Effects of PGE$_2$ in guinea pig colonic myenteric ganglia

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Received 11 April 2002; accepted in final form 25 July 2002

Manning, Brian P., Keith A. Sharkey, and Gary M. Mawe. Effects of PGE$_2$ in guinea pig colonic myenteric ganglia. Am J Physiol Gastrointest Liver Physiol 283: G1388–G1397, 2002. First published August 21, 2002; 10.1152/ajpgi.00141.2002.—PGE$_2$ is a proinflammatory mediator that can influence many cell types. This study was conducted to determine whether PGE$_2$ alters the electrical activity of distal colonic myenteric neurons, because colitis is typically associated with altered motility and changes in neural signaling may be involved. The electrical properties of intact myenteric neurons were evaluated with intracellular microelectrodes. Acute application of PGE$_2$ elicited a prolonged depolarization in both AH and S neurons with little effect on input resistance or electrical excitability. PGE$_2$ effects were suppressed by tetrodotoxin (TTX) or neurokinin (NK) receptor antagonists, indicating that PGE$_2$ acts directly and indirectly to depolarize colonic neurons. PGE$_2$-evoked depolarization was concentration dependent (≈3 μM EC$_{50}$) and was attenuated by the E prostanoid (EP)1/2 receptor antagonist, AH-6809. When preparations were maintained for 48 h in the presence of the stable PGE$_2$ analog PGE$_2$-ethanolamide (10 μM), neurons exhibited a significant membrane depolarization and enhanced excitability. These results suggest that PGE$_2$ can play a role in altered motility in colitis by evoking changes in the electrical properties of myenteric neurons.

motility; inflammation; colitis; innervation; enteric nervous system

THE NERVOUS SYSTEM OF THE bowel, which is known as the enteric nervous system (ENS) regulates visceromotor, secretomotor, and vasomotor activities in the wall of the intestines. Given that much of the morbidity associated with inflammatory bowel disease is caused by disordered gastrointestinal motor function and that colonic smooth muscle function is regulated by the ENS, it is quite possible that alterations in the excitability of the enteric neurons contribute to intestinal dysmotility. A variety of immune-mediated inflammatory disorders exist, including Crohn’s disease and ulcerative colitis, and dysmotility, hypersecretion, and enhanced perception of pain are common features (17, 22, 23, 46). It is now clear that cutaneous and visceral inflamations lead to enhanced excitability of extrinsic primary afferent neurons and altered reflex activity (5, 8, 14, 48). Changes may also occur in the afferent components of intrinsic enteric neural circuitry, and such changes could contribute to altered motor reflex activity in the inflamed bowel.

In guinea pig bowel, it is possible to identify the functional role of a given neuron on the basis of consensus knowledge regarding the electrical, morphological, chemical coding, and axonal projection patterns of these cells. For example, a class of cells known as AH neurons, on the basis of electrical properties, functions as the afferent limb of the peristaltic reflex circuit (19) and is considered to have roles as intrinsic primary afferent neurons and interneurons (for review, see Ref. 30). These cells have a Dogiel type II morphology (multiple processes) and extend projections to other ganglia and to the lamina propria (7, 34, 40, 42), where they could respond to stimuli originating at the mucosal surface. Therefore, changes in the electrical properties of AH cells would likely alter motility and secretory patterns in the gut. Other neurons of the myenteric plexus, consisting of interneurons and motor neurons, have a Dogiel type I morphology (single process) and share a common electrophysiological phenotype (7, 34). These cells are known as S cells and project to other enteric neurons and to intestinal smooth muscle (35, 41). Because intestinal inflammation is typically centered in the lamina propria of the mucosal layer, components of the reflex circuitry that are most likely to be affected by inflammatory mediators are those that pass into this region. In the case of motility reflex circuits, this includes the processes of AH cells, which, as described above, project to the mucosa.

During the inflammatory process, a plethora of compounds are released that have actions on many cell types, including neurons. A common feature of intestinal inflammation is the upregulation of the cyclooxygenase-2 (COX-2) enzyme, leading to increased synthesis of several eicosanoids (16, 46, 47, 50), including prostaglandin E2 (PGE$_2$). Within guinea pig submucosal plexus neurons in the colon, PGE$_2$ application has been shown to lead to membrane depolarization and increased action potential frequency, as well as increased chloride ion secretion (18). PGE$_2$ has also been shown to activate myenteric plexus neurons in the...
guinea pig ileum (14) and augment acetylcholine release from myenteric neurons of the small intestine (9, 13) and colon (27, 39). Moreover, in ileal myenteric AH cells, activation of adenylyl cyclase increases excitability by causing a membrane depolarization and suppression of the prolonged afterhyperpolarization (45), consistent with the known effects of Trichinella-induced inflammation in the small intestine (44).

In the present study, we conducted studies designed to elucidate the effects of PGE$_2$ on colonic myenteric neurons in an effort to resolve whether PGE$_2$-induced changes in neuronal activity could contribute to changes in motility that are associated with colitis. Intracellular voltage recordings were obtained from randomly impaled colonic myenteric neurons in acutely dissected and organ-cultured preparations. Responses to PGE$_2$ were evaluated after pressure microejection or addition to the circulating Krebs solution. To assess the effects of prolonged exposure of myenteric neurons to PGE$_2$, longitudinal muscle-myenteric plexus preparations were maintained in organ culture conditions in the presence or absence of the stable PGE$_2$ analog, PGE$_2$ ethanola.

METHODS

Tissue Preparation

Adult guinea pigs of either sex, weighing 250–300 g, were anesthetized with isoflurane and exsanguinated. This method has been reviewed and approved by the Institutional Animal Care and Use Committee of the University of Vermont. The distal colon was removed and immediately placed in an iced Krebs solution [chemical components (in mM): 121 NaCl, 5.9 KCl, 2.5 CaCl$_2$, 1.2 MgCl$_2$, 25 NaHCO$_3$, 1.2 NaH$_2$PO$_4$, and 8 glucose, aerated with a 95% O$_2$-5% CO$_2$]. Nifedipine (5 μM) and atropine (200 nM) were added to the Krebs solution to eliminate smooth muscle contraction. The colon preparation was dissected by pinning both free ends, then carrying a longitudinal incision along the mesenteric border. Tissue was then pinned flat in a Sylgard polymer-lined dish while being continuously perfused by circulating iced Krebs. The mucosal and submucosal layers of the colon were removed under a dissecting microscope, exposing the underlying muscularis externa. Circular muscle bundles of the muscularis externa were subsequently removed, exposing the myenteric ganglionated plexus. This dissected tissue was then placed into a tissue chamber for electrophysiological recording.

Electrophysiological Recordings

Methods for electrophysiological recording were similar to those previously described (36). The colon preparation was pinned flat in a low-volume (6-ml) tissue chamber and continuously perfused with warm Krebs solution (37°C, aerated by 95% O$_2$-5% CO$_2$). Ganglia were visualized using Hoffman modulation contrast microscopy.

Intracellular recordings were done using an Axoclamp 2B electrometer (Axon Instruments, Foster City, CA) with 2 M KCl-filled microelectrodes. Tips of the microelectrodes were backfilled with 1% (wt/vol) neurobiotin (Vector Laboratories, Burlingame, CA) to allow for subsequent reacquisition and immunohistochemical and morphological characterization. These microelectrodes had resistances between 60 and 80 MΩ for voltage recording. Capacitance neutralization was adjusted as needed for optimum current injection and voltage recording. Results were monitored on a separate oscilloscope, whereas data acquisition and analysis were performed using MacLab Chart software to emulate oscilloscope readings (MacLab hardware and software; ADInstruments, Castle Hills, NSW, Australia). Electrodes used to stimulate fiber tracts were extracellular monopolar Teflon-coated platinum wires (25-μm diameter) placed against interganglionic fiber bundles and used to stimulate synaptic transmission to impaled cells. Activation of fast excitatory postsynaptic potentials (EPSPs) involved a single 0.5-ms current pulse applied to interganglionic fiber bundles.

Baseline neuronal excitability was assessed by determining the threshold current necessary to activate at least one action potential in a given cell (threshold strength in nA) during a 500-ms current pulse. Potential alterations in neuronal excitability were assessed by determining the number of action potentials generated during a depolarizing current pulse delivered at two times the threshold strength.

Compounds to be tested on colonic myenteric neurons were applied by pressure microejection or addition to the circulating Krebs solution. Pressure ejections of compounds involving glass micropipettes (15- to 20-μm tip diameter) placed 50–100 μm from the target ganglia were induced by pulses of nitrogen gas (300 kg/cm$^2$; 900 ms). The distance of pressure ejection micropipette was maintained at 50–100 μm from the tissue, if selection of a new target ganglion was necessary. Washout periods (>15 min) were done between subsequent administrations of test compounds to allow for the recovery of responses in the event of receptor desensitization.

Classification of Impaled Neurons

Originally, neurons in the myenteric plexus of the guinea pig distal colon were called type 1 or 4 if they exhibited multiple or single action potentials, respectively, with no after-spike hyperpolarization, and they were called type 2 neurons if they exhibited a single action potential on depolarization and did display a prolonged postspike hyperpolarization (53). Subsequent investigations combined evaluations of electrophysiological and morphological properties and concluded that single axonal Dogiel type I neurons exhibited properties similar to S cells of the small intestine, whereas multicellular Dogiel type II neurons exhibited AH cell properties (35, 49).

In the present study, we used the same classification scheme used to describe neurons in the guinea pig small intestine, because, as in the small intestine, there are two primary types, S or AH, of spiking neurons in the myenteric plexus of the colon (35, 37, 49). The most important criteria for identifying a given cell as an AH neuron were the presence or absence of a shoulder on the repolarizing phase of the action potential, its morphology, and the duration of the prolonged afterhyperpolarization (usually lasting seconds). Particular attention was paid to the repolarizing phase of the action potential, because the shoulder caused by an inward Ca$^{2+}$ conductance is retained in activated AH neurons, although their other electrical properties become indistinguishable from S neurons when they are activated synaptically or pharmacologically (6, 31, 44). S neurons exhibited action potentials after the onset of a depolarizing current pulse, often exhibited anodal break spikes on the offset of a hyperpolarizing current pulse, and, in some cases, generated spontaneous action potentials. Prostaglandin actions were evaluated in AH and S cells. All cells exhibiting a mean input resistance of <50 MΩ were excluded from data analysis.
Immunohistochemical Labeling

After intracellular recording, tissues were fixed in a 2% paraformaldehyde, 0.2% picric acid solution at 4°C overnight. Tissues were then blocked with a 4% goat serum in 100 mM PBS/Triton-X (0.5%) for 30 min at room temperature. Primary antisera to the calcium-binding protein, calbindin (Swant, Bellinzona, Switzerland), were used at 1:1,000 diluted in 4% goat serum/PBS-Triton-X and incubated at 4°C overnight. Tissues were then washed 3 × 15 min each and placed in secondary antibodies. Neurobiotin-filled cells were visualized by adding streptavidin-conjugated FITC (1:400; Jackson Laboratories) to the secondary antibody solution. Immunohistochemical labeling was then visualized using an Olympus fluorescent microscope, and images were captured using an Olympus digital camera utilizing Magnafire digital imaging software (Optronics, Goleta, CA).

In Vitro Organ Culture

To determine the effects of prolonged exposure to PGE2, colon explants were placed in organ culture for ~48 h before electrophysiological recording. Organ culture techniques were similar to those previously reported (28). In these experiments, colon was removed from experimentally naive animals, as above. This tissue was then dissected under sterile conditions using Krebs solution containing 5µM nifedipine filtered through a 0.22-µm-diameter Fisherbrand filter disc (Fisher Scientific, Springfield, NJ). Tissue was pinned flat in a Sylgard-lined petri dish sterilized before dissection by soaking in 100% ethanol, and mucosal and submucosal layers were removed. After dissection, the tissue was bathed with a DMEM/F-12 culture medium containing 10% horse serum, antibiotic-antimycotic (1 ml/100 ml), gentamicin (10 mg/100 ml), amphotericin B (12.5 µg/ml), and nifedipine (1 µM) (all compounds from Sigma, St. Louis, MO). Experimental tissues were bathed in culture media containing 10 µM PGE2-ethanolamide (Cayman, Ann Arbor, MI), a synthetic analog of endogenous PGE2 that exhibits higher stability and slower degradation in aqueous environments than the endogenous form. Culture medium was replaced at ~12-h intervals in all experiments. After organ culture for 48 h, preparations were washed with normal Krebs solution, the circular layer of the muscularis externa was removed, and the underlying myenteric plexus was exposed. Preparations were then repinned in an electrophysiology culture dish. Myenteric neurons were then tested for passive and active electrical properties and the presence and/or absence of synaptic inputs. Measures of input resistance, number of action potentials per depolarizing current pulse, threshold to action potential, presence of anodal break action potentials, and presence or absence of fast or slow excitatory postsynaptic potentials were recorded. Impaled cells were iontophoretically labeled with neurobiotin, and preparations were immunohistochemically stained with rabbit calbindin antibody to determine cell morphology and calbindin immunoreactivity. Control tissues were dissected and maintained in organ culture as above, but no PGE2-ethanolamide was added to the culture media.

Compounds

PGE2, AH-6809, and PGE2 ethanolamide were all obtained from Cayman. PGE2, AH-6809, and PGE2 ethanolamide were dissolved in 100% ethanol to 60 mM, 60 mM, and 100 mM stock solutions, respectively, and serially diluted in normal Krebs to final working concentrations. Ethanol concentrations were never >0.1% in final solutions. Stock solutions (1 mM) of SR-140333 and SR-142801 (courtesy of Sanofi Recherche, Montpellier, France) were prepared in DMSO, and these compounds were serially diluted in normal Krebs to a final working concentration of 100 nM. Tetrodotoxin (TTX) was obtained from Sigma and diluted in 100% ethanol to a stock concentration of 0.5 mM. Final working concentrations were made by diluting the stock in normal Krebs.

Statistical Analysis

Averaged values are presented as means ± SE. Statistical analysis was performed using paired or unpaired Student’s t-test when appropriate to determine the difference between control and experimental values (Prism v. 3.0a for Macintosh; Graphpad Software, La Jolla, CA). Differences were considered to be significant if P < 0.05.

RESULTS

In the present study, intracellular recordings were obtained from a total of 120 neurons from 52 preparations. Out of a total of 120 neurons, 77 neurons were S cells (49.4%) and 43 were AH cells (27.6%). Of the 77 S neurons, 73% were successfully characterized morphologically, and all of these were classified as having Dogiel type I morphology, with the remainder of cells not classified due to insufficient iontophoretic filling of the cell. None of the S/Dogiel type I cells were calbindin immunoreactive. Of the 43 AH cells, all of the cells successfully filled with neurobiotin (88%) were classified as Dogiel type II cells. Seventy-four percent of the cells identified electrophysiologically and morphologically as AH/Dogiel type II cells were calbindin immunoreactive (Fig. 1). No cells exhibiting S cell electrophysiological properties were Dogiel type II in morphology, and no AH cells exhibited a Dogiel type I morphology.

In recordings from S cells, resting membrane potential was −57.9 ± 1.7 mV and resting membrane input resistance was 211 ± 40 MΩ (n = 77). In AH cells, resting membrane potential was −58.9 ± 2.1 mV and resting membrane input resistance was 105 ± 23 MΩ (n = 43).

PGE2 Depolarizes AH and S Cells

AH cells. When PGE2 was applied to AH cells by pressure microejection (10 µM in pipette, 900 ms), 20 of 21 AH cells responded with a maximal depolarization that had an amplitude of 8.9 ± 1.1 mV and a duration to half-maximal repolarization (dur50) of 49.4 ± 13.6 s (n = 20) (Fig. 2). Membrane input resistances were unaffected during this depolarization (control = 96 ± 22 MΩ, PGE2 = 97 ± 21 MΩ; P is not significant, n = 8). The threshold strength to elicit action potentials by intracellular depolarizing current in AH cells was not altered after acute PGE2 application and application of direct current to return the membrane potential to resting levels (control, 0.3 ± 0.07 nA; PGE2, 0.3 ± 0.05 nA). Similarly, there was no difference in the number of action potentials generated at two times threshold strength (control, 2 ± 1 action potential; PGE2, 2 ± 1
action potential). Of the AH cells responding to PGE2, only one generated spontaneous action potentials during the sustained depolarization. This cell also fired anodal break action potentials after PGE2 application, and generated more action potentials in response to a depolarizing current pulse of the same amplitude.

Prolonged afterhyperpolarization of AH cells was not significantly altered by brief application of PGE2 (amplitude: control $6.9 \pm 1.9$ mV, PGE2 $4.7 \pm 1.2$ mV, $n = 7$; $dur_{50}$: control $1.9 \pm 1.0$ s, PGE2 $0.6 \pm 0.3$ s; $n = 7$; total duration: control $2.9 \pm 0.5$ s, PGE2 $1.7 \pm 0.4$ s; $n = 7$). Prolonged afterhyperpolarizations were evaluated at the resting membrane potential for a given neuron after brief (<5 ms) depolarizing current pulses.

$S$ cells. In $S$ cells, PGE2 evoked a prolonged depolarization with an amplitude of $6.9 \pm 0.8$ mV and a $dur_{50}$ of $27.6 \pm 6.0$ s (Fig. 2). Membrane input resistances were unaffected during PGE2-mediated depolarization (control, $261 \pm 76$ MΩ; PGE2, $231 \pm 39$ MΩ; $P$ is not significant, $n = 12$). No difference was detected in threshold strength (control, $0.3 \pm 0.04$; PGE2 0.3 ± 0.06) or number of action potentials generated by doubling threshold strength (control $2 \pm 1$, PGE2 $2 \pm 1$) after acute application of PGE2. Of the 38 $S$ cells that responded to PGE2 application, three exhibited an increase in excitability as demonstrated by the generation of spontaneous action potentials.

**Effects of TTX on PGE2-Mediated Depolarization**

To determine whether the PGE2-induced depolarization was due to a direct action of the prostanoid on myenteric neurons, responses to PGE2 were measured...
in the presence of the sodium channel blocker TTX (0.5 mM) added to the perfusing solution. In these experiments, 22 neurons from 14 preparations were studied. In 12 AH cells tested, TTX inhibited both the amplitude and the duration of the PGE2-evoked depolarization to ~40–50% of control values (P < 0.05 for both; Fig. 3). In 10 S cells, both the amplitude and the duration of the PGE2-evoked depolarization were significantly inhibited in the presence of TTX, resulting in an approximate inhibition of 65–75% control values (P < 0.05 for both; Fig. 3).

To eliminate the possibility that receptor desensitization contributed to the reduction in responsiveness to PGE2, two sequential applications of PGE2 were performed on impaled neurons in normal Krebs solution. Washout periods of ~10–15 min were done between prostaglandin applications in a given cell. In the five AH cells from two preparations tested, initial application of 10 μM PGE2 resulted in a maximal depolarization amplitude of 10.7 ± 4.6 mV and duration of 32.7 ± 5.1 s determined by comparing resting membrane potential with the maximum depolarization amplitude after PGE2 application. A second application of PGE2 after the washout period resulted in a depolarization of statistically similar mean amplitude, as determined by paired Student’s t-test (6.9 ± 2 mV, n = 5) and duration (25.6 ± 6 s, n = 5). In 8 S cells from three preparations, initial and second applications of PGE2 were also statistically similar (amplitude: initial = 6.4 ± 2 mV, second = 6.5 ± 2 mV, n = 8; duration: initial = 41.6 ± 26 s, second = 28.7 ± 12 s, n = 6).

**PGE2-Induced Depolarization was Concentration-Dependent**

Concentration-effect curves were generated in AH cells by adding PGE2 to the bathing solution at concentrations ranging from 100 nM to 30 μM with washout of ~10 min between each application. These experiments were conducted only on AH cells. PGE2 application resulted in a concentration-dependent depolarization with an effective concentration resulting in half-maximal response (mean EC50) of 3.3 ± 1.4 μM and a calculated Hill slope of 2.2 (Fig. 4). This value was determined by using a least squares nonlinear regression analysis of the data (R² = 0.93 ± 0.01, n = 7; Prism v. 3.0a for Macintosh, Graphpad Software). In the presence of TTX, the mean EC50 for the PGE2-induced depolarization was 2.1 ± 1.0, with a calculated Hill slope of 1.8 (R² = 0.92 ± 0.05) (Fig. 4). There was no significant difference between the EC50 and the Hill slope in the presence of TTX, but the maximal response to bath-applied TTX was attenuated in the presence of TTX, which was consistent with the effects of TTX on responses to microejection of PGE2 demonstrated above.

**Prostanoid Receptor Antagonist AH-6809 Attenuates PGE2-Induced Depolarizations**

PGE2 acts as an agonist at E prostanoid (EP) receptors. The specific EP receptor(s) responsible for the effects of PGE2 in the myenteric plexus is currently unknown, but evidence suggests the presence of the EP1 receptor mRNA within rat myenteric plexus (15,

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**Fig. 3.** The PGE2-mediated membrane depolarization was significantly inhibited by tetrodotoxin (TTX) in both S and AH cells. A: PGE2-induced membrane depolarization of an AH cell before and during TTX administration. B: PGE2-induced membrane depolarization of an S cell before and during TTX administration. Arrows (in A and B) indicate point of application of PGE2. C: graphs demonstrating that PGE2-induced membrane depolarization was altered by TTX in AH cells *P < 0.05. D: graphs demonstrating that in S cells both the amplitude and duration of the PGE2-induced membrane depolarization were significantly inhibited in the presence of TTX. *P < 0.05.
EC50
NK Receptor Antagonists Significantly Inhibit PGE2-Mediated Depolarizations in AH Cells

Previous investigations have determined that NK1 and NK3 receptors mediate prolonged depolarizations in the myenteric plexus of the intestine and that tachykinins likely contribute to slow EPSPs in myenteric neurons (1, 25, 26). Because the result of the experiments in the presence of TTX indicated that a component of the PGE2-mediated depolarization may be indirect and due to release of neuroactive compounds from nearby nerve terminals, additional experiments were conducted to investigate a possible role of tachykinins in the generation of the indirect or presynaptic effects of PGE2 observed in myenteric AH cells. In these experiments, antagonists of neurokinin receptors NK1 and NK3 (SR-140333 and SR-142801, respectively, 100 nM for each) were applied to impaled AH cells after a control application of PGE2 (10 μM in pipette, 900 ms). Application of the NK receptor antagonists significantly inhibited PGE2-mediated depolarization amplitude (control = 5.4 ± 0.4 mV, NK antagonists = 2.9 ± 0.5 mV, range = 36–54% decrease; n = 8, P < 0.05) and dur50 (control = 40 ± 10 s, NK antagonists = 26 ± 7 s, range = 30–48% decrease; n = 8, P < 0.05).

Effects of Prolonged Exposure to PGE2-Ethanolamide

Inflammation is typically a long-lasting condition associated with a prolonged elevation of PGE2 levels. Therefore, we conducted additional experiments to determine the effects of prolonged exposure of myenteric neurons to PGE2 by maintaining colonic longitudinal muscle-myenteric plexus preparations in organ culture for 48 h in the presence or absence of the relatively stable PGE2 analog PGE2-ethanolamide (10 μM). Proportions of impaired S and AH cells cultured in the absence of PGE2-ethanolamide did not differ from those evaluated in acutely dissected preparations (S: naive = 64%, cultured = 63%; AH: naive = 36%, cultured = 20%).

Significantly depolarized resting membrane potentials were detected in PGE2-ethanolamide-cultured AH cells compared with cultured controls (control = −60.5 ± 1.7 mV, n = 11; PGE2-ethanolamide = −50.8 ± 3.0 mV, n = 9). No difference was observed in the threshold to initiate an action potential (threshold strength in nA) in AH cells of either treatment group (control, n = 11; PGE2-ethanolamide, n = 9), although there was a significantly higher number of action potentials elicited at two times threshold strength in PGE2-ethanolamide-cultured AH cells (Fig. 6). Spontaneous action potentials were observed in 67% of PGE2-ethanolamide-cultured AH cells, whereas spontaneous action potentials were not detected in any of the AH cells studied in the control preparations. Peak amplitude of the prolonged afterhyperpolarization in AH cells was significantly diminished in the PGE2-ethanolamide-cultured AH cells compared with controls (control = 19.6 ± 2.3 mV, n = 9; PGE2-ethanolamide = 10.8 ± 3.2 mV, n = 9). No change in membrane input resistances was detected in the presence of AH-6809.
amide-cultured cells (control, n = 11; PGE$_2$-ethanolamide, n = 9) (Fig. 7). Time to the peak of the prolonged afterhyperpolarization was also significantly attenuated in PGE$_2$-ethanolamide-cultured AH cells (control, n = 11; PGE$_2$-ethanolamide, n = 9) (Fig. 7). The $t_{50}$ of the prolonged afterhyperpolarization was also significantly inhibited (control, n = 11; PGE$_2$-ethanolamide, n = 9). The total duration of the prolonged afterhyperpolarization was also significantly diminished (control, n = 11; PGE$_2$-ethanolamide, n = 9). No difference was detected between control and PGE$_2$-ethanolamide-cultured AH cells in input resistance (control = 108 ± 19 MΩ, n = 11; PGE$_2$ = 115 ± 19 MΩ, n = 7) or action potential amplitude (control = 72 ± 4 mV, n = 7; PGE$_2$ = 64 ± 9 mV, n = 7).

PGE$_2$-ethanolamide-cultured S cells had a significantly depolarized resting membrane potential compared with control cultured cells (control = 55 ± 2 mV, n = 34; PGE$_2$ = 42 ± 3 mV, n = 8). Mean input resistances were significantly decreased in PGE$_2$-ethanolamide-cultured S cells (control = 175 ± 22 MΩ, n = 34; PGE$_2$ = 67 ± 17 MΩ, n = 8). Action potential amplitude was also significantly decreased (control = 66 ± 3 mV, n = 34; PGE$_2$ = 41 ± 3 mV, n = 8). No observable differences were seen between control and PGE$_2$-ethanolamide-cultured S cells in threshold resistance.
strength, maximum number of action potentials per depolarizing current pulse generated at two times threshold strength, or presence of spontaneous action potentials.

**DISCUSSION**

The present study was conducted to test whether PGE₂ can alter the electrical properties of colonic myenteric neurons as a means of contributing to changes in motility typically associated with colitis. Acute application of PGE₂ elicited a depolarization of myenteric neurons but did not alter the excitability of either S or AH cells. Acute PGE₂ application typically did not lead to the spontaneous generation of action potentials in myenteric neurons, did not increase the number of action potentials in response to a depolarizing current pulse, and did not alter the prolonged afterhyperpolarization in AH cells. However, chronic application of PGE₂ to longitudinal muscle-myenteric plexus explants resulted in increased excitability of AH cells, with a membrane depolarization, an increase in the detection of spontaneous action potentials, more action potentials generated per depolarizing current pulse, and diminished prolonged afterhyperpolarization. These results suggest that myenteric neurons express receptors for PGE₂ and that continuous activation of these receptors leads to enhanced excitability in AH cells.

Increase in AH cell excitability caused by prolonged exposure to PGE₂ may contribute to the dysmotility associated with inflammatory bowel disease. Because AH cells are thought to act as intrinsic primary afferent neurons and interneurons in the motor reflex circuitry of the intestines, alteration of active and passive membrane properties of AH cells would affect the afferent portion of the peristaltic reflex arc (35, 40). AH cells have processes that extend into the lamina propria, as well as projections to other myenteric neurons (2–4, 40). Thus, because the inflammatory response is typically centered in the lamina propria of the mucosal layer in colitis, the processes of AH cells would be directly exposed to proinflammatory mediators such as PGE₂. Consistent with this model, we have recently found that the excitability of AH cells is increased in trinitrobenzenesulfonic acid (TNBS)-induced colitis (32).

Although the PGE₂-mediated depolarization in AH cells was significantly reduced in the presence of TTX, it was not completely inhibited. This suggests the possibility that the PGE₂-mediated depolarization of colonic myenteric neurons involves actions of PGE₂ both directly on AH cells and indirectly through the release of neuroactive compounds from nearby nerve terminals onto the impaled neurons. The family of bioactive peptides known as the tachykinins has been implicated in the generation of slow EPSPs in colonic myenteric ganglia (1, 25, 26). Present results suggest that tachykinin receptor blockade can alter PGE₂-mediated depolarizations in myenteric AH cells, suggesting that the indirect effects of PGE₂ may be due to the release of endogenous tachykinins, such as substance P.

Acute and chronic application of PGE₂ depolarized S cells with negligible alteration of neuronal excitability. S cell depolarization in response to acute PGE₂ application was also inhibited by TTX, suggesting that PGE₂ also depolarizes S cells through direct and indirect mechanisms. S cells exhibit Dogiel type I morphology with single axonal processes that typically contact the circular or longitudinal muscle bundles, but S/Dogiel type I cells do not exhibit processes that extend into the lamina propria (10, 35, 40, 54). It is therefore unlikely that S cells are directly altered by the inflammatory response in colitis, but their activity may change due to upstream changes in the neuronal reflex circuitry. In support of this model, we have recently found that, whereas AH cells are hyperexcitable in the inflamed colon, the electrical properties of S cells in the inflamed colon are not different from those in the normal colon (32). The only change detected in S cells in colitis was an increase in the proportion that received slow excitatory synaptic input, which is consistent with a downstream effect of inflammation on primary afferent neurons.

In the myenteric ganglia of the distal colon, acute application of PGE₂ evokes a depolarization that was not associated with a change in input resistance or neuronal excitability. In other regions of the bowel, brief application of PGE₂ elicited a depolarization that is often accompanied by the generation of action potentials. This type of response has been observed in the myenteric plexus of the ileum (14) as well as the submucosal plexus of the distal colon (18). By contrast, application of PGE₂ to gallbladder neurons results in a complex response typified by rapid membrane hyperpolarization, followed by depolarization and another, prolonged, hyperpolarization (24). Taken together, these findings indicate that the actions of PGE₂ are tissue specific, and acute exposure of distal colonic myenteric neurons to PGE₂ has a modulatory effect, but it does not itself activate the generation of action potentials.

The EP₁–4 receptors are most sensitive to PGE₁ and PGE₂. In the present study, we found that the dual EP₁/EP2 receptor antagonist AH-6809 attenuated the PGE₂-mediated depolarization. EP₁ and EP2 receptor mRNA is present in multiple physiological systems (for review see Ref. 12), but of these, only the EP₁ receptor message is present within the digestive tract in any appreciable level (38). The EP₁ receptor is linked to the heterotrimeric G protein G₁₃⁻α₁, which activates phospholipase C after prostaglandin binding and generates inositol-1,4,5-trisphosphate [Ins(1,4,5)P₃] and diacylglycerol (DAG). In addition, EP3 receptor mRNA has been detected in the muscularis of the rat colon (15), indicating that it is possible that myenteric neurons express this prostanoid receptor as well. This receptor is alternatively coupled either to G₆⁻α₁, which activates phospholipase C after prostaglandin binding and generates inositol-1,4,5-trisphosphate [Ins(1,4,5)P₃] and diacylglycerol (DAG). However, to date, no subtype-selective EP3 receptor antagonist has been developed. Therefore, barrier the development of higher-specificity receptor ago-
nists and antagonists for EP receptors, uncoupling of the receptor-intracellular signaling cascade may be one way of defining the receptor responsible for the actions of PGE2 in the myenteric plexus of the colon.

Evidence from the present study indicates that PGE2 likely acts on more than one type of receptor to depolarize AH cells in this system. A slight PGE2-induced depolarization persisted in the presence of the dual EP1/EP2 receptor antagonist AH-6809. Dissociation studies involving the displacement of [3H]PGE2 from EP receptors by AH-6809 have demonstrated $K_i$ of ~350 nM (11, 29), suggesting that the concentration used in the present study (5 μM) would be sufficient to block the majority of EP1 receptors. The present experiments also failed to detect any change in membrane input resistance in response to acute PGE2 application. This result could be explained by activation of receptors coupled to opposing intracellular signaling cascades.

Upregulation of COX-2 activity and PGE2 synthesis are common features of intestinal inflammation (47), and PG levels typically remain elevated for an extended period during chronic inflammation (21, 47, 51, 52). We have recently found that inhibition of COX-2 activity reverses the increases in myenteric neuronal excitability seen in TNBS-induced colitis (33), suggesting that increases in PG synthesis that accompany inflammation may lead to altered myenteric neuronal activity. Results of the present study suggest that prolonged exposure of AH neurons to an analog of PGE2 has a dramatic effect on AH neuronal excitability. This result is important because it suggests that desensitization of myenteric neurons does not occur rapidly in the presence of PGE2, allowing PGE2 to maintain its effects throughout chronic inflammation. Furthermore, these findings indicate that changes observed in response to prolonged exposure to the agonist may involve changes in gene expression and protein synthesis, because acute application of PGE2 did not alter neuronal excitability. AH neurons normally have a steady-state Ca$^{2+}$-activated K$^+$ conductance that holds the membrane at a more negative potential, and this conductance also contributes to the prolonged afterhyperpolarization characteristic of these cells (20, 43). It is possible that prolonged exposure to the analog of PGE2 leads to a decrease in the Ca$^{2+}$-activated K$^+$ conductance in these cells. Alternatively, PGE2 could have similar effects by disrupting Ca$^{2+}$ entry and/or mobilization of intracellular Ca$^{2+}$ stores in AH cells. Further studies will be required to test whether changes in protein synthesis occur in response to prolonged exposure to PGE2, and to determine what changes in these neurons are responsible for the changes in excitability that were observed.

Results of this study support the theory that PGE2 can contribute to dysmotility in colitis by modulating the activity of myenteric neurons in the distal colon. Although acute application of PGE2 simply caused a depolarization of myenteric neurons, chronic application, which more closely reflects the inflamed condition, depolarized the neurons and significantly increased the excitability of AH cells. Inhibition of the prolonged afterhyperpolarizing potential and decrease in action potential accommodation of myenteric AH cells, which act at the afferent limb of the reflex circuitry, would allow these neurons to respond more vigorously to luminal stimuli, and in turn, alter motor patterns, resulting in dysmotility.

We thank Dr. David Linden for valuable discussion. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-62267, National Institute of Neurological Disorders and Stroke Grant NS-28995, and the Crohn’s and Colitis Foundation of Canada (to K. A. Sharkey and G. M. Mawe). K. A. Sharkey is an Alberta Heritage Foundation for Medical Research Medical Scientist.

REFERENCES


