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Functional And Pharmacological Characterisation Of Potassium Channel Subtypes In The Mouse Intestine

Gastrointestinal (GI) tract motility relies on highly coordinated contractions of its longitudinal and circular smooth muscles. Disruption to this physiological process is associated with long term motility disorders such as irritable bowel syndrome (IBS). Although much is known about the ionic conductances responsible for GI smooth muscle contraction, comparably little is known about the underlying potassium (K^+) channels responsible for suppressing smooth muscle contractility and promoting GI tract relaxation [(1)]. Here we utilise pharmacological techniques to dissect the distribution and contribution of various subtypes of K^+ channels in regulating intestinal motility in the mouse.

Adult C57BL/6 mice (20-30 g) were euthanised by carbon dioxide asphyxiation and cervical dislocation. Distal ileum and colon segments (2-3 cm) were dissected and mounted on an aerator and suspended from a force transducer in an organ bath containing Krebs solution at either 32 or 37°C. Changes in tension were measured using Lab Chart® software (AD Instruments). Tissue viability was assessed by responses to carbachol (CCh; 1 nM - 300 µM). The effects of a variety K^+ channel activators and inhibitors (used at maximal concentrations)were assessed on sub-threshold CCh pre-contracted tissue (32°C) and during spontaneous activity (37°C). Data are displayed as mean ± SEM and statistical analysis were performed using theStudents paired t-test, and deemed significant at $p<0.05$.

Under basal conditions at 32 °C, the broad spectrum K^+ channel inhibitors tetraethyl ammonium (TEA; 10 mM) and barium chloride ($BaCl_2$; 10 mM) and the selective KCNQ channel inhibitor XE-991 (100 µM) all promoted significant increases in tension in both ileum and colon (n = 6 each condition). Sensitivity to $BaCl_2$ was significantly less in colon versus ileum (p = 0.0035). Exposure to 4-aminopyridine (4-AP, 5 mM) did not markedly effect tension in either ileum or colon (n = 6 each). Physiological activity measured at 37 °C revealed TEA, $BaCl_2$ and XE-991 significantly increased the force of spontaneous contractions in both ileum and colon but were without effect on the frequency of contraction (n= 6 for each).

In tissues pre-contracted with sub maximal concentrations of carbachol (CCh, 10 µM) at 32 °C, the KCNQ channel agonist Retigabine (1-100 µM) induced a concentration-dependent decrease in muscle tension in both ileum and colon. Relaxation induced by 100 µM Retigabine was not maximal but differed significantly between ileum and colon (54.3 ± 1.9 % *vs* 35.4 ± 3.1 %, p =0.0004). The K_{2P} channel agonist Riluzole (100 µM) which specifically activates the mechanosensitive K_{2P} channels TREK-1, TREK-2 and TRAAK [(2)]caused a significant reduction in pre-contracted muscle tension in both ileum (37.1 ± 3.9 %, n = 14, $p<0.0001$) and colon (18.9 ± 4.4 %, n = 11, p =0.0023). Similarly, the mechanosensitive K_{2P} channel activator, BL-1249, induced a concentration-dependent decline in muscle tension in both ileum and colon. Relaxation induced by 60 µM BL-1249 was not maximal but differed significantly between ileum and colon (93.1 ± 1.2 % *vs*56.1 ± 4.2 %, $p<0.001$). As expected, 100 µM Retigabine and 100 µM Riluzole significantly decreased the force of spontaneous contractions at 37 °C in both ileum and colon ($p<0.05$, n = 5-6). Interestingly, 30 µM BL-1249 only decreased contraction force in ileum (p =0.0030) and not colon (p=0.0917). However, all K^+ channel agonists were without effect on contraction frequency.

These data begin to reveal the heterogeneous expression of K^+ channel subtypes along the mouse gastrointestinal tract and highlight K^+ channels as potential therapeutic targets for treating gastrointestinal motility disorders such as irritable bowel syndromes.

(1) Beyder&Farrugia (2012) *Ther Adv Gastroenterol* ***5:*** *5-21.*

(2) Cadaveira-Mosquera, A. *et al*., (2011) *J Neurosci* ***31:*** *1375-85.*