Distinct expression of intermediate-conductance calcium-activated potassium (IK) channels in intrinsic primary afferent neurons of the rat gastrointestinal tract

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Intrinsic primary afferent neurons (AH neurons) in the intrinsic ganglia of the small intestine have a broad action potential that is followed by early and late afterhyperpolarising potentials (AHP). Our laboratory has reported electrophysiological evidence consistent with intermediate-conductance calcium-activated potassium channels (IK channels) being responsible for the AHP in enteric primary afferent neurons (Vogalis *et al.*, 1992), however the molecular expression of IK channels has not been reported in these cells. This study was undertaken to investigate whether IK channels are expressed in the enteric nervous system and whether their expression corresponds to those cells known to express AHP currents.

To localise the IK channels, an antibody was generated in rabbits against the N-terminal 15 amino acids of the rat IK channel and immunohistochemistry performed on whole mount preparations of rat gastrointestinal tract. Evidence for specificity of the antibody was shown in Western blots where it was found to recognise a single band of 160kD in HEK 293 cells transfected with IK cDNA plasmid but not in cells transfected with vector alone or vector containing SK2 cDNA, or on blots probed with pre-immune serum.

IK channel immunoreactivity was found in specific nerve cell bodies throughout the gastrointestinal tract, from the esophagus to the rectum. The majority of immunoreactive neurons had Dogiel type II morphology and in the myenteric plexus of the ileum almost all immunoreactive neurons were of this shape. Intrinsic primary afferent neurons in the rat small intestine are Dogiel type II neurons that are immunoreactive for calretinin, and it was found that almost all the IK channel immunoreactive neurons were also calretinin immunoreactive. IK channel immunoreactivity also occurred in calretinin-immunoreactive, Dogiel type II neurons in the caecum. Within immunoreactive cells, the initial segments of the axons contained the highest density of sites, but not axon terminals. No immunoreactivity was found in surrounding muscle or glia.

Molecular evidence for IK expression was determined by RT-PCR analysis using oligonucleotide probes based on the rat IK sequence (Neylon *et al.*, 1990). RT-PCR cloning from a highly enriched myenteric ganglion extract revealed an mRNA sequence that was identical to the IK channel mRNA expressed in other cell types.

It is concluded that IK channels are expressed on specific neurons of the gastrointestinal tract. They are almost exclusively located on cell bodies and proximal parts of axons of intrinsic primary afferent neurons. From functional studies, these IK channels are predicted to control the excitability states of the enteric nervous system.

Neylon, C.B., Lang, R.J., Fu, Y., Bobik, A. & Reinhart, P.H. (1999) *Circulation Research,* 85, e33-e43.
Vogalis, F., Harvey, J.R. & Furness, J.B. (2002) *Journal of Physiology,* 538, 421-33.