Basal Activation of ATP-Sensitive Potassium Channels in Murine Colonic Smooth Muscle Cell

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ABSTRACT The function and molecular expression of ATP-sensitive potassium (K_{ATP}) channels in murine colonic smooth muscle was investigated by intracellular electrical recording from intact muscles, patch-clamp techniques on isolated smooth muscle myocytes, and reverse transcription polymerase chain reaction (RT-PCR) on isolated cells. Lemakalim (1 μM) caused hyperpolarization of intact muscles (17.2 $\pm$ 3 mV). The hyperpolarization was blocked by glibenclamide (1–10 μM). Addition of glibenclamide (10 μM) alone resulted in membrane depolarization (9.3 $\pm$ 1.7 mV). Lemakalim induced an outward current of 15 $\pm$ 3 pA in isolated myocytes bathed in 5 mM external K^+ solution. Application of lemakalim to cells in symmetrical K^+ solutions (140/140 mM) resulted in a 97 $\pm$ 5 pA inward current. Both currents were blocked by glibenclamide (1 μM). Pinacidil (1 μM) also activated an inwardly rectifying current that was insensitive to 4-aminopyridine and barium. In single-channel studies, lemakalim (1 μM) and diazoxide (300 μM) increased the open probability of a 27-pS K^+ channel. Openings of these channels decreased with time after patch excision. Application of ADP (1 mM) or ATP (0.1 mM) to the inner surface of the patches reactivated channel openings. The conductance and characteristics of the channels activated by lemakalim were consistent with the properties of K_{ATP}. RT-PCR demonstrated the presence of K_{ir} 6.2 and SUR2B transcripts in colonic smooth muscle cells; transcripts for K_{ir} 6.1, SUR1, and SUR2A were not detected. These molecular studies are the first to identify the molecular components of K_{ATP} in colonic smooth muscle cells. Together with the electrophysiological experiments, we conclude that K_{ATP} channels are expressed in murine colonic smooth muscle cells and suggest that these channels may be involved in dual regulation of resting membrane potential, excitability, and contractility.

INTRODUCTION

In 1983, Noma identified a K^+ current in cardiac myocytes that was inhibited by intracellular ATP. Many studies have since demonstrated the presence of ATP-sensitive K^+ conductances (K_{ATP}) in a variety of tissues. Currents attributed to K_{ATP} channels have been observed in a number of vascular and visceral smooth muscle preparations, including rabbit mesenteric artery (Nelson et al., 1990), saphenous vein (Nakashima and Vanhoutte, 1995), rabbit portal vein (Kitamura and Kuriyama, 1994), guinea pig trachealis (Murray et al., 1989), canine bronchial smooth muscle (Kamei et al., 1994), guinea pig urinary bladder (Bonev and Nelson, 1993), guinea pig gall bladder, (Zhang et al., 1994), and pig proximal urethra (Teramoto and Brading, 1996). Currents arising from K_{ATP} are inwardly rectifying K^+-selective currents that are regulated by the metabolic state of the cell (Quayle et al., 1995). These channels are inhibited by high concentrations of intracellular ATP, insensitive to changes in intracellular Ca^{2+}, activated by certain K^+ channel agonists (e.g., pinacidil (PNC), cromakalim, and diazoxide (DZX)), and inhibited by glibenclamide (GBC). GBC is a selective K_{ATP} channel blocker at submicromolar concentrations (Bray and Quast, 1992; Edwards and Weston, 1993; Kitamura and Kuriyama, 1994; Teramoto and Brading, 1996).

A number of studies have suggested that K_{ATP} plays an important role in the regulation of resting membrane potential and membrane excitability in tissues such as the heart (Noma, 1983) and pancreatic β-cells (Ashcroft and Kakei, 1989), but the physiological role of K_{ATP} in smooth muscle has been more controversial. For example, Quast and Cook (1989) reported that K_{ATP} channels were not activated under basal conditions in vascular smooth muscle because GBC did not increase muscle tone. In other reports GBC (1–10 μM) caused marked depolarization of resting membrane potential in smooth muscles (Murray et al., 1989; Nelson et al., 1990; Itoh et al., 1992; Kamei et al., 1994; Nakashima and Vanhoutte, 1995; Teramoto and Brading, 1996). There also appear to be significant differences in the pharmacological and electophysiological profiles of K_{ATP} in various smooth muscles. For example, the sensitivity of K_{ATP} to compounds such as DZX and lemakalim (LMK) varies, and the unitary conductance of K_{ATP} channels differs between preparations (Standen et al., 1989; Kajioka et al., 1991; Miyoshi et al., 1992). These findings suggest that various species of K_{ATP} may be expressed in smooth muscles.

Recent molecular studies have shown that functional K_{ATP} channels are formed by the combination of a sulfonylurea receptor (SUR) and an inward rectifier K^+ channel subunit of the $K_{ir}6$ family (Inagaki et al., 1995a,b; Sakura et al., 1995; Ammala et al., 1996; Isomoto et al., 1996; Shyng and Nichols, 1997). Two genes have been identified that encode the SUR subunits (i.e., SUR1 and SUR2). Alternative splicing of exon 38 results in two species of SUR2 (A

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and B), which differ in their carboxy terminal amino acid sequences (Isomoto et al., 1996). Various combinations of $K_{ir}6$ and SUR subunits convey the heterogeneity in channel properties observed in native cells. For example, combination of SUR2A and Kir6.2 results in "cardiac-like" channels that are inhibited by ATP at concentrations greater than 100 μM and by the sulfonylurea compound glibenclamide. These channels are activated by PNC and cromakalim, but not by DZX. SUR2B/$K_{ir}6.1$ has been proposed as the molecular entity responsible for K_{ATP} in vascular smooth muscles (Isomoto et al., 1996; Yokoshiki et al., 1998). Expression of these subunits results in channels that are stimulated by low concentrations of ATP (0.1–100 μM) and inhibited by higher concentrations (1–3 mM). These channels are activated by pinacidil and DZX, and have characteristics similar to those of nucleotide diphosphate K^+ channels (K_{NDP}) reported in vascular smooth muscles (Kajioka et al., 1991; Zhang and Bolton, 1996). The molecular entity responsible for K_{ATP}-like currents in gastrointestinal smooth muscles is currently unknown.

In the present study we have sought to identify the molecular species responsible for K_{ATP} in murine colonic smooth muscle and determine the function of this conductance. We investigated the role of K_{ATP} in the regulation of membrane potential and excitability, using intracellular electrical recording from intact colonic muscles, characterized the properties of K_{ATP} and the channels that contribute to K_{ATP} in colonic myocytes with patch clamp techniques, and determined the expression of specific isoforms of $K_{ir}6$ and SURs in isolated myocytes by reverse transcription polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Intracellular electrical recording

Colonic smooth muscle cells were isolated from 20- to 30-day-old *Balb/C* mice of either sex. Mice were anesthetized with chloroform and sacrificed by cervical dislocation, and the proximal colon was quickly removed. The colon was opened along the myenteric border. The mucosa was removed, leaving the intact tunica muscularis. Strips of muscle (3 × 10 mm) were cut and pinned to the floor of an electrophysiological chamber that was constantly perfused with oxygenated Krebs-bicarbonate solution of the following composition (mM): 118.5 NaCl, 4.5 KCl, 1.2 $MgCl_2$, 23.0 $NaHCO_3$, 1.2 KH_2PO_4, 11.0 dextrose, 2.4 $CaCl_2$ (pH 7.4), at 37 ± 0.5°C and left to equilibrate for at least 1 h. The pH of this solution was 7.3–7.4 when bubbled with 95% O_2/5% CO_2. Intracellular electrical recordings were made using conventional capillary glass microelectrodes (1.2 mm o.d., 0.6 mm i.d.; FHC Inc.) that were filled with 3 M KCl and had resistances ranging from 30 MΩ to 70 MΩ. Impalements were accepted based on previously discussed criteria (Smith et al., 1987). Membrane potential was measured with a high input impedance electrometer (WPI Duo 773; WPI, Sarasota, FL), and outputs were displayed on an oscilloscope (Nicolet 3091; Nicolet, Madison, WI). Analogue electrical and mechanical signals were reproduced on chart paper (Gould 2200) as well as digitized and recorded on a video cassette recorder (Panasonic Hi-Tech 4). Data were also stored and analyzed by computer (Micron Millenia Pro2), using a data acquisition program (AcqKnowledge III; Biopac Systems, Santa Barbara, CA). In some experiments, nifedipine (1 μM) was included in the perfusion solution to reduce contraction and facilitate impalements of cells. Nifedipine had no effect on responses of the muscles to GBC (10 μM) or LMK (1 μM).

Cell preparation

Strips of colonic muscle were immersed in Ca^{2+}-free Hanks' solution containing (in mM): 125 NaCl, 5.36 KCl, 15.5 NaOH, 0.336 Na_2HCO_3, 0.44 KH_2PO_4, 10 glucose, 2.9 sucrose, 11 HEPES. Mucosa and submucosa were removed. Small pieces of muscle (5 × 15 mm) were cut and incubated in a Ca^{2+}-free Hanks' solution containing 230 units collagenase (Worthington Biochemical Co.), 2 mg fatty acid-free bovine serum albumin (Sigma Chemical Co.), 2 mg trypsin inhibitor (Sigma Chemical Co.), and 0.11 mg ATP (Sigma Chemical Co.), and 0.1 mg protease (Sigma Chemical Co.). Incubation in the enzyme solution was carried out at 37°C for 8–12 min, and then the tissues were washed with Ca^{2+}-free Hanks' solution. Gentle trituration resulted in the liberation of isolated myocytes. Dispersed cells were kept at 4°C in Ca^{2+}-free Hanks' solution supplemented with S-MEM media (Sigma) and 0.5 $CaCl_2$, 0.5 $MgCl_2$, 4.17 $NaHCO_3$, and 10 HEPES, which prevented adherence of the cells to the storage vessel. Before electrophysiological experiments, a drop of the cell suspension was pipetted into an electrophysiological chamber (0.3 ml) on the stage of an inverted microscope. After 5 min, cells adhered to the bottom, and patch-clamp experiments were begun. These experiments were performed at room temperature (22–24°C). All recordings were made within 6 h of dispersing the cells.

Voltage-clamp experiments

Patch-clamp experiments using the dialyzed whole-cell and cell attached and excised patch configurations were performed on colonic smooth muscle cells. Currents were amplified with a List EPC-7 amplifier and digitized with a 12 bit A/D converter (TL-1, DMA interface; Axon Instruments). Data were stored on videotape or digitized on line with pClamp software (version 5.5.1 or 6.03; Axon Instruments). Data were sampled at 5 kHz and low-pass filtered at 1 kHz with an 8-pole Bessel filter. Probability density plots were obtained by scaling the amplitude histograms so that the total area beneath the curve equaled 1. Data were expressed as mean ± standard error, and a value of $p < 0.05$ was considered statistically significant.

Solutions

For the recordings of K^+ currents with the dialyzed whole-cell technique, the external MnPSS contained (in 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 composition of the internal solution for dialysed cells was (in mM): 110 K-gluconate, 20 KCl, 5 $MgCl_2$, 5 HEPES, 1 EGTA, 0.1 Na_2ATP, and 0.1 NaADP adjusted to pH 7.2 with Tris. For recording K^+ channel currents in cell-attached or excised patches, the bath solution contained (in mM): 140 KCl, 1 EGTA, 0.61 $CaCl_2$, and 10 HEPES, adjusted to pH 7.4 with Tris. The pipette solution was identical to the bath solution, except that 200 nM charybdotoxin (ChTx) was added to inhibit BK channels in the majority of cell-attached experiments. $[K^+]$ gradients are given as $[K^+]_{external}/[K^+]_{internal}$. LMK (Smith Kline Beecham), pinacidil (RBI), and GBC (Sigma) were dissolved with dimethyl sulfoxide, and DZX was dissolved in 1 M NaOH. The final concentration of dimethyl sulfoxide and NaOH was less than 0.1% and 0.1 mM, respectively.

Molecular biological techniques

Colonic smooth muscle cells, isolated by the procedure described above, were collected individually for molecular studies. Micropipettes were constructed from borosilicate glass (Sutter Instruments, Novato, CA) with 40- to 50-μm diameter tips. Cells were transferred to the stage of a phase contrast microscope and allowed to stick lightly to the glass coverslip bottom of a small chamber for 10 min. Then the cells were perfused with sterile phosphate-buffered saline to remove cellular debris. Single smooth muscle cells were identified and collected by positioning the tip of the micropipette near the cell and applying light suction. Approximately 60 smooth muscle cells were collected from each dispersion. After selection,

the cells were expelled from the pipette into an RNase-free microcentrifuge tube, flash-frozen in liquid nitrogen, and stored at −80°C until use.

Total RNA was prepared from colonic smooth muscle cells with SNAP Total RNA Isolation kits (Invitrogen, Carlsbad, CA), following the procedures of the manufacturer. Because only ~60 smooth muscle cells were used in each RNA isolation, 20 μg of polyinosinic acid (a carrier of RNA) was added to the lysates. First-strand cDNA was synthesized from the RNA preparations with a Superscript II RNase H Reverse Transcriptase kit (Gibco BRL, Gaithersburg, MD); RNA (1 pg) was reverse transcribed by use of an oligo$(dT)_{12-18}$ primer (500 μg/μl). To perform PCR, the following sets of primers were used: K_{ir} 6.1 forward, nucleotides 789–807, and reverse, 1300–1320 (gene accession number D4222145); K_{ir} 6.2 forward, nucleotides 698–717, and reverse, 1364–1383 (D50581); SUR1 forward, nucleotides 1424–1443, and reverse, 1653–1672 (L40624); and SUR2 forward, nucleotides 4300–4319, and reverse, 4512–4531 (D86038). SUR2 primers were design to amplify both SUR2A and SUR2B, and their presence would be demonstrated by the generation of a 407-bp or 231-bp fragment, respectively. PCR primers for β-actin (forward, nucleotides 2383–2402, and reverse, 3071–3091; V01217) were used to assess the viability of RNA samples as well as to detect genomic DNA contamination, whereby the primers were designed to span an intron in addition to two exons. Complementary DNA (20% of the first-strand reaction) was combined with sense and antisense primers (20 μM), 1 mM deoxynucleoside triphosphates, 60 mM Tris-HCl (pH 8.5), 15 mM $(NH_4)SO_4$, 1.5 mM $MgCl_2$, 2.5 units of TAQ (Promega), 1 Ampliwax Gem 100 (Perkin-Elmer), and RNase-free water to a final volume of 50 μl. The reaction occurred in a COY II Themal Cycler under the following conditions: 32 cycles at 94°C for 1 min; 57°C for 30 s, 72°C for 1 min, with a final extension step at 72°C for 10 min. Five microliters of the first-round PCR product was then added to a new reaction mixture containing all of the components listed above, and 32 additional cycles of PCR were then performed. PCR reactions in the presence of SUR2A/2B primers were performed as follows: an initial denaturation at 94°C for 4 min, followed by 30 cycles at 94°C for 45 s, 55°C for 1 min, 72°C for 2 min, with a final extension step at 72°C for 8 min (Isomoto et al., 1996). PCR products were separated by 2% agarose gel electrophoresis.

RESULTS

Effects of K^+-channel opener and GBC on membrane potentials

The murine proximal colon exhibited spontaneous electrical activity consisting of slow electrical oscillations with superimposed action potentials, as previously described (Ward et al., 1996). The average resting membrane potential (RMP) of the tissues was −52.8 ± 1.8 mV ($n = 17$). Perfusion with LMK (1 μM) induced significant hyperpolarization (17.0 ± 2.1 mV, $n = 7$), decreased slow wave and spike frequency, and eventually blocked excitable events (Fig. 1 *A*). GBC (1–10 μM), an antagonist of K_{ATP} channels, inhibited the LMK-induced hyperpolarization and restored spontaneous spike potentials (Fig. 1 *B*). In the presence of LMK, GBC also induced a small depolarization relative to control RMP (4.8 ± 0.6; $n = 8$ impalements from six muscle strips; $p < 0.05$). GBC (1–10 μM) applied to tissues in the absence of LMK caused depolarization (i.e., 9.3 ± 1.7 mV, $n = 6$; $p < 0.05$).

Whole-cell recordings

LMK (1 μM) evoked a sustained outward current (15 ± 3 pA, $n = 3$) in dialyzed cells held at −50 mV and perfused with MnPSS (with a physiological K^+ gradient; Fig. 2 *A*). In a symmetrical K^+ gradient (140/140 mM), LMK (1 μM) induced an inward current averaging 97 ± 5 pA ($n = 4$) at a holding potential of −50 mV. This holding potential was used because it mimicked the normal resting potentials of the cells in situ and minimized activation of voltage-dependent K^+ channels. GBC (1 μM) suppressed the current activated by LMK (Fig. 2 *B*).

The current activated by LMK was studied with voltage step and ramp protocols. Membrane currents were recorded in response to steps from a holding potential of 0 mV to test

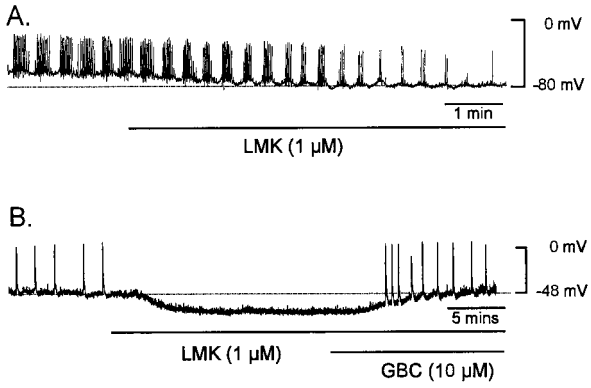

FIGURE 1 Effects of LMK on resting membrane potential and spontaneous excitability of murine proximal colon. (*A*) A representative intracellular recording of typical slow wave activity with superimposed spike potentials. Addition of LMK (1 μM; *black bar*) induced hyperpolarization and inhibited slow waves and spikes. The dashed line indicates −80 mV potential, which is reached during exposure to LMK. (*B*) A recording from another muscle that was also spontaneously active. However, in this example slow waves were much less apparent. LMK (1 μM) caused hyperpolarization and cessation of spiking. Addition of GBC (10 μM) reversed the effects of LMK. In this trace the dashed line indicates the original resting potential of −48 mV.

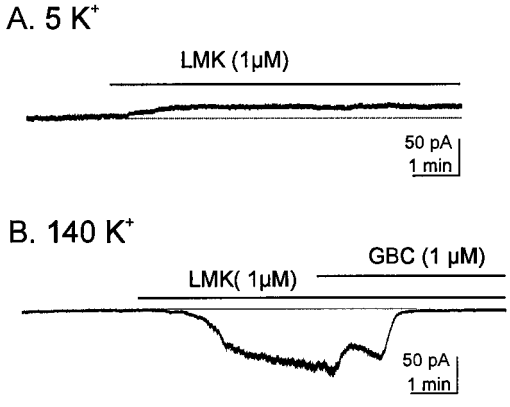

FIGURE 2 Effects of LMK on whole-cell currents of murine colonic myocytes. (*A*) LMK(1 μM) evoked outward current at a holding potential of −50 mV in dialyzed cells with a physiological K^+ gradient (5 mM K^+ in bath solution). (*B*) LMK evoked inward current at a holding potential of −50 mV in symmetrical K^+ gradients (140 mM in pipette and bath solutions). GBC (1 μM) reversed the effects of LMK. The dotted line indicates the current level at a holding potential of −50 mV.

potentials from −120 mV to +10 in 10-mV increments. These experiments were performed in a symmetrical K^+ gradient (140/140 mM). Control currents, shown in Fig. 3, *A* and *E*, showed weak inward rectification. We tested whether a portion of the inwardly rectifying current was due to a Ba^{2+}-sensitive current. Ba^{2+} (100 μM) blocked a small portion of the constitutive inward current in these cells (Fig. 3, *B* and *E*). PNC (1 μM), added in the continued presence of Ba^{2+}, activated inward current that exceeded the amplitude of the control current (Fig. 3, *C*, *E*, and *F*). In additional experiments, PNC (1 μM) activated a current of similar magnitude in the presence of 4-aminopyridine (5 mM; data not shown).

We also studied the current activated by PNC by using ramp voltage protocols. In symmetrical K^+ gradients (140/140 mM), cells were ramped from positive (+40 mV) to negative (−120 mV) potentials from a holding potential of 0 mV. After stepping from 0 to +40 mV, the cells were held at +40 mV for 100 ms to inactivate most of the delayed rectifier component of current before ramping to −120 mV. However, PNC activated an inwardly rectifying inward current at negative potentials (Fig. 4, *A* and *B*). GBC (1 μM) blocked the current activated by PNC. The block of current by GBC exceeded the magnitude of the current activated by PNC (i.e., a portion of the control current was also blocked; Fig. 4, *A* and *C*). Therefore, the GBC-sensitive current, averaging 243 ± 7 pA at −80 mV ($n = 4$), was significantly greater than the current activated by PNC (i.e., 135 ± 4 pA at −80 mV, $n = 4$) in these cells ($p < 0.05$).

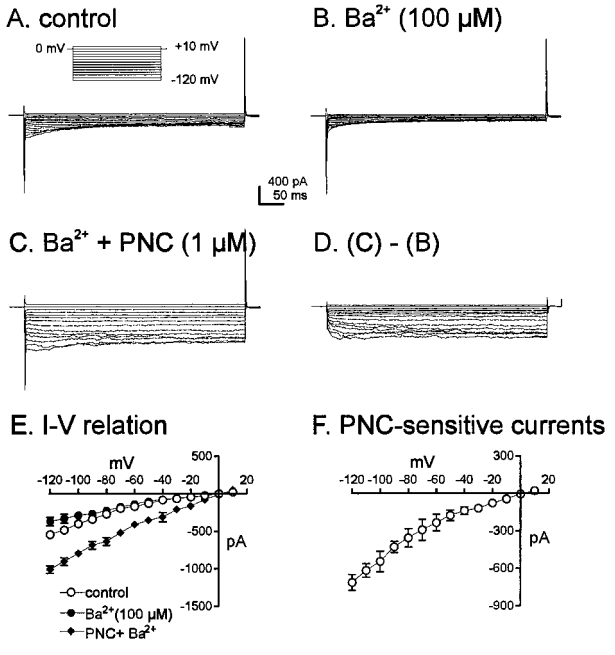

FIGURE 3 The effect of barium on PNC-induced currents. (*A*) Currents obtained by stepping a cell from a holding potential of 0 mV to −120 mV through +10 mV in 10-mV increments. The cells was studied in a symmetrical K^+ gradient (140/140 mM). Inward currents were induced at negative potentials. (*B*) The effects of barium (100 μM), which inhibited a portion of the inward current. (*C*) The currents after the addition of PNC in the continued presence of Ba^{2+}. A substantial inward current was activated, and this is shown in *D* as the difference current (*traces* in *B* subtracted from *traces* in *C*). *E* and *F* summarize experiments from five cells. (*E*) *I-V* relationships for control (○), Ba^{2+} (●), and Ba^{2+} and PNC (◆). (*F*) The *I-V* relationship for the current activated by PNC. The current is inwardly rectifying and reverses at the K^+ equilibrium potential.

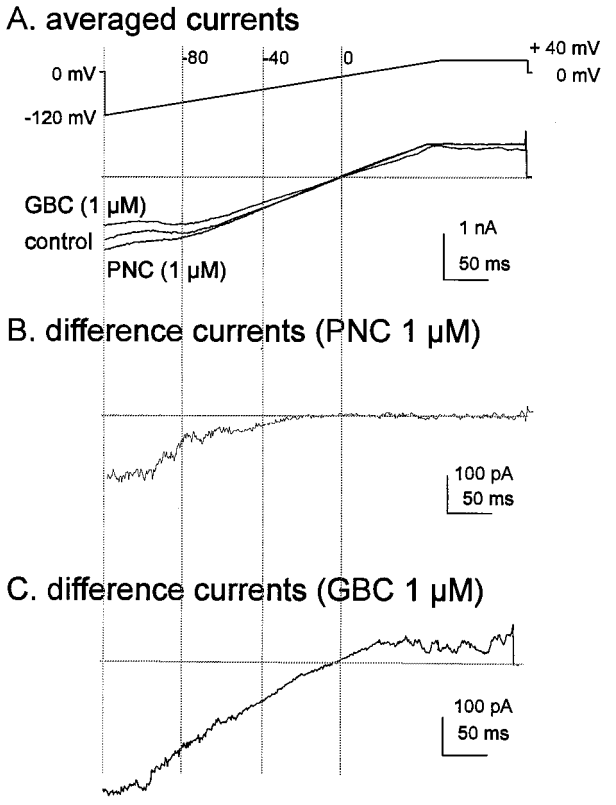

FIGURE 4 Effects of GBC on PNC-induced currents during a ramp protocol. (*A*) Averaged currents obtained from 15 episodes of the ramp protocol (i.e., 0 mV holding potential, step to +40 mV, and ramp to −120 mV in 500 ms). PNC (1 μM) induced inward current at negative potentials. E_K was 0 mV in the symmetrical K^+ (140/140 mM) solution. The current induced by PNC (1 μM) was inhibited by GBC (1 μM). (*B*) Difference currents obtained by subtracting the current after PNC from the control current. (*C*) The GBC-sensitive current obtained by subtracting current in the presence of PNC from the current after PNC and GBC.

Single-channel recordings

The properties of the channels activated by K^+ channel openers were investigated with single-channel experiments in cell-attached patches. At a patch potential of −50 mV (cell and patch in symmetrical K^+ gradients; 140/140 mM), occasional openings of a channel with a conductance of 27 pS ($NP_o = 0.14 \pm 0.04$; $n = 5$) were observed. LMK (1 μM) increased the open probability (NP_o) of these channels to 0.53 ± 0.09 ($n = 5$). The channels activated by LMK showed burst-like openings. After washout of LMK, open probability returned to control levels (Fig. 5, *A*, *B*, and *D*). The amplitude of this channel was 1.35 ± 0.3 pA at −50 mV ($n = 5$). Application of DZX, an agonist that has been

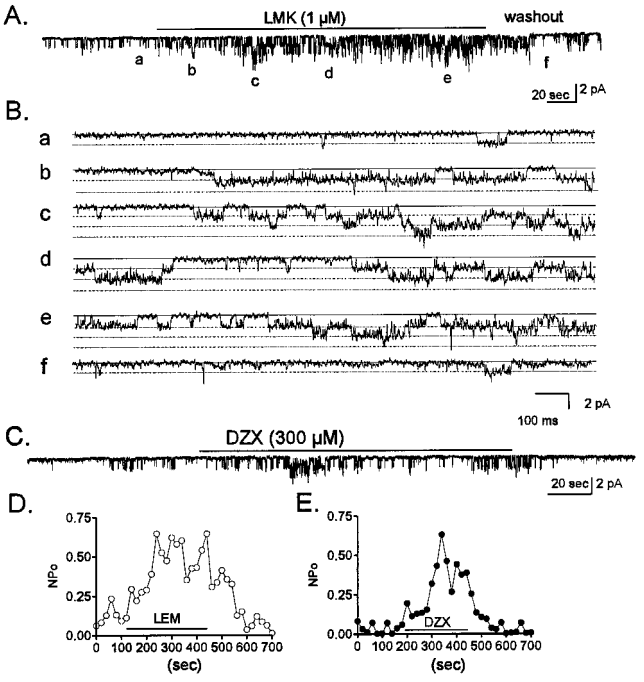

FIGURE 5 Effects of LMK on single K^+-channel currents in the cell-attached patch configuration. (*A*) LMK (1 μM) added to the bath increased openings of channels (downward defections; holding potential −50 mV and symmetrical 140/140 mM K^+ gradient). This effect was reversed after washout of LMK at a holding potential of −50 mV. (*B*) Portions of the record in *A* at an expanded time scale, denoted by *a–f*. (*C*) The DZX (300 μM) also increases channel openings in on cell patches. (*D* and *E*) Changes in open probability before, during, and after exposure to LMK and DZX (as labeled). Changes in NP_o (number of channels × open state probability) were calculated every 20 s.

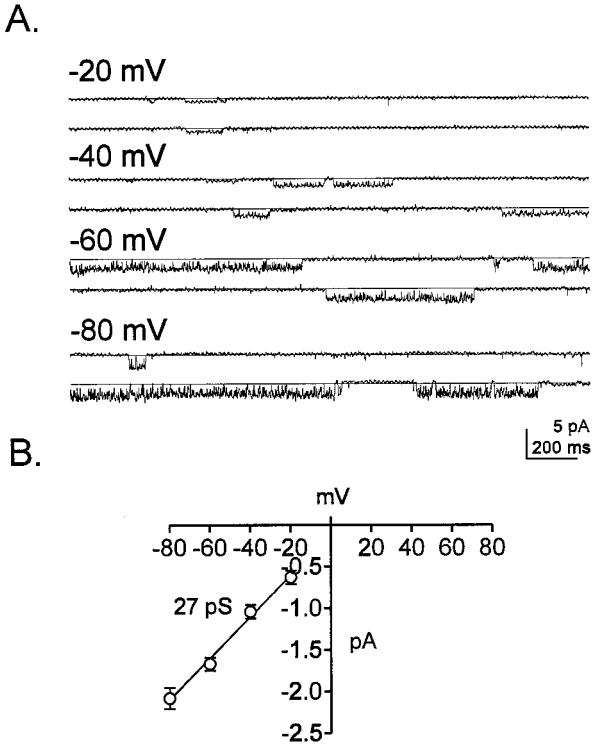

FIGURE 6 *I-V* relation for single K_{ATP} channels in murine colonic smooth muscle cells. (*A*) Single-channel openings at test potentials from −20 to −80 mV in a symmetrical K^+ gradient (140/140 mM) after treatment with LMK (1 μM). The dashed line indicates the closed state. (*B*) Summary *I-V* relationships from five patches. The amplitudes of single-channel currents were obtained from all-points amplitude histograms. The slope conductance, fitted by linear regression, was 27 pS.

demonstrated to activate SUR2B and SUR1 and not SUR2A at the concentration tested (300 μM; Isomoto et al., 1996), also increased the NP_o of the 27-pS channels. In these experiments NP_o was 0.12 ± 0.08 under control conditions and increased to 0.35 ± 0.09 in response to DZX at a holding potential of −50 mV (Fig. 5, *C* and *E*; $n = 6$, $p < 0.05$). The current-voltage relationships for the channels activated by LMK obtained from five cells are shown in Fig. 6. The slope conductance calculated from the *I-V* relationship was 27 ± 0.5 pS ($n = 5$) in symmetrical K^+ (140/140 mM) solution.

When patches were excised from the cells, the openings of the 27-pS channels decreased and the channels demonstrated further "run-down" with time. For example, NP_o averaged 0.11 ± 0.3 ($n = 5$) 5 min after patch excision, and openings were very rare 10 min after patch excision. Application of ADP (1 mM) in the continued presence of LMK restored the open probability of the 27-pS channels (Fig. 7, *A* and *A′*) to an average open probability of 0.36 ± 0.08 ($n = 6$). Application of ATP (0.1 mM) to the intracellular surface of the patch in the continued presence of LMK also increased NP_o (0.31 ± 0.09, $n = 6$, Fig. 7, *B* and *B′*). However, application of higher concentrations of ATP (i.e., >1 mM) did not restore NP_o (Fig. 7 *C*).

Molecular expression of K_{ATP} in murine colonic smooth muscle cells

RT-PCR was performed on collections of colonic myocytes, using K_{ir} 6.1, K_{ir} 6.2, SUR1, or SUR2A/SUR2B gene specific primers. RT-PCR detected transcripts for K_{ir} 6.2 as well as SUR2B in mRNA isolated from murine proximal colon smooth muscle cells; however, transcripts for K_{ir} 6.1, SUR1, and SUR2A were not detected within the same cell preparations. Results of these studies are shown in Fig. 8.

DISCUSSION

Resting membrane potential and spontaneous electrical activity in the mouse colon are similar to the electrical patterns recorded from human colonic smooth muscles (Huizinga et al., 1985; Rae et al., in press; Ward and Jackson, 1996). Therefore, the ionic conductances expressed by murine colonic muscles may serve as a model to enhance our understanding of the electrical activity in the human colon. In the present study we have demonstrated the expression and function of ionic channels that participate in the regulation of membrane potential and excitability in murine colonic muscles. GBC, a drug considered relatively specific for K_{ATP} (Ashcroft and Ashcroft, 1990), blocked a conductance

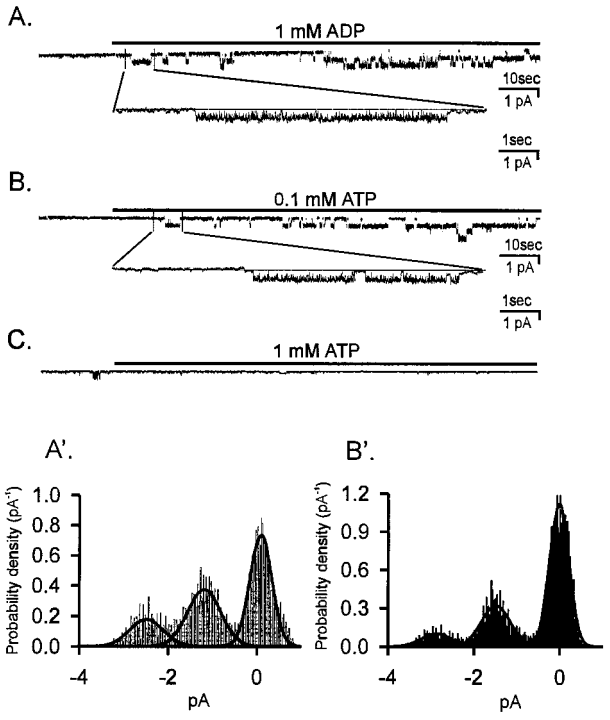

FIGURE 7 Effects of ADP and ATP on the activity of the K_{ATP} channels. The open probability of K_{ATP} channels decreased in excised patches as a function of time after excision (rundown). (*A*) Application of ADP (1 mM; *black bar*) in the presence of LMK (1 μM; *throughout trace*) restored the activity of K_{ATP} channels in an excised patch after rundown. The holding potential in all traces was −50 mV. An expanded trace (*inset*) showing the region of the record demarcated by the dotted lines is shown below the main trace. The dotted line in the inset shows the closed state. (*B*) Another record from an excised patch after rundown. The addition of 0.1 mM ATP (*black bar*) in the continued presence of LMK restores some openings of K_{ATP}. The inset below the main trace shows an expanded trace of the region demarcated by the dotted lines. (*B′*) An all-points amplitude histogram after the addition of ATP (0.1 mM). (*C*) The application of ATP (1 mM) after rundown of K_{ATP} channels. ATP at this concentration did not restore openings of K_{ATP} channels. In all records, there is also a small channel present that is unaffected by any of the treatments. This channel has previously been identified as a small-conductance Ca^{2+}-activated K^+ channel (see Koh et al., 1997).

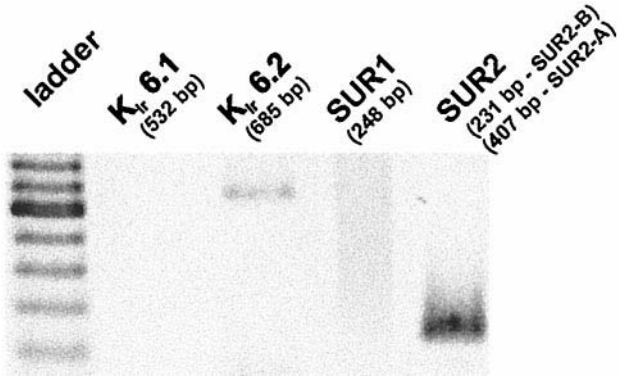

FIGURE 8 Molecular identity of subunits that may be responsible for K_{ATP} in colonic smooth muscle cells. PCR was performed in the presence of gene-specific primers for K_{ir} 6.1, K_{ir} 6.2, SUR1, or SUR2A/2B. After amplification, PCR products were resolved by 2% ethidium bromide-agarose electrophoresis; size markers were used to indicate the size of the experimental fragments (*lane 1*). RT-PCR detection yielded visible amplification products for K_{ir} 6.2 (*lane 3*, 685-bp fragment) and SUR2B (*lane 5*, 407-bp fragment) in mRNAs isolated from murine proximal colonic smooth muscle cells. PCR fragments for K_{ir} 6.1 (*lane 1*, 532-bp fragment) SUR1 (*lane 4*, 248-bp fragment), and SUR2A (*lane 5*, 231-bp fragment) were not observed.

that was activated under basal conditions. This conductance yielded outward current in isolated myocytes in physiological K^+ gradients and contributed to the membrane potentials of muscle cells within intact tissues. Additional GBC-sensitive current was activated in colonic myocytes by K^+ channel agonists such as LMK and PNC. These agents hyperpolarized intact muscles and blocked the spontaneous discharge of action potentials. The channels activated by LMK or DZX had properties consistent with K_{ATP} channels that have been observed in a number of preparations (see review, Wellman and Quayle, 1997). Molecular studies showed the expression of K_{ir}6.2 and SUR2B in colonic myocytes. Combination of these subunits produces a conductance with the properties of K_{ATP} (Isomoto et al., 1996). Taken together, these observations suggest that Kir6.2/SUR2B channels may be responsible for the 27-pS channels and GBC-sensitive conductance observed in colonic smooth muscle cells.

There are several reports describing the unitary conductance of K_{ATP} channels in smooth muscle cells. For example, small-conductance (10–30 pS) K_{ATP} channels have been identified in smooth muscle cells from portal vein (Kajioka et al., 1991), cultured coronary cells (Miyoshi and Nakaya, 1991; Miyoshi et al., 1992), guinea pig urinary bladder (Bonev and Nelson, 1993), and guinea pig proximal urethra (Teramoto and Brading, 1996). Larger conductance K_{ATP} channels (130 pS) are present in smooth muscle cells from mesenteric arteries (Standen et al., 1989), rat tail artery (Furspan, 1990), and canine aorta (Kovacs and Nelson, 1991). Channels activated by LMK and DZX in murine colonic smooth muscle cells had a slope conductance of 27 pS in symmetrical K^+ gradients (140/140 mM). This conductance is similar to the 30-pS channels observed in porcine coronary artery (Miyoshi et al., 1992).

We found that currents activated by K^+ channel agonists in colonic myocytes showed weak rectification properties similar to the properties of K_{ATP} in skeletal muscle (Quayle et al., 1988). Most studies in smooth muscle have demonstrated rather linear properties for K_{ATP} (see review, Nelson and Quayle, 1995). Therefore, we tested whether the inward currents activated at negative potentials were contaminated by other inwardly rectifying conductances (i.e., Ba^{2+}-sensitive currents). Application of Ba^{2+} reduced current at negative potentials, suggesting the possible expression of Ba^{2+}-sensitive inward rectifier channels in murine myocytes. This conductance was not characterized in the present study. Even in the presence of Ba^{2+} (100 μM), K^+-channel agonists activated an inward current in symmetrical K^+ solution. This suggests that the pharmacology of K_{ATP} in

murine colon may differ from the equivalent conductance in guinea pig urinary bladder (Bonev and Nelson, 1993), because the latter was relatively sensitive to block by external Ba^{2+}.

Activation of K_{ATP} channels under basal conditions was previously observed in pig proximal urethra (Teramoto and Brading, 1997). We found basal activation of K_{ATP} channels at −50 mV, and that the open probability was increased by K^+ channel openers. Whole-cell voltage-clamp experiments showed that the current blocked by GBC was larger than the current activated by PNC, suggesting that basally active K_{ATP} channels may contribute to the resting conductance of colonic myocytes. This finding is consistent with the report of den Hertog et al. (1989), in which GBC caused depolarization of guinea pig taenia coli. Findings from the current study demonstrate that basal activation of these channels contributes to membrane potential in colonic muscles. Our data also suggest that K_{ATP} can contribute to dual regulation of membrane conductance and generate either depolarization or hyperpolarization, depending upon the open probability of K_{ATP} channels.

We found that transcripts for SUR2B and K_{ir} 6.2 are expressed in mouse colonic smooth muscle cells. Our findings are consistent with those of Isomoto et al. (1996), which demonstrated the expression of SUR2B but not SUR2A in the murine colon. Whereas Isomoto et al. (1996) evaluated the types of SUR2 transcripts present in the murine colon, they did not determine the expression level of K_{ir} 6 channel subfamily members or SUR type 1. Furthermore, in the previous study, RNA was isolated from heterogeneous populations of cells within whole tissues. Therefore, it was impossible to know whether expression of SUR2B occurred in smooth muscle or other cell types present. Our data show that SUR2B and K_{ir} 6.2 mRNAs are expressed in murine colonic smooth muscle myocytes. Combination of these subunits may result in the molecular entity responsible for K_{ATP} in murine colonic muscles. The observation that DZX activated K_{ATP} channels in murine colonic smooth muscle is consistent with this hypothesis, because DZX has been shown to be capable of activating SUR1 or SUR2B, but not SUR2A (Isomoto et al., 1996). In the future, gene knock-out experiments may be able to test the role of specific K_{ATP} subunits more directly.

Although we have shown profound effects on membrane potential and excitability from K_{ATP} in colonic muscles, at the present time we are not sure how physiological regulation occurs. Intracellular ATP regulates these channels, but levels of ATP are normally in the mM range in smooth muscle myocytes (Bonev and Nelson, 1993; Quayle et al., 1994). Nucleotide diphosphates may also contribute to the regulation of open probability (Kamouchi and Kitamura, 1994). For example, we found that after run-down of channels in excised patches, either high concentrations of ADP (1 mM) or low concentrations of ATP (0.1 mM) applied to the intracellular surface of inside-out patches could restore openings of K_{ATP} channels. It is possible, therefore, that the ratio of ADP/ATP may be an important factor regulating these channels (see review, Yokoshiki et al., 1998). It is also possible that regulation of K_{ATP} is due primarily to stimulation by agonists. For example, vasodilating substances such adenosine and CGRP activate K_{ATP} via the cAMP pathway in vascular smooth muscles (Nelson et al., 1990; Nakashima and Vanhoutte, 1995). On the other hand, angiotensin II inhibits the K_{ATP} channels in rat arterial smooth muscle cell through protein kinase C (Kubo et al., 1997). Regulation of K_{ATP} in colonic muscles will be the subject of additional studies in the future.

In conclusion, our data are consistent with the hypothesis that K_{ATP} is composed of K_{ir} 6.2 and SUR2B subunits in colonic muscles. These channels appear to have a significant open probability under basal conditions, creating a conductance that contributes to resting potential and excitability. Dual regulation of these channels may participate in physiological regulation of membrane potential and excitability in the colon.

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