Mechanism of active repolarization of inhibitory junction potential in murine colon

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Baker, Salah A., Violeta Mutafova-Yambolieva, Kevin Monaghan, Burton Horowitz, Kenton M. Sanders, and Sang Don Koh. Mechanism of active repolarization of inhibitory junction potential in murine colon. Am J Physiol Gastrointest Liver Physiol 285: G813–G821, 2003; 10.1152/ajpgi.00115.2003.—Enteric inhibitory responses in gastrointestinal (GI) smooth muscles involve membrane hyperpolarization that transiently reduce the excitability of GI muscles. We examined the possibility that an active repolarization mechanism participates in the restoration of resting membrane potential after fast inhibitory junction potentials (IJPs) in the murine colon. Previously, we showed these cells express a voltage-dependent nonselective cation conductance (NSCC) (11, 12). In this study, we examined the effects of Ni2+ and Ni2+ and mibebradil, both blockers of the NSCC, on responses to locally applied ATP. We also tested the effects of Ba2+, Ni2+, and mibebradil, all blockers of the NSCC, on responses to locally applied ATP. Spritzes of ATP caused transient hyperpolarization, and the durations of these responses were significantly increased by the blockers of the NSCC. We considered whether NSCC blockers might affect ATP metabolism and found that Ni2+ decreased ATP breakdown in colonic muscles. Mibebradil had no effect on ATP metabolism. Because both Ni2+ and mibebradil had similar effects on prolonging responses to ATP, it appears that restoration of resting membrane potential after ATP spritzes is not primarily due to ATP metabolism. Neurally released enteric inhibitory transmitter and locally applied ATP resulted in transient hyperpolarizations of murine colonic muscles. Recovery of membrane potential after these responses appears to involve an active repolarization mechanism due to activation of the voltage-dependent NSCC expressed by these cells.

gastrointestinal motility; adenosine 5′-triphosphate; voltage-dependent nonselective cation current

GASTROINTESTINAL (GI) motility is orchestrated by the enteric nervous system activating and inhibiting GI muscles in orderly patterns. The inhibitory component of neuromuscular regulation comes from release of several neurotransmitter substances, including ATP, NO, and peptides (4, 10, 21). In GI muscles such as human, murine, and guinea pig colon, release of ATP during nerve stimulation evokes a fast inhibitory junction potential (IJP), which transiently takes the membrane potentials of smooth muscle cells toward the equilibrium potential for the K+ ionic gradient (hyperpolarization) (8, 22). Release of NO during the same period results in a smaller amplitude and longer-duration hyperpolarization phase that can outlast the fast phase of the IJP by several times. The fast hyperpolarization arises from activation of apamin-sensitive, small-conductance Ca2+-activated K+ channels (SK) (14), and the slow phase of the IJP is due to NO-dependent stimulation of cyclic GMP production, activation of protein kinase G, and activation of NO-dependent K conductance(s) (5, 26, 28, 29). In most preparations, repolarization from the IJP results in a depolarization phase (rebound excitation) through activation of chloride (10) and nonselective cation conductance (NSCC) (11, 12).

There has never been a clear explanation for the rapid repolarization of IJPs, and questions remain about the factors making IJPs so brief (~0.5–1.5 s). This is an important question, however, because it directly bears on postjunctional responses and the regulation of excitability of GI smooth muscles by neurotransmitters. An understanding of this repolarization mechanism might be exploited to develop drugs to regulate motility. The repolarization phase of IJPs has been ignored because most investigators consider it to be a passive process that is due to the metabolism and/or dilution by diffusion of neurotransmitters (ATP or NO). For example, as transmitter concentration falls near postjunctional receptors, the K+ channels (SK or NO-dependent K conductances) activated during IJPs may close, producing passive restoration of resting membrane potential. Because the actual spatiotemporal profiles of neurotransmitters concentrations after nerve stimulation are unknown, it is not clear that transmitter depletion can fully explain IJP repolarization. We have considered the possibility that a conductance responsible for an inward current with voltage-dependent properties that render it unavailable at the resting potential might activate at the negative potentials reached during IJPs. Such a conductance could participate in “active repolarization” that would tend to rapidly terminate IJPs.

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Our laboratory recently characterized two components of voltage-gated, inward currents in murine colonic myocytes (15). One component had properties of an L-type \( \text{Ca}^{2+} \) current and was inhibited by dihydropyridines. The second component did not “run down” during cell dialysis and was resistant to nicardipine (up to \( 10^{-6} \) M). The nicardipine-insensitive current was a low-voltage-activated current, and it was inhibited by \( \text{Ni}^{2+} \) (50% inhibitory concentration = \( 1.4 \times 10^{-5} \) M), mibefradil (10\(^-6\) to \( 10^{-5} \) M), and extracellular \( \text{Ba}^{2+} \). Our experiments suggested that the second component of voltage-dependent inward current in murine colonic myocytes appeared to be a novel, voltage-gated nonselective cation current. The properties of this conductance suggest it could contribute to active repolarization of IJPs in GI muscles, and in the present study we designed experiments to test this hypothesis. We have compared the effects of blockers of voltage-gated nonselective cation current on IJPs with responses to locally applied ATP.

**METHODS**

**Intracellular microelectrode recordings.** BALB/c mice (20–30 days old, Harlan Sprague Dawley, Indianapolis, IN) were anesthetized by chloroform inhalation and decapitated after cervical dislocation. Proximal colons, 1 cm from the ileocecal sphincter, were removed and opened along the mesenteric border. The contents of the colon were removed by washing with Krebs-Ringer bicarbonate (see below), and the mucosa was removed. The remaining tunica muscularis was cut into 10 x 6 mm sheets and pinned out in a Sylgard-coated dish with the mucosal surface of the circular muscle layer facing upward.

Muscles were maintained at 37.5 ± 0.5°C by a flowing Krebs-Ringer bicarbonate solution containing (in mM) 120.4 NaCl, 5.9 KCl, 15.5 NaHCO\(_3\), 11.5 glucose, 1.2 MgCl\(_2\), 1.2 NaH\(_2\)PO\(_4\), and 2.5 CaCl\(_2\). The solution was bubbled with 97% \( \text{O}_2 \)-3% \( \text{CO}_2 \) and had a pH of 7.3–7.4. Circular muscle cells were impaled with glass microelectrodes filled with 3 M KCl having resistances between 50 and 80 MΩ. Transmembrane potential was measured with a high input impedance amplifier (WPI S-7071, Sarasota, FL). Tissues were equilibrated for ~1 h before recordings were begun.

**Transmural stimulation and picospritzing of ATP.** Two platinum wires were placed parallel to the long axis of the circular muscle layer and were connected to a square wave stimulator (Grass 588, Grass, Quincy, MA) through stimulus isolation unit (Grass SIU 5A). Electrical field stimulation was applied as single and multiple pulses (0.5-ms duration and 150-V intensity at frequencies from 1 to 30 Hz). In some experiments, small puffs of ATP-containing solution were administered directly to the muscles by means of a pressurized application device (Picospritzer; General Valve, East Hanover, NJ). In these experiments, a micropipette (8- to 10-μm diameter) filled with 0.1 M ATP was placed as close as possible to the site of the intracellular microelectrode used to record responses. Pressure pulses at 12 lb/in.\(^2\) and 60-ms duration were applied to deliver ATP to the site of recording. Tetraethylammonium, \( \text{Ba}^{2+} \), and \( \text{Ni}^{2+} \) (as Cl– salts) were purchased from Sigma (St. Louis, MO). Mibebradil was a gift from Dr. Eva-Maria Gutknecht and Dr. Pierre Weber (Hoffmann-La Roche, Basel, Switzerland).

**Measurement of ectonucleotidase activity.** 1\(^{\text{N}}\)H-etheno (\( \text{e} \))-modified nucleotides, have been previously used as exogenous substrates for ATPases or AMPases (13, 17, 20, 24). Moreover, \( \text{e} \)-nucleotides can be detected at fmol concentration range, providing a sensitive approach of measuring small amounts of the degradation products. Stock solution of 50 μM eATP was prepared from ATP as previously described (6). Briefly, 100 μl of a citrate phosphate buffer (pH 4.0) containing 62 parts of 0.1 M citric acid and 38 parts of 0.2 M Na\(_2\)HPO\(_4\) and 10 μl of 3-chloroacetaldehyde were added to 200 μl of the nucleotide solution (final concentration of nucleotide, 50 μM) in a borosilicate glass culture tube (Fisher Scientific), and the samples were heated for 40 min at 80°C in a dry bath incubator (Fisher Scientific) to produce the fluorescent derivative eATP. Isolated segments of mouse colon (19.2 ± 0.2 mg wet wt; \( n = 12 \)) were placed in 200-μl BRANDEL superfusion chambers, as described previously (18, 23). Chambers were mounted vertically in a thermostatic block (at 36.5°C), and the tissue segments were superfused from bottom to top with oxygenated Krebs solution containing (in mM) 118.5 NaCl, 4.2 KCl, 1.2 MgCl\(_2\), 23.8 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 11.0 dextrose, and 1.8 CaCl\(_2\), and was maintained at 36.5°C at a flow rate of 1 ml/min. After 45 min of equilibration, tissues were subjected to perfusion with eATP (50 nM) for 10 min, and samples from the superfusate were collected from the solution before the tissue (S1) and after 10 min of contact with the tissue (S2). All samples were collected in ice-cold test tubes, and the reaction was stopped by immersing the sample vials in liquid nitrogen. The difference of the \( \text{e} \)-nucleotide content between S1 and S2 was taken as a measure for ectonucleotidase activity. In some experiments, tissues were superfused with 0.2 mM Ni\(^{2+}\) or 1 μM mibebradil for 5 min before the perfusion with the eATP was started. Both Ni\(^{2+}\) and mibebradil were present along with eATP during the 10-min perfusion before S2 was collected.

**High-performance liquid chromatography analysis of \( \text{e} \)-nucleotides and \( \text{e} \)-ADO.** Samples were analyzed for eATP, eADP, eAMP, and eADO content by reverse high-performance liquid chromatography system (HP1100 LC; Agilent Technologies, Wilmington, DE). The mobile phase comprised 0.1 M KH\(_2\)PO\(_4\) (pH 6.0 adjusted with 1 M KOH) as buffer A and solvent B consisted of 35% methanol and 65% buffer A. Gradient elution was employed according to the following linear program:

- **t** time 0–0.5 min of contact with the tissue (S1) and after 10 min of contact with the tissue (S2). All samples were collected in ice-cold test tubes, and the reaction was stopped by immersing the sample vials in liquid nitrogen. The difference of the e-nucleotide content between S1 and S2 was taken as a measure for ectonucleotidase activity. In some experiments, tissues were superfused with 0.2 mM Ni\(^{2+}\) or 1 μM mibebradil for 5 min before the perfusion with eATP was started. Both Ni\(^{2+}\) and mibebradil were present along with eATP during the 10-min perfusion before S2 was collected.

**High-performance liquid chromatography analysis of e-nucleotides and e-ADO.** Samples were analyzed for eATP, eADP, eAMP, and eADO content by reverse high-performance liquid chromatography technique in conjunction with fluorescence detection. Thus the e-nucleotides were separated with an ODS-AM C\(_18\) column (Waters, Milford, MA) in a gradient high-performance liquid chromatography system (HP1100 LC; Agilent Technologies, Wilmington, DE). The mobile phase comprised 0.1 M KH\(_2\)PO\(_4\) (pH 6.0 adjusted with 1 M KOH) as buffer A and solvent B consisted of 35% methanol and 65% buffer A. Gradient elution was employed according to the following linear program: time 0, 0% solvent B; 18 min, 100% solvent B. Flow rate was 1 ml/min, run time was 20 min, and postrun time was 5 min. Column temperature was ambient, whereas the autosampler temperature was set at 4°C. Fluorescent signals were detected with a programmable fluorescence detector (HP1100) at an excitation wavelength of 230 nm and an emission wavelength of 420 nm at gain 17. The amounts of adenylic nucleotides and nucleosides in each sample were calculated from calibration curves of nucleotide standards run simultaneously with every set of unknown samples. Results were normalized for sample volume and tissue wet weight, and the overflow of nucleotides was expressed in pmol/mg tissue.

**Statistics.** All values are expressed as means ± SE, and statistical significance was determined by paired t-test.

**RESULTS**

**Ba\(^{2+}\) effect on IJP pattern.** Electrical recordings were obtained from circular and longitudinal smooth muscle layers. First, we examined the effects of Ba\(^{2+}\) on IJPs recorded from circular smooth muscle cells (Fig. 1). Electrical field stimulation (0.5-ms pulse width; 150 V)
with frequencies ranging from 1 to 30 Hz elicited frequency-dependent, fast IJPs in the presence of \(N^\omega\)-nitro-L-arginine (L-NA; 100 \(\mu\)M) and atropine (1 \(\mu\)M; Fig. 1A). The mean peak amplitude of fast IJPs at 1, 10, and 30 Hz were 25 ± 1.2, 25 ± 1.2, and 26 ± 1.6 mV (\(n = 6\)), respectively. The averaged durations of IJPs (measured from onset to 90% repolarization) were 0.96 ± 0.12, 0.99 ± 0.11, and 1.01 ± 0.09 s (\(n = 6\)) at 1, 10, and 30 Hz, respectively. Resting membrane potentials remained unchanged between stimulations at various frequencies.

We tested the effect of external \(\text{Ba}^{2+}\) (100 \(\mu\)M to 1 mM) on IJP profiles (Fig. 1, B–D). Application of \(\text{Ba}^{2+}\) (100 \(\mu\)M) did not show significant effect on IJP pattern. However, higher concentrations of \(\text{Ba}^{2+}\) (500 \(\mu\)M) significantly increased IJP duration (1.28 ± 0.11 s, \(n = 6\), \(P < 0.01\); values from-10 Hz stimulation) and amplitude (30 ± 1.1 mV, \(n = 6\), \(P < 0.05\); Fig. 1, E and F). \(\text{Ba}^{2+}\) (500 \(\mu\)M) also significantly depolarized resting membrane potential (i.e., from -55.5 ± 2.1 to -50.3 ± 2.3 mV in the presence of \(\text{Ba}^{2+}\), \(P < 0.001\), \(n = 6\)).

In the presence of \(\text{Ba}^{2+}\), there was significant rebound excitation after repolarization of the IJP. The rebound excitation was characterized by depolarization and a burst of action potentials (see Fig. 1, C and D). The large rebound excitation may somewhat obscure the repolarization phase of the IJP, so additional experiments were performed in the presence of nicardipine (1 \(\mu\)M), which totally abolished both the action potentials and the rebound excitation depolarization. In the presence of nicardipine, \(\text{Ba}^{2+}\) (500 \(\mu\)M) significantly increased IJP duration (from 1.03 ± 0.06 to 1.36 ± 0.23 s, \(n = 6\), \(P < 0.05\); values from 10-Hz stimulation; Fig. 2) and amplitude (from 27 ± 1.8 to 31 ± 1.5 mV, \(n = 6\), \(P < 0.05\)). \(\text{Ba}^{2+}\) (500 \(\mu\)M) also significantly depolarized resting membrane potential (i.e., from -53.4 ± 2.2 to -48.0 ± 2.9 mV in the presence of \(\text{Ba}^{2+}\), \(P < 0.05\), \(n = 6\)).

Two additional blockers of the voltage-dependent NSCC channel in colonic myocytes, \(\text{Ni}^{2+}\) and mibebradil, were also tested, but these compounds have generalized blocking effects on a variety of \(\text{Ca}^{2+}\) channels. Application of these compounds, therefore, may affect neurotransmitter release, and we observed indications of this because both compounds decreased IJP amplitudes. For example, in experiments in which \(\text{Ni}^{2+}\) was tested, the mean peak amplitudes of fast IJPs at 1, 10, and 30 Hz were 24.3 ± 0.5, 21.5 ± 1.9, and 22.3 ± 2.19 mV at 1, 10, and 30 Hz, respectively. In tests with mibebradil, the mean peak amplitudes of fast IJPs at 1, 10, and 30 Hz under control conditions were 28.7 ± 4.7, 27.7 ± 4.6, and 27.5 ± 4.9 mV, respectively. After mibebradil (5 \(\mu\)M), amplitudes of IJPs significantly decreased (to 22.0 ± 1.5, 21.5 ± 1.76, and 22.3 ± 2.19 mV at 1, 10, and 30 Hz, respectively). Thus it is likely that \(\text{Ni}^{2+}\) and mibe-
fradil are not useful in assessing the kinetics of the IJP because of possible prejunctional effects.

**Effects of NSCC blockers on responses to locally applied ATP.** To avoid problems with NSCC blockers on transmitter release, we performed experiments in which ATP, the putative transmitter responsible for the fast IJP, was directly applied to the muscle by pressure ejection. ATP (0.1 M loaded in a micropipette) was picospritzed with a single 60-ms pulse. These experiments were performed in the presence of tetrodotoxin (0.5–1 μM), L-NA (100 μM), and atropine (1 μM). In these tests, resting membrane potential averaged $-46.4 \pm 0.9$ mV ($n = 7$). The amplitude and duration of ATP-induced hyperpolarizations were $21.8 \pm 1.2$ mV and $49.4 \pm 5.2$ s ($n = 5$), respectively (Fig. 3). While maintaining the same impalement and without moving the pressure ejection pipette, we tested the effects of Ba$^{2+}$ effects on ATP-induced hyperpolarizations. Ba$^{2+}$ (500 μM) did not significantly affect the resting membrane potential or the amplitude of ATP-induced hyperpolarization (i.e., $-44.2 \pm 0.97$ and $24.6 \pm 1.57$ mV, respectively; Fig. 3, C and D). These
values were not significantly different from control value. The durations of ATP-induced hyperpolarizations were significantly prolonged by Ba $^{2+}$ (to 65.0 ± 4.97 s; Fig. 3, A and B).

Using the same technique, we tested the effects of Ni$^{2+}$ on ATP-induced hyperpolarizations (Fig. 4). In these tests, the resting membrane potential was $-48.3 \pm 0.9$ mV ($n = 6$), and the amplitude and duration of ATP-induced hyperpolarizations were $20.8 \pm 0.9$ mV and $69.5 \pm 12.3$ s ($n = 6$), respectively. While the same impalement was maintained and without moving the pressure ejection pipette, addition of Ni$^{2+}$ (500 μM) did not affect resting membrane potential or the maximum amplitude of ATP-induced hyperpolarizations ($-49.0 \pm 0.7$ and $22.2 \pm 1.0$ mV, $P > 0.05$; Fig. 4, C and D). However, the duration of ATP-induced hyperpolarization was significantly increased after Ni$^{2+}$ (to $111.7 \pm 21.6$ s; Fig. 4B).

We also tested the effects of mibefradil on ATP-induced hyperpolarizations (Fig. 5). In these experiments, the resting membrane potential was $-49.0 \pm 0.7$ mV ($n = 4$). The amplitude and duration of ATP-
induced hyperpolarization were 21.3 ± 0.8 mV and 41.5 ± 2.2 s (n = 4), respectively. While the same impalements were maintained, mibefradil (1 μM) did not affect resting membrane potential or the amplitudes of ATP-induced hyperpolarizations (−48.0 ± 0.7 and 21.3 ± 0.5 mV, respectively, P > 0.1). However, the duration of ATP-induced hyperpolarizations was significantly increased after mibefradil (to 72 ± 4.2 s, P < 0.001; Fig. 5, A and B).

**Ni**\(^{2+}\) and mibefradil effect on ATPase activity. One possible explanation for the increase in the duration of ATP-induced hyperpolarization could be that the compounds used might block tissue ATPase activity and thus prolong the actions of ATP. Therefore, we tested the effects of **Ni**\(^{2+}\) and mibefradil on colonic tissue ATPase activity.

A typical chromatogram of eATP consists of a major eATP peak with a retention time of 10.5 ± 0.4 min and a small eADP peak (−6% of the eATP peak), with no signals corresponding to eAMP or eADO (S1; Fig. 6A). eATP was not degraded for a period of 30 min at 36.5°C in the absence of tissue (data not shown). With the tissue present, however, a 10-min incubation with the eATP resulted in decreased eATP, increased eADP, and appearance of eAMP and eADO (collectively called e-product) (Fig. 6, A and B), suggesting that eATP undergoes degradation during a 10-min contact period with colonic muscles, presumably due to the action of membrane-bound nucleotidases (25). No significant reduction of eATP or enhancement of the e-products occurred in the mouse colon treated with **Ni**\(^{2+}\) (0.2 mM) (Fig. 6C), suggesting that **Ni**\(^{2+}\)-inhibited ATPase activity. In the presence of mibefradil (1 μM), however, eATP was degraded to the same level as in control tissues (Fig. 6D).

**DISCUSSION**

ATP is thought to be a prominent enteric inhibitory neurotransmitter in the GI tract that is involved in the descending inhibition responses (1–3, 9). ATP and intrinsic inhibitory nerve stimulation elicit responses that are reduced or blocked by apamin and thus are considered to be due to activation of SK channels (19, 32). The inhibitory actions of ATP are thought to be mediated by P2Y receptors (10, 19), and we have recently described a mechanism of how activation of P2Y receptors can couple to activation of apamin-sensitive SK channels in the murine colonic smooth muscle cells (14). In the present study, we have investigated a mechanism of active repolarization that appears to participate in the restoration of resting membrane potentials after apamin-sensitive (fast) IJPs and responses to localized application of ATP. We have previously described the properties of a voltage-dependent NSCC in murine colonic smooth muscle cells that is weakly available at the resting potential of colonic myocytes, but the availability of this current increases as a function of hyperpolarization (15). We suggest that at the negative potentials reached during IJPs (i.e., close to the equilibrium potential for K\(^+\) ions), NSCC channels would reset. We have previously shown that full recovery from inactivation of the voltage-dependent NSCC can occur within 300 ms at 31°C, and this rate would be expected to increase at physiological temperatures (15). Activation of these channels during the repolarization phase of IJPs would tend to steepen the trajectory of the repolarization because this conductance provides inward current. We used inhibitors of this conductance to show that recovery from IJPs and localized ATP application was delayed when the NSCC was blocked. Thus our data show there is an active repolarization mechanism that hastens recovery from fast IJPs in GI muscles.

None of the other known inward current conductance(s) in colonic myocytes is likely to significantly contribute to the active repolarization mechanism. Neuronal stimulation in the presence of dihydropyri-
dine to block L-type Ca\(^{2+}\) channels did not change the profiles of IJPs in the present study or in previous investigations of IJPs (7). This observation tends to exclude the involvement of L-type Ca\(^{2+}\) channels or Ca\(^{2+}\)-activated Cl\(^{-}\) channels, which depend, in part, on Ca\(^{2+}\) entry for activation in the active repolarization of IJPs. Additionally, the voltage range for activation of resolvable L-type Ca\(^{2+}\) channels (i.e., positive to \(-50\) mV) is too positive for these channels to be a factor in IJP repolarization (16, 27).

An active repolarization mechanism would require a conductance that activates at negative potentials (greater than \(-60\) mV) and exhibits voltage dependence. One class of channels that satisfies this criterion is the T-type Ca\(^{2+}\) channels, which are low-voltage-activated Ca\(^{2+}\) channels. There have been reports in the literature about T-type Ca\(^{2+}\) conductance in GI smooth muscle cells (30, 31, 33, 34). The well-described characteristics of T-type Ca\(^{2+}\) currents are 1) low threshold activation, 2) rapid inactivation, 3) negative potentials for half-inactivation, 4) slow deactivation, 5) resistance to dihydropyridine, and 6) equal Ca\(^{2+}\) and Ba\(^{2+}\) permeability. Our previous voltage-clamp studies of murine colonic muscles showed that the voltage-dependent NSCC is a discrete (non-T-type) conductance. The properties of the voltage-dependent NSCC include 1) low threshold activation at negative potentials, 2) current reversal at 0 mV, 3) fast recovery from inactivation, and 4) block by Ni\(^{2+}\), Ba\(^{2+}\), and mibebradil. The low threshold activation of the NSCC suggests that this conductance could provide a mechanism for active repolarization of IJPs.

Having identified several blockers of the NSCC in our laboratory’s previous study of the voltage-dependent NSCC (15), we used these agents to study the involvement of this conductance in enteric inhibitory responses and responses to ATP. Ba\(^{2+}\) was the most useful agent to study the role of the NSCC in IJPs elicited by electrical field stimulation, reducing the rate of repolarization without any significant effect on amplitude. We found that other blockers of the NSCC (i.e., Ni\(^{2+}\) and mibebradil) reduced the amplitude of EJPs. Because these compounds are nonspecific Ca\(^{2+}\)-channel blocking agents, it is possible that they interfered with transmitter release, which is Ca\(^{2+}\) dependent. Ba\(^{2+}\) can block some of the conductance(s) present in smooth muscle cells (e.g., inward rectifier K\(^{+}\) conductance), and application of 500 \(\mu\)M Ba\(^{2+}\) induced depolarization of resting membrane potential by a few millivolts.

There are two inward rectifier-type conductance(s) that could be involved. First, activation of an "I\(_{\text{f}}\)-like" current (an inwardly rectifying cation conductance) could participate in repolarization of IJPs. However, we have never observed an I\(_{\text{f}}\)-like conductance in colonic cells, thus activation of such a conductance during the IJP is unlikely. Second, it is possible that inactivation of a K\(^{+}\) conductance could participate in repolarization of the IJP. However, how an inwardly rectifying K\(^{+}\) conductance could increase the rate of repolarization is unclear. At the peak of the fast IJP, these channels would produce outward current that would summate with the influence of SK channels (main conductance activated during the fast IJP), and, during repolarization of the IJP, inwardly rectifying K\(^{+}\) channels would tend to impede (not enhance) repolarization. A pharmacological inactivation of inwardly rectifying K\(^{+}\) channels might contribute, but there is no evidence that ATP or other inhibitory transmitters affect inwardly rectifying K\(^{+}\) channels. Thus the mechanism for inactivation of a K\(^{+}\) conductance and the rationale for considering the involvement of such channels is vague. Therefore, the prolongation of IJPs would have to be due to activation of an outward current or inhibition of an inward current component. The only conductance we are aware of in murine colonic myocytes with these characteristics is the voltage-dependent NSCC.

Stimulation of enteric inhibitory nerves releases at least two neurotransmitters, ATP and NO. Thus a potential prejunctional effect of inhibitors of NSCC makes it difficult to directly assess the role of the NSCC in postjunctional neural responses. Therefore, we applied ATP directly, via picospritzing, in the presence of tetrodotoxin to block release of other neurotransmitters from neural activation by ATP. In these studies, we were able to compare several disparate blockers of NSCC. Spritzing ATP on colonic muscles resulted in transient hyperpolarizations that varied somewhat in amplitude and duration from experiment to experiment due to control resting potential, spritz pipette characteristics, and placement of the tip of the pipette. To avoid problems in interpreting our data, we conducted these experiments by comparing responses before and after application of NSCC blockers without moving the spritz pipette and without changing impalements. Under these conditions, we observed consistent significant increases in the duration of the hyperpolarization responses after application of the three NSCC blockers tested. Ni\(^{2+}\) and mibebradil are known to also be blockers of T-type Ca\(^{2+}\) channels, but we have never found evidence of expression of T-type Ca\(^{2+}\) channels in colonic myocytes (e.g., see Ref. 15). In our laboratory’s previous report (15), we found that a low concentration (300 \(\mu\)M) of Ni\(^{2+}\) inhibited voltage-dependent NSCC. These data confirm the observations made with nerve-evoked IJPs and suggest that this conductance is activated during ATP-dependent hyperpolarization responses. Thus the data suggest that the voltage-dependent NSCC participates in active repolarization of IJPs in colonic muscles.

We also tested the effect of the NSCC conductance blockers on the breakdown of ATP. A variety of extracellular enzymes participate in degrading extracellular ATP and its metabolites, including ecto-ATPase and ecto-ATP diphosphohydrolases or ADPase. These enzymes are activated by Ca\(^{2+}\) or Mg\(^{2+}\) and are inhibited by millimolar concentrations of Cu\(^{2+}\), Zn\(^{2+}\), or La\(^{3+}\) (35). Therefore, we tested the idea that part of the prolongation of IJPs or ATP responses due to NSCC
Blockers could be due to inhibition of ATP metabolism. Mouse colon tissues demonstrated detectable ecto-ATPase activity, and Ni²⁺ significantly inhibited this activity. Mibefradil, however, did not significantly affect ATP metabolism. Because Ni²⁺ and mibefradil had the same effect on hyperpolarizations due to locally applied ATP, it is likely that ATP metabolism was not the primary factor in ending these responses. It is possible that, after the hyperpolarization response to ATP develops, the NSCC is activated and tends to counteract the same effect on hyperpolarizations due to locally applied Ca²⁺-dependent K⁺ channels activated by ATP in murine colonic smooth muscle. Am J Physiol Cell Physiol 273: C2010–C2021, 2001.

In summary, the fast IJP component of the enteric inhibitory response in murine colonic muscles recovers too quickly to be simply a passive result of shutting off of the K⁺ conductance(s) responsible for the hyperpolarization. We have described a voltage-dependent NSCC that, although unavailable at the resting membrane potential, can reset during the IJP. Activation of the conductance during the repolarization response hastens recovery of resting membrane potential. In the presence of blockers of the NSCC, the ATP responses were prolonged and may have lasted until the ATP concentration fell due to diffusion from the site of application.

Disclosures
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References


