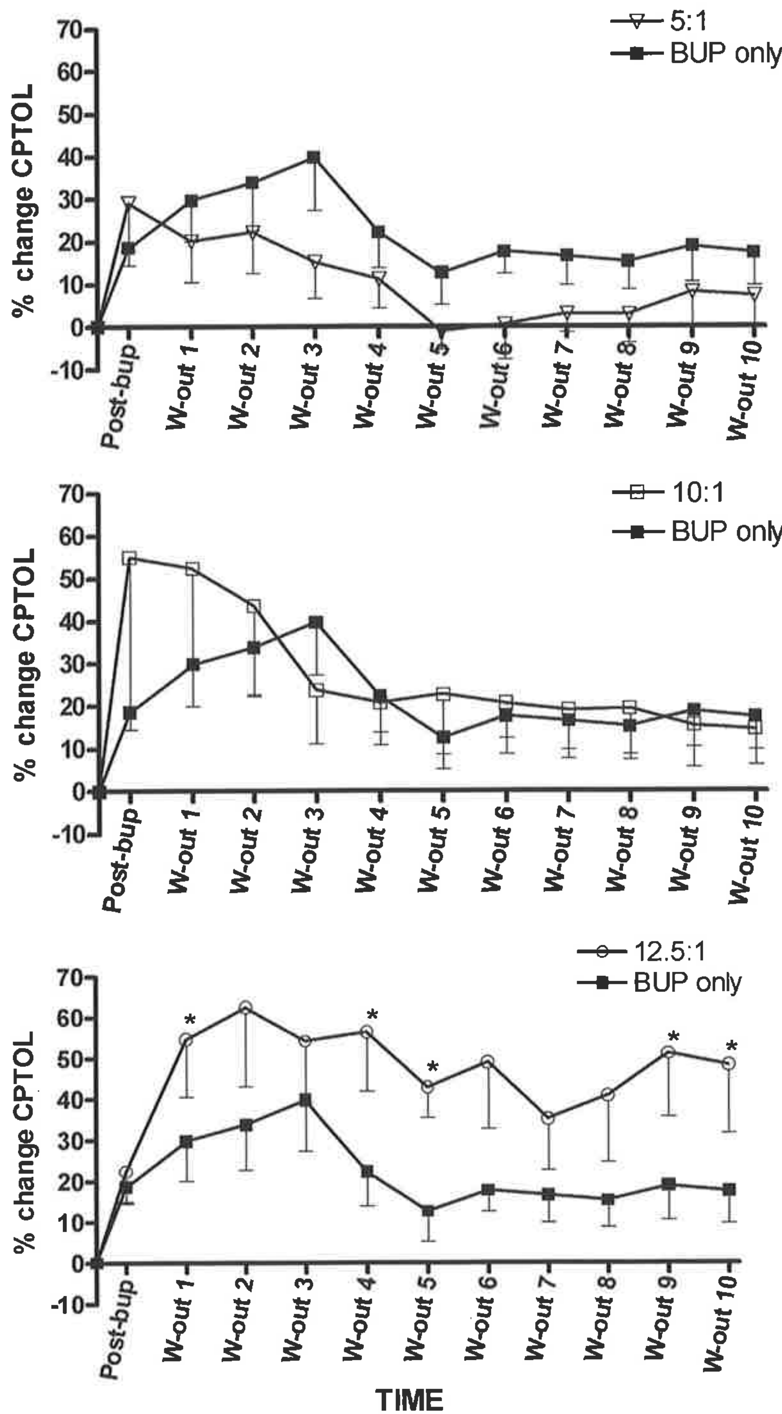

Figure 6-4. Mean (±SEM) CPTOL percent change from Baseline for BUP(0.5μg/kg):NLX ratios (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5μg/kg, n=7) across all time points (Baseline, Post-bup, and hourly over BUP washout period to 10 hrs) among healthy volunteers. *p<0.05, significant difference from BUP only, Wilcoxon signed ranks. Note that data for the BUP only condition are presented in all 3 graphs.

6.2.4.3. Difference between % change$_{RATIO}$ and % change$_{BUPONLY}$

The percent change from baseline CPTOL associated with each BUP:NLX ratio minus the change associated with BUP only (i.e. % change$_{BUP\ only}$ - % change$_{RATIO}$) is presented for each ratio at each time point in the upper panel of Figure 6-5. The area under the percent change curve for these data is presented in the lower panel. Wilcoxon signed rank tests between the AUC of the ratios revealed a significantly greater increase in CPTOL associated with the 12.5:1 ratio compared to the 5:1 ratio ($z=-2.201$, $p=0.028$). There were no significant differences in AUC between the 12.5:1 and 10:1 ratios ($z=-0.656$, $p=0.499$) or between the 10:1 and 5:1 ratios ($z=-1.153$, $p=0.249$).

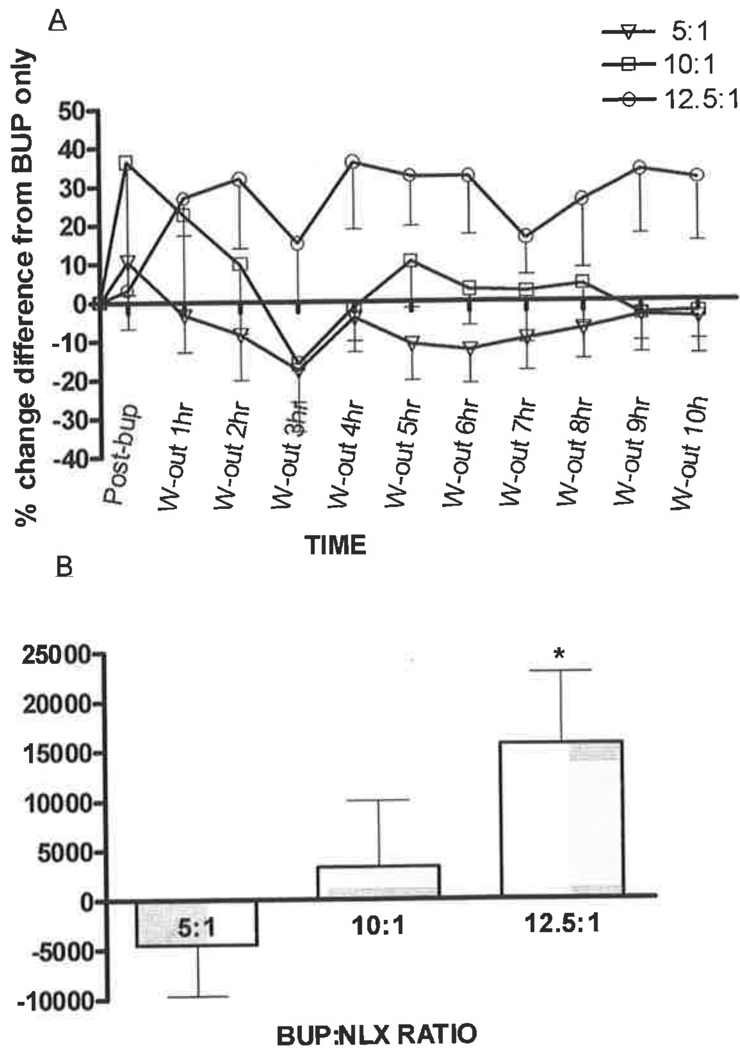

Figure 6-5. A. Mean (±SEM) CPTOL percent change difference from BUP only (0.5 µg/kg, n=7) for each BUP(0.5 µg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) across all time points (Baseline, Post-bup, and hourly over BUP washout to 10 hrs) among healthy volunteers. B. Mean (±SEM) CPTOL AUC percent change difference from BUP only for each BUP:NLX ratio. *p<0.05, 12.5:1 ratio significantly greater than 5:1 ratio, Wilcoxon signed ranks.

6.2.5. Respiration

6.2.5.1. Effect of each condition

Mean (±SEM) breaths per minute at each time point for each condition are displayed in Figure 6-6. Repeated measures analysis of variance over the first three time points revealed a significant decrease in breaths per minute in the BUP only ($F_{2,12}$=10.25,

p=0.003) and 5:1 ratio ($F_{2,12}$=8.39, p=0.005) conditions. Post-hoc tests (Tukey's Multiple Comparison Test) revealed a significant effect between Baseline and Washout 1hr for both the BUP only (p<0.01) and 5:1 ratio (p<0.01) conditions.

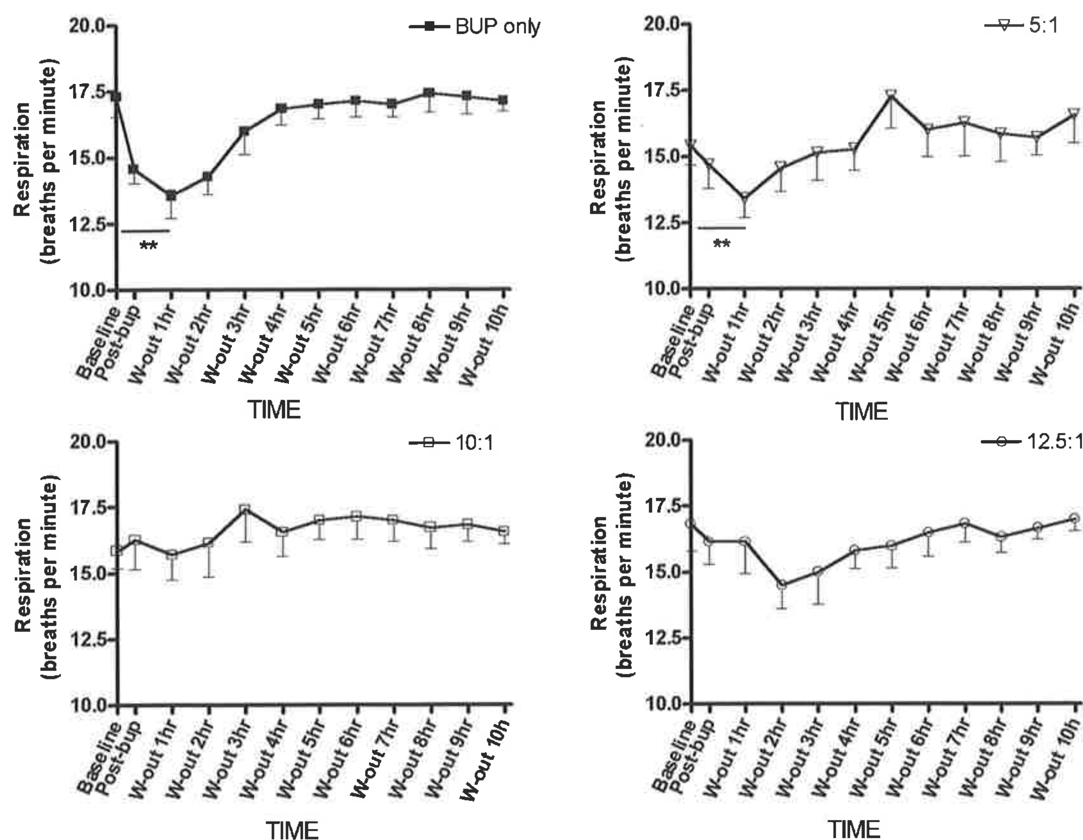

Figure 6-6. Mean (±SEM) breaths per minute for BUP only (0.5 µg/kg, n=7) and each BUP(0.5 µg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) across all time points (Baseline, Post-bup, and hourly over BUP washout to 10 hrs) among healthy volunteers. **p<0.01, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

6.2.5.2. Effect of BUP:NLX ratio compared to BUP alone

Percent decrease in breaths per minute was significantly greater in the BUP only condition than associated with all BUP:NLX ratios at one or more time points (see Figure 6-7). BUP only was associated with significantly greater reduction in breaths per minute at Post-bup testing than the 5:1 ratio (-15.31±2.85 vs. –4.93±2.02, t(6)=-3.162, p=0.02), the 10:1 ratio (-15.31±2.85 vs. 2.61±5.02, t(6)=-2.528, p=0.045) and the 12.5:1 ratio (-12.86±1.73 vs. –

3.37±3.70, $t(6)=-2.599$, $p=0.048$). A significantly greater decrease in breaths per minute was associated with BUP only condition at Washout 1hr testing compared to the 10:1 ratio (-20.55±5.84 vs. –0.93±4.54, $t(6)=-2.611$, $p=0.04$). By Washout 4hr-6hr, respiration rate had returned to baseline levels in all conditions.

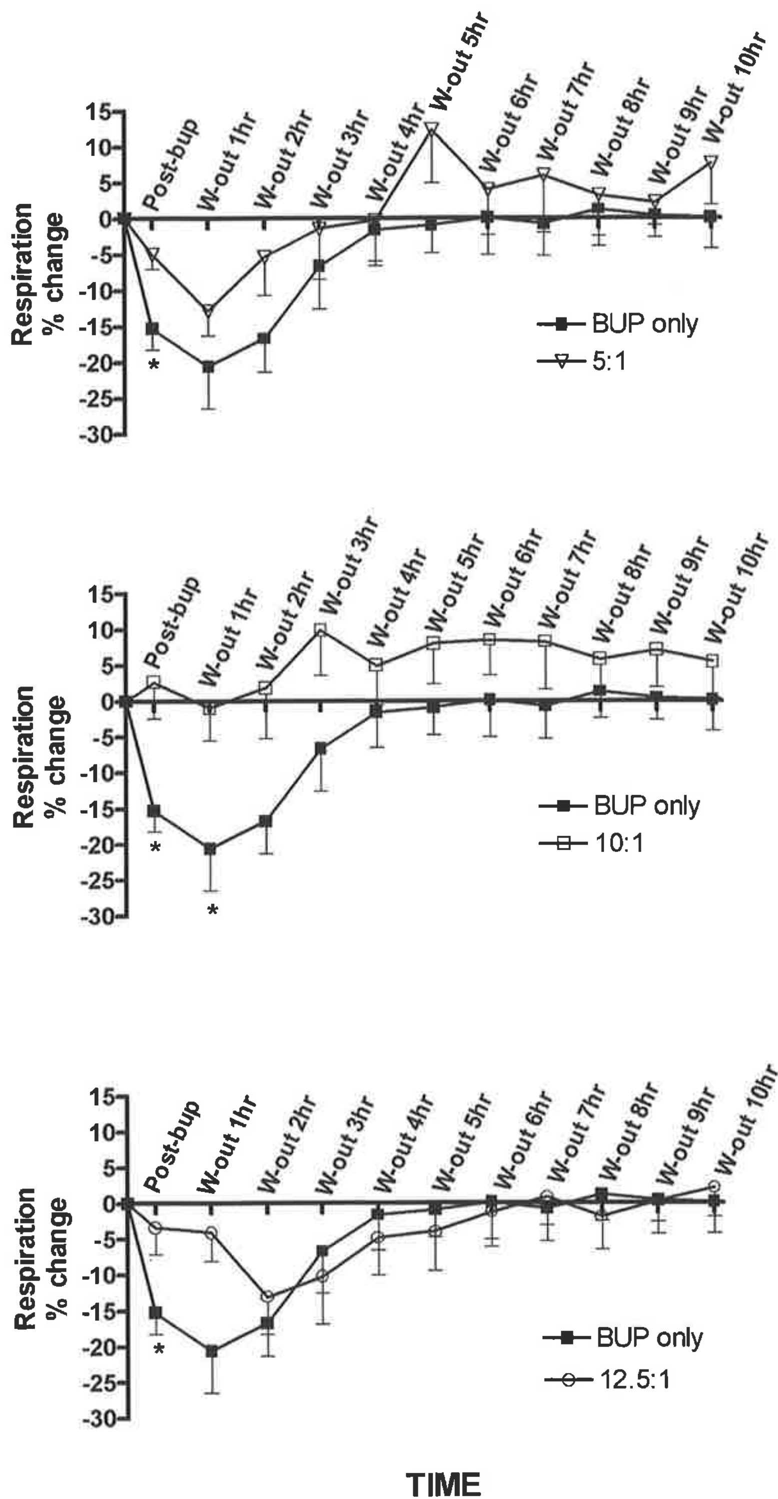

Figure 6-7. Mean (±SEM) percent change from Baseline breaths per minute for each BUP(0.5 μg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5 μg/kg) across all time points (Baseline, Post-bup, and hourly over BUP washout to 10 hrs) among healthy volunteers. *p<0.05, significant difference from BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

6.2.6. Heart rate

6.2.6.1. Effect of each condition

Figure 6-8 displays mean (±SEM) heart rate at each time point for each condition. There were no significant differences over Baseline, Post-bup and Washout 1hr for any condition.

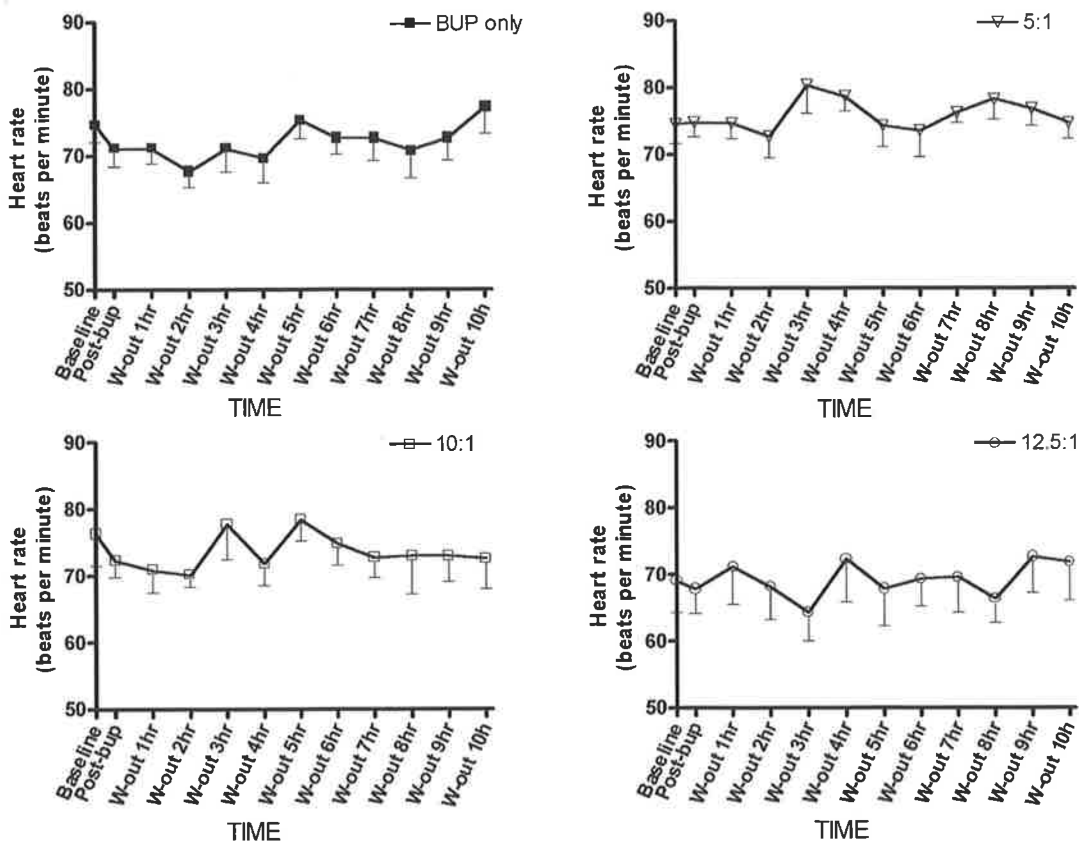

Figure 6-8. Mean (±SEM) heart rate (beats per minute) for each condition (BUP only {0.5 μg/kg, n=7}, BUP{0.5 μg/kg}:NLX in a 5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6} ratio) over all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. p>0.05, no significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

6.2.6.2. Effect of BUP:NLX ratio compared to BUP alone

There were no significant differences in heart rate between BUP only and any BUP:NLX ratio across all time points (see Figure 6-9).

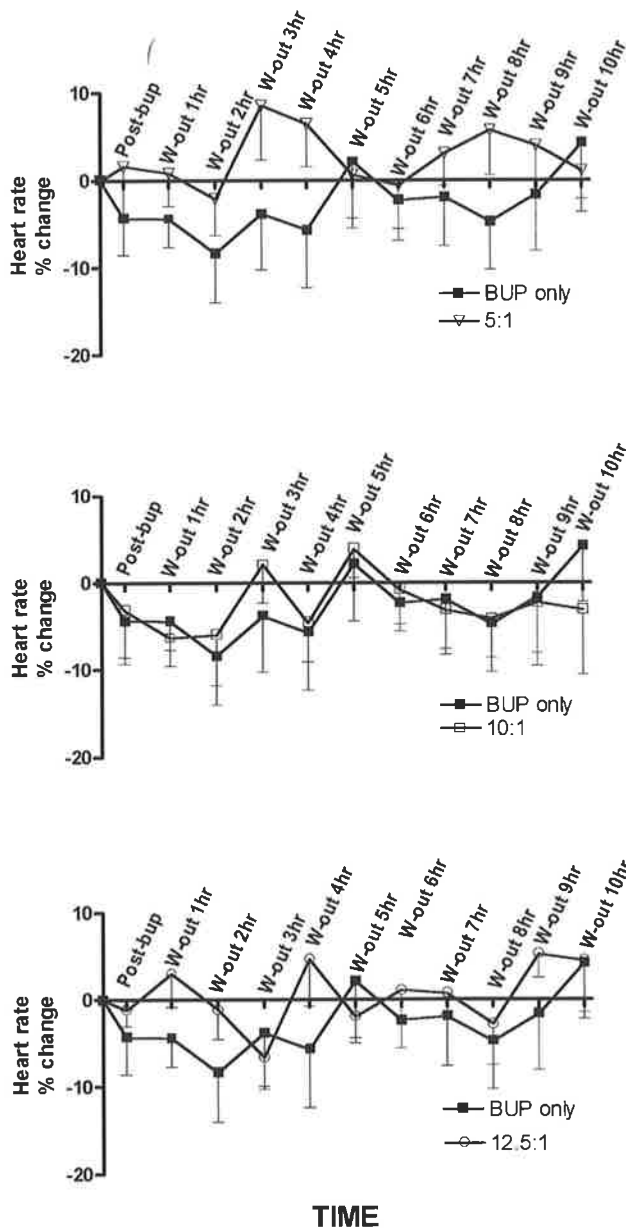

Figure 6-9. Mean (±SEM) percent change from Baseline heart rate for each BUP(0.5 µg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5 µg/kg, n=7) over all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. p>0.05, no significant differences between ratios and BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

6.2.7. Oxygen saturation

6.2.7.1. Effect of each condition

Arterial oxygen saturation significantly decreased in the 5:1 BUP:NLX condition over Baseline, Post-bup and Washout 1hr ($F_{2,12}=4.30$, $p=0.039$). Post-hoc analyses revealed a significant difference between Baseline and Washout 1hr ($p<0.05$). There were no significant differences in oxygen saturation over these time points in any other condition (see Figure 6-10); however, the BUP only condition was approaching significance ($p=0.0527$).

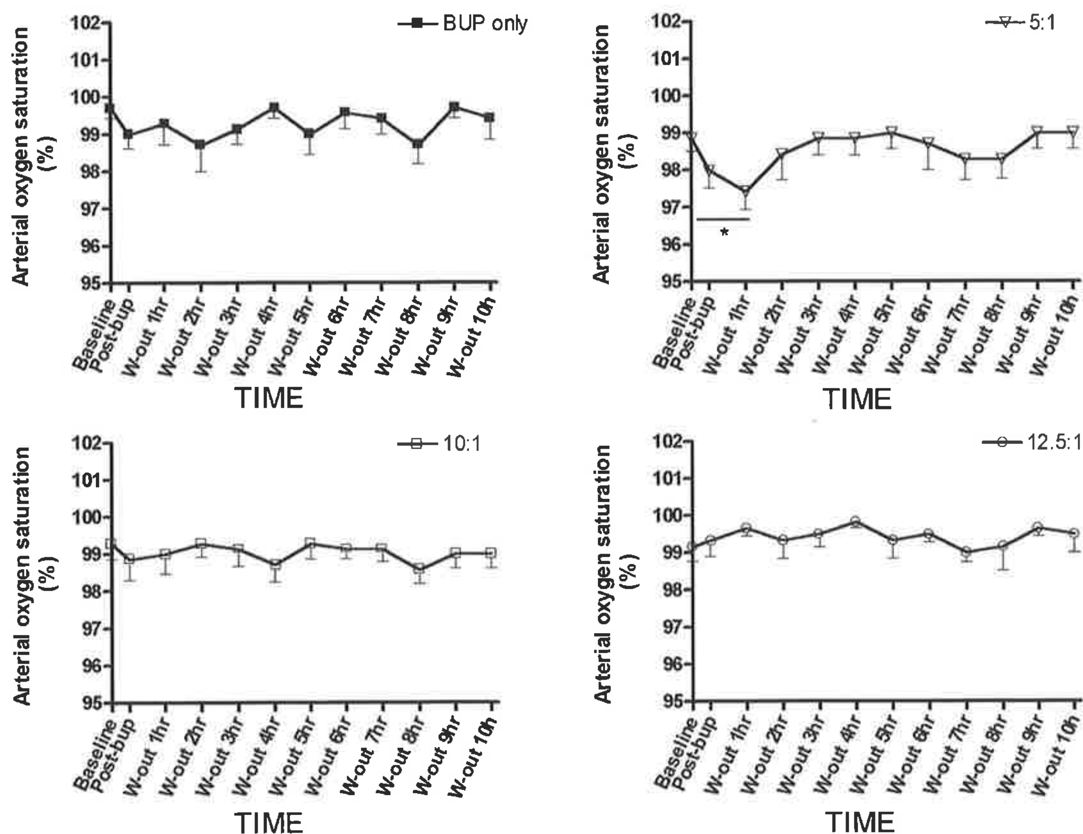

Figure 6-10. Mean (±SEM) arterial oxygen saturation(%) for BUP only (0.5 µg/kg, n=7) and each BUP(0.5 µg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) over all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. p<0.05, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

6.2.7.2. Effect of BUP:NLX ratio compared to BUP alone

There was a significant difference between BUP only (-0.84±0.167) and the 12.5:1 BUP:NLX ratio (0.17±0.168) at the Post-bup time point ($t(5)=-3.87$, $p=0.012$) (see Figure 6-11). There were no other significant differences between BUP only and any other ratio ($p>0.05$).

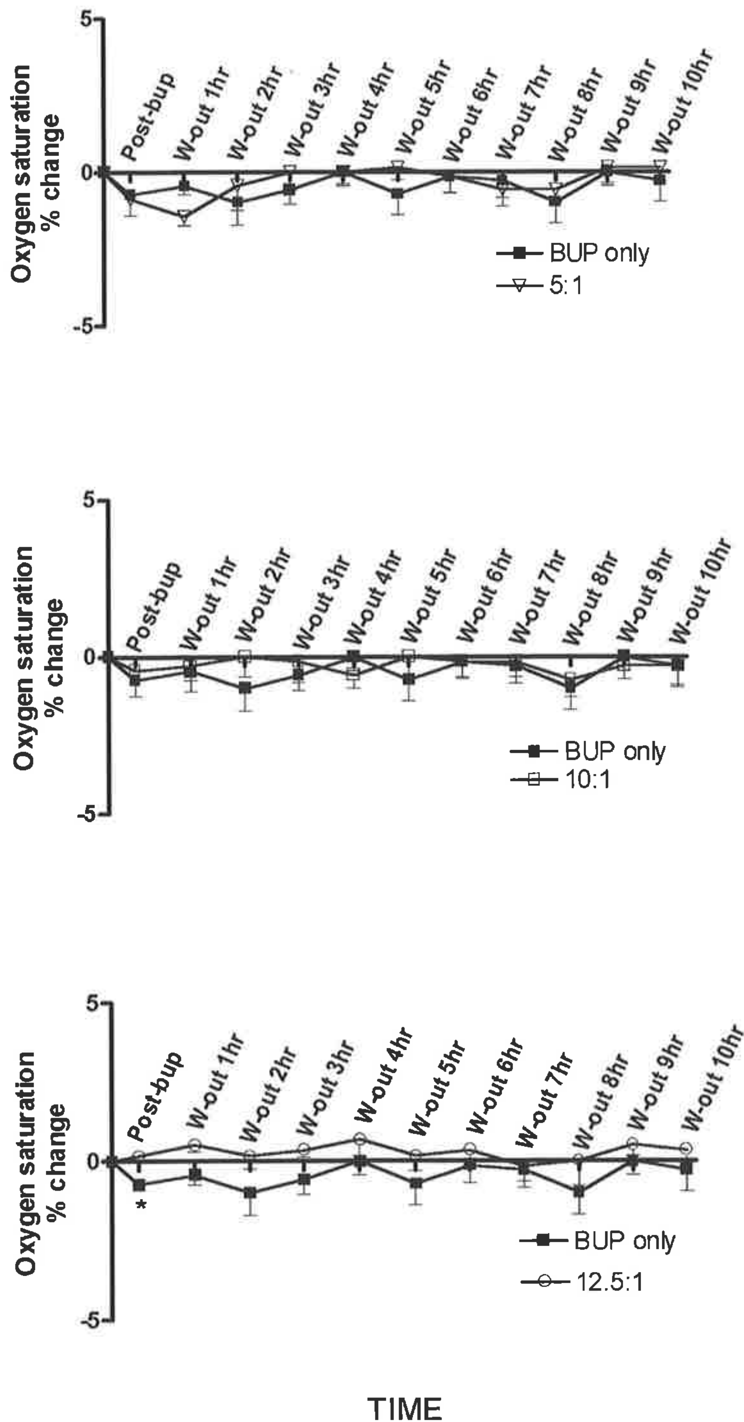

Figure 6-11. Mean (±SEM) percent change from Baseline arterial oxygen saturation for BUP(0.5 µg/kg):NLX ratios (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5 µg/kg, n=7) across all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. *p<0.05, significant difference from BUP only, paired samples t-test. Note that data for the BUP only condition are presented in all 3 graphs.

6.2.8. Blood pressure

6.2.8.1. Effect of each condition

There was a significant increase in systolic blood pressure over Baseline, Post-bup and Washout 1hr in the BUP only condition ($F_{2,12}$=3.90, p=0.0496), and a decrease over these time points in the 5:1 condition ($F_{2,12}$=5.54, p=0.0197) (Figure 6-12). A significant decrease in diastolic blood pressure was observed in the 12.5:1 ratio over these three time points ($F_{2,10}$=5.35, p=0.0263) (Figure 6-12).

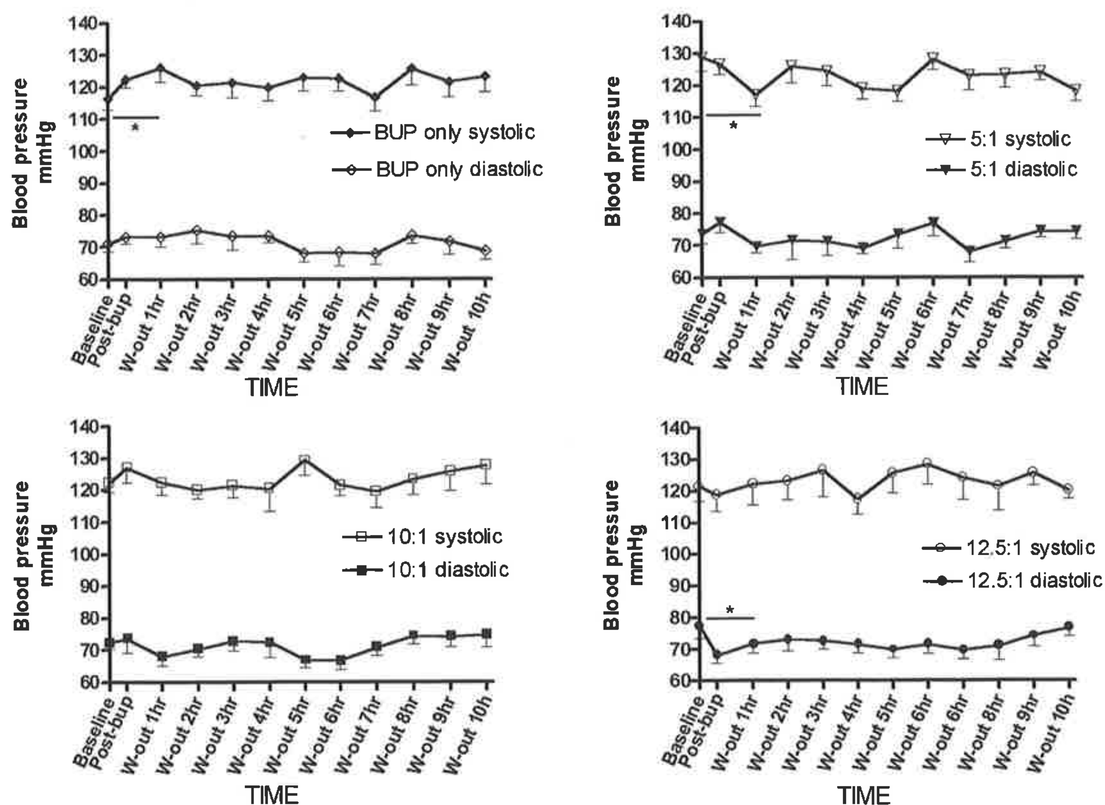

Figure 6-12. Mean (±SEM) blood pressure (mmHg) for BUP only (0.5 µg/kg, n=7) and each BUP(0.5 µg/kg):NLX ratio (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) across all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. *p<0.05, significant variance over Baseline, Post-bup and Washout 1hr, one-way repeated measures ANOVA.

6.2.8.2. Effect of BUP:NLX ratio compared to BUP alone

There was a significant difference in systolic blood pressure between BUP only and 5:1 at Washout 1hr ($t(6)=3,76$, $p=0.009$); Washout 4 hr ($t(6)=2.95$, $p=0.026$), Washout 5 hr ($t(6)=3.10$, $p=0.021$) and Washout 10 hr ($t(6)=3.35$, $p=0.015$) (Figure 5-18). There were no significant differences in diastolic blood pressure between BUP only and BUP:NLX ratios in percent change from baseline (Figure 6-13).

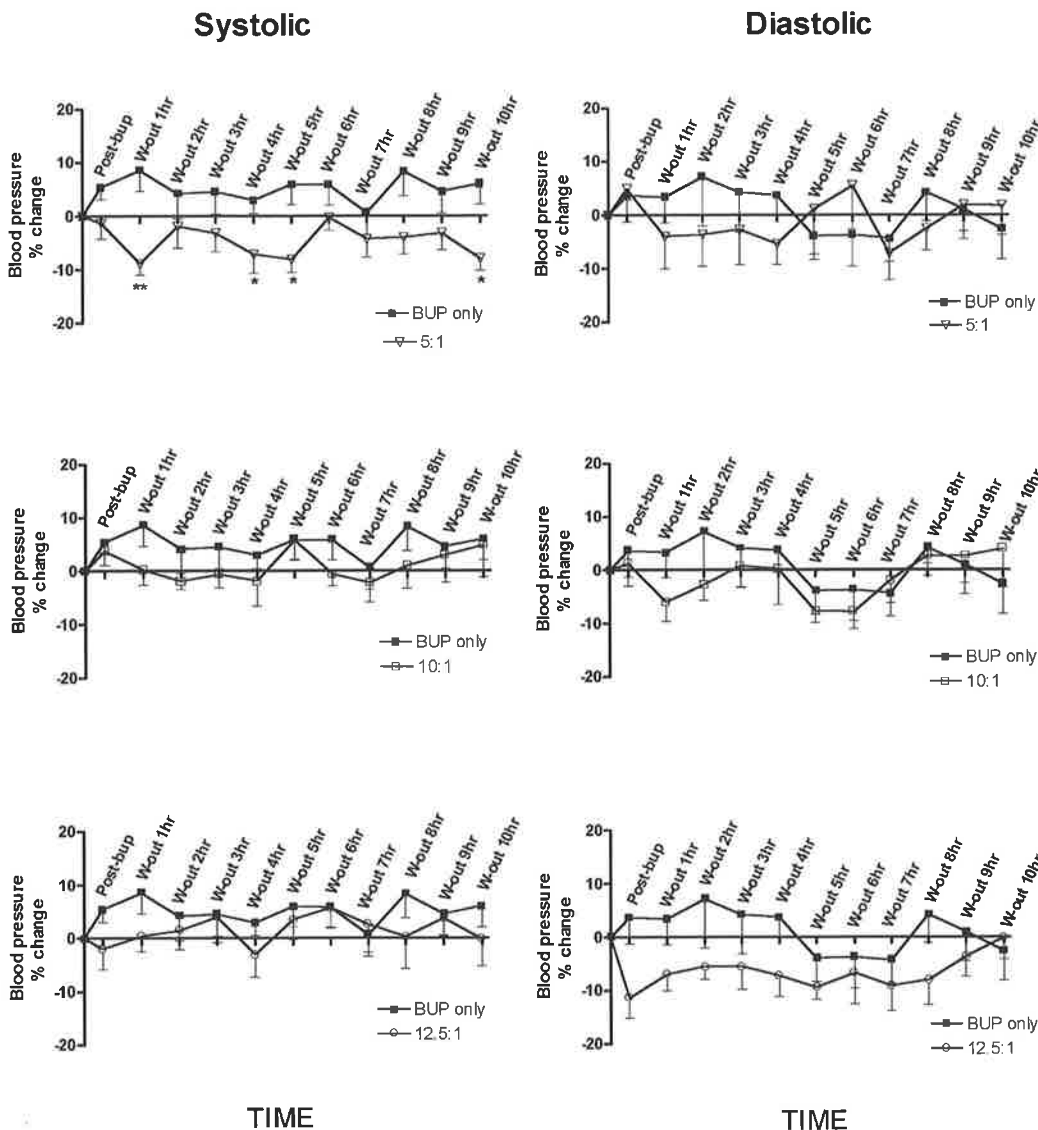

Figure 6-13. Mean (±SEM) percent change from Baseline blood pressure for BUP(0.5 µg/kg, n=7):NLX ratios (5:1 {n=7}, 10:1 {n=7} and 12.5:1 {n=6}) compared to BUP only (0.5 µg/kg) across all time points (Baseline, Post-bup and hourly over BUP washout to 10 hrs) among healthy volunteers. *p<0.05, **p<0.01, significant difference from BUP only, paired samples t-test. Note that systolic and diastolic data for the BUP only condition are presented in all graphs on the left and right panel, respectively.

6.2.9. Subjective effects

The incidence of subjective effects is outlined in Table 6-7 according to subject and drug condition. Consistent with the subjective effects experienced in the first BUP:NLX ratio study, there was no greater prevalence of effects among subjects during the BUP:NLX ratio conditions than during the BUP only condition.

BUP only	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**% of subjects**
Sedation*		W-out 1hr W-out 7hr	W-out 1hr			W-out 1hr W-out 2hr W-out 3hr	W-out 2hr	57
Nausea*		W-out 2hr				W-out 1hr		29
Pleasant feeling						Post-bup		14
Dizziness	W-out 1hr							14
Difficulty concentrating					W-out 7hr			14
BUP:NLX 5:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**% of subjects**
Sedation*	W-out 2hr	W-out 1hr				W-out 1hr		43
Nausea*						W-out 1hr		14
Pleasant feeling					Post-bup W-out 1hr			14
Dizziness	W-out 1hr							14
Difficulty concentrating								
BUP:NLX 10:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**% of subjects**
Sedation*		W-out 7hr W-out 8hr	W-out 4hr		W-out 1hr W-out 5hr	W-out 4hr		57
Nausea								
Pleasant feeling	W-out 1hr							14
Dizziness	W-out 1hr							14
Difficulty concentrating							W-out 1hr	14
BUP:NLX 12.5:1	**Subject 1**	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**% of subjects**
Sedation*	W-out 7hr	W-out 1hr W-out 6hr	W-out 4hr					50
Nausea								
Pleasant feeling				W-out 1hr	W-out 1hr			33
Dizziness								
Difficulty concentrating		W-out 1hr						17

* All cases of sedation and nausea rated as 1, mild. Nausea subsided without need for metaclopramide. No episodes of vomiting.

Table 6-7. Incidence of subjective effects among healthy volunteers following IV infusion of BUP only (0.5 μg/kg) or BUP:NLX in a 5:1, 10:1 or 12.5:1 ratio. Effects are detailed according to subject, drug condition and time point(s) at which effect was experienced (see Table 6-4 for time point descriptions). Shaded section represents missing data due to opioid positive urine.

6.3. Combined CPTOL results for ratio studies 1 and 2

The CPTOL results from both ratio studies (Chapters 5 and 6) have been combined to faciliate visual comparison of findings. Figure 6-14 displays the mean area under the curve of the percent change from baseline associated with each BUP:NLX ratio minus the effect of BUP only for each subject. As the monitoring period for the second ratio study was extended to 10 hrs post-infusion (compared to the 6 hr period of the first ratio study),

to facilitate comparison between studies, calculation of AUC for the ratios from the second study (5:1, 10:1 and 12.5:1) excluded values after the 6 hr time point.

This effect on CPTOL across the ratio range displays a bell-shaped curve. The 5:1 ratio produced less antinociception than BUP alone, suggesting that at this NLX dose antagonised the effect of BUP. These data indicate that the lower limit of the effective ratio range has been identified. The 12.5:1 and 15:1 ratios were associated with an equivalent increase in the magnitude of CP antinociception above the effect of BUP alone. Antinociception then decreased with the 20:1 ratio, with a slight increase observed with the 25:1 ratio.

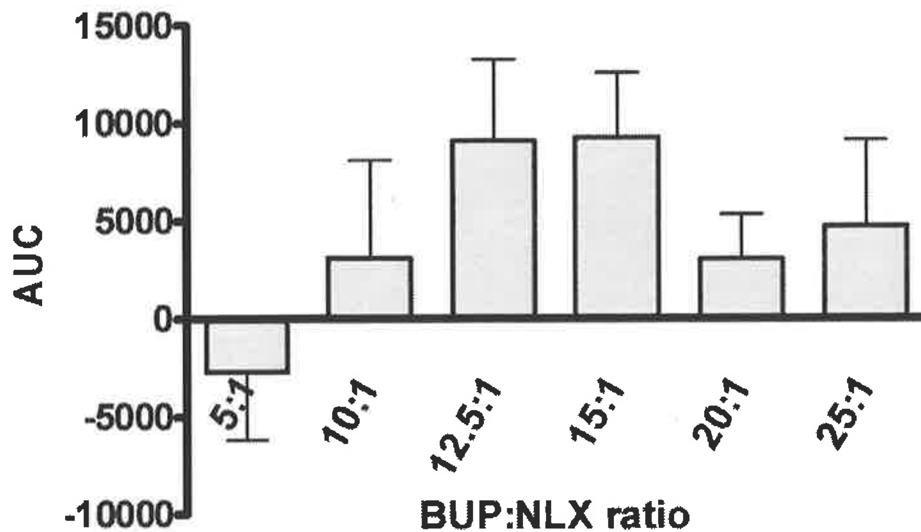

Figure 6-14. Mean (±SEM) area under the difference in %change from baseline CPTOL for each BUP:NLX ratio (5:1 {n=7}, 10:1 {n=7}, 12.5:1 {n=6}, 15:1 {n=6}, 20:1 {n=5}, 25:1 {n=6}) and %change from baseline CPTOL for BUP only curve (AUC).

6.4. Discussion

The aim of the current study was to investigate further the effect of BUP:NLX combinations on antinociception and adverse effects, with BUP:NLX ratios of 5:1, 10:1 and 12.5:1. Findings from this study are consistent with those of the first BUP:NLX ratio study described in the previous chapter. A significantly greater increase in CPTOL was observed with one of the BUP:NLX ratios (12.5:1) compared to the effect of BUP alone. Also in line with the previous study, this enhancement occurred *without* a concomitant increase in adverse effects, and indeed with a *decrease* in respiratory depression compared to BUP alone.

CPTOL increased significantly in the early post-drug period in both BUP only and the BUP:NLX 12.5:1 ratio. However, the mean increase in CPTOL associated with the 12.5:1 ratio was significantly greater than that associated with BUP alone at several time points. The greatest difference between BUP only and the 12.5:1 ratio in percent change from baseline CPTOL occurred at the Washout 4 hr time point, with the 12.5:1 condition producing a mean increase from baseline of 56% compared to the 22% associated with BUP only (a difference of 34% of baseline CPTOL, p=0.046). This difference is of a similar magnitude to that associated with the BUP:NLX 15:1 ratio described in the previous chapter, which produced an increase of 37% of baseline CPTOL above the effect associated with BUP only at the same time point.

The BUP:NLX 10:1 ratio also produced a marked mean increase in CPTOL at the Post-bup time point (55%); however, this change was not significantly different from the effect of BUP only (18%) due to the large degree of variability associated with the ratio.

Importantly, the enhancement of CP antinociception occurred without a simultaneous increase in adverse effects. A significant decrease in breaths per minute was evident over the first three time points (Baseline, Post-bup and Washout 1 hr) in the BUP only and BUP:NLX 5:1 ratio. Moreover, significantly *greater* respiratory depression was observed with the BUP only condition than with *any* of the BUP:NLX ratio conditions. These significant differences occurred early in the post-infusion period. At the Post-bup time point the percent decrease from baseline breaths per minute associated with BUP only (15% decrease) was significantly greater than was observed for the BUP:NLX 5:1 (5% decrease), 10:1 (no decrease) and 12.5:1 (3% decrease) ratios. A significantly greater decrease in arterial oxygen saturation was also observed for the BUP only condition compared to the 12.5:1 ratio (p=0.012).

There was no greater incidence of adverse effects, including nausea and sedation, in the BUP:NLX ratio conditions compared to BUP only. Consistent with the previous study (see 5.8), each subject generally experienced a similar side effect profile during each condition. The greatest prevalence of nausea occurred in the BUP only condition (29%), while no subjects experienced nausea in the 10:1 or 12.5:1 ratio conditions. The prevalence and severity of sedation was similar across all drug conditions.

As described previously (see section 5.8), these results are consistent with research demonstrating a reduction in opioid adverse effects with the administration of ultra-low dose antagonist (Korbon et al. 1983; Rawal et al. 1986; Gueneron et al. 1988; Cepeda et al. 2004) or enhanced analgesia with either no increase or a reduction in side effects (Gan et al. 1997; Gear et al. 2000; Cruciani et al. 2003; Schmidt et al. 2003).

As a significant antinociceptive effect of the BUP:NLX 15:1 ratio compared to BUP only was observed close to the end of the 6-hour monitored washout period in the previous study, the current experiment was extended to 10 hours post-infusion to determine the duration of antinociceptive effect. Even at 9 and 10 hours post-infusion, the 12.5:1 BUP:NLX ratio was associated with significantly greater antinociception than the BUP only condition. This may be considered to support findings from the previous study (Chapter 5) and earlier reports (Levine et al. 1988; Gear et al. 2003; Schmidt et al. 2003), of prolonged analgesia with the addition of ultra-low dose antagonist to agonist treatment.

However, the reason for the lack of significant effect (compared to BUP only) at the Washout 6hr, 7hr and 8hr time points while a significant difference was observed at both 9hr and 10hr time points is unclear. The lack of significant effect at these time points may be attributed in part to greater variation in response. One potential explanation may be that subjects were experiencing what is commonly referred to as the "post-lunch dip", that is, an increased level of sleepiness in the early afternoon (Carskadon and Dement 1992). While level of sleepiness has not directly been associated with changes in pain response, poor sleep and sleep deprivation have been associated with heightened pain sensitivity (Hakki Onen et al. 2001; Onen et al. 2001). However, it is unlikely that this or other environmental or physiological factors caused the apparent decline in antinociception over these time points as this effect was not observed in the other drug conditions, nor was it observed among subjects receiving saline in the dose-ranging study (see 4.10.3.2). Furthermore, circadian rythyms have not been reported to impact upon response to experimental pain (Koltyn et al. 1999).

Of greater interest, perhaps, is the reason for the *increase* in antinociception observed at the Washout 9hr time point. Given that the mechanism for enhanced analgesia with opioid

agonist:antagonist combinations is not well understood (discussed in detail below), it is difficult to speculate why the enhanced antinociception in the current study is apparent up to 10 hours post-drug administration, particularly when the duration of action of NLX is approximately one hour, and BUP in the comparatively higher doses used for analgesia (0.3 – 0.6 mg) is typically required 6-8 hourly. A better understanding of the mechanisms involved in enhanced antinociception with opioid agonist:antagonist combinations will provide greater insight into the time course and dose response for BUP combined with NLX. It would, however, be useful for future investigations of these drug combinations to extend the monitoring period beyond 10 hours in order to define the duration of effect.

Findings from both ratio studies described are in line with earlier reports (Gear et al. 2003; Cougnon-Aptel et al. unpublished) that dose ratio is critical in observing the antinociceptive potentiation in agonist:antagonist combinations. In the current study, BUP:NLX in a 12.5:1 ratio was associated with significantly greater CP antinociception than the 5:1 ratio. This highlights the importance of dose ratio in achieving antinociceptive potentiation, particularly as the difference in the dose of NLX administered for each of these ratios would have been small (a 70 kg subject would have been administered 7 μg NLX in the 5:1 ratio compared to 2.8 μg NLX administered in the 12.5:1 ratio). The results of the ratio studies described suggest that even a minor change in BUP:NLX ratio will impact upon the effect observed (Figure 6-14). These findings further account for the failure by several clinical investigations of opioid agonist:antagonist analgesia to observe an enhanced effect with the combination. This again underscores the advantages of assessing this combination initially in an experimental pain model to define the effective dose ratio prior to testing in a clinical pain population (see 1.9.3), but also has implications for the clinical use of these combinations in terms of achieving the required dose ratio to manage pain. These and other implications are discussed below.

In the current study, a significant increase in CPTHR was observed in the BUP only condition over the Baseline, Post-bup and Washout 1hr time points. While non-significant due to the large variation in response, increases in CPTHR were observed in all ratios across the testing day with the lowest mean values occurring at baseline. The greater effect of CPTHR observed in the current study compared to the previous study may be attributed to subjects in the current study being more responsive to the antinociceptive effects of opioids. Indeed, the CPTOL response in the current study demonstrated a response curve similar to that of the previous study, but in the current study the magnitude of response was in the order of 20 to 40% greater than baseline in the early post-drug period compared with the increase of 15% and lower observed in the previous study. Consistent with the previous study, however, no significant differences in CPTHR were observed between BUP only and the BUP:NLX ratios in CPTHR.

The findings from both BUP:NLX ratio studies described indicate that significantly enhanced antinociception may be achieved with the combination of BUP and ultra-low dose NLX, and that the adverse side effects that often limit the use of opioids are either unchanged or reduced. The mechanisms that underlie antinociceptive potentiation with agonist:antagonist combinations have not yet been elucidated. Based on our current understanding of opioid receptor binding and transduction, it is possible to describe a number of potential mechanisms that may be involved. However, it should be noted that there is currently a paucity of evidence to adequately explain all aspects of the observed potentiation, and thus the following discussion of potential mechanisms is highly speculative.

Given the evidence for the biphasic effect of NLX described previously (specifically, that it may be antinociceptive in low doses and pronociceptive in high doses, see section

1.9.2.2), it should initially be considered whether the increased effect represents the additive effect of BUP and NLX antinociception, rather than an interaction between them. The study by Cougnon-Aptel and colleagues revealed that the BUP and NLX administered together produced greater antinociception than the combined effects of the two drugs administered individually. Furthermore, the results suggest that the effect of NLX is influenced by the dose of BUP, as the antinociceptive potentiation in that study was observed only when the BUP dose alone was *not* antinociceptive. Based on these results, it may be considered that the increase in antinociception is not simply an additive effect of BUP and NLX, and thus a NLX-only condition was not included in the current BUP:NLX ratio studies.

As detailed in section 1.9.2.4, the primary mechanism that has been proposed to account for the enhanced antinociception observed with morphine/antagonist combinations is the bimodal opioid receptor model. Classically, opioid agonists exert their effects by binding with inhibitory PTX-sensitive G_i/G_o-coupled opioid receptors. In low doses, it has been demonstrated that opioid agonists bind with a divergent, excitatory G_s-coupled mode of receptor, the anti-analgesic effects of which are typically masked by the inhibitory analgesic effects observed at higher, therapeutic doses (Crain and Shen 2000). When present in extremely low (pM) concentrations, agents such as NLX and NTX have been shown to selectively antagonise excitatory, but not inhibitory, opioid-receptor mediated functions in dorsal root ganglion (Crain and Shen 2000). The bimodal opioid receptor model postulates that the enhanced antinociception associated with low or ultra-low doses of an opioid antagonist is a result of this selective blockade of excitatory, anti-analgesic opioid effects.

Given the unique pharmacology of BUP, it is interesting to speculate whether the mechanisms that underlie BUP:antagonist antinociceptive potentiation are the same as those underlying morphine or other agonist:antagonist potentiation. For example, a potential limitation of the bimodal opioid receptor model in explaining the potentiation observed with the BUP:NLX combination is that BUP has been reported to interact preferentially with PTX-*insensitive* G-protein coupled receptors, only interacting with PTX-sensitive proteins at higher doses (Wheeler-Aceto and Cowan 1991).

An interesting finding from the animal study by Cougnon-Aptel and colleagues was that BUP:NLX potentiation was only observed when BUP itself did not produce significant antinociception. Potentiation with sub-maximal agonist doses has not been reported with other μ-opioid receptor agonists that have been investigated in combination with an antagonist; however, it should also be noted that these combinations have not been rigorously tested over a range of doses and ratios. It has been reported that the opioid analgesic nalbuphine, which is thought to produce analgesia primarily through interaction with the κ-receptor, produces marked analgesia when administered in low doses and combined with 0.4 mg naloxone (Gear et al. 2003).

ORL1 receptor activation is thought to compromise BUP analgesia, and animal evidence suggests that BUP's bell-shaped dose-response curve may result from interaction with this receptor. Lutfy and colleagues recently reported that the administration of an ORL1 receptor antagonist both eliminated BUP's bell-shaped curve and enhanced antinociception using the tail-flick assay (Lutfy et al. 2003). It would seem plausible then to contend that NLX antagonises interaction with the ORL1 receptor, particularly as pretreatment with NLX symmetrically shifts the BUP dose-response curve to the right (Dum and Herz 1981), suggesting that NLX has the same binding affinity as BUP for the receptor or effector

system producing the bell-shaped curve. However, NLX has not demonstrated any binding affinity for the ORL1 receptor. Furthermore, the involvement of ORL1 receptor binding in the BUP dose-response in humans has not been clearly demonstrated and, as described previously (see 4.2.1), animal studies have indicated that BUP has a very low affinity for the ORL1 receptor (Huang et al. 2001) and is unlikely to interact with this receptor type at the lower concentrations required for analgesia.

Another possible mechanism of potentiation may involve BUP's interaction with the κ-receptor. While BUP has classically been reported to antagonise κ-receptor activation (Cowan 1995), other investigations have reported low-efficacy partial agonist activity at this receptor type (Zhu et al. 1997; Huang et al. 2001). The effect of κ-receptor activation on nociception is also unclear. Kappa-receptor agonists can have analgesic action by inhibiting glutamate synaptic currents (Ackley et al. 2001), and indeed there are several opioids that are thought to produce analgesia primarily via agonist interaction with κ-receptors (nalbuphine, pentazocine and butorphanol) (Gutstein and Akil 2001). As described previously, while it is generally considered that BUP's analgesic effects are mediated by the μ receptor, one report also contends that agonist activity at κ3 receptors contributes to the drug's analgesic effects (Pick et al. 1997). However, κ-receptor activation can antagonise μ-receptor mediated effects (Bie and Pan 2003), and κ-receptor activity has been associated with the development of hyperalgesia (Wu et al. 1983). Gear and colleagues have proposed that the κ-receptor agonist nalbuphine may exert both analgesic and anti-analgesic effects, and that the enhanced analgesia observed with the combination of low-dose nalbuphine and naloxone may result from selective antagonism by naloxone of the anti-analgesic effects (Gear et al. 2003). It is possible that ultra-low dose NLX antagonises anti-analgesic κ-receptor mediated effects produced by BUP or endogenous ligand interaction with this receptor.

Notwithstanding, the possible mechanisms described above do not readily explain why the potentiation observed in the Cougnon-Aptel study occurred only when BUP itself was not antinociceptive. There is evidence that receptor interaction of BUP and other opioid ligands can be dose-dependent. For example, as described, BUP only binds with PTX-sensitive G-protein coupled receptors at higher doses (Wheeler-Aceto and Cowan 1991). Moreover, if the trough observed in the BUP dose-response curve is related to ORL1 receptor interaction as proposed by Lutfy and colleagues, it follows that this interaction is dominant or only occurs at higher doses. It is conceivable then, that the nature of BUP's interaction with the κ-receptor is dose-dependent, and that NLX selectively antagonises anti-analgesic effects of BUP κ-receptor activation, thus enhancing antinociception. Interestingly, the Cougnon-Aptel study also investigated the effect of BUP combined with naltrexone and found that this combination was not as effective as BUP combined with NLX. This may provide support for the notion that κ-receptor interaction underlies the potentiation, as NLX binds with high potency to both μ- and κ-receptors, while naltrexone has a greater binding affinity for μ- than κ-receptors.

Previous reports have suggested that modulation of opioid effects by addition of ultra-low dose antagonist may be selective, with some opioid effects being enhanced or reduced while others are unaffected (for example, Gan et al. 1997, Cepeda et al. 2004, Cruciani et al. 2003). Indeed, findings from both ratio studies described herein reveal that antinociception to CP stimulation can be significantly enhanced with the administration of BUP:NLX without a simultaneous increase in adverse effects. None of the potential mechanisms described account for the selective potentiation of antinociception without potentiation of other measured effects.

It has been proposed that the antagonism of adverse opioid effects with ultra-low dose antagonist without antagonism of analgesia relates to different concentration-response curves for antagonism of different opioid effects (that is, a lesser concentration of antagonist is required to antagonise adverse effects than is required to antagonise analgesia) (Gan et al. 1997). Thus, both the excitatory effects of Gs-coupled receptor binding and the adverse opioid effects are antagonised by (ultra)-low antagonist concentration, resulting in potent analgesia as well as a reduction in adverse effects.

An alternative explanation may be that the mechanism underlying selective potentiation of antinociception is brain-region specific, occurring only in areas of the brain involved in pain processing. Indeed *in vitro* opioid binding to excitatory Gs-protein coupled receptors (the basis for the bimodal opioid receptor model) has been investigated only on nociceptive types of dorsal root ganglion neurones. Neuromodulation specific to certain brain regions has previously been demonstrated in opioid systems. For example, as described previously, upregulation of opioid systems upon cessation of chronic opioid antagonist administration is brain region specific (Marley et al. 1995; Unterwald et al. 1998). In addition, opioid system downregulation occurring with chronic exposure to opioids has also been reported to be brain region specific (Bhargava and Gulati 1990). These examples demonstrate that receptor activity and adaptations are not homogeneous throughout the brain and spinal cord, thus it is possible that the mechanism underlying antinociceptive potentiation is specific to the brain regions involved in pain transmission and modulation.

In summary, the current study reveals significantly greater antinociception to CP stimulation with the combination of BUP and NLX in a 12.5:1 ratio compared to the same dose of BUP alone. This BUP:NLX ratio was associated with an equivalent incidence of

adverse effects, and significantly *less* respiratory depression, than the BUP alone. These findings support the results of the first ratio study, which together provide evidence for the potential clinical utility of this combination.

6.5. Limitations associated with the BUP studies

There are several limitations associated with the BUP studies described that must be addressed in consideration of the findings. Many of these limitations pertain not only to the study described in the current chapter, but to all BUP studies detailed in this thesis (Chapters 4, 5 and 6).

Numerous practical limitations are encountered in conducting research of this nature. As described previously (see 3.6), pain is a subjective and private experience, which may be modulated by many variables, and for which there is no reliable, objective marker (Kumar et al. 2002). Determining the level of pain experienced by an experimental subject or by a clinical pain patient is, for the most part, dependent on the report of the individual in pain. While the research environment and procedures are standardised, there are numerous factors that are difficult or impossible to control. For example, it was not possible to control subjects' amount or quality of sleep the night before testing days. As mentioned previously, poor sleep or sleep deprivation have been associated with reduced tolerance of pain (Hakki Onen et al. 2001; Onen et al. 2001), and a poor sleep the night before would also be likely to contribute to the level of sedation during testing days, which were lengthy and featured prolonged periods of inactivity. This is of particular importance given that sedation is monitored as an adverse opioid effect and was one of the outcome measures. One approach to controlling this potentially confounding variable would be to have subjects report the number of hours and rate the quality of their sleep on the previous night.

While subjects were instructed to eat a light breakfast before each testing day, they often reported having no breakfast due to the testing days commencing at such an early hour. Similarly, there was no control over food intake throughout the testing days. Previous research has investigated a link between palatable food intake and activation of endogenous opioid systems, and there have been reports that the intake of palatable foods can enhance morphine antinociception in rats (Kanarek et al. 1997; Kanarek and Homoleski 2000). While this has not been demonstrated in humans, food intake the morning of and during testing days may have impacted upon nociceptive test performance. To address this issue, subjects may be asked to fast overnight prior to each testing day and food intake over the course of each day could be standardised. Similarly, while caffeine consumption was minimised during each testing day (typically a coffee or tea was offered during the morning and again during the afternoon), consumption of these beverages by each subject was not recorded. While the normative study described in Chapter 3 found no significant impact of average daily caffeine intake on CP or ES test performance, there have been reports that pain response may be altered in the period immediately following intake of a large dose (250 mg) of caffeine (Keogh and Witt 2001).

Another potential source of variation was subjects' level of activity during each testing day. Testing days were long (in the second ratio study subjects attended for approximately 12 hours on each day), with relatively long intervals (approximately 50 minutes) of inactivity between assessment time points. Between time points subjects were permitted to read magazines, books, or other material (including study, as many participants were university students), and were also permitted to engage in other sedentary activities such as needlework. Watching television and receiving visitors was not permitted during testing days. Some subjects maintained alertness and interest by engaging in activities of personal relevance (such as bringing their own book to read or studying for exams), while others

would fluctuate between reading magazines, engaging research staff in conversation and sleeping. Level and type of activity varied not only between subjects, but within subjects across testing days. These varying levels of activity, and thus alertness and boredom, may also have potentially introduced extra variation in nociceptive test performance and level of alertness. Similar to the problem of food intake, one approach would have been to standardise level and type of activity between time points, for example tasks could be designed to engage subjects' attention during the inter-testing periods. One practical limitation that may be problematic in standardising these factors however is subject retention. Prohibiting subjects from engaging in activities of personal interest throughout the numerous and lengthy inter-testing periods would most likely compromise recruitment and increase the rate of subject withdrawal.

A further potential source of error that must be considered in studies such as these is a subject response bias. It has previously been demonstrated that research subjects may alter their response or performance on experimental measures in order to please the investigator or comply with perceived norms or expectations. This has been reported to occur primarily in face-to-face research situations (for example, Chestnutt et al. 2004). This response bias may be of particular concern in regard to the ratio studies, for which numerous lengthy data collection sessions were conducted with each subject and a rapport is established between the investigator/research staff and the subject. This may have impacted upon subjects' motivation in the conduct of the nociceptive tests, possibly by enhancing the desire to be positively regarded (i.e. endure the tests for longer), or conversely by making the subject feel more comfortable and less concerned about maintaining a stoic impression. However, it is considered that this response bias would not have had a marked impact on results due to the randomisation of drug conditions.

A final limitation to consider is the method by which subjective and adverse effects were monitored throughout the testing days. While sedation and nausea scales were used to rate the occurrence and severity of these effects, other subjective effects were, in general, recorded as either present or not. It would have been preferable to have included a questionnaire requiring subjects at each time point to rate the subjective and adverse effects experienced since the previous time point. Such an approach would have facilitated better quantification of the incidence and severity of subjective effects.

7. Conclusions, clinical implications and future directions

The key aim of this thesis was to investigate the potential for the addition of ultra-low doses of the opioid antagonist naloxone to enhance the antinociceptive effect of buprenorphine without increasing the incidence or severity of adverse effects in healthy humans. In order to achieve this aim a normative study of the experimental nociceptive tests to be used, the cold pressor and electrical stimulation tests, was initially conducted. The purpose of this normative study was three-fold: (1) to establish normative data for the tests; (2) to establish upper and lower limits of response for subject inclusion in subsequent studies; and (3) to determine the factors that contributed to inter-individual variability in test performance.

Subsequent to the normative study, a dose-ranging study of IV buprenorphine in healthy volunteers was conducted in order to determine whether these nociceptive tests were sensitive to IV doses of buprenorphine, and to select an appropriate dose for use in the subsequent studies.

Two studies investigating the combined effects in healthy volunteers of IV buprenorphine (0.5 μg/kg over 30 minutes) and ultra-low dose naloxone were then carried out. The first study assessed the antinociceptive, physiological and subjective effects of buprenorphine alone and buprenorphine administered simultaneously with naloxone in buprenorphine: naloxone ratios of 15:1, 20:1 and 25:1. The second study replicated the procedures, but with lower ratios (5:1, 10:1 and 12.5:1).

The following discussion will summarise conclusions from each study, outline the clinical implications of the findings and propose directions for future research.

7.1. Overview of findings

7.1.1. Normative data and inter-individual variability in experimental pain testing

While the cold pressor and electrical stimulation tests are commonly used techniques for experimental nociceptive stimulation, the methods associated with each of these techniques had not been standardised. This limited both our understanding of the normal response to these techniques and the potential for meaningful comparisons between findings from different investigative groups (Eccleston 1995). The normative study of the cold pressor and electrical stimulation tests described in Chapter 3 has defined normal response to test performance according to the methods used by our research group. This is the first normative study of the electrical stimulation test, and while a normative model of the cold pressor test had previously been published (Walsh et al. 1989), this study had used an earlier cold pressor technique that lacked many of the features that have since been incorporated in order to reduce response variability and reduce mean pain tolerance (Garcia de Jalon et al. 1985; Eckhardt et al. 1998; Doverty et al. 2001).

Due to the maximum limit associated with the cold pressor and electrical stimulation tests it was also imperative that the upper and lower limits of test performance be identified for subject inclusion in the subsequent buprenorphine studies. When evaluating an analgesic agent, a subject's baseline response to a nociceptive test must be sufficiently below the maximum limit on the test to allow a significant increase to be observed. Reducing mean pain tolerance may be achieved by modification of the test method, as has been described for the tests used in the current investigations (see section 3.6), and by excluding subjects whose baseline response is too close to the cut-off point. As the latter approach may induce a bias in subject selection, an alternative is to exclude all potential subjects at the extreme high or extreme low ends of the distributions. This approach has been employed

previously (Eckhardt et al. 1998); however, the range in baseline response for subject inclusion was not based on normative data. The normative study described herein facilitated the identification of upper and lower boundaries of baseline response for inclusion in the subsequent buprenorphine studies described. Upper and lower boundaries of baseline cold pressor tolerance were established at 21 seconds and 85 seconds for subject inclusion in the three subsequent buprenorphine studies described. Findings from these buprenorphine studies suggest that the limits established were appropriate, with censored data due to the maximum cold pressor limit being observed at few time points over the course of testing. Furthermore, significant cold pressor antinociception was observed in all studies indicating that the upper limit of baseline response for subject inclusion (85 seconds) was sufficiently below the test cut-off to observe a statistically significant effect in a cohort of as few as 6 individuals.

A further important issue in experimental pain induction is that the sensitivity of a test in detecting a drug effect can be compromised by inter- and intra-individual variation in pain response. While the test method may be adapted to reduce this variability, there are numerous factors that have been reported to contribute to variability in test performance between individuals. Such factors have included, but not been limited to, sex, ethnicity, age and psychological or cognitive parameters. Reports of the impact of each of these variables on test performance have varied widely, which may be attributed to the wide range of nociceptive tests used, differences in outcome measure and subject group, and the potential source of variability under investigation. Furthermore, these reports have typically focused on the contribution of only one or a small number of variables to test performance. Consequently, there was a poor understanding of the relative contribution of these variables to experimentally induced pain, and little indication of the degree to which these factors should be controlled in the design of experimental pain studies. The

normative study described assessed the contribution of a large range of variables (11 in cold pressor performance and 10 in electrical stimulation performance) to variability in test performance within the one cohort of subjects. Using Cox proportional hazards, fear of pain and sex were identified as significant contributors to variability in cold pressor performance, and fear of pain and the interaction between sex and ethnicity were found to contribute significantly to electrical stimulation performance.

The findings of this study provide data on the response of healthy volunteers to the nociceptive tests, providing a basis for comparison with future data from different subject groups and populations. These data will also assist in the design of future studies with these techniques, firstly by allowing baseline test performance limits for subject selection to be established, and secondly by establishing which variables may be controlled to reduce inter-subject variability in test performance.

7.1.2. Buprenorphine in experimental pain

While the antinociceptive effects of buprenorphine have been well-characterised in animal models of experimental noxious stimulation (for example, Skingle and Tyers 1980) and the analgesic properties of the agent are well recognised clinically, no reported studies had evaluated buprenorphine in a human experimental pain paradigm. In contrast, the antinociceptive actions of many other agents, including opioids and NSAIDs, have been evaluated with a range of nociceptive tests, including the cold pressor and electrical stimulation tests.

The dose-ranging study assessed the antinociceptive effects of IV buprenorphine in a cumulative dosing schedule in a cohort of healthy volunteers using the cold pressor and

electrical stimulation tests. Findings demonstrated that buprenorphine is associated with significant antinociception in both cold pressor and electrical stimulation.

Peak increase in mean tolerance from baseline in this study was 37.6% (±10.3) for cold pressor tolerance, and 18.3(±1.6)% for electrical stimulation tolerance). In the current study buprenorphine was administered in doses lower than would normally be used for pain management (300-600 μg by slow IV injection every 6-8 hours). Subjects received a total of 1.875 μg/kg over a 2-hour period, equating to a total dose in a 70 kg subject of 131.25 μg. Using the same cold pressor method, a plasma morphine concentration of 11 ng/ml increased mean cold pressor tolerance by approximately 31%, while a plasma morphine concentration of 33 ng/ml increased mean cold pressor tolerance by approximately 97% (Athanasos et al. 2002). A plasma morphine concentration of 15 ng/ml is required for minimum effective post-operative pain relief (Dahlstrom et al. 1982; Gourlay et al. 1986), while a concentration of 50 ng/ml is sufficient to provide relief from moderate to severe post-operative pain (Berkowitz et al. 1975). It may therefore be concluded that buprenorphine is consistent with other opioids in producing significant antinociception to cold and electrical pain in healthy volunteers.

7.1.3. Buprenorphine combined with ultra-low dose naloxone

7.1.3.1. "Proof of concept"

The most significant contribution of this thesis is the demonstration that buprenorphine combined with ultra-low dose naloxone can significantly enhance cold pressor antinociception compared to buprenorphine alone, with a simultaneous reduction in respiratory depression and no greater incidence of other adverse effects. Previous human studies of agonist:ultra-low dose antagonist combinations have produced inconsistent

findings (Levine et al. 1988; Gear et al. 2000; Cepeda et al. 2002; Cruciani et al. 2003; Cepeda et al. 2004). These inconsistencies may be related to differences in trial methodology, in particular to a failure to study a range of agonist:antagonist dose ratios. Previous reports suggest that the dose ratio is critical to observe enhanced analgesia/antinociception (Gear et al. 2003; Cougnon-Aptel et al. unpublished), an observation that is supported by the findings of the investigations described herein. The current studies represent the first reported investigation of buprenorphine combined with ultra-low doses of an opioid antagonist, and the first investigation of agonist:antagonist combinations in human experimental pain. By investigating the buprenorphine:naloxone combination in an experimental pain paradigm with healthy, pain-free volunteers, intra-individual response to a range of buprenorphine:naloxone ratios compared to buprenorphine alone could be assessed, and a significant advantage of buprenorphine:naloxone in two ratios (12.5:1 and 15:1) has been demonstrated. The 15:1 and 12.5:1 ratios were observed to be approximately equivalent, maximally increasing cold pressor antinociception by a further 37% and 34% of baseline response, respectively, above the effect associated with buprenorphine alone.

The electrical stimulation test was used in the first ratio study as a secondary nociceptive test, and as a comparison of the effect of buprenorphine on a tonic (cold pressor) versus a phasic (electrical stimulation) test. Previous reports have demonstrated that opioid antinociception can be observed in electrical stimulation response, however the cold pressor test has been found to be a more sensitive test for opioid effect (Luginbuhl et al. 2001; Athanasos et al. 2002). While the dose-ranging study demonstrated that significant changes in electrical stimulation response may be observed with buprenorphine, the magnitude of this effect was not as great as was associated with the cold pressor test. Similarly, buprenorphine alone or in any buprenorphine:naloxone ratio was not associated

with a significant effect on electrical stimulation response in the first ratio study (Chapter 5), and was therefore not included in the subsequent study.

7.1.3.2. Clinical implications of the findings

The results of the two buprenorphine:naloxone ratio studies have demonstrated that this combination can produce a statistically significant increase in antinociception in humans. While proof of concept was the primary objective of the current studies the clinical relevance of the findings is an important consideration. It is, however, difficult to quantify how meaningful is a given percent increase in tolerance to experimental noxious stimulation. A recent study of 700 post-operative pain patients reported that a 20% change in pain score on a numeric rating scale was indicative of minimal improvement in analgesia, a 35% change indicative of much improvement, and a 45% change indicative of very much improvement (Cepeda et al. 2003). It has further been reported that the percent change in pain ratings following a given dose of fentanyl is equivalent in clinical and experimental pain (Price et al. 1986). While it may be proposed, then, that a mean increase in cold pressor tolerance of about 35% *above the effect of buprenorphine alone* observed in both the 15:1 and 12.5:1 buprenorphine:naloxone ratios is indicative of a clinically significant improvement, it is unclear to what extent a given percentage decrease in pain rating relates to a given percentage increase in cold pressor pain tolerance.

An alternative approach is to consider the percent change in cold pressor tolerance typically associated with therapeutic doses of the prototypic opioid analgesic, morphine. A plasma morphine concentration of 15 ng/ml is required for minimum effective post-operative pain relief (Dahlstrom et al. 1982; Gourlay et al. 1986). Using the same cold pressor method, a mean plasma morphine concentration just below this (11 ng/ml)

increased mean tolerance by approximately 31% (Doverty et al. 2001), while a mean plasma morphine concentration of 23 ng/ml increased mean tolerance by 52% (Athanasos et al. 2002). Based on this evidence then, it may be argued that an increase from baseline cold pressor tolerance of approximately 35% beyond the effect of buprenorphine alone represents a clinically significant improvement.

Notwithstanding, the key outcome from these ratio studies has been the demonstration that this combination can potentiate antinociception in humans without a simultaneous increase in adverse effects. In order to clarify the clinical significance of the magnitude of change, this combination should undergo testing in a clinical pain setting.

7.1.3.3. Directions for future research

The findings from the current buprenorphine:naloxone ratio studies indicate that this combination warrants further investigation. As described, further elucidation of the mechanisms involved in opioid agonist:antagonist analgesia would contribute significantly to work in the area. In addition to this objective, however, based on the results presented herein there are a number of experimental and clinical research directions that would complement and expand our understanding of the effects of this drug combination.

The initial steps towards developing our understanding of buprenorphine and ultra-low dose naloxone would involve further experimental work with healthy volunteers. The current studies have established a dose-ratio response to the cold pressor test over a range of buprenorphine:naloxone ratios (5:1–25:1). This ratio response forms a bell-shaped curve, with the optimal ratios of those tested being 12.5:1 and 15:1. However, it is interesting to note that at the 25:1 ratio an increase in response is again observed, which may be the beginning of a second increasing arm of the ratio response curve. It would be

valuable, then, to initially replicate the ratio studies over a higher range of buprenorphine:naloxone ratios in order to define the full range of effective buprenorphine:naloxone ratios in experimental pain. This would further guide the selection of dose ratios to be tested clinically.

It would also be useful to assess the most effective buprenorphine:naloxone ratios with a lower dose of buprenorphine. A submaximal buprenorphine dose was selected based on the findings of Cougnel-Aptel and colleagues (unpublished) that enhanced antinociception with this combination occurred only when the buprenorphine alone was *not* antinociceptive. The 0.5 μg/kg dose of buprenorphine was selected on the basis of the dose-ranging study (see 4.9.4). This dose alone did produce significant antinociception among one cohort of subjects (ratio study 2, see 6.2.4.1), suggesting that it would be worthwhile investigating the combination using a lower dose of buprenorphine.

While the current studies have demonstrated that the combination of buprenorphine and naloxone can enhance cold pressor antinociception, an interesting area of investigation for future studies would be to determine the factors that contribute to inter-individual variation in response to this drug combination. This would be of particularly interest in light of growing evidence of the role of biological factors, such as sex (Fillingim and Gear 2004) and baseline sensitivity to nociceptive stimuli (Elmer et al. 1998; Hutchinson et al. 2004), in modulating response to opioids.

Following these proposed experimental studies, the most logical and appropriate action would be to trial the most effective dose ratios in a cohort of clinical pain patients. This could initially be undertaken with post-operative pain patients, with patients randomised to receive buprenorphine alone according to therapeutic guidelines or the

buprenorphine:naloxone combination in one of several selected ratios. Treatment outcome could be measured by factors such as (1) need for/time to rescue medication, (2) patient pain rating scales (Farrar et al. 2000), and (3) incidence and severity of side effects (observer and patient rated scales, need for medication to alleviate side-effects).

7.2. Summary

In conclusion, the major contributions and findings of this thesis are as follows:

The normative distributions of 2 commonly used nociceptive tests have been established with standardized methods, allowing comparison with future work in other populations. Importantly, several factors have been identified to contribute or not contribute significantly to inter-individual variability in test performance, and may thus be appropriately controlled for in the design of experimental pain studies with healthy volunteers.

The effect of IV buprenorphine on performance on these 2 tests has been established, demonstrating that buprenorphine may be considered to produce similar effects on these tests as other opioids. The cold pressor test, in particular, has been demonstrated to be a sensitive assay for the antinociceptive effects of buprenorphine, though considerable variability is evident in response to the cold pressor test relative to the electrical stimulation test.

The most significant finding from this thesis is that the combination of buprenorphine and ultra-low dose naloxone can significantly enhance and prolong cold pressor

antinociception with no greater prevalence of adverse effects, and in the case of respiratory depression a significant decrease, compared to buprenorphine alone.

These findings contribute significantly to the body of work in this area, being the first investigation of buprenorphine in combination with ultra-low or low-dose opioid antagonist, the first investigation in a model of experimental pain, and the first to demonstrate enhanced antinociception and a clear simultaneous decrease in respiratory depression without the potentially confounding variables encountered in research with clinical pain.

By enhancing analgesia and reducing adverse effects, the combination of buprenorphine and ultra-low dose antagonist has the potential to overcome barriers that limit the use of opioids in pain management. These findings may lead to improved patient comfort and quality of life, and reduce the cost and impact of pain on the individual and the community.

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