THE EXPRESSION AND FUNCTION

OF

STRETCH-ACTIVATED

2P-4TMD K^+ CHANNELS IN THE HEART

A THESIS SUBMITTED TO THE UNIVERSITY OF ADELAIDE AS THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY

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SUMMARY

2-Pore-4-Transmembrane domain (2-P-4-TMD) potassium channels are first cloned in mouse and have been shown to be expressed in a widely variety of animals. To the humans, 2P potassium channels are not only expressed in central nervous system but are also found in peripheral organs. 2P potassium channels are the members of KCNK gene family that control background potassium conductance for regulating resting membrane potential and cell excitability. Similar arrangement of conservative amino acid sequence (T-x-G-x-G) is shown on the pore structure, which contributes to a typical K^+-selective ionic propensity. Based on the unique physiological and pharmacological characteristics, TREK-1 (Twik-RElated K^+ channel; KCNK2), TREK-2 (KCNK10) and TRAAK (Twik-Related Arachidonic Acid-stimulated K^+ channel; KCNK4) are distinguished from other members of 2P potassium channels.

TREK-1, TREK-2 and TRAAK are sensitive to membrane stretch, pH, temperature and polyunsaturated fatty acids. TREK-1 is also activated by lysophospholipids and inhaled anaesthetics. The functions of TREK-1 are regulated by neurotransmitters and receptor-dependent signalling mechanisms, which include TREK-1 activity is enhanced by phosphorylating Ser-351 of C-terminus by a cGMP-activated protein kinase (PKG); TREK-1 is inactivated by phosphorylating Ser-300 and Ser-333 of C-terminus by protein kinase A (PKA) and C; The action of $G\alpha_s$-couple receptors pathway preventing TREK-1 activity is similar as PKA whereas inhibition of $G\alpha_q$-couple receptors to TREK-1 is similar as PKC. TREK-1 is also

largely inhibited by prostaglandin E_2 (PGE_2). In addition, TREK-1 is notably insensitive to typical potassium channel inhibitors such as tetraethylammonium (TEA), 4-aminopyridine (4-AP) and Ba^{2+}. Furthermore, TREK-1 is not inactivated by Gd^{3+}. TREK-1 knockout mouse shows that polyunsaturated fatty acid-activated neuroprotection is weakened in ischemia area and the sensitivity of TREK-1 to volatile anaesthetics is reduced. 3 splice variants have been identified for TREK-2 including Variant A (Va) (Genebank AF279098), Variant B (Vb) (AF385399) and Variant C (Vc) (AF385400). The coding areas of Va, Vb and Vc encode 539, 544 and 544 amino acids respectively. TREK-2 has 7 exons (as does TREK-1), and the splice variants arise because of alternative sequences in the first exon. However, distribution of TREK-2 in human tissues is different from TREK-1 and TRAAK. Va is mainly expressed in kidney and pancrease, and Vb is largely expressed in kidney and pancreas whereas Vc mainly in brain. Vb and Vc expressed in human embryonic kidney 293 cell line show similar characteristics as a single channel and alternative sensitivity to antagonist of PKC and PKA. In spite of sharing 78% sequence homology, the distribution of TREK-1 and TREK-2 in human brain is different.

With TREK-1 expressed in cardiomyocytes of mice and rat and TREK-1-like potassium currents found in human atrial tissue, these observations strongly indicate the existence of TREK-1, TREK-2 or TRAAK in human heart. To investigate the expression of TREK-1, TREK-2 and TRAAK in human, PCR, nested PCR and sequencing are essential to be used in the illustration of the homology of TREK channels in human heart and other TREK-expressed organs. Sequencing is applied to

decide whether TREK in human heart is identical to any published TREK sequence in Genebank. To TREK-1 and TRAAK, primer design focuses on showing at least one pore structure in sequencing. To TREK-2, primer design is concentrated on exhibiting the unique arrangement of exon 1 of each variant in sequencing. Due to the likely functions of TREK channels under different physiological and pathological conditions, Western blot, real-time PCR and immunohistochemistry are applied in the quantification and localization of TREK channels in normal and diseased human heart.

The results presented in this thesis show the existence of TREK-1, variant A and C of TREK-2 and TRAAK in human heart; the localization of TREK-1 and variants of TREK-2 on the membrane and in cytoplasmic areas of human cardiomyocyte; the notably high-level expression of TREK-1 in diseased human heart; the reverse expression of variant A and C of TREK-2 in normal and diseased human heart. These observations strongly indicate TREK channels play important roles in arrhythmia genesis and TREK-sensitive cardiac remodeling within the development of cardiac hypertrophy, ischemia cardiomyopathy and idiopathic dilated cardiomyopathy.

Despite TREK channels are first discovered in human heart and the current lack of TREK-specific agonists and antagonists have restricted the research of the function of TREK channels in human heart, it is postulated that TREK may be a practical target for clinically relevant applications.