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GENE EXPRESSION OF THE RENIN-ANGIOTENSIN SYSTEM IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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ABSTRACT

The principal aim of the studies described in this thesis was to examine the gene expression of the renin-angiotensin system (RAS) in the spontaneously hypertensive rat (SHR) and the normotensive Wistar-Kyoto rat (WKY).

(1) The technique of *in situ* hybridisation was used in order to determine the cellular localisation of the gene expression of the RAS in kidney and liver in the pre-hypertensive and established phases of hypertension and to determine whether the sites of expression of the RAS in vascular tissue were similar in hypertensive and normotensive rats. The developmental regulation of kidney renin gene expression in the SHR was similar to that seen in the WKY. No differences in the cellular localisation of angiotensinogen mRNA in liver was noted at any age in both SHR and WKY. ACE mRNA expression in liver and kidney was also similar it the two rat strains. In the mesenteric vascular bed of both SHR and WKY, angiotensin converting enzyme (ACE) was expressed in the endothelium and smooth muscle layer of the blood vessels as well as in the surrounding adipose tissue. Angiotensinogen expression was noted in the smooth muscle and surrounding adipose tissue, while renin mRNA was inconsistently demonstrated in the smooth muscle layer only. ACE mRNA was also demonstrated in the adipocytes of human subcutaneous and extra-peritoneal adipose tissue.

(2) The mRNA expression for renin, angiotensinogen and ACE was determined in the kidneys and livers from SHR and WKY during chronic treatment with captopril and following its withdrawal. Chronic captopril treatment was associated with a dramatic rise in renin mRNA in the kidney and an elevation in mRNA for ACE in the liver. The release from captopril treatment was associated with a reversal of the increase in kidney renin mRNA but no reversal of the sustained elevation of ACE mRNA in the liver. *In situ*

hybridisation revealed a localisation of renin to the area of the juxtaglomerular apparatus in the kidneys from untreated animals, but recruitment of vascular sites of renin expression in kidneys from captopril-treated animals. In kidneys from released animals, renin mRNA expression was once again confined to the juxtaglomerular apparatus. ACE mRNA was expressed in hepatocytes throughout the livers from animals in all treatment groups. The results highlight a differential effect of captopril withdrawal upon the gene expression of the components of the renin-angiotensin system in kidney and liver.

(3) Neonatal sympathectomy did not influence the gene expression of the RAS components in the kidneys, livers and the mesenteric vascular bed from adult SHR and WKY. ACE activity in plasma, kidney, liver, aorta, lung, skeletal muscle and brain from adult animals was also not affected by neonatal sympathectomy. The accumulation of AII into aorta, mesenteric artery and its branches, kidney and skeletal muscle was not influenced by innervation, hyperinnervation or sympathectomy which indicates that, unlike adrenaline, the facilitation of sympathetic transmission by AII does not involve a process of uptake of the peptide into sympathetic nerves.

(4) The ACE gene was expressed at a similar level in livers and kidneys from both SHR and WKY, despite the fact that the corresponding ACE activity was at least an order of magnitude lower in livers when compared with kidneys. The possibility that endogenous ACE inhibitory activity was present in the liver was investigated by examining the effect of liver homogenates on plasma ACE activity. The effect of hepatotoxicity on plasma ACE activity was also studied, in order to determine whether the liver was a source of circulating ACE. Liver homogenates from both WKY and SHR, when added to plasma, significantly decreased the measurable ACE activity in it by 41% and 50% respectively. When liver homogenates were pre-incubated with the sulphydryl-blocking drug 5,5'-dithiobis-(2-nitro benzoic acid) (DTNB, 5 mM), the inhibitory effect of the homogenate

on plasma ACE was significantly reduced. Plasma ACE activity was not influenced by carbon tetrachloride-induced hepatotoxicity in either rat strain. In contrast, there was a four-fold increase in liver ACE activity which was not associated with a significant change in the ACE mRNA levels or a significant change in the inhibitory activity of liver homogenates. The results indicate that hepatic ACE activity is subject to endogenous inhibition and suggest that there is an uncoupling of ACE gene expression and activity in the liver.

CHAPTER 4

THE EFFECT OF CHRONIC CAPTOPRIL TREATMENT AND ITS WITHDRAWAL ON THE GENE EXPRESSION OF THE RAS IN THE SHR

CHAPTER 5

THE EFFECT OF NEONATAL SYMPATHECTOMY ON THE RAS IN ADULT SHR