A pH-sensitive potassium conductance (TASK) and its function in the murine gastrointestinal tract

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The excitability of smooth muscles is regulated, in part, by background K^+ conductances that determine resting membrane potential. However, the K^+ conductances so far described in gastrointestinal (GI) muscles are not sufficient to explain the negative resting potentials of these cells. Here we describe expression of two-pore K^+ channels of the TASK family in murine small and large intestinal muscles. TASK-2, cloned from murine intestinal muscles, resulted in a pH-sensitive, time-dependent, non-inactivating K^+ conductance with slow activation kinetics. A similar conductance was found in native intestinal myocytes using whole-cell patch-clamp conditions. The pH-sensitive current was blocked by local anaesthetics. Lidocaine, bupivacaine and acidic pH depolarized circular muscle cells in intact muscles and decreased amplitude and frequency of slow waves. The effects of lidocaine were not blocked by tetraethylammonium chloride, 4-aminopyridine, glibenclamide, apamin or MK-499. However, depolarization by acidic pH was abolished by pre-treatment with lidocaine, suggesting that lidocaine-sensitive K^+ channels were responsible for pH-sensitive changes in membrane potential. The kinetics of activation, sensitivity to pH, and pharmacology of the conductance in intestinal myocytes and the expression of TASK-1 and TASK-2 in these cells suggest that the pH-sensitive background conductance is encoded by TASK genes. This conductance appears to contribute significantly to resting potential and may regulate excitability of GI muscles.

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Smooth muscle cells have negative resting potentials that are important in regulating the overall excitability and contractile properties of tissues utilizing these cells for force development. In the gastrointestinal (GI) tract smooth muscle cells display a relatively large range of resting potentials, from approximately −40 to −80 mV. In phasic regions of the GI tract electrical slow waves, generated by interstitial cells of Cajal (ICC) (see Ward *et al.* 1994; Dickens *et al.* 2000), are periodic depolarizations that are superimposed upon the resting potentials of smooth muscle cells (Horowitz *et al.* 1999). Thus, slow waves induce periods of enhanced open probability for L-type Ca^{2+} channels in smooth muscle cells, periodic Ca^{2+} entry, and phasic contractions (Horowitz *et al.* 1999). The amplitude of slow waves recorded from smooth muscle cells and therefore the efficiency of these events to activate L-type Ca^{2+} channels is highly dependent upon the input resistance and the resting membrane potentials of the smooth muscle cells (Kito *et al.* 2005). Opening of K^+ channels hyperpolarizes smooth muscle cells and diminishes the degree of depolarization achieved during slow waves. At present, the mechanisms for setting and regulating membrane potential, including the precise contributions of a variety of K^+ channel subtypes expressed in GI myocytes, are not fully understood. Most K^+ conductances expressed by these cells that have been characterized previously contribute only a few millivolts, if at all, to resting membrane potentials in intact muscles (Horowitz *et al.* 1999), and the major background conductance(s) responsible for the resting K^+ conductance in GI muscles has not been identified.

In addition to voltage-dependent and inward rectifier K^+ channels, smooth muscles express two-pore K^+ channel gene products. For example, we have reported expression of stretch-dependent K^+ channels that appear to be encoded by TREK-1 in GI smooth muscle cells. These channels contribute to resting membrane potential, responses to changes in cell length, responses to nitrergic stimulation, and the ability of GI organs to serve a reservoir function (Koh *et al.* 2001; Koh & Sanders, 2001). Other members of the two-pore K^+ channel family,

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such as TASK, may also be expressed and contribute to membrane potentials (Eglen *et al.* 1999; Kindler *et al.* 1999; Gurney *et al.* 2003). TASK channels are pH sensitive and inhibited by local anaesthetics, such as lidocaine and bupivicaine (Leonoudakis *et al.* 1998; Reyes *et al.* 1998). Interestingly, channels encoded by TASK-2 display time-dependent activation and no voltage-dependent inactivation (Kindler *et al.* 2003). In the present study we have explored the hypothesis that TASK channels are present in GI smooth muscle cells and contribute in a significant way to the compliment of background K^+ conductances in these cells. We have characterized a pH-sensitive conductance in murine intestinal myocytes and found evidence with pharmacological, molecular and morphological techniques that the pH-sensitive conductance may be encoded by TASK genes in these cells.

Methods

Intracellular microelectrode recordings

BALb/c mice between the ages of 30–60 days were used for these studies. Animals were obtained from the Jackson Laboratory (Bar Harbour, MN, USA). Mice were anaesthetized with isoflurane (Baxter, Deerfield, IL, USA) prior to cervical dislocation and exsanguinated by decapitation. The animals were maintained and the experiments performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Institutional Animal Use and Care Committee at the University of Nevada approved all procedures used.

After animals were killed, small intestinal segments 2 cm from the ileocecal valve were used for electrophysiological experiments. The tissues were removed and placed in Krebs'-Ringer bicarbonate solution (KRB). The bowel was opened along the mesenteric border, luminal contents were removed with KRB, and the tissues were pinned to the base of a Sylgard-coated (Dow Corning Corp., Miland, MI, USA) dish. The mucosa was removed by sharp dissection. Segments of the tunica muscularis (approximately 10 mm × 4 mm) were placed in a recording chamber with the circular muscle (muscosal aspect) facing upward. Tissues of the proximal colon were also used after similar dissection and preparation in some experiments. Impalements of cells were made with glass microelectrodes having resistances of 80–120 MΩ. Transmembrane potentials were recorded with a standard electrometer (Duo 773; World Precision Instruments, Sarasota, FL, USA). Data were recorded on a PC running Acknowledge data acquisition software (Biopac systems, Inc.). Several electrical parameters were analysed: (i) resting membrane potential (RMP); (ii) slow wave amplitude; (iii) slow wave frequency.

Preparation of isolated myocytes

Isolated myocytes were also prepared from the small and large intestines. Segments of the small intestine and colon were opened along the longitudinal axes of the organs, pinned out in a Sylgard (Dow Corning)-lined dish, and washed with Ca^{2+}-free solution containing (mM): 125 NaCl, 5·36 KCl, 15·5 NaOH, 0·336 Na_2HPO_4, 0·44 KH_2PO_4, 10 glucose, 2·9 sucrose and 11 Hepes, adjusted to pH 7·4 with tris (hydroxymethyl)aminomethane (Tris). The tunica muscularis was incubated in a Ca^{2+}-free solution supplemented with 4 mg ml^{-1} fatty acid-free bovine serum albumin, 2 mg ml^{-1} papain, 1 mg ml^{-1} collagenase and 1 mM dithiothreitol at 37°C for 8–12 min. After enzymatic digestion the muscles were washed with Ca^{2+}-free solution and gently agitated to create a cell suspension. Dispersed cells were stored at 4°C in Ca^{2+}-free solution. Experiments were performed at room temperature within 6 h of cell dispersion.

Voltage-clamp patch experiments

The whole-cell patch-clamp technique was used to record membrane currents from dissociated murine colonic and ileal smooth muscle cells. Currents were amplified with a List EPC-7 (List Electronic, Darmstadt, Germany) or Axopatch 200B (Axon Instruments). Data were digitized with either a 12- or 16-bit analog-to-digital converter (Digidata 1322A and Digidata 1320A, respectively, Axon Instruments). Data were stored directly and digitized on-line using pCLAMP software (version 8.0, Axon Instruments). Current-clamp ($I = 0$) experiments were also performed to measure the effects of pH on membrane potentials of freshly dispersed colonic myocytes. Data were sampled at 4 kHz and filtered at 1 kHz and analysed using pCLAMP (version 8.0, Axon Instruments), and Graphpad Prism (version 3.0, San Diego, CA, USA). Pipette resistances were 1–4 MΩ. In most experiments, the uncompensated series resistance was between 2 and 4 MΩ, and thus voltage errors could have approached 12 mV. Voltage errors were much smaller during steps to negative potentials.

Drugs and solutions for electrophysiological experiments

In sharp electrode experiments the tissue chamber housing muscles was constantly perfused with oxygenated KRB of the following composition (mM): NaCl 118.5; KCl 4.5; $MgCl_2$ 1.2; $NaHCO_3$ 23.8; KH_2PO_4 1.2; dextrose 11.0; $CaCl_2$ 2.4. The pH of the KRB was 7.3–7.4 when bubbled with 97% O_2–3% CO_2 at 37.0 ± 0.5°C. After pinning, the muscles were left to equilibrate for at least 1 h before experiments were begun.

Nifedipine was obtained from Sigma and dissolved in ethanol at a stock concentration of 10 mM before being

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added to perfusion solution at a final concentration of 1 μM. All experiments were performed in the presence of tetrodotoxin (1 μM). Membrane-impermeable pH buffers, MES ((2-(*N*-morpholino) ethanesulphonic acid; Sigma)) and Tris (Sigma) were used to adjust the KRB to acidic and alkaline pH, respectively. Osmolarity did not exceed more than 325 mosmol l^{-1}. Lidocaine and bupivacaine were also obtained from Sigma and dissolved in de-ionized H_2O before being diluted in Krebs' solution to the final concentration. Tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), apamin and glibenclamide were all purchased from Sigma. *N*-[1′-(6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl)-3,4-dihydro-4-hydrooxyspiro-2H-1-benzopyran-2,4′-piperidine]-6-yl-(+)-methanesulphonamide monohydrochloride (MK-499) was kindly donated by Merck. TEA, 4-AP and apamin were dissolved in de-ionized water and glibenclamide and MK-499 dissolved in dimethyl sulfoxide (final concentration of DMSO was less than 0.1%).

In patch-clamp experiments smooth muscle myocytes were bathed in a Ca^{2+}-free physiological salt solution (MnPSS) containing (mM): 5 KCl, 135 NaCl, 2 $MnCl_2$, 10 glucose, 1.2 $MgCl_2$, and 10 HEPES adjusted to pH 7.4 with Tris. The pipette solution contained (mM): 110 potassium gluconate, 20 KCl, 5 $MgCl_2$, 2.7 K_2ATP, 0.1 Na_2GTP, 2.5 creatine phosphate disodium, 5 HEPES and 10 BAPTA, adjusted to pH 7.2 with Tris to minimize large-conductance Ca^{2+}-activated K^+ currents. 4AP, TEA, or lidocaine (1 mM) were added as described in Results.

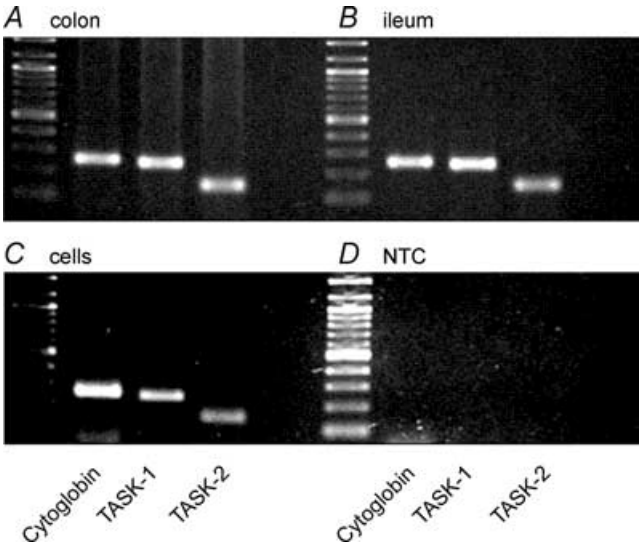

Figure 1. RT-PCR analysis of mRNA expression in tissues and cells
PCR products were generated through the use of gene specific primers. Cytoglobin primers were used to confirm that the products generated were representative of RNA (251 bp band). TASK-1 and TASK-2 transcripts were detected in murine colon (*A*), ileum (*B*) and in isolated colonic myocytes (*C*). NTC (*D*), non-template control.

Expression of TASK-2 channels in *Xenopus* oocytes

X. laevis oocytes were isolated as previously described (Overturf *et al.* 1994). Stage V and VI oocytes were injected with 50 nl of cRNA encoding TASK2 (1.9 μg μl^{-1}; GenBank accession number AF319542) using a Drummond Nanoject microinjector (Drummond Scientific Co., Broomall, PA, USA). Whole-cell currents were recorded using the two-microelectrode voltage-clamp technique. Glass microelectrodes were filled with 3 M potassium aspartate and had resistances of 1–3 MΩ. Oocytes were superfused with a low chloride Ca^{2+}-free solution designed to minimize the endogenous Ca^{2+}-activated Cl^- current. This solution contained 96 mM sodium isethionate, 2 mM KCl, 2.8 mM $MgCl_2$, 5 mM HEPES, and 0.05 mM niflumic acid, pH 7.4. Reagents were either applied to the bath (volume, 0.5 ml) via a gravity-fed perfusion system or injected into the oocyte. The mixing time to exchange bath solutions was approximately 30 s. Each experiment was performed at room temperature (24–28°C) on oocytes collected from two or more frogs. Voltage protocols were applied using pCLAMP 9.0 software. In short, 400-ms voltage steps were applied from a holding potential of −80 mV to

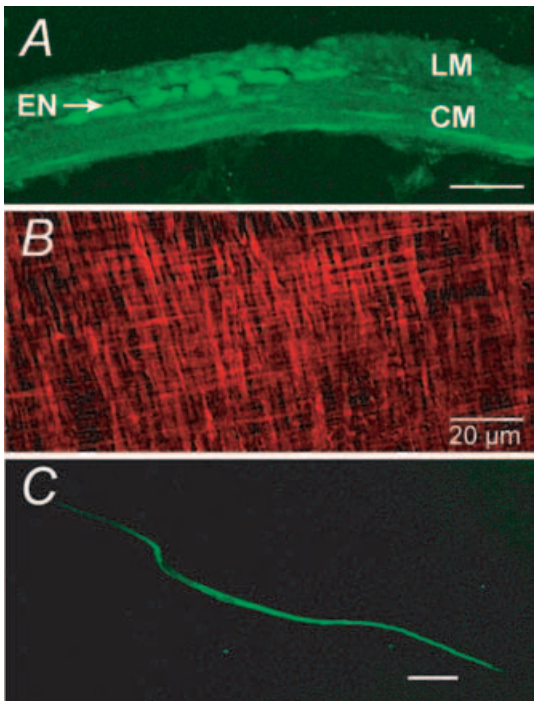

Figure 2. TASK-2-like immunoreactivity in small intestine
A, in cryostat sections, TASK-2-like immunoreactivity was observed in both circular (CM) and longitudinal (LM) muscle layers of small intestine and in a subpopulation of enteric neurones (EN). *B*, whole-mount preparation showed that TASK-2-like immunoreactivity was expressed in both muscle layers. *C*, enzymatically dispersed colonic myocytes also revealed TASK-2-like immunoreactivity. Scale bar in all panels, 20 μm.

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test potentials ranging from −150 to +50 mV in 20 mV increments. Linear leak and capacitance currents were removed using a P/5 protocol.

Analysis of electrophysiological data

Data are expressed as means ± S.E.M. Student's *t* test was used where appropriate to evaluate differences in the data. *P* values less than 0.05 were taken as statistically significant differences.

Molecular studies

Total RNA was prepared from murine small and large intestinal muscles and isolated smooth muscle cells using the SNAP Total RNA isolation kit (Invitrogen, San Diego, CA, USA) as per manufacturer's instruction including the use of polyinosinic acid (20 μg) as an RNA carrier. First strand cDNA was prepared from the RNA using the Superscript II Reverse Transcriptase kit with 500 μg μl^{-1} of oligo dT primers to reverse transcribe the RNA sample. The cDNA reverse transcription product was amplified with channel-specific primers by PCR. The amplification profile for these primer pairs was: an initial step to 95°C for 10 min to activate the amplitaq polymerase, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. The amplified products (5 μl) were separated by electrophoresis on a 4% agarose/1 × TAE (Tris, acetic acid, EDTA) gel, and the DNA bands visualized by ethidium bromide staining. RT-control on each RNA sample was a cDNA reaction for which the reverse transcriptase was not added, controlling for genomic DNA contamination in the source RNA. These negative controls were subjected to a second round of amplification to assure specificity of the reactions and the quality of the reagents. The following PCR primers were used (the genebank accession number is given in parentheses for the reference nucleotide sequence used): TASK-1 (KCNK3) (accession no. AF006824) nt 305–329 and 523–547: amplicon, 243 bp; TASK-2 (accession no. AF319542) nt 136–155 and 264–283: amplicon, 148 bp. Cytoglobin primers that spanned two exons and an intron were used to confirm that the products generated were representative of RNA. Any cDNA preparation that amplified the cytoglobin intron (amplicon, 550 bp) was discarded. Cytoglobin (Cygb) (accession no. NM030206) nt 663–684 and 892–914: amplicon, 251 bp.

Immunohistochemistry

Immunohistochemistry was performed using an antibody raised against TASK-2 (Alomone Laboratories, Israel). Small intestinal tissues used for immunohistochemical studies were fixed with 4% paraformaldehyde (w/v) in PBS

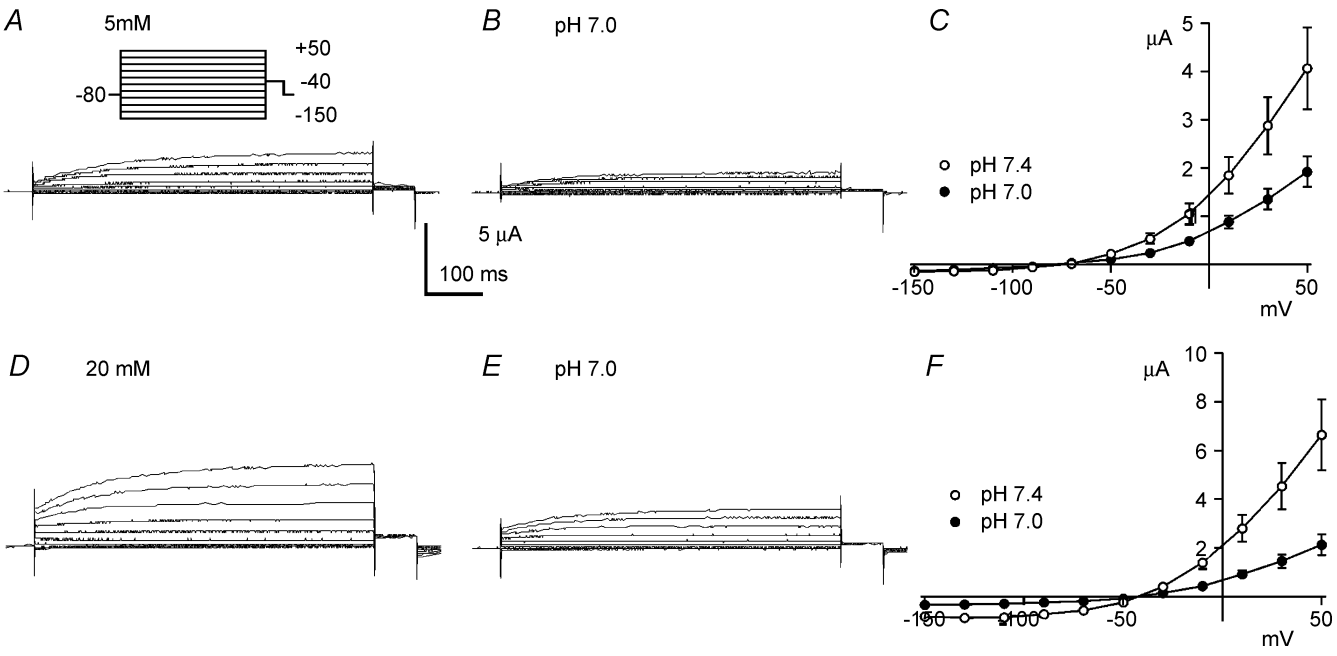

Figure 3. TASK-2 currents expressed in oocytes
TASK-2 cloned from murine colon was expressed in oocytes. The cells were held at −80 mV and stepped to test potentials ranging from −150 to +50 mV in 20 mV intervals (see inset). *A*, control traces in 5 mM $[K^+]_o$. *B*, exposure to pH 7.0 solution decreased outward currents. *C*, summary of the current–voltage (*I–V*) relationship before (○) and after (●) exposure to pH 7.0 solution ($n = 4$). *D*, control traces in 20 mM $[K^+]_o$. *E*, exposure to pH 7.0 solution in the presence of 20 mM $[K^+]_o$. *F*, summary of *I–V* relationship before (○) and after (●) exposure to pH 7.0 solution in the presence of 20 mM $[K^+]_o$ ($n = 4$). Reversal potentials for the outward current shifted as a function of external K^+ as predicted by Nernst relationship for the K^+ gradient.

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for 30 min at room temperature. Tissues were subsequently washed (4 × 15 min) in PBS and cryoprotected in a graded series of sucrose solutions (5, 10, 15 and 20% w/v made up in PBS, 1 h each). Tissues were then embedded in TissueTek embedding medium (Sakura Finetek, Torrance, CA, USA) and 20% sucrose in PBS (1 part/2 parts v/v) and rapidly frozen in isopentane pre-cooled in liquid nitrogen. Cryosections was cut using a Leica CM 3500 cryostat at a thickness of 10 μm and collected on vectabond-coated microscope slides. For whole-mount preparations tissues were pinned to the Sylgard floor of a dissecting dish and stretched to 110% of its resting length and the mucosa removed before being fixed in 4% paraformaldehyde (w/v) made up in PBS for 30 min at room temperature. Following fixation tissues were washed (4 × 15 min) in PBS.

For whole mounts and cryosections, non-specific antibody binding was blocked using bovine serum albumin (BSA, 1% in PBS, Sigma) for 1 h at room temperature. Tissues were incubated with primary antibody to TASK-2 (1: 200) overnight at 4°C. After labelling with primary antibody, tissues and sections were washed for 2 h in PBS before being incubated in secondary antibody (Alexa Fluor 488-coupled goat anti-rabbit IgG) for 1 h at room temperature. Secondary antibody was obtained from Molecular Probes and diluted to 1 : 200 in PBS. After washing with PBS, specimens were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA). Control tissues were prepared by either omitting primary or secondary antibodies from the incubation solutions.

Tissues were examined with a Zeiss LSM 510 META confocal microscope with an excitation wavelength of 488 nm. Confocal micrographs shown are digital composites of *Z*-series scans of several optical sections through a depth of 20–30 × 0.5 μm for full or partial thickness of the musculature and 10 × 0.5 μm for isolated cells. Final images were constructed with Zeiss LSM 510 META software.

Results

TASK channels are expressed in the small and large intestine

RT-PCR was performed using specific primer pairs for TASK-1 and TASK-2 channels on extracts of small intestinal and colonic muscles. Figure 1*A* and *B* demonstrate that TASK-1 and TASK-2 mRNAs were expressed in these muscles. GI muscles contain a variety of cell types, including neurones, interstitial cells of Cajal (ICC), macrophages, mast cells and smooth muscle cells. Therefore, we also examined expression of TASK channel mRNAs in smooth muscle cells. In these experiments cells were enzymatically dispersed and 30–50 smooth muscle cells were carefully collected with a blunt suction

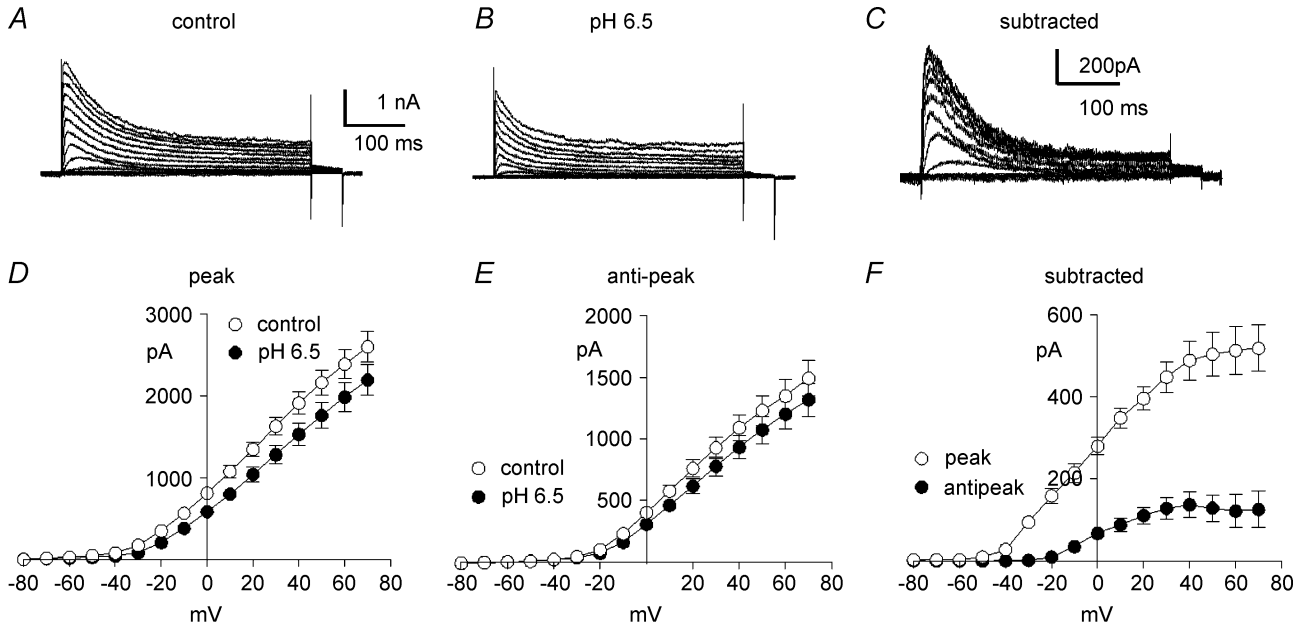

Figure 4. The effect of acidic pH on outward currents in colonic myocytes
Cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals. *A*, control traces. *B*, exposure to pH 6.5 solution. *C*, difference currents in which traces in *B* were digitally subtracted from currents in *A*. *D*, summary *I–V* relationship for peak currents before (○) and after (●) exposure to pH 6.5 solution ($n = 16$). *E*, summary *I–V* relationship for anti-peak (end of test pulse) currents before (○) and after (●) exposure to pH 6.5 solution. *F*, summary *I–V* relationship for peak (○) and anti-peak (●) currents from subtracted currents.

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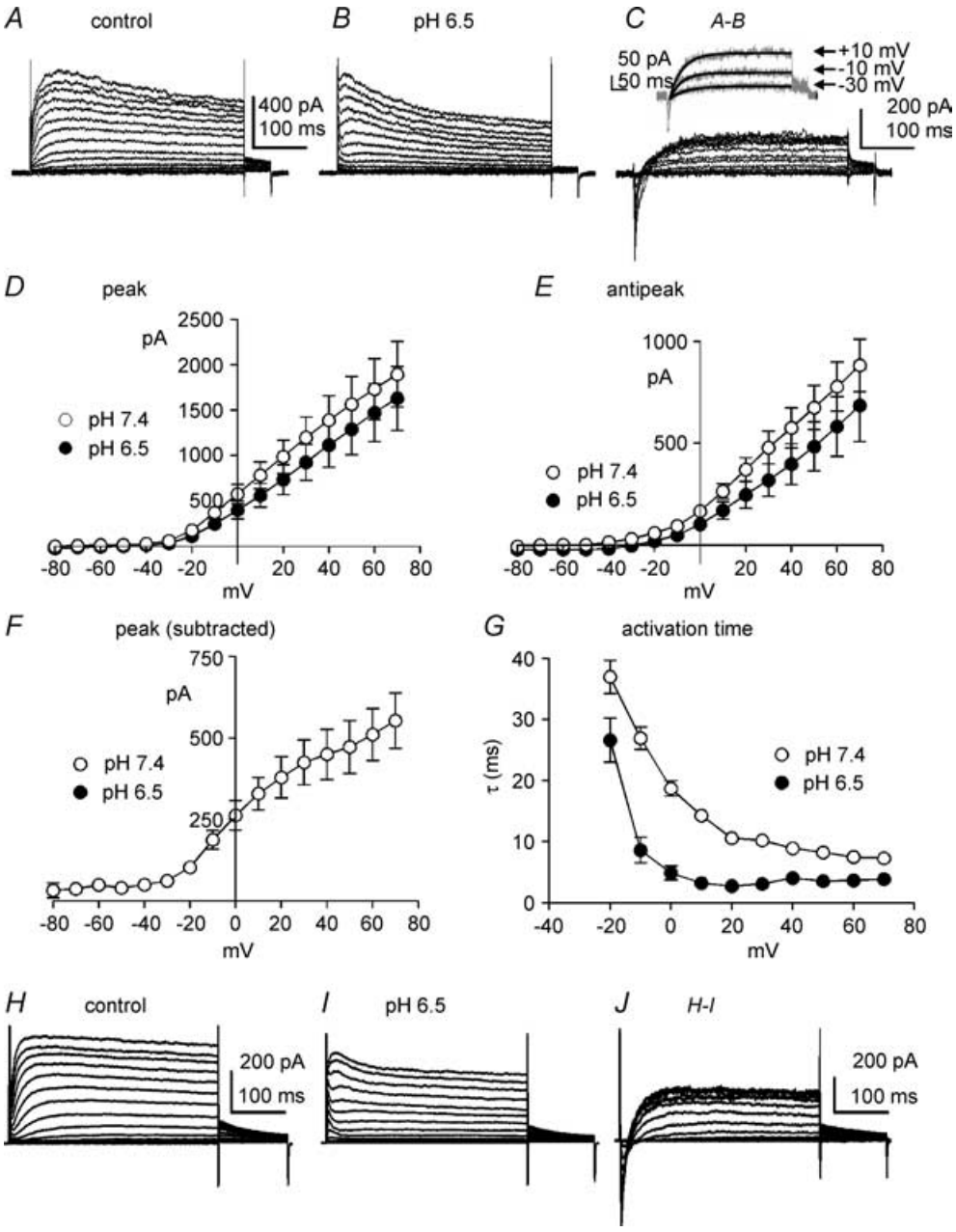

Figure 5. Effects of extracellular acidic pH on outward currents in the presence of 4-AP and TEA in colonic myocytes (*A*–*G*) and ileal myocytes (*H*–*J*)

Cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals. *A*, control traces from colonic myocytes are shown in the presence of 4AP (5 mM) and TEA (10 mM). *B*, exposure to pH 6.5 solution decreased outward current in the presence of 4-AP (5 mM) and TEA (10 mM). *C*, difference currents obtained by subtracting currents obtained at pH 6.5 (*B*) from control currents (*A*). Inset shows exponential fits (continuous lines) of outward currents generated by steps to −10, 0 and +10 mV to obtain time constants of activation. *D*, summary of *I*–*V* relationship for peak currents before (○) and after (●) exposure to pH 6.5 solution in the presence of 4-AP (5 mM) and TEA (10 mM) ($n = 7$). *E*, summary of *I*–*V* relationship for anti-peak (end-pulse) currents before (○) and after (●) exposure to pH 6.5 solution. *F*, summary of *I*–*V* relationship for difference currents representing the slowly activating and non-inactivating outward current blocked by acidic pH. *G*, activation time constants as a function of voltage for net outward currents before (○) and after (●) exposure to pH 6.5 in the presence of 4-AP (5 mM) and TEA (10 mM) ($n = 7$). *H*, control currents obtained in an ileal myocyte in the presence of 4-AP (5 mM) and TEA (10 mM). *I*, currents after exposure of the ileal cell to pH 6.5 solution in the presence of 4-AP (5 mM) and TEA (10 mM). *J*, difference currents obtained by subtracting currents in pH 6.5 (*I*) from control currents (*H*). Acidic pH blocked a conductance with similar properties in both ileal and colonic myocytes.

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pipette (6–10 μm tip). PCR products from isolated smooth muscle cells are shown in Fig. 1*C*.

TASK-2 protein expression was studied by immunohistochemistry using a rabbit polyclonal antibody (see Methods). After labelling, cryostat cross-sections were examined with confocal microscopy and revealed TASK-2-like immunoreactivity in the circular and longitudinal muscle cells of the small intestine (Fig. 2*A*). A subpopulation of myenteric neurones also expressed TASK-2-like immunoreactivity (Fig. 2*A*). In whole-mount preparations TASK-2-like immunoreactivity was also apparent in circular and longitudinal muscle cells, and to a lesser extent, within myenteric neurones (Fig. 2*B*). Antibodies for TASK-1 (Alomone Laboratories, and Santa Cruz Biotech, CA, USA) were also used; however, these did not give suitable labelling for immunofluorescence in intact muscles.

We also tested whether TASK-2 proteins are present in colonic myocytes. Labelling of dispersed colonic myocytes with polyclonal antibodies to TASK-2 revealed expression (Fig. 2*C*).

Properties of TASK-2 channels expressed in oocytes

Previous studies have demonstrated a characteristic profile and pharmacology for TASK channels (see Kindler *et al.* 2003). We cloned TASK-2 channels from murine colon and expressed channels in *Xenopus laevis* oocytes to confirm the kinetics and pH sensitivity of TASK-2 channels from GI muscles. Oocytes injected with cRNA encoding TASK-2 displayed voltage-dependent, slowly activating, and non-inactivating outward currents in response to depolarization. Cells were held at −80 mV and stepped to test potentials ranging from −150 to +50 mV in 20 mV intervals. The current–voltage (*I–V*) relationship was plotted for peak currents (Fig. 3*A–C*). The mean peak outward current at −10 mV and +50 mV was 1.25 ± 0.18 and 4.07 ± 0.99 μA, respectively, under control conditions (pH 7.4, $n = 4$). The activation time constants at −10 and +50 mV were 83 ± 3 and 159 ± 6 ms, respectively. These values were not different from a previous report (e.g. time constant = 147 ± 5 ms, measured at +40 mV; see Kindler *et al.* 2003). Exposure to pH 7.0 solution decreased peak outward current (e.g. at −10 and +50 mV was 0.58 ± 0.16 and 2.38 ± 0.71 μA, respectively ($P < 0.05$ compared with control values). Figure 3*C* shows a summary of the *I–V* relationships before and after pH 7.0 (5 mM external K^+). We also tested the effects of pH 7.0 in the presence of external high K^+ concentration (20 mM) (Fig. 3*D–F*) to examine the K^+ selectivity. Reversal potentials for the outward current shifted as a function of external K^+ as predicted by the Nernst relationship for the K^+ gradient. Exposure to pH 7.0 solutions decreased outward currents, suggesting that acidic conditions blocked TASK-2 currents in the oocytes.

A pH-sensitive K^+ conductance with properties similar to TASK conductances in murine intestinal myocytes

Voltage-dependent outward currents were recorded from colonic and ileal myocytes using voltage-step protocols

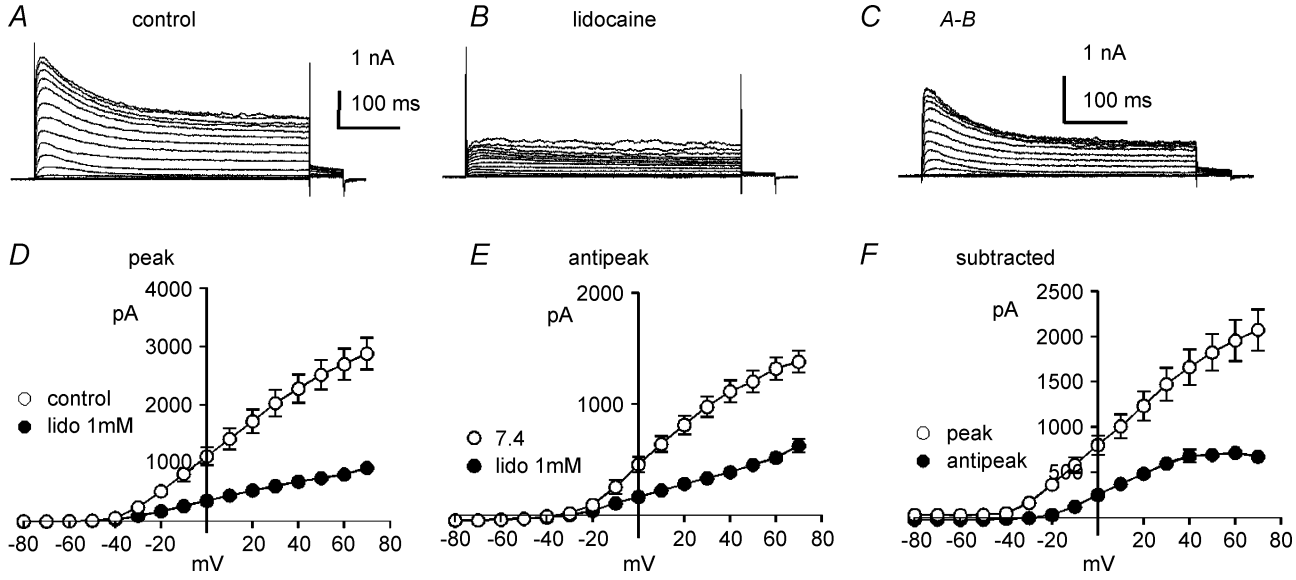

Figure 6. Effects of lidocaine on outward currents in colonic myocytes
Cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals. *A*, control traces. *B*, lidocaine (1 mM). *C*, difference currents obtained by subtraction of currents in the presence of lidocaine (*B*) from control currents (*A*). *D*, summary of *I–V* relationship for peak currents before (○) and after (●) application of lidocaine ($n = 5$). *E*, summary of *I–V* relationship for anti-peak (end-pulse) currents before (○) and after (●) application of lidocaine. *F*, summary of *I–V* relationship for peak (○) and anti-peak (●) currents from subtracted currents.

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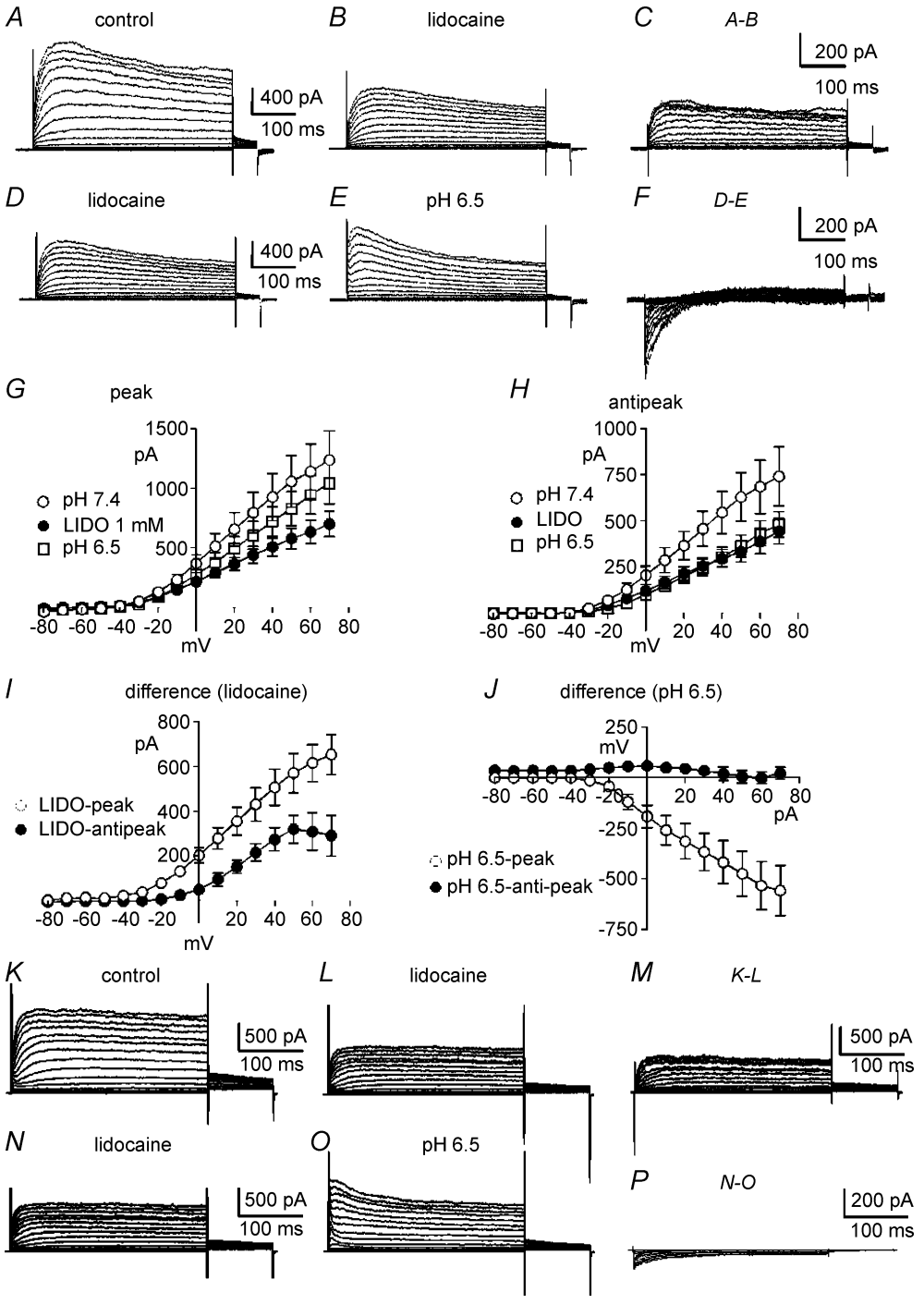

Figure 7. Effects of extracellular acidic pH on outward currents in colonic myocytes (*A–J*) and ileal myocytes (*I–O*) in the presence of lidocaine

Cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals. *A*, control traces in the presence of 4-AP (5 mM) and TEA (10 mM) in colonic myocytes. *B*, application of lidocaine in the presence of 4-AP (5 mM) and TEA (10 mM). *C*, difference currents obtained by subtracting responses in the presence of lidocaine (*B*) from control currents in the presence of 4-AP and TEA (*A*). *D*, control traces in the presence of lidocaine (1 mM), 4-AP (5 mM) and TEA (10 mM). *E*, exposure to pH 6.5 solution in the continued presence of lidocaine (1 mM), 4-AP (5 mM) and TEA (10 mM). *F*, difference currents obtained by subtracting currents in *E* from those in *D*. *G*, summary *I–V* relationship for peak currents in control (○) in the presence of 4-AP and TEA, lidocaine (●) and pH 6.5 (□) in the presence of lidocaine ($n = 5$). *H*, summary *I–V* relationship for anti-peak (end-pulse) currents in control (○) in the presence of 4-AP and TEA, lidocaine (●) and pH 6.5 (□) in the presence of lidocaine. *I*, summary *I–V* relationship for anti-peak (end-pulse) currents before (○) and after (●) application of lidocaine

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under whole-cell patch clamp conditions. Contamination of outward currents with large-conductance Ca^{2+}-activated K^+ channels was minimized by replacement of external Ca^{2+} (2 mM) with equimolar Mn^{2+} (MnPSS, see Methods) and dialysis with 10 mM BAPTA (free Ca^{2+} level ~10 nM). Cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals under these conditions. The *I–V* relationship was plotted for peak currents from colonic myocytes (Fig. 4*A* and *D*). The mean peak outward current at 0 and +50 mV was 813 ± 62 and 2164 ± 154 pA, respectively, and mean anti-peak current (i.e. measured at the ends of test potentials) at 0 and +50 mV was 399 ± 31 and 1229 ± 120 pA, respectively, under control conditions ($n = 16$, Fig. 4*A* and *E*). Changing the bath solution to one of the same composition, but adjusted to pH 6.5, decreased the mean peak outward current (e.g. at 0 and +50 mV was 587 ± 54 and 1761 ± 155 pA, respectively; $P < 0.01$ compared with control values, Fig. 4*B* and *D*) and anti-peak outward current (e.g. at 0 and +50 mV was 303 ± 29 and 1072 ± 110 pA, respectively; $P < 0.01$ compared with control values, Fig. 4*B* and *E*). *I–V* relationships under control conditions and in the presence of pH 6.5 solution were statistically different (ANOVA, $P < 0.001$). Figure 4*C* shows difference currents describing the rapidly activating and inactivating conductance that was blocked by pH 6.5. Summary *I–V* curves were constructed from the difference currents in each experiment and are shown in Fig. 4*F*. Outward currents recorded from ileal myocytes were similarly affected by pH 6.5 (data not shown).

The effects of extracellular acidic pH on outward currents in the presence of 4-AP and TEA

In a previous report (Koh *et al.* 1999), we isolated two types of delayed rectifier K^+ currents in colonic myocytes using pharmacological techniques and voltage protocols. TEA isolated an A-type current that was blocked by 4-AP. Treatment with 4-AP in the absence of TEA revealed a classical, sustained delayed rectifier K^+ current. In the current study cells were held at −80 mV and stepped to test potentials ranging from −80 to +70 mV in 10 mV intervals in the presence of both 4-AP (5 mM) and TEA (10 mM). The resulting *I–V* relationship was plotted for peak currents from murine colonic myocytes (Fig. 5*A*, *D* and *E*). The peak outward current at 0 and +50 mV was 573 ± 107 and 1565 ± 304 pA, respectively, and the anti-peak current (measured at the end of the test potential) at 0 and +50 mV was 166 ± 23 and 675 ± 108 pA, respectively, under control conditions ($n = 7$). Exposure to pH 6.5 solution did not affect the peak outward current significantly (e.g. at 0 and +50 mV was 395 ± 98 and 1288 ± 284 pA, respectively ($P > 0.05$ compared with control values, Fig. 5*B* and *D*) but the anti-peak current was reduced (e.g. at 0 and +50 mV was 90 ± 29 and 434 ± 130 pA, respectively ($P < 0.01$ compared with control values, Fig. 5*B* and *E*). Figure 5*C* shows difference currents comparing control *versus* pH 6.5. Exposure to pH 6.5 reduced a slowly activating and non-inactivating current. Summary *I–V* curves showing the current inhibited at pH 6.5 (pH 6.5-sensitive currents) are presented in Fig. 5*F*. These data suggest that the decrease in net outward current elicited by low pH was caused by a decrease in a slowly activating and non-inactivating K^+ current.

Solutions at pH 6.5 also appeared to increase the initial part of the delayed rectifier K^+ currents. However, the amplitude of the peak current was not affected by pH 6.5 (see Fig. 5*D*). Therefore, we analysed the activation time constants before and in the presence of pH 6.5 (Fig. 5*G*). In acidic condition, the activation time constant was significantly decreased from 26.9 ± 1.8 to 8.6 ± 2.1 ms at −10 mV ($P < 0.001$, $n = 7$). In difference currents, this enhancement in activation rate is reflected by an initial transient downward deflection in the presence of acidic pH (downward deflection in Fig. 5*C*). The effects of pH on the A-type component of the delayed rectifier current, which are encoded by the Kv4 family of K^+ channels in colonic myocytes (Amberg *et al.* 2003), were not further investigated in this study. The subtracted currents in Fig. 5*C* followed by fast activation revealed slow activation kinetics. The slow activation time constants from pH-sensitive currents were 43 ± 3 and 56 ± 6 ms at −10 and 10 mV, respectively ($n = 7$, see inset in Fig. 5*C*). These activation time constants were similar to previously reported values in TASK-2 expressed in mammalian cells (Reyes *et al.* 1998).

We also tested the effects of acidic pH in ileal myocytes in the presence of 4-AP and TEA. Changing the pH from 7.4 to 6.5 had similar effects on ileal myocytes as on colonic myocytes. (Fig. 5*H–J*). The peak outward current at 0 and +50 mV was 231 ± 22 and 857 ± 91 pA, respectively, and the anti-peak current (measured at

in the presence of 4AP and TEA (see *C*). *J*, summary *I–V* relationship for peak (○) and anti-peak (●) currents after exposure to pH 6.5 solution in the presence of lidocaine, 4-AP and TEA (see *F*). *K*, control traces in the presence of 4-AP (5 mM) and TEA (10 mM) in ileal myocytes. *L*, application of lidocaine in the presence of 4-AP (5 mM) and TEA (10 mM). *M*, difference currents obtained by subtraction of currents in the presence of lidocaine (*L*) from control currents (*K*). *N*, control traces in the presence of lidocaine (1 mM), 4-AP (5 mM) and TEA (10 mM) in ileal myocytes. *O*, exposure to pH 6.5 solution in the presence of lidocaine (1 mM), 4-AP (5 mM) and TEA (10 mM). *P*, difference currents obtained by subtraction of currents in *O* from *N*.

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the end of the test potential) at 0 and +50 mV was 191 ± 21 and 709 ± 89 pA, respectively, under control conditions ($n = 7$). Exposure to pH 6.5 solution did not affect the peak outward current significantly, but the anti-peak current was reduced (e.g. at 0 and +50 mV was 113 ± 9 and 539 ± 73 pA, respectively ($P < 0.01$ compared with control values). Figure 5*J* shows difference currents comparing control *versus* pH 6.5. Exposure to pH 6.5 reduced a slowly activating and non-inactivating current.

The effects of extracellular acidic pH on outward currents in the presence of lidocaine

Kindler *et al.* (2003) reported that lidocaine blocked TASK-2 channels. Thus, we tested the effects of lidocaine on the outward current in colonic myocytes. Application of lidocaine (1 mM) significantly decreased peak and anti-peak currents through all tested potentials (−80 to +70 mV) (Fig. 6*A*, *B*, *D* and *E*). The peak current (1712 ± 206 pA in control conditions) significantly decreased to 527 ± 51 pA after lidocaine treatment at +20 mV ($P < 0.01$, $n = 5$). Difference currents showed that both peak and anti-peak outward currents were decreased (Fig. 6*C* and *F*). The shape of the difference currents revealed time-dependent inactivation, suggesting that lidocaine has non-specific effects on delayed rectifier K^+ currents. Therefore, we further tested the effects of lidocaine in the presence of 4-AP and TEA to eliminate delayed rectifier K^+ currents. Under these conditions the peak outward current was 369 ± 76 and 1057 ± 217 pA in control conditions at 0 and +50 mV,

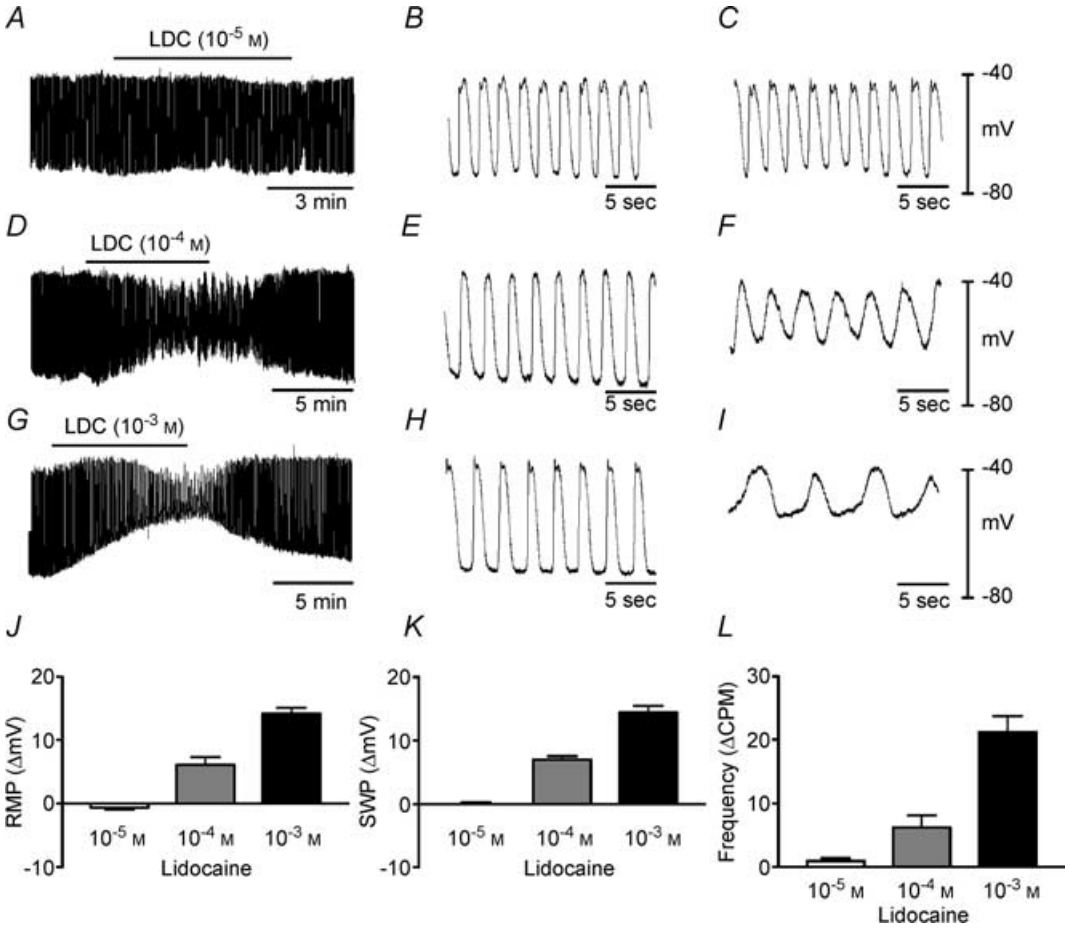

Figure 8. Effects of lidocaine (LDC) on membrane potential of murine ileum
Intracellular microelectrode recordings were made from circular muscle cells of intact ileal muscles in the presence of tetrodotoxin (10^{-6} M). *A*, low concentration (10^{-5} M) of lidocaine did not affect the membrane potentials. *B* and *C*, expanded scale of control and maximal effect of lidocaine (10^{-5} M). *D*, lidocaine (10^{-4} M) depolarized murine ileal tissue. *E* and *F*, expanded scale of control and maximal effect of lidocaine (10^{-4} M). *G*, lidocaine (10^{-3} M) further depolarized murine ileal tissue. *H* and *I*, expanded scale of control and maximal effect of lidocaine (10^{-3} M). *J*, summary of changes in resting membrane potential (RMP) with different concentrations of lidocaine ($n = 5$). *K*, summary of changes in slow wave potentials (SWP) with different concentrations of lidocaine. *L*, summary of changes in slow wave frequency with different concentrations of lidocaine.

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respectively (Fig. 7*A* and *G*, $n = 5$). Peak current at these potentials was significantly reduced to 217 ± 29 and 578 ± 87 pA by lidocaine ($P < 0.05$, Fig. 7*B* and *G*). Anti-peak current was also reduced by lidocaine (i.e. from 203 ± 50 and 627 ± 133 pA in control at 0 and +50 mV to 118 ± 24 and 331 ± 56 pA, respectively; $P < 0.05$). Subtracted currents are shown in Fig. 7*C* and *I*. Under these conditions, the lidocaine-sensitive current showed no time-dependent inactivation, suggesting that lidocaine inhibited a conductance other than a delayed rectifier.

We also tested the effects of pH 6.5 in the presence of lidocaine on colonic myocytes (Fig. 7*D*–*F*). Solutions at pH 6.5 did not inhibit the anti-peak current after treatment of cells with lidocaine (i.e. 96 ± 20 and 360 ± 58 pA at 0 and +50 mV, respectively, $P > 0.05$ when values were compared with currents in the presence of lidocaine, Fig. 7*F*, *H* and *J*), suggesting lidocaine inhibited the pH-sensitive outward current in these cells. Interestingly, pH 6.5 increased peak but not anti-peak currents (Fig. 7*F*, *G* and *J*) suggesting that effects of acidic pH on A-type currents (see Fig. 5) were not affected by lidocaine.

We repeated the experiments to test the effects of lidocaine on ileal myocytes (Fig. 7*K*–*P*). The effects of lidocaine and of pH 6.5 in the presence of lidocaine, 4AP and TEA were similar to colonic myocytes. Application of lidocaine (1 mM) significantly decreased peak and anti-peak currents at all tested potentials (−80 to +70 mV; $n = 7$; $P < 0.05$). In the presence of lidocaine, 4AP and TEA anti-peak current at 0 and +50 mV was 108 ± 14 and 344 ± 74 pA, respectively. Solutions at pH 6.5 did not inhibit the anti-peak current after pretreatment of cells with lidocaine (i.e. 117 ± 25 and 453 ± 91 pA at 0 and +50 mV, respectively; $n = 6$; $P > 0.05$ compared with currents recorded in the presence of lidocaine at pH 7.4, Fig. 7*N* and *P*), suggesting lidocaine inhibited the pH-sensitive outward current in these cells. Anadamide (3 μM) and Zn^{3+} (30 μM) also inhibited the pH-sensitive currents ($n = 3$ each; data not shown).

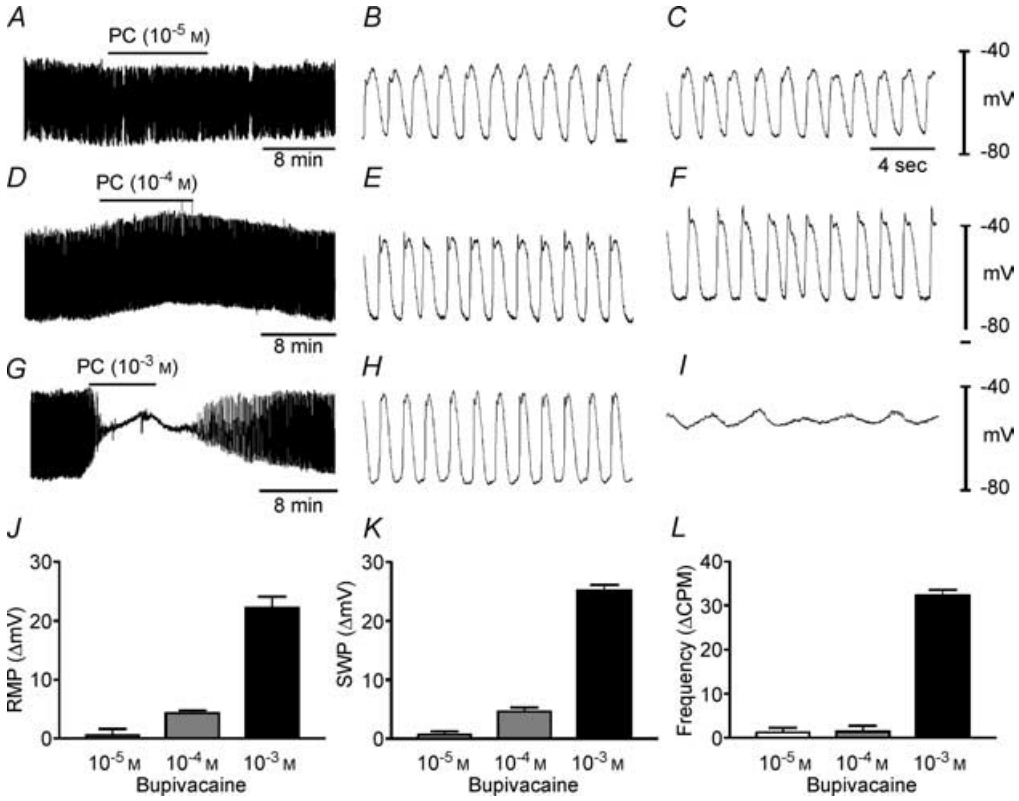

Figure 9. Effects of bupivacaine (PC) on membrane potential of murine ileum
Intracellular microelectrode recordings were made from circular muscle cells of intact ileal muscle strips in the presence of tetrodotoxin (10^{-6} M). *A*, low concentration (10^{-5} M) of bupivacaine did not affect the membrane potentials. *B* and *C*, expanded scale of control and maximal effect of bupivacaine (10^{-5} M). *D*, bupivacaine (10^{-4} M) depolarized murine ileal tissue. *E* and *F*, expanded scale of control and maximal effect of bupivacaine (10^{-4} M). *G*, bupivacaine (10^{-3} M) further depolarized murine ileal tissue. *H* and *I*, expanded scale of control and maximal effect of bupivacaine (10^{-3} M). *J*, summary of changes in resting membrane potential (RMP) with different concentrations of bupivacaine ($n = 5$). *K*, summary of changes in slow wave potentials (SWP) with different concentrations of bupivacaine. *L*, summary of percentage changes in slow wave frequency with different concentrations of bupivacaine.

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Contribution of the lidocaine- and bupivacaine-sensitive conductance to resting potentials of intact intestinal muscles

Intracellular microelectrode experiments were performed to examine the effects of local anaesthetics on the electrical activity of intact small and large intestinal muscles. To exclude the effects of lidocaine on enteric neurones, all experiments were performed in the presence of tetrodotoxin (10^{-6} M). Low concentrations of lidocaine (up to 10^{-5} M) had no effect on resting membrane potentials of circular muscle cells in murine ileum (Fig. 8*A–C*). At 10^{-4} M lidocaine induced depolarization from -73 ± 2 to -66 ± 3 mV ($n = 5$, $P < 0.01$, Fig. 8*D–F*). This effect was associated with decreased slow wave amplitude (from 26 ± 1 to 19 ± 1 mV; $P < 0.001$) and reduced slow wave frequency (from 0.58 ± 0.017 to 0.48 ± 0.017 Hz; $P < 0.05$; $n = 5$). Higher concentrations of lidocaine (10^{-3} M) produced further membrane depolarization (from -71 ± 1 to -57 ± 1 mV; $n = 5$; $P < 0.001$, Fig. 8*G–I*). Slow wave amplitude decreased from 26 ± 1 to 11 ± 1 mV ($P < 0.001$), and slow wave frequency decreased from 0.57 ± 0.017 to 0.22 ± 0.017 Hz ($P < 0.001$). Summarized data from these experiments are shown in Fig. 8*J–L*). Bupivacaine, a derivative of lidocaine, caused similar effects as lidocaine on the resting membrane potential, slow wave amplitude and frequency (Fig. 9). Summarized data for bupivacaine are shown in Fig. 9*J–L*. At higher concentrations, bupivacaine (10^{-3} M) produced effects on slow wave parameters that exceeded the effects of lidocaine at the same concentration.

Effects of lidocaine in the presence of K^+ channel blockers

Lidocaine is known to have effects on a variety of ion channels (e.g. Ye *et al.* 1999; Kutchai & Geddis, 2001; Charpentier, 2002; Xu *et al.* 2003), and it is possible that the depolarizing effects of this compound could be due to blockade of multiple K^+ conductances. To evaluate the effects of lidocaine on other K^+ conductances we retested the effects of lidocaine after pre-exposure of muscles to compounds known to block various K^+ conductances expressed by murine GI muscles. TEA (10 mM), an inhibitor of large and intermediate conductance Ca^{2+}-activated K^+ channels and the sustained component of the delayed rectifier K^+ conductance in murine intestinal myocytes (Koh *et al.* 1999), had no significant effect on membrane potential (from -69 ± 5 to -64 ± 4 mV in the presence of TEA; $n = 4$, $P > 0.05$) in murine ileum. Application of lidocaine in the presence of TEA depolarized the membrane potential to -53 ± 5 mV ($n = 4$, $P < 0.05$ compared with in the presence of TEA, Fig. 10*A*). 4-AP (5 mM), an inhibitor of the transient delayed rectifier current in intestinal myocytes (A-type current), induced a depolarization from -64 ± 1 to -56 ± 1 mV ($n = 4$, $P < 0.01$). Exposure to lidocaine in the presence of 4-AP caused further depolarization to -47 ± 2 mV ($P < 0.05$ compared with RMP in the presence of 4-AP, Fig. 10*B*). Glibenclamide (10^{-5} M), an inhibitor of K_{ATP} channels, did not alter resting membrane potential. In the presence of glibenclamide, lidocaine caused depolarization from -64 ± 1 to -54 ± 2 mV ($n = 4$, $P < 0.01$, Fig. 10*C*).

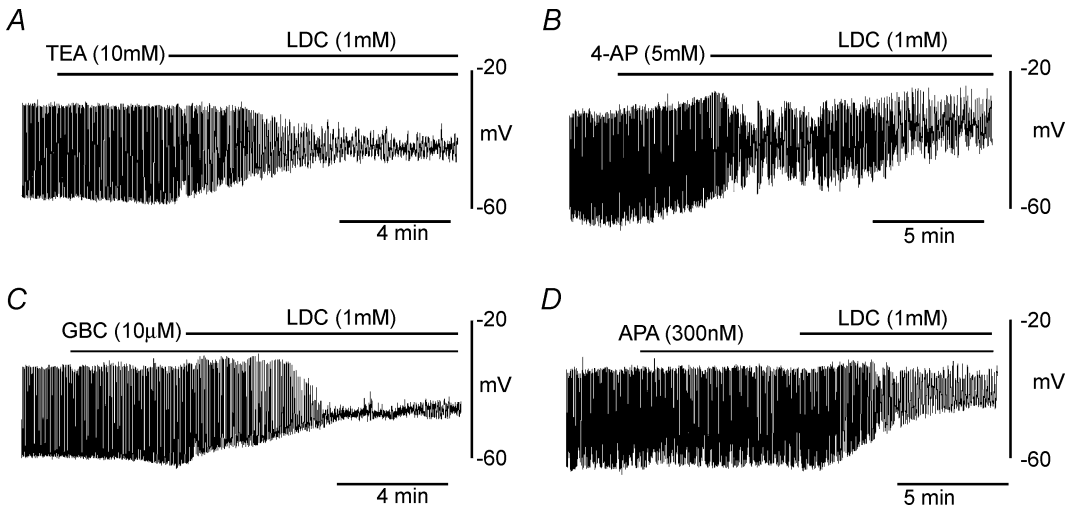

Figure 10. Effects of lidocaine on membrane potential in the presence of K^+ channel blockers
A, lidocaine induced depolarization in the presence of TEA (10 mM). *B*, 4-AP (5 mM) caused depolarization. Lidocaine after 4-AP caused further depolarization. *C*, lidocaine induced depolarization in the presence of glibenclamide (GBC, 10 μM). *D*, lidocaine induced depolarization in the presence of apamin (APA, 300 nM).

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Neither apamin (300 nM), a blocker of small conductance Ca^{2+}-activated K^+ conductance, nor MK499, a blocker of KCNH2 channels, had any effect on membrane potential. Application of lidocaine in the presence of apamin or MK499, depolarized cells from -67 ± 4 and -66 ± 1 mV to -47 ± 1 mV ($n = 3$, Fig. 10*D*) and -58 ± 2 mV ($n = 4$, $P < 0.01$, data not shown), respectively.

Effects of pH and lidocaine on resting membrane potential

We hypothesized that the effects of lidocaine on resting membrane potentials could be due to blocking of the pH-sensitive K^+ channels expressed by these cells. When the pH of the bath solution was changed from 7.4 to 6.5 and 6.0 cells depolarized from -75 ± 1 mV to -68 ± 2 and -51 ± 1 mV ($n = 5$, $P < 0.05$ and $P < 0.001$), respectively (Fig. 11*A–C*). Alkalization hyperpolarized cells from -74 ± 2 mV to -77 ± 2 mV (pH 8.0, $n = 5$, $P > 0.05$) and -78 ± 2 mV (pH 8.5, $P < 0.01$, Fig. 11*A–C*). Slow wave amplitude and frequency were also reduced by acidification (to pH 6.5 and 6.0), but alkalization (pH 8.0 and 8.5) had no effect. For example, changing the pH to 6.0 decreased slow wave amplitude from 36 ± 2 mV to 3 ± 1 mV ($n = 5$, $P < 0.001$, Fig. 11*A*, *B* and *D*) and slow wave frequency from 0.6 ± 0.017 to 0.13 ± 0.1 H_2 ($n = 5$, $P < 0.001$, Fig. 11*A*, *B* and *E*). Data from experiments in which the pH was changed are summarized in Fig. 11*C–E*.

When muscles were exposed to pH 6.0 solutions, lidocaine had no further effect on membrane potential (Fig. 12*A*). When the order of application of these drugs

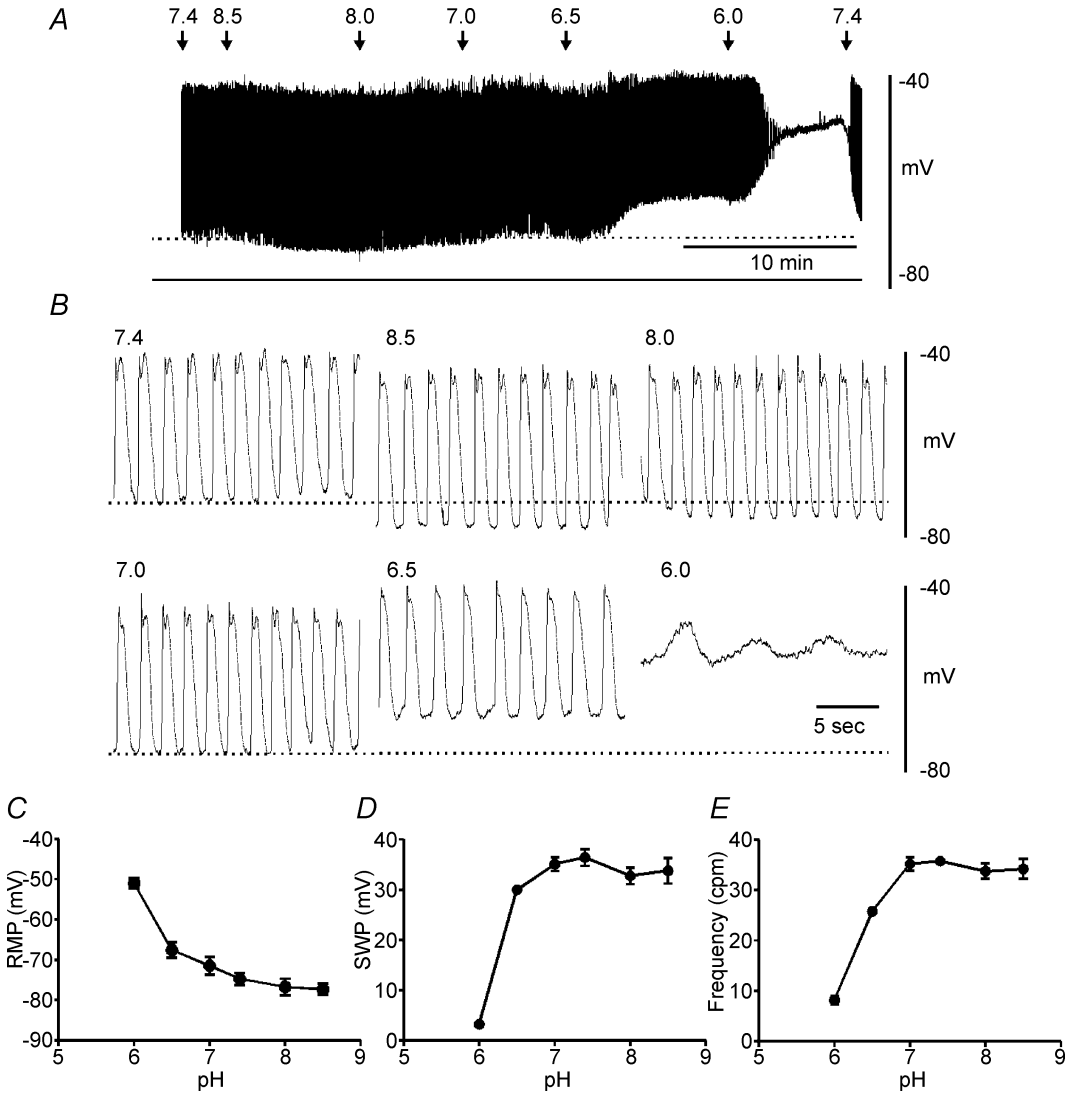

Figure 11. Effects of extracellular pH on membrane potentials of ileal muscles
A, application of solutions at different pH (from 8.5 to 6.0) affected resting membrane potentials of ileal muscles. *B*, expanded time scales showing effects of different pH solutions on membrane potential (data from *A*). *C–E*, summary of the effects of external pH on resting membrane potentials (RMP), slow wave amplitude (SWP) and slow wave frequency ($n = 5$).

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was reversed, lidocaine depolarized cells from -60 ± 3 mV to -52 ± 6 mV ($n = 4$), but acidic pH (6.0) had no further effect on membrane potential (Fig. 12*B*). Alkaline pH (8.5) induced hyperpolarization from -67 ± 1 mV to -71 ± 1 mV ($n = 4$). Additional application of lidocaine depolarized the membrane potential to -56 ± 1 mV (Fig. 12*C*). In reverse order experiments, lidocaine induced depolarization from -69 ± 1 mV to -56 ± 4 mV ($n = 4$, Fig. 12*D*), but subsequent addition of alkaline pH (8.5) solution failed to cause hyperpolarization (i.e. -56 ± 2 mV). These data suggest that the effects of both acidification and alkalization occur through a conductance that was inhibited by lidocaine.

We also tested pH effects on resting membrane potential in murine colon in the presence of TTX (10^{-6} M). The application of pH 6.5 solution into the bath induced rapid depolarization from -47 ± 1 mV in control to -33 ± 1 mV in pH 6.5 ($n = 16$, $P < 0.001$) that was reversible upon restoration of control pH (Fig. 13*A*). Pretreatment with TEA (10 mM) did not affect resting membrane potentials (-47 ± 2 mV in control and -47 ± 2 mV in TEA, $n = 6$) and did not inhibit pH effects on resting membrane potentials (-33 ± 3 mV with pH 6.5, $P < 0.001$, Fig. 13*B*). Pretreatment with 4-AP (5 mM) induced depolarization from -51 ± 1 mV to -45 ± 1 mV ($n = 5$, $P < 0.01$), and pH 6.5 further depolarized colonic tissue in the presence of 4-AP to -34 ± 1 mV ($P < 0.001$, Fig. 13*C*). Pretreatment with both TEA and 4-AP induced depolarization from -51 ± 1 mV to -46 ± 1 mV ($n = 5$, $P < 0.01$) and did not block the effects of pH 6.5 on resting membrane potentials (-32 ± 1 mV with pH 6.5, $P < 0.001$, Fig. 13*D*). Lidocaine (1 mM) depolarized colonic tissue from -50 ± 1 mV to -35 ± 2 mV ($n = 6$, $P < 0.001$), and this effect was reversible upon removal of the drug (Fig. 13*E*). Application of pH 6.5 solution in the continued presence of lidocaine did not cause further depolarization (i.e. -35 ± 1 mV with lidocaine and pH 6.5, $n = 6$).

We also performed the current-clamp experiments on isolated colonic myocytes. The resting membrane potentials under control conditions (MnPSS dialysis with 10 mM BAPTA) was -36 ± 2 mV ($n = 5$). In the same cells, both 4-AP and TEA depolarized cells to -33 ± 1 mV. In the presence of 4-AP and TEA, pH 6.5 solution further depolarized cells to -26 ± 1 mV ($P < 0.01$; Fig. 13*F*).

Discussion

Two-pore K^+ channels (also named KCNK channels according to the HUGO Gene Nomenclature Committee) include TWIK, TREK, TASK, TALK and TRAAK. TASK channels are sensitive to changes in extracellular pH. TASK-1 encodes an acid- and anaesthetic-sensitive background K^+ conductance that sets the resting membrane potential in some cells, such as cerebellar granule neurones, somatic motoneurones, locus coeruleus cells and rat heart (Lesage, 2003). TASK-2 has a low sequence similarity to other two-pore domain

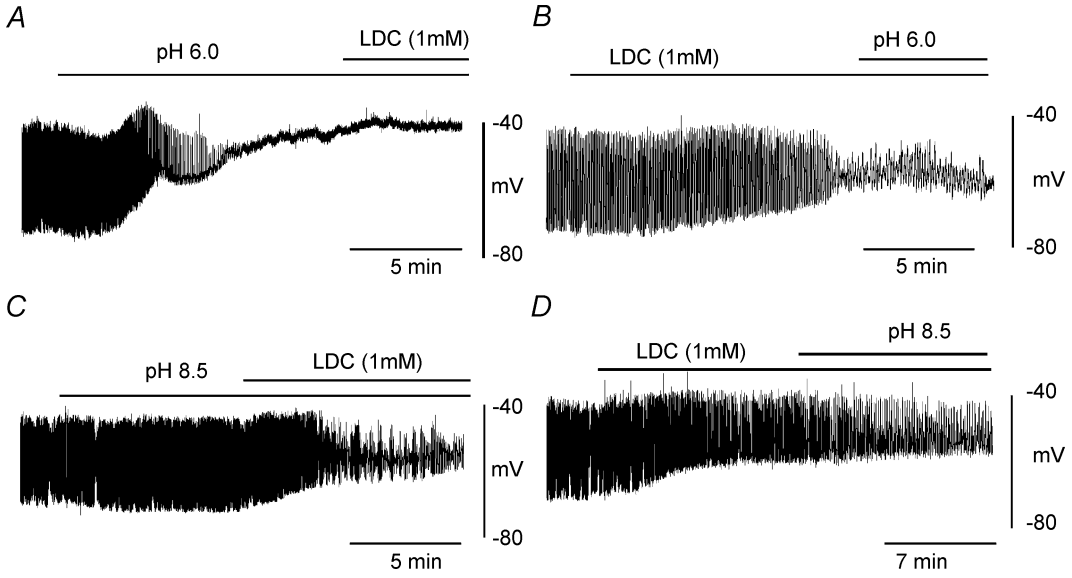

Figure 12. Effects of acidic and alkaline pH before and after lidocaine treatment of murine ileum
A, exposure of ileal muscles to pH 6.0 caused depolarization. Application of lidocaine (1 mM) after pH 6.0 solution did not induce further depolarization. *B*, in reverse order, lidocaine caused depolarization, but addition of acidic pH (6.0) in the continued presence of lidocaine had no further effect on membrane potential. *C*, exposure of muscles to pH 8.5 solution caused hyperpolarization. Addition of lidocaine (1 mM) after exposure to pH 8.5 caused depolarization. *D*, in reverse order, lidocaine induced depolarization, but subsequent addition of alkaline pH (8.5) solution failed to cause hyperpolarization.

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K^+ channels, such as TWIK-1, TREK-1, TASK-1 and TRAAK (18–22% of amino acid identity), but TASK-2 channels have similar topology consisting of four membrane-spanning domains and similar pH sensitivity as TASK-1. TASK-2 currents display time-dependent activation and are non-inactivating throughout second-long voltage pulses (Reyes *et al.* 1998; Kindler *et al.* 2003).

In this study we have demonstrated expression of TASK-1 and -2 mRNAs and proteins in smooth muscle cells of murine small and large intestine. We found a pH-sensitive conductance in ileal and colonic myocytes that had properties similar to TASK channels. The native conductance was voltage dependent, slowly activating, non-inactivating, inhibited by acidic solutions, and blocked by lidocaine. We also found evidence for a pH- and lidocaine-sensitive background conductance in intact small and large intestinal muscles that contributes significantly to resting potentials. Our findings are consistent with the hypothesis that TASK channels are responsible for the pH-sensitive, voltage-independent background conductance in GI muscles. These channels may contribute to setting of membrane potential and regulation of excitability in these cells.

A similar conductance, attributed to TASK-1 expression, has also been reported in a study of pulmonary artery smooth muscle cells (Gurney *et al.* 2003). The arterial conductance showed similar pH sensitivity as the conductance we observed in intestinal muscles. Halothane has been reported to activate TASK channels (Patel *et al.* 1999). While it is difficult to compare the kinetics of the conductance in vascular muscle with that observed in the present study because difference currents were not displayed in the previous study, similarities in the pH-sensitive currents in the two muscle types suggest that TASK family channels may be generally important in both vascular and visceral smooth muscles as regulators of membrane potential.

The pH-sensitive conductance in murine intestinal myocytes was reasonably well-isolated by blocking A-type and sustained delayed rectifier K^+ currents in colonic myocytes with 4-AP and TEA (see Koh *et al.* 1999). The remaining pH-sensitive current (Fig. 2*C*) displayed slow, time-dependent activation kinetics similar to TASK-2 currents (Kindler *et al.* 2003). Similar slow activation kinetics were observed with currents of TASK-2 channels expressed in oocytes, and this conductance also showed pH sensitivity similar to native conductances in smooth muscle myocytes. TASK-3 currents are highly sensitive to extracellular pH, but TASK-3 channels exhibit time-independent activation and non-inactivating K^+ currents (Kim *et al.* 2000). TASK-4 is sensitive to extracellular pH, but, in contrast to other TASK channels, pH sensitivity is shifted toward more alkaline pH values. TASK-4 currents are only weakly inhibited by 1 mM concentrations of quinine, bupivacaine and lidocaine (Decher *et al.* 2001). TASK-5 did not functionally express in COS-7 cells (Kim & Gnatenco, 2001). Thus, TASK-3, -4 and -5 are less likely to contribute to the pH-sensitive conductance we have observed in intestinal myocytes.

We also found that a component of the A-type current in colonic myocytes was sensitive to pH; however, characterization of the pH effects on this conductance were beyond the scope of the current study. We have

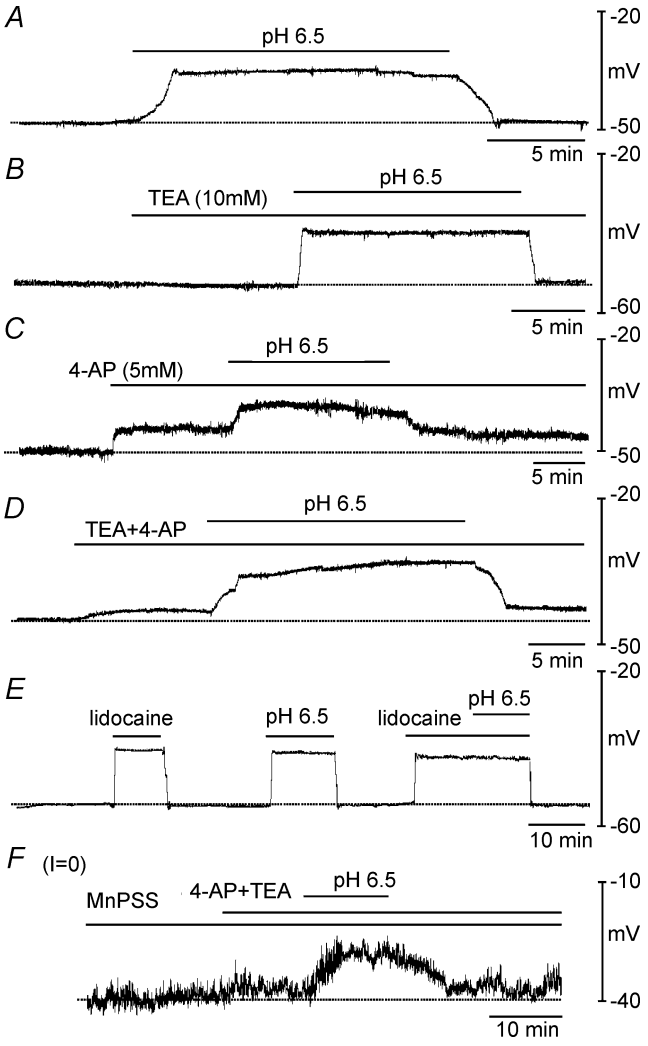

Figure 13. Effects of pH and lidocaine on membrane potentials of murine colon

A, exposure of colonic muscles to pH 6.5 induced rapid membrane depolarization. *B*, depolarization in response to pH 6.5 solution occurred in the presence of TEA (10 mM). *C*, 4-AP (5 mM) caused depolarization. The application of pH 6.5 solution in the presence of 4-AP caused further depolarization. *D*, pH 6.5 induced membrane depolarization in the presence of both TEA and 4-AP. *E*, lidocaine and pH 6.5 induced depolarization. In the presence of lidocaine, pH caused no further depolarization. *F*, pH 6.5 depolarized isolated colonic myocytes in the presence of both 4-AP and TEA under current-clamp conditions ($I = 0$).

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previously shown that the A-type current is likely to be due to expression of Kv4 family channels in colonic myocytes (Amberg *et al.* 2003). A previous report has suggested that the effects of pH on A-type currents are blocked by 4-AP (Singarayar *et al.* 2003), but close inspection of the data shows that acidic pH enhanced the *rate* of activation of this conductance. Further studies performed on expressed Kv4 channel currents will be needed to fully characterize the effects of pH on activation.

Local anaesthetics are inhibitors of a variety of channels, including Na^+ channels, voltage-gated K^+ channels such as hKv1.5, Kv2.1, Kv4.3 and KvLQT1, and G protein-coupled inward rectifying K^+ channels at micromolar concentrations (Gonzalez *et al.* 2001; Zhou *et al.* 2001). Local anaesthetics are also inhibitors of two-pore K^+ channels (Kindler *et al.* 1999). Both TASK-1 and -2 channels are inhibited by the local anaesthetics lidocaine and bupivicaine, bupivicaine being the more potent blocker ($IC_{50} = 68\ \mu$M for TASK-1 and 81% of inhibition of TASK-2 at 1 mM) (Leonoudakis *et al.* 1998; Reyes *et al.* 1998; Kindler *et al.* 1999). We tested the effects of lidocaine (1 mM) on the pH-sensitive currents in murine colonic myocytes. Lidocaine also inhibits Atype currents and the sustained delayed rectifier current in intestinal myocytes (not shown), but in our experiments these conductances were blocked with 4-AP and TEA. In the presence of these blockers and lidocaine, pH 6.5 had no effect. These data suggest that lidocaine is a blocker of the pH-sensitive conductance in GI myocytes.

Experiments were performed on intact muscles, using the pharmacology and pH sensitivity of the conductance we identified in single myocytes. Membranes of cells in intact muscles were depolarized by lidocaine; however, pretreatment with acidic pH blocked the effects of lidocaine, suggesting that acidic pH and lidocaine exert effects through a similar conductance. Taken together, expression of TASK channels, kinetic property and pharmacology of the native pH-sensitive conductance in isolated myocytes, and responses of intact muscle to conditions that block the native pH-sensitive conductance, suggest that TASK channels provide an important contribution to the K^+ conductances responsible for setting the negative resting potentials in GI smooth muscles. Since membrane potential is a potent determinant of the overall excitability of smooth muscle tissues and particularly the response of GI muscles to slow wave depolarization, this conductance is likely to be an important factor in maintenance of normal physiological activity. At this point it is unclear whether these conductances are also subject to regulation by physiological agonists and second messenger systems, making them targets for the effects of neurotransmitters and other biological agonists.

Blood pH never drops to values as low as those used in the present study to block the TASK-like conductance, but local changes in pH due to pathophysiological conditions such as ischaemia, inflammation or gastrointestinal obstruction (stasis) may result in tissue acidosis. These conditions can also induce acute pain by lowering thresholds of mechanoreceptors in enteric sensory neurones. Our data suggest that effects on acid-sensing ion channels may also contribute to some of the responses of GI muscle to localized acidosis. Additional studies on the electrical activity of intact muscles and organs, using the pharmacology and pH conditions established here for TASK channels, may help elucidate the role of these channels in GI excitability and may help determine the role of TASK-like conductances in normal and pathological conditions.

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