A-9

Alteration of voltage-dependent activation by a single point mutation of a putative nucleotide-binding site in large-conductance Ca^{2+}-activated K^+ channel

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BK_{Ca} channels were suggested to contain one or more domains of the 'regulator of K^+ conductance' (RCK) in their cytosolic carboxyl termini (Jiang et al.2001). It was also shown that the RCK domain in mammalian BK_{Ca} channels might sense the intracellular Ca^{2+} with a low affinity (Xia et al. 2002). We aligned the amino acid sequence of the α-subunit of rat BK_{Ca} channels (rSlo) with known RCK domains and identified a second region exhibiting about 50% homology. This putative domain, RCK2, contains the characteristic amino acids conserved in other RCK domains. We wondered whether this second domain is involved in the domain-domain interaction and the gating response to intracellular Ca^{2+} for rSlo channel, as revealed in the structure of RCK domain of *E. coli* channel (Jiang et al. 2001). In order to examine the possibility, site-directed mutations were introduced into the RCK2 domain of rSlo channel and the mutant channels were expressed in *Xenopus* oocytes for functional studies. One of such mutation, G772D, in the putative nucleotide-binding domain resulted in the enhanced Ca^{2+} sensitivity and the channel gating of rSlo channel. These results suggest that this region of BK_{Ca} channels is important for the channel gating and may form an independent domain in the cytosolic region of BK_{Ca} channels. In order to obtain the mechanistic insights of these results, G772 residue was randomly mutagenized by site-directed mutagenesis and total 17 different mutant channels were constructed. We are currently investigating these mutant channels by electrophysiological techniques.