Inflammation modulates in vitro colonic myoelectric and contractile activity and interstitial cells of Cajal

G. Lu, X. Qian, I. Berezin, G. L. Telford, J. D. Huizinga, and S. K. Sarna. Inflammation modulates in vitro colonic myoelectric and contractile activity and interstitial cells of Cajal. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1233–G1245, 1997.—Inflammation suppresses phasic contractile activity in vivo. We investigated whether inflammation also suppresses in vitro phasic contractile activity and, if so, whether this could be due to the alteration of specific slow wave characteristics and morphology of the interstitial cells of Cajal (ICC). Circular muscle strips were obtained from normal and inflamed distal canine colon. Inflammation was induced by mucosal exposure to ethanol and acetic acid. The amplitudes of spontaneous, methacholine-induced, substance P-induced, and electrical field stimulation-induced contractions were smaller in inflamed muscle strips than in normal muscle strips. Inflammation reduced the resting membrane potential and the amplitude and duration of slow waves in circular muscle cells. Inflammation did not affect the amplitude of inhibitory junction potentials but did decrease their duration. Ultrastructural studies showed expansion of the extracellular space between circular muscle cells, reduction in the density of ICC and associated neural structures, damage to ICC processes, vacuolization of their cytoplasm, and blebs of the plasma membrane. We conclude that inflammation-induced alterations of slow wave characteristics contribute to the suppression of phasic contractions. These alterations may, in part, be due to the damage to ICC. Inflammation impairs both the myogenic and neural regulation of phasic contractions.

slow waves; electrical control activity; intracellular recording; inhibitory junction potential; colitis; substance P; acetylcholine

COLONIC SLOW WAVES REGULATE excitation-contraction coupling of phasic contractions in vivo and in vitro (2, 26, 27a, 32, 34). The phasic contractions occur only when the membrane depolarizes beyond an excitation threshold during slow waves. However, the precise electrical events associated with phasic contractions vary, depending on the amplitude of these contractions under different experimental conditions. The amplitude of contractions recorded concurrently with intracellular electrophysiological tracings depends primarily on the amplitude and duration of the plateau potential of the slow wave (25). The amplitude of these contractions is typically in the range of 0.25 to 1 g (1.5 to 3 × 10 mm muscle tissue). These contractions are not associated with any spike bursts during the plateau potential and are called spike-independent contractions (25). When the in vitro contractions are stimulated by the addition of an agonist such as acetylcholine or substance P to the muscle bath and the electrical recordings are made concurrently with extracellular electrodes or sucrose gap, the phasic contractions are associated with spike bursts superimposed on the plateau potential of the slow waves (7, 9, 11). These contractions are typically 3–5 g in amplitude (1.5 to 3 × 10 mm muscle tissue). The intracellular electrodes cannot be maintained inside the cells with these stronger contractions. Similarly, the phasic contractions recorded in vivo in the intact animals or in the ex vivo colon are always accompanied by spike bursts recorded by extracellular electrodes (17, 26, 28, 29). These are spike-dependent contractions. These contractions are typically 75–150 g in amplitude (5 × 15 mm muscle tissue). The amplitude of spike-dependent contractions is correlated with the number, frequency, and duration of the spike bursts.

Previous reports have indicated that spike-dependent in vivo phasic contractions in patients with ulcerative colitis (15, 35) as well as in animal models of inflammation (31) are suppressed during inflammation. The generation of isometric tone in response to stretch and cholinergic stimulation is also decreased in muscle strips taken from patients with ulcerative colitis and from animal models of inflammation (8, 10, 33). However, the increase in isometric tone is a sustained contraction and it does not depend on rhythmic slow wave depolarizations. The excitation-contraction coupling and signal transduction for the tonic and phasic contractions are likely to be different. It is not known whether colonic inflammation also suppresses the in vitro spike-independent phasic contractions. If so, does inflammation alter the slow wave characteristics that may account, at least partially, for the suppression of phasic contractions?

Our objectives in this study were to determine the effects of colonic inflammation on spontaneous and agonist-induced in vitro phasic contractions as well as the intracellular electrical activity. The pacemaker cells for slow waves in the colon are thought to be the interstitial cells of Cajal (ICC) associated with a specialized layer of circular muscle cells near the submucosal border (1, 20, 30, 40, 44). These cells are interconnected by a network of gap junctions. We therefore also investigated whether inflammation damages the ICC and their processes, which could affect the characteristics of colonic slow waves and the excitation-contraction coupling in the circular muscle layer.

EXPERIMENTAL METHODS

Intracellular recordings and contractile activity. The dogs were anesthetized with 30 mg/kg pentobarbital sodium. We removed 5-cm-long segments of the distal colon, 5–10 cm orad
to the peritoneal reflection, from 25 normal dogs and 17 dogs in which colonic inflammation was induced by mucosal exposure to ethanol and acetic acid. In three additional dogs, the tissues were removed 30 min after the mucosal exposure to ethanol and acetic acid to determine if mucosal injury itself had any effect on slow wave and contractile parameters. The mucosa was removed, and a full-thickness (1.5 × 10 mm) muscle strip was dissected parallel to the long axis of the circular muscle cells. The strip was pinned down in a muscle bath with the cross-sectional surface facing up. One-half of the length of the muscle strip was immobilized by pinning along the edges to record intracellular electrical activity, as described previously (23). The loose end of the other half was connected to an isometric force transducer (Grass Instruments, Quincy, MA) to record contractile activity concurrently.

The muscle bath volume was 2 ml. The bath was perfused continuously with prewarmed and oxygenated Krebs solution at 5 ml/min (137.4 mM Na+, 5.9 mM K+, 2.5 mM Ca2+, 1.2 mM Mg2+, 134 mM Cl–, 15.5 mM HCO3–, 1.2 mM H2PO4–, and 11.5 mM glucose). The solution was bubbled with 95% O2-5% CO2 to maintain a pH of 7.4. The bath temperature was maintained at 38 ± 0.5°C. The muscle strips were stretched to 1 g and left to equilibrate for at least 2 h. Thereafter, the muscle strips were stretched incrementally until the amplitude of spontaneous phasic contractions was maximal.

Circular smooth muscle cells were impaled with glass microelectrodes (20–35 MΩ) filled with 3 M KCl. Recordings were accepted when a sharp drop in voltage greater than −55 to −35 mV was observed, depending on the distance of the electrode from the border of the circular muscle layer and the submucosa. Membrane potentials were amplified (model Duo 773, World Precision Instruments, New Haven, CT) and displayed on an oscilloscope (Tektronix 5111A, Tektronix, Beaverton, OR). The myoelectric and contractile activities were recorded on an FM tape recorder (Hewlett-Packard 3968A, Santa Clara, CA) for later off-line analysis. A square wave stimulator (model S48, Grass Instruments) and a stimulus isolation unit (model SIUSB, Grass Instruments) were used to apply electrical field stimulation (EFS) to the muscle strips. The EFS was applied through two platinum electrodes dipped into saline and placed parallel to the muscle strips.

Induction of colonic inflammation. An intraluminal catheter (ID, 2.6 mm; OD, 4.9 mm) was implanted surgically in the proximal colon under general pentobarbital sodium anesthesia. The dogs were allowed to recover for at least 7 days. The colon was cleansed by infusing 1 liter Colyte through the proximal intraluminal catheter. This method induces diffuse colitis that lasts for 10 days (31). Two days after the induction of inflammation, the dogs were anesthetized with 10 mg/kg Telazol (Fort Dodge Laboratories, Fort Dodge, IA), and 75 ml of 95% ethanol were infused intraluminally at 5 ml/min. Ten minutes later, 10 ml of 75% acetic acid were infused over a 1-min period. Simultaneously, the same volumes of ethanol and acetic acid were infused through a Silastic tube inserted anally. The end of the anal tube was 15 cm from the external anal sphincter. Five minutes after acetic acid infusions concluded, 100 ml of 0.9% saline were infused through the proximal intraluminal catheter. This method induces diffuse pan colitis that lasts for ~10 days (31). Two days after the induction of inflammation, the dogs were anesthetized with 30 mg/kg pentobarbital sodium and the tissue was harvested from the distal colon, as described above. All dogs had diarrhea on the day of tissue harvesting. The dogs were given 500 ml of 5% dextrose in Ringer by daily intravenous infusion to compensate for fluid loss by diarrhea.

Electron microscopy studies. Three additional dogs in which inflammation was induced as described above were anesthetized with 30 mg/kg pentobarbital sodium. The abdomen was opened along the midline, and the mesenteric artery supplying blood to a 5-cm-long segment in the proximal colon was identified. An Angiocath was inserted into the artery, and the perfused segment was identified by infusing 0.9% saline. The mesenteric vein draining blood from this segment was identified and cut. The segment was then fixed by perfusing a solution of 2% glutaraldehyde in 0.075 M cacodylate buffer containing 4.5% sucrose and 1 mM CaCl2, pH 7.4, as rapidly as possible and until the segment became rigid. The segment was then removed, opened lengthwise, cleansed, and submerged in the same glutaraldehyde fixative in a petri dish (19). The tissue was cut into strips along the circular muscle axis (1.5 × 2 to 3 cm long) and fixed further in the fixative for 4–5 h at room temperature. The tissue was then washed two to three times in 0.1 M cacodylate buffer containing 6% sucrose and 1.25 mM CaCl2, pH 7.4. Tissues from three normal colons fixed as described above were used as control.

Measurement of myeloperoxidase activity. Myeloperoxidase (MPO) was measured as described by Castro et al. (5). Tissue samples from six normal dogs and six dogs with colonic inflammation were chosen randomly from the whole group. In addition, the five tissue samples that showed no spontaneous slow wave activity in muscle strips were also processed for MPO activity. The MPO was determined in the lamina propria scraped with a glass slide and in the muscularis externa separately. The tissues were weighed and homogenized with hexadecyltrimethylammonium bromide (HTAB) buffer [0.5% HTAB in 50 mM phosphate buffer, pH 6.0, 4°C; 1:20 (wt/vol)]. The homogenate was freeze thawed three times and then centrifuged at 35,000 g for 30 min. The supernatant was processed for MPO as described previously (5). One unit of MPO activity was defined as that degrading 1 μM hydrogen peroxide per minute at 25°C. The MPO activity was expressed per gram of wet tissue.

Test substances. The following agents were used: acetyl-[b](−) methacholine chloride, atropine sulfate, phentolamine hydrochloride, propranolol-[b]-hydrochloride, and N-nitro-L-arginine methyl ester (L-NAME). All these substances were purchased from Sigma Chemical (St. Louis, MO), dissolved in a stock solution of 0.9% saline, and diluted in Krebs solution. [Sar9,Met(O2)11]-substance P was purchased from Research Biochemicals International (Natick, MA) and also dissolved as described above.

Data analysis. The contractile response was quantified as the mean amplitude of phasic contractions. Each dose of the agonist was infused for 5 min, and the mean amplitude of contractions was determined during the 5th min of the infusion and expressed as percent wet weight of the tissue. The amplitudes were measured from the baseline before the start of infusion of the test substance. The amplitudes of the spontaneous phasic contractions were also averaged over 1-min periods. All data are expressed as means ± SE. One-way analysis of variance with repeated measures was used for normally distributed data. Kruskal-Wallis one-way analysis of variance on ranks was used if the data failed the normality test. Multiple comparisons were done by Student-Newman-Keuls method. P ≤ 0.05 was considered statistically significant. This study was approved by the Animal Care Committee at the Zablocki Veterans Affairs Medical Center.

**RESULTS**

Effect of mucosal injury on slow wave and contractile parameters. All muscle strips taken from the colon 30 min after mucosal exposure to ethanol and acetic acid exhibited spontaneous slow waves and contrac-
tions. The slow waves and contractile parameters of these muscle strips were not significantly different from those of strips taken from the normal dogs (Table 1). For all further work, the strips from normal colon were used as control.

Inflammatory modulation of in vitro phasic contractile activity. All muscle strips from the normal colon exhibited spontaneous phasic contractions after a 2-h equilibration period (Fig. 1A). Twelve of seventeen muscle strips from the inflamed colon also exhibited spontaneous phasic contractions (Fig. 1B). The remaining five muscle strips did not show any identifiable contractile activity (Fig. 1C).

The mean weight of the normal muscle strips (6.6 ± 0.2 mg; n = 25) was not different from that of the inflamed muscle strips (7.0 ± 0.3 mg; n = 17; P > 0.05). The mean amplitude of the spontaneous phasic contractions in the inflamed muscle strips (17.7 ± 2.6 mg/mg wet tissue wt; n = 12) was significantly less than that in the normal muscle strips (77.2 ± 3.6 mg/mg wet tissue wt; n = 25; P < 0.05). However, the frequency of the spontaneous phasic contractions was not different between the inflamed and the normal muscle strips (4.3 ± 0.3 vs. 4.2 ± 0.3 cycles/min, respectively).

Methacholine at 10⁻⁹ to 10⁻⁵ M and substance P at 10⁻¹¹ to 5 × 10⁻⁶ M dose dependently increased the amplitude of phasic contractions in both normal and inflamed muscle strips (Fig. 2). The response in the inflamed muscle strips was significantly less than that in the normal strips (Fig. 2, P < 0.05 for methacholine as well as substance P).

Inflammatory modulation of slow wave activity. The thickness of the circular muscle layer in normal muscle strips was 1.5 ± 0.04 mm (n = 16). The thickness increased to 1.9 ± 0.06 mm (n = 11, P < 0.05) in the inflamed muscle strips. The location of the recording site in the circular muscle layer was normalized as the percent distance from the submucosal border. Slow waves were recorded at 5%, 50%, and 95% of the circular muscle thickness from the submucosal border. The resting membrane potential in the inflamed muscle strips was significantly less than that in the normal muscle strips at 5% and 50% locations, but not at the 95% location in the circular muscle layer (Fig. 3A). The percent decrease in the resting membrane potential from the 5% to 95% distance from the submucosal border was significantly greater in the normal (38 ± 3%; n = 4) than in the inflamed muscle strips (25 ± 2%; n = 4; P < 0.05).

Whereas all normal muscle strips exhibited spontaneous slow wave activity (Fig. 1A), the slow waves were absent in 5 of 17 inflamed muscle strips (Fig. 1C). The resting membrane potentials in these strips at 5%, 50%, and 95% distances were, however, not significantly different from those in the 12 muscle strips that exhibited spontaneous slow waves (Table 2, P > 0.05). The muscle strips that had no slow waves also showed no spontaneous contractions (Fig. 1C).

### Table 1. Effect of acute mucosal injury on slow wave and contractile parameters

<table>
<thead>
<tr>
<th></th>
<th>Normal Colon</th>
<th>Colon 30 min After Acute Mucosal Injury</th>
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<tbody>
<tr>
<td>Thickness of circular muscle layer, mm</td>
<td>1.50 ± 0.04</td>
<td>1.48 ± 0.03</td>
</tr>
<tr>
<td>Frequency of slow waves, cycles/min</td>
<td>4.2 ± 0.3</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>Amplitude of slow waves at 50% distance, mV</td>
<td>9.1 ± 1.0</td>
<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>Duration of slow waves, s</td>
<td>7.5 ± 0.14</td>
<td>7.1 ± 0.90</td>
</tr>
<tr>
<td>Resting membrane potential at 50% distance, mV</td>
<td>−59.7 ± 1.5</td>
<td>−60.0 ± 2.0</td>
</tr>
<tr>
<td>Amplitude of spontaneous contractions, mg/mg wet tissue wt</td>
<td>77.2 ± 3.6</td>
<td>75.9 ± 7.0</td>
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Values are means ± SE.

Fig. 1. Intracellular recordings indicate reduction in resting membrane potential and amplitude and duration of slow waves in inflamed colon (B) compared with normal colon (A). The amplitude of spontaneous phasic contractions in inflamed colon was also decreased. Tracings in C show absence of slow wave activity and spontaneous contractions observed in 5 of 17 inflamed muscle strips.
The mean amplitude of the slow waves in the inflamed muscle strips was significantly less than that in the normal muscle strips at the 5%, 50%, and 95% distance in the circular muscle layer (Table 3). The mean maximum attained level of depolarization decreased significantly at the 5% distance in the circular muscle layer during inflammation, but there was no significant change at the 50% and 95% distances (Table 3).

The mean duration of slow waves at 50% amplitude was significantly shorter in the inflamed strips than in the normal strips at the 5% and 50% distances in the circular muscle layer (Table 3). The slow wave duration at the 95% distance could not be measured reliably due to the instability and small amplitude of slow waves at this location. However, the frequency of slow waves in the inflamed muscle strips (4.3 ± 0.3 cycles/min) was not different from that in the normal strips (4.2 ± 0.3 cycles/min). The slow waves associated with spontaneous contractions were not superimposed with spikes in normal or inflamed muscle strips.

Table 2. Resting membrane potentials in inflamed muscle strips with and without spontaneous slow waves

<table>
<thead>
<tr>
<th>Distance from Submucosal Border</th>
<th>Inflamed Muscle Strips With Spontaneous Slow Waves, mV</th>
<th>Inflamed Muscle Strips Without Spontaneous Slow Waves, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>−61.3 ± 2.8</td>
<td>−56.8 ± 2.2</td>
</tr>
<tr>
<td>50%</td>
<td>−50.0 ± 2.5</td>
<td>−45.5 ± 4.5</td>
</tr>
<tr>
<td>95%</td>
<td>−44.0 ± 3.1</td>
<td>−42.0 ± 3.7</td>
</tr>
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</table>

Values are means ± SE.

Effect of inflammation on EFS-induced contractions. EFS at 5, 10, and 15 Hz, with pulse duration of 0.35 ms, amplitude of 150 V, and train duration of 1 min, stimulated phasic contractions that increased in amplitude with frequency (Fig. 4A). The amplitude of EFS-induced contractions in inflamed muscle strips was significantly less than that in normal muscle strips at all frequencies of stimulation (Fig. 4A). Atropine (1 µM) significantly reduced the amplitude of contractions in both normal and inflamed muscle strips (Fig. 4, B and C). In contrast, L-NAME (1 µM) had no significant effect on the contractile response to EFS in either case (Fig. 4, B and C).

Fig. 3. A: resting membrane potentials decreased from the 5% to the 95% distance in circular muscle layer from submucosal border in both normal and inflamed muscle strips. Inflammation also decreased resting membrane potentials at the 5% and 50% distances but not at the 95% distance. B: tracings of slow wave activity from circular muscle at the 5% (a), 50% (b), and 95% (c) distances from myenteric border.
Effect of inflammation on inhibitory junction potentials. Under nonadrenergic, noncholinergic (NANC) conditions (1 µM atropine, 1 µM phentolamine, and 1 µM propranolol), EFS at frequencies of 1, 5, 10, 15, 20, 25, and 30 Hz, with pulse duration of 0.35 ms, amplitude of 150 V, and train duration of 1 s, induced inhibitory junction potentials (IJPs) in all normal muscle strips and in 12 of 17 inflamed muscle strips (Fig. 5, A and B). The IJPs were blocked almost completely by 1 µM L-NAME, as has been reported previously in the canine colon (Ref. 39; data not shown). The amplitude (Fig. 6A) and duration (Fig. 6B) of the IJPs were frequency dependent in both the normal and the inflamed muscle strips. There was no significant difference in the amplitude of IJPs between the normal and the inflamed muscle strips. However, the duration of IJPs was shorter in the inflamed muscle strips compared with the normal muscle strips. The 5 of 17 muscle strips that did not show slow waves (Fig. 1C) had no IJPs at any frequency of stimulation (Fig. 5C).

Effect of inflammation on MPO activity. The MPO activity in the muscularis externa and the lamina propria of the inflamed muscle strips that exhibited spontaneous slow waves with altered characteristics was significantly greater than that in normal muscle strips (Table 4). The MPO activity in the muscularis externa of the inflamed muscle strips without spontaneous slow waves was significantly greater than that in the muscularis externa of normal muscle strips, as well as in the inflamed muscle strips with slow waves (Table 2). The MPO activity in the lamina propria of muscle strips without slow waves was greater than in normal strips but not different from those that had slow waves (Table 2).

Effect of inflammation on ICC and immunocyte infiltration. At the submucosal surface of the circular muscle layer of the proximal dog colon, a dense network of overlapping ICC and nerve fibers adjoined the circular muscle cells (Fig. 7), as has been reported previously (3). ICC were interconnected to one another and to adjacent innermost circular muscle cells by gap junctions and close apposition contacts (Figs. 7B, 8B, and 9C). Elsewhere in the circular muscle layer, the gap junctions were seen extremely rarely, but these were present between innermost circular muscle cells (within the 1–7 innermost layers of cells) (Fig. 9B). ICC differed from smooth muscle cells by a branched pattern of cellular processes and the presence of numerous mitochondria, free ribosomes, bundles of intermediate filaments, and caveolae (Fig. 7B). Submucosal fibroblasts were distinguished from ICC by a lack of caveolae and the presence of a well-developed cisterna of rough endoplasmic reticulum (Fig. 8A). ICC were situ-

### Table 3. Effect of inflammation on slow wave characteristics

<table>
<thead>
<tr>
<th>Distance from Submucosal Border</th>
<th>Slow Wave Amplitude, mV</th>
<th>Slow Wave Duration, s</th>
<th>Amplitude of Maximum Membrane Depolarization, mV</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Inflamed</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=4)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>5%</td>
<td>20.4 ± 1.2</td>
<td>10.3 ± 2.5*</td>
<td>7.5 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(8)</td>
<td>(16)</td>
</tr>
<tr>
<td>50%</td>
<td>9.1 ± 0.97</td>
<td>5.8 ± 0.79*</td>
<td>4.6 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>95%</td>
<td>2.8 ± 0.37</td>
<td>1.6 ± 0.24*</td>
<td>1.4 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
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Values are means ± SE; nos. in parentheses are n values. Slow wave duration at 95% distance could not be measured reliably due to instability and small amplitude of depolarizations. *P < 0.05 vs. normal.

Fig. 4. A: EFS at 5, 10, and 15 Hz frequency dependently increased the amplitude of phasic contractions in normal and inflamed muscle strips. Response in inflamed strips was significantly less than in normal muscle strips. B and C: atropine significantly inhibited amplitude of phasic contractions in both normal (B) and inflamed (C) strips, but N-nitro-L-arginine methyl ester (L-NAME) had no significant effect.
ated in close proximity to nerve fibers (Fig. 7) and frequently formed close contacts with them.

Infiltration of neutrophils and macrophages occurred in the circular muscle layer and the submucosal region of all sections of the inflamed tissues (Figs. 8A and 9A). Structural changes were observed in some circular muscle cells, indicating cell injury. Damaged smooth muscle cells were mostly restricted to regions with an extensive infiltration of neutrophils and macrophages (Fig. 8). The structural abnormalities in injured muscle cells consisted of the appearance of many secondary lysosomes, large lipid droplets, and empty membrane-bounded vacuoles (Fig. 8). Some smooth muscle cytoplasm displayed the proliferation of ribosomes, indicating that a moderate myofibroblast transformation occurred in some cells (Fig. 8B). In most sections, there were a few electron lucent smooth muscle cells, indicating irreversible injury. They were characterized by dark, necrotic nuclei, prominent vacuolization of cytoplasm, and the partial depletion of myofilaments, resulting in reduction of electron density of the cytoplasm (Fig. 8A). In some samples, there was an expansion of the extracellular space between circular muscle cells.

Table 4. MPO activity in muscularis externa and lamina propria of normal and inflamed colon and colon with acute injury

<table>
<thead>
<tr>
<th></th>
<th>Muscularis externa</th>
<th>Lamina propria</th>
</tr>
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<tbody>
<tr>
<td>Normal colon</td>
<td>0.09 ± 0.008</td>
<td>0.42 ± 0.1</td>
</tr>
<tr>
<td>Inflamed colon with spontaneous slow waves</td>
<td>1.2 ± 0.43*</td>
<td>2.6 ± 0.83*</td>
</tr>
<tr>
<td>Inflamed colon with no slow waves</td>
<td>3.0 ± 1.76*</td>
<td>6.2 ± 1.92*</td>
</tr>
<tr>
<td>Muscle strips with acute injury</td>
<td>0.57 ± 0.19*</td>
<td>1.11 ± 0.15*</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05 vs. normal colon.

Fig. 5. Inhibitory junction potentials (IJPs) in normal (A) and inflamed (B) muscle strips in frequency range of 1–30 Hz are shown. C: 5 of 17 inflamed muscle strips with no slow wave activity also did not show IJPs.

Fig. 6. A: amplitude of IJPs increased dependently with frequency in normal and inflamed muscle strips. There was no difference in IJP amplitude between normal and inflamed strips at any frequency. B: duration of IJPs also increased dependently with frequency in normal and inflamed muscle strips. However, duration of IJPs in inflamed muscle strips was significantly less than that in normal strips at all frequencies.
which might be due to edema and may account for the increase in the total thickness of the circular muscle layer.

In all sections of the inflamed tissues, there was an apparent reduction in the density of ICC at the inner border of the circular muscle layer (Figs. 8A, 9A, and 10A) compared with control tissue (Fig. 7A). The network of ICC with associated neural structures was disrupted. The density of nerve fibers in the submucosal plexus appeared qualitatively to be decreased. In most sections, only small groups of ICC processes were detected (Figs. 8 and 9, A and B). In other sections, several ICC were undamaged, with normal ultrastructure of their cell bodies (Fig. 10A) and cellular processes (Figs. 8B and 9C). They formed gap junctions (Fig. 9C) and close apposition contacts with each other.

Fig. 7. Cross section through circular muscle-submucosa interface of control dog colon. A: a low-magnification micrograph shows a typical dense network of interstitial cells of Cajal (ICC) processes (arrows) and nerves (N) closely associated with innermost circular muscle layer (CM) present in dog colon. At low magnification, ICC are distinguished by a high concentration of mitochondria (m) and the branched character of their processes. Note absence of immune cells in submucosa. F, fibroblast. Original magnification, ×5,280. Bar, 2 µm. B: high-magnification micrograph showing arrangement between small and large overlapping ICC processes and nerves in inner border of circular muscle layer. ICC processes interconnected by gap junction (large arrow) and close apposition contacts (small arrows) were positioned at close proximity to nerve and muscle cells. ICC is recognized by its branched profile, presence of numerous mitochondria, and plasma membrane caveolae (arrowheads). Original magnification, ×21,150. Bar, 1 µm.
Fig. 8. Cross section through the circular muscle-submucosa interface of inflamed dog colon. A: a low-magnification micrograph shows an infiltration of neutrophils (Nu) into circular muscle layer (CM) and the effect of inflammation on ICC network and ultrastructure of smooth muscle cells. There is injury to some smooth muscle cells. A damaged muscle cell (dCM) is characterized by a dark electron-dense nucleus (Nu), light cytoplasm containing secondary lysosomes (L), and confluent cytoplasmic vacuoles (-x-). Extracellular space between cells is enlarged and filled with collagen (Co). Note accumulation of single or confluent lipid droplets (arrows) in some smooth muscle cells (arrows). Most of the ICC network at the inner border of circular muscle layer is absent in this section. Only a small group of ICC processes is seen (boxed area). F, fibroblast. Original magnification, ×5,870. Bar, 2 µm. B: high-magnification micrograph of ICC seen in boxed area in A. There are no obvious changes to these ICC with respect to mitochondria (m), filaments, and caveolae (arrowheads). ICC processes still form close apposition contacts (arrows) with neighboring circular muscle cells, similar to control tissue. The most conspicuous structural change in these ICC processes is the presence of membrane-bounded structures between two ICC process (-x-). Note cytological abnormalities in two neighboring smooth muscle cells: presence of a large lipid droplet (L) in cell at top and proliferation of ribosomes (R) in cell at left. Original magnification, ×27,600. Bar, 1 µm.
and with adjacent smooth muscle cells (Fig. 8B). Most of the structural abnormalities in ICC were seen in the cellular processes, consisting of the vacuolization of the cytoplasm (Figs. 9, A and B, and 10A), blebbings of the plasma membrane, and the appearance of multiple membranes in the zones of contacts between ICC processes (Figs. 8B and 10B). A few ICC processes showed irreversible damage, which was characterized by a large number of ruptured mitochondria, the depletion of many thin and intermediate filaments, the presence of large lipid droplets, and empty membrane-bounded vacuoles (Fig. 10C). Direct contacts or close appositions between ICC and nerves were rare in the inflamed colon.

Fig. 9. A: low-magnification micrograph of the inner border of circular muscle layer (CM). No apparent injury to smooth muscle cells is seen in this region. However, most of the ICC network at the inner border of the CM has disappeared. Note a group of injured ICC processes (boxed area) adjacent to innermost circular muscle cells. N, nerve. Nu, a neutrophil in submucosa. Original magnification, ×6,700. Bar, 2 µm. B: high-magnification micrograph of boxed area in A shows structural abnormalities in injured ICC processes: single and multiple merging membrane-bounded vacuoles (v) and plasma membrane blebbings (*). Mitochondria (m) and filament organization appear normal. ICC processes are connected by close apposition contacts (arrows). Co, collagen, E, elastin. Original magnification, ×17,100. Bar, 1 µm. C: gap junction (arrow) between two ICC processes from lesion area. Original magnification, ×63,150. Bar, 200 nm.
Fig. 10. A: Longitudinal section through the circular muscle-submucosa interface from another inflamed colonic segment showing normal ICC cell body and injured processes (diICC). No structural abnormalities are seen in ICC body (ICC). The cytoplasm of a damaged ICC process is dominated by multiple membrane-bounded empty vacuoles (*). N, nerve; Co, collagen; CM, circular muscle cell. Original magnification, ×11,790. Bar, 1 µm. B: A group of overlapping ICC processes at close proximity to muscle cells (CM). Note gap junction contact (large arrow) between two innermost circular muscle cells, and small close apposition contacts (small arrows) between partially injured ICC processes. Original magnification, ×33,210. Structural abnormalities in ICC processes include multiple membrane-bounded vacuoles (*) merging with plasma membrane present in zones of contacts between ICC processes. Bar, 500 nm. C: Irreversibly injured ICC process is characterized by presence of many ruptured mitochondria (dm), partial depletion of filaments (*), empty vacuoles (v), and a large lipid droplet (L). An ICC process is recognized by the presence of numerous caveolae. Original magnification, ×19,225. Bar, 1 µm.
MPO activity. The MPO activity in the tissue removed 48 h after the mucosal exposure to ethanol and acetic acid was significantly greater than that in normal tissue (Table 4). The MPO activity was increased in both the lamina propria and the muscularis externa. There was no significant difference in MPO activity between the tissue that exhibited spontaneous slow waves and the tissue that had no slow waves. The MPO activity in the tissue that was removed 30 min after the mucosal exposure to ethanol and acetic acid was also significantly greater than that in the normal tissue (Table 4), but it was not significantly different from that in the tissue removed after 48 h in both the muscularis externa and the lamina propria (Table 4).

**DISCUSSION**

Previous studies have reported that spontaneous in vivo spike-dependent phasic contractions of colonic circular muscle cells are suppressed in patients with ulcerative colitis (15, 35) as well as in animal models of colonic inflammation (31). Our present findings show that spontaneous in vitro spike-independent phasic contractions of colonic circular muscle strips are also suppressed significantly during inflammation. The suppression of these contractions is associated with a significant reduction in the resting membrane potential and in the amplitude and duration of slow waves.

The resting membrane potential in circular muscle cells from normal muscle strips exhibited a gradient of ~29 mV from the submucosal to the myenteric border, as has been reported previously (20). This gradient decreased significantly during inflammation to ~10 mV. The decrease in the gradient was due to depolarization of the resting membrane potential at 5% and 50% distances in the circular muscle layer. There was no significant change in membrane potential at the 95% distance from the submucosal border. The precise mechanisms underlying the generation of the resting membrane potential or its gradient are not understood completely. However, two hypotheses have been proposed. According to the first hypothesis (19), the circular muscle cells have a uniform intrinsic resting membrane potential of about ~60 mV. The coupling of circular muscle cells near the submucosal border with the ICC pushes up the resting membrane potential of circular muscle cells in the inner one-third of the layer. Similarly, the coupling of the circular muscle cells with the longitudinal muscle cells near the myenteric border pulls down the resting membrane potential of the cells in the outer one-third, giving rise to the observed gradient in the full-thickness intact muscle strips. The intrinsic resting membrane potential of the longitudinal muscle cells is about ~44 mV. In support of this hypothesis, removal of the ICC and longitudinal muscle layers produces a uniform membrane potential in the remaining circular muscle layer (19). The removal of the ICC layer alone flattens the gradient only in the inner one-third of the circular muscle layer (19).

Ultrastructural findings in our study showed that the ICC and their processes were partially damaged by the inflammatory response. Whereas the physical removal of the entire ICC layer may completely eliminate the gradient of resting membrane potential in the submucosal one-third of the circular muscle layer, partial damage reduced the gradient partially but significantly. The gradient in the outer one-half of the circular muscle layer was not affected, because this is maintained by the interaction between the longitudinal and circular muscle layers (19) and this did not seem to be affected by inflammation. There was little or no damage to the longitudinal muscle cells during inflammation. Recently, Rumessen (24) also reported damage to ICC in the colon of ulcerative colitis patients similar to that observed in the acetic acid model of colonic inflammation. The density of ICC is also decreased in the aganglionic segment of patients with Hirschsprung's disease (42), resulting in a loss of slow waves (18).

The second hypothesis is that the resting membrane gradient is maintained by differences in the activities of electrogenic Na\(^+-K^+\) pump. Inhibition of the Na\(^+-K^+\) pump by ouabain or removal of K\(^+\) from the extracellular medium decreases the resting membrane potential of circular muscle cells near the submucosal border and in the middle but not near the myenteric border (4). However, the expression of the Na\(^+\) pump in the circular muscle layer is not affected during inflammation (14). It seems, therefore, that the changes in the resting membrane potential in colonic circular muscle cells during inflammation may primarily be due to the damage to the ICC, rather than due to the decreased expression of the Na\(^+\) pump.

Five of the seventeen inflamed muscle strips exhibited no slow waves at any location in the circular muscle layer and no spontaneous phasic contractions in vitro. The MPO activity in the muscle strips without slow waves was, however, not significantly different from that in inflamed muscle strips that showed slow waves. Physical removal of the ICC layer in muscle strips (19), the destruction of ICC by methylene blue and exposure to light (20), the reduction of these cells in BALB/c mice (22), or lack of development of ICC (12, 43) also lead to the absence of slow waves and spontaneous in vitro contractions. However, the resting membrane potential in the inflamed muscle strips without spontaneous slow waves was not different from that in muscle strips with slow waves. Thus additional factors, such as the increased distance between circular muscle cells and direct injury to circular muscle cells noted during inflammation, may also be involved.

Colonic inflammation reduced the amplitude of slow waves at 5% and 50%, but not 95%, thickness of the circular muscle layer from the submucosal border. Previous studies (6, 19, 32) have noted a direct relationship between the resting membrane potential and the slow wave amplitude in colonic circular smooth muscle cells. The amplitude of slow wave depolarizations decreases as the resting membrane potential decreases. Therefore, it is likely that the reduction of slow wave amplitude during inflammation is secondary to the depolarization of the membrane potential. The lack of a change in slow wave amplitude at the 95% distance correlates with no change in the resting membrane potential at this location. Due to the concurrent decrease in resting membrane potential and slow wave...
amplitude during inflammation, the maximum depolarization at the slow wave plateau in the inflamed colon at the 50% location was not different from that in the normal colon. However, the maximum amplitude of depolarization at the 5% location was significantly greater than that in the normal colon. Although it is known that the slow waves regulate contractile activity by depolarizing the membrane above an excitatory threshold potential (38), it is not known whether this excitatory threshold is a fixed potential or if it is a function of the resting membrane potential. Therefore, the precise effects of the concurrent decrease of the resting membrane potential and slow wave amplitude on excitation-contraction coupling cannot be predicted at this time.

Inflammation had no significant effect on the frequency of colonic slow waves or spontaneous in vitro phasic contractions. Koch et al. (16) also reported no change in the frequency of colonic contractions in muscle strips taken from patients with ulcerative colitis. Our findings are also in agreement with those of Cohen et al. (8), who reported depolarization of the resting membrane potential in the circular muscle cells of the rabbit colon during inflammation.

Inflammation reduced the duration of slow waves at 50% amplitude, which represents a reduction in the duration of the plateau potential. This reduction may be a major factor in the reduction of phasic contractile activity during inflammation. The amplitude of spike-independent in vitro phasic contractions is correlated directly with the amplitude and duration of the plateau potential. The precise mechanisms of reduction of the plateau potential during inflammation are not known. However, removal of the ICC layer from muscle strips has been reported to reduce the duration of the plateau potential (19). Damage to the ICC and their processes noted in inflamed muscle strips may therefore contribute to the reduction in the duration of the plateau potential.

Inflammation decreased the amplitude of the phasic contractile response induced by methacholine and substance P in muscle strips. We have reported a similar decrease in the contractile response to close intrarterial infusions of methacholine and substance P during inflammation in conscious dogs (21, 41) (S. R. Jadcherla and S. K. Sarna, unpublished results). The phasic contractions in vivo are spike dependent (25, 26, 27). These data indicate that inflammation may have similar effects on in vivo spike-dependent and in vitro spike-independent contractions.

Inflammation had no significant effect on the amplitude of the IJP’s stimulated by EFS in the frequency range of 1–30 Hz in the strips that displayed spontaneous slow waves. However, the duration of the IJP was reduced significantly at all frequencies in the above range. The colonic IJP’s in our study as well as in those reported previously (39) were largely nitronergic: they were blocked by L-NAME. The reduction in the duration of IJP suggests a reduction in the synthesis of neuronal NO. Therefore, the inhibition of phasic contractions during inflammation is unlikely to be due to an excessive nitronergic inhibitory input from the NANC neurons. The reduction in the synthesis or the release of neuronal nitric oxide is supported by our findings that the “on response” to EFS that depends on the release of acetylcholine was also reduced. Ultrastructural observations also indicated damage to the enteric neurons during inflammation.

The precise mechanisms by which mucosal exposure to ethanol and acetic acid induces inflammation have not yet been established (36, 45). However, a protonated form of acid is required to induce inflammation, because mucosal exposure to HCl of pH similar to that of acetic acid does not induce inflammation (37, 46). The factors involved in inducing inflammation by acetic acid include breakdown of the mucosal barrier, subsequent infiltration of luminal antigens, and, perhaps, the dissociation of acetic acid within the epithelium to liberate protons (45). Our data indicate, however, that the changes in slow wave and contractile parameters are not due to initial mucosal injury induced by acetic acid but due to the subsequent inflammatory response. Our data also suggest that the damage to the neuromuscular apparatus for motility may require an increase in immunocyte infiltration beyond a certain threshold and a prolonged exposure of the inflammatory response mediators to the enteric neurons and smooth muscle cells. The MPO activity was increased significantly 30 min after the mucosal exposure to ethanol and acetic acid, but no significant change in slow wave or contractile parameters was observed until 48 h after the beginning of the inflammatory response.

In conclusion, our findings show that inflammation alters specific parameters of slow waves in the colon and reduces the amplitude of spontaneous spike-independent in vitro phasic contractions. The changes in the electrical activity of the cell membrane, including the depolarization of the resting membrane potential and reduction in the amplitude and duration of slow waves, may be related to the damage to the ICC and their processes. Inflammation also suppresses the contractions induced by muscarinic and neurokinin receptor stimulation. The duration of IJP in response to EFS in the colon was decreased significantly by the inflammatory response, indicating a reduction in the neuronal release of NO.

This study was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-32346 (S. K. Sarna), Veterans Affairs Medical Research Service and Medical College of Wisconsin Research Affairs Committee Grant (G. Lu), and the Advisory Board of the Digestive Disease Research Center (S. K. Sarna).

Portions of this work have been presented previously in abstract form (Gastroenterology 110 (6): A710, 1996).

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Received 19 November 1996; accepted in final form 7 August 1997.

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