A link to two-pore domain potassium channel regulation

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The two-pore domain potassium (K2P) channel family play an important role in the regulation of the resting membrane potential and excitability of cells. So named as each α-subunit of these channels contains two pore domains, the K2P channels also contain an unusually large extracellular pre-pore linker called the M1P1 loop.

There are currently 15 members of this family, which can be divided into 6 sub-families on the basis of structural and functional properties. Among these subfamilies is the Tandem pore Acid Sensitive K subfamily (TASK1 (K2P 3.1), TASK3 (K2P9.1) & TASK5 (K2P 15.1)). As their name suggests, the TASK channels are sensitive to extracellular acidification, with a histidine at position 98 shown to be crucial for the pH sensitivity of TASK1 and TASK3 channels (see Kim, 2005). We have shown previously that zinc is a selective blocker of TASK3 channels with little effect on TASK1 in physiological conditions (Clarke *et al.*, 2004). This selective block involves both H98 and a glutamate residue (E70) within the M1P1 loop, suggesting that the M1P1 loop may lie close to the pore and play an important role in channel regulation (Clarke *et al.*, 2004). The long extracellular M1P1 loop is not conserved in potassium channels with known crystal structures. However, a structural homology model of TASK1 based on known potassium channel structures was recently published (Yuill *et al.*, 2007) where the M1P1 loops are assumed to lie in close association at position N53, a position homologous to TWIK1 C69, an M1P1 cysteine responsible for channel dimerization (Lesage *et al.*, 1996).

In this study we create cysteine mutants and M1P1 chimaeric channels using standard PCR techniques. The cDNA of these mutants was injected into *Xenopus laevis* oocytes and 1-3 days later used for two-electrode voltage clamp recordings exactly as previously described (Clarke *et al.*, 2004). All experiments were approved by the Animal Ethics Committee of the University of Sydney. We demonstrate that while engineered cysteines at TASK3 N53 on opposing M1P1 loops do not appear to form disulphide bonds, cysteines at positions 70 and 98 are able to form both inter- and intra-subunit spontaneous disulphide bonds therefore the M1P1 loops of TASK3 channels lie in close apposition to the pore. Through NMR Spectroscopy studies we show that the M1P1 loops are likely to form alpha helices and chimaeric exchange of the M1P1 loops between TASK channels demonstrates that this unusual linker plays a role in the TASK channel pH sensing mechanism.

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