Intermediate-conductance calcium-activated potassium channel expression and function in the enteric nervous system

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Intermediate-conductance calcium-activated potassium channels (IK, KCNN4, KCa3.1) are important in many cellular functions including lymphocyte activation, erythrocyte volume regulation, vascular dilatation and transepithelial secretion. Despite this broad range of cell types, IK channels have so far not been found in the central nervous system suggesting they may not play a role in regulating neuronal excitability. Neurons in the enteric nervous system display a long-lasting post-spike afterhyperpolarization (AHP) that has many of the characteristics of an IK-like current, however until recently definitive characterization of the channel responsible has not been possible. In the present study, we used a series of antibodies raised against the IK channel to demonstrate its localization to Dogiel Type II intrinsic primary afferent neurons (IPANs) of the rat, mouse and human enteric nervous systems. We showed that the long-lasting AHP in these neurons is blocked by the IK channel antagonist clotrimazole (5μM). Western blotting of myenteric ganglion extracts revealed an immunoreactive band at 49kDa. A similar protein band was detected using the anti-V5 antibody in HEK293 cell lysates expressing the V5 epitope-tagged IK channel indicating that this band corresponds to the IK channel subunit. To determine whether neuronal IK channels are regulated by phosphorylation as they are in other systems, we examined the role of protein kinase A (PKA). Using GST fusion proteins, we found that PKA phosphorylates predominantly serine332 in the intracellular region of the IK channel. A related ion channel, SK2, is not phosphorylated by PKA. By site-directed mutagenesis, we identified a PKA binding motif located near this major phosphorylation site on the IK channel. In conclusion, we present evidence that the channel underlying the AHP in enteric neurons is the IK channel. These neuronal IK channels are likely to be important in control of excitability states of the enteric nervous system.