Different types of contractions in rat colon and their modulation by oxidative stress

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Gonzalez, Asensio, and Sushil K. Sarna. Different types of contractions in rat colon and their modulation by oxidative stress. Am J Physiol Gastrointest Liver Physiol 280: G546–G554, 2001.—The aim of this study was to investigate the modulation of in vitro rat colonic circular muscle contractions by dextran sodium sulfate (DSS)-induced inflammation and in spontaneous inflammation in HLA-B27 rats. We also examined the potential role of hydrogen peroxide (H2O2) in modulating excitation-contraction coupling. The muscle strips from the middle colon generated spontaneous phasic contractions and giant contractions (GCs), the proximal colon strips generated primarily phasic contractions, and the distal colon strips were mostly quiescent. The spontaneous phasic contractions and GCs were not affected by inflammation, but the response to ACh was suppressed in DSS-treated rats and in HLA-B27 rats. H2O2 production was increased in the muscle strip of the inflamed colon. Incubation of colonic circular muscle strips with H2O2 suppressed the spontaneous phasic contractions and concentration and time dependently reduced the response to ACh; in the middle colon, it also increased the frequency of GCs. We conclude that H2O2 mimics the suppression of the contractile response to ACh in inflammation. H2O2 also selectively suppresses phasic contractions and increases the frequency of GCs, as found previously in inflamed dog and human colons.

colonic inflammation; dextran sodium sulfate; giant contraction; hydrogen peroxide; giant migrating contractions

IN SEVERAL SPECIES, in vivo studies (38–40) have shown that the small intestinal and colonic circular muscle cells generate three distinct types of contractions: rhythmic phasic contractions, giant migrating contractions (GMCs), and tone. The cellular mechanisms of initiation of these contractions and their motility functions are markedly different (41–43). The rhythmic phasic contractions are regulated by slow waves superimposed with spikes. Their maximum frequency is the same as that of slow waves. These contractions partially or completely occlude the lumen, propagate over short distances, and cause the mixing and propulsive movements in the fasting and the postprandial states (9). The GMCs are severalfold longer in duration and larger in amplitude than the phasic contractions, and in dogs and humans they propagate uninterrupted over very long distances, sometimes over the entire length of the small intestine or the colon (15, 17, 20, 30, 37). Strong occlusion of the lumen coupled with long distances of uninterrupted propagation by these contractions causes mass movements (17, 23). GMCs are not regulated by slow waves, as their duration is much longer than that of a single slow wave cycle (37). The tone regulates the lumen size, and the tone is also not regulated by rhythmically occurring slow waves. The precise role of tone in propulsion is not known, but a decrease in the lumen size may enhance the efficacy of phasic contractions in mixing and propulsion.

Rodents are now increasingly used in gastrointestinal motility studies because of the availability of transgenic and knockout models. However, it is not known whether the colonic circular muscle of these species generates the same types of contractions as the higher species, such as dogs and humans, in vivo and in vitro. The stooling habits and stool shape and texture in rats and mice differ from those in dogs and humans.

Inflammation suppresses rhythmic phasic contractions and tone and, at the same time, increases the frequency of GMCs (19, 31, 33, 44). The suppression of phasic contractions prevents the normal mixing and propulsive movements of the gut, whereas frequent mass movements due to the increased frequency of GMCs cause diarrhea. The differences in cellular mechanisms that differentially suppress phasic contractions and tone, while at the same time stimulating GMCs, are not completely understood. However, it is thought that these alterations in cellular function are due to the production of reactive oxygen species (ROS), cytokines, and lipid mediators in the muscularis during inflammation. Main et al. (29) and Hurst and Collins (18) reported that some cytokines, such as tumor necrosis factor-α and interleukin-1β (IL-1β) can damage the enteric neurons to produce dysfunction similar to that seen in inflammation. In contrast, the effects of ROS on smooth muscle contractility are not completely understood. It is known that the levels of ROS are elevated in the mucosa of patients with inflammatory bowel disease as well as in experimental models of inflammation (13, 22, 46). If ROS contribute

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to smooth muscle and enteric neural dysfunction in inflammation, their production also must be increased in the muscularis. It is unlikely that the elevation of ROS levels in the mucosa can directly affect smooth muscle or enteric neural function.

The objectives of this study were to 1) define the types of contractions the rat colon generates in a muscle bath, 2) determine whether the production of hydrogen peroxide (H$_2$O$_2$) is increased in the muscularis of dextran sodium sulfate (DSS)-treated rats and transgenic HLA-B27 rats that develop spontaneous inflammation, and 3) examine the effect of incubation of muscle strips with H$_2$O$_2$ on the occurrence of different types of colonic contractions.

**MATERIALS AND METHODS**

**Animal model of inflammation.** Male Sprague-Dawley rats (200–250 g, Harlan, Indianapolis, IN) were used. Colonic inflammation was induced by giving 5% (wt/vol) DSS (mol wt >40,000, ICN Biochemicals, Aurora, OH) in drinking water for 7 days. The mortality rate was 8.3%. Transgenic HLA-B27 rats from a Fisher 344 genetic background, which develop spontaneous enterocolitis (1), were purchased from Taconic (Germantown, NY). By 28–32 wk of age, rats had developed loose stools and were killed. Age-matched Fisher 344 rats, also purchased from Taconic, were used as controls for transgenic HLA-B27 rats.

**Muscle bath studies.** The whole colon from the cecum to the pelvic brim was removed after asphyxiation of the animal with CO$_2$ and immediately immersed in warm, carbogented Krebs solution (in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1 NaH$_2$PO$_4$, 1.2 MgCl$_2$, 11 d-glucose, and 25 NaHCO$_3$). Segments (3 cm long) of the proximal (~1 cm from the cecum), middle (~7 cm from the cecum), and distal colon (~15 cm from the cecum) were removed, opened along the mesenteric border, cleaned, and pinned flat on a petri dish with Sylgard base. The mucosal layer, facing up, was removed under a magnifying glass. Strips (2 mm × 10 mm) were cut along the circular muscle axis and mounted in 3-ml muscle baths filled with carbogented Krebs solution at 37°C. Contractions were measured with Grass isometric force transducers connected to a Grass polygraph (Quincy, MA) that was interfaced to a computer. The strips were stretched in steps until the ACh-induced contractions were of maximal amplitude. They were left to equilibrate for at least 50 min while the bath solution was replaced every 10–15 min.

The effect of H$_2$O$_2$ on ACh-induced contractions was tested by first obtaining a control response to 2 μM ACh that was taken as 100%. Then H$_2$O$_2$ was added to the bath to give final concentrations of 0.3, 1, and 3 mM. The bathing solution was replaced every 15 min to compensate for H$_2$O$_2$ degradation. Control strips without H$_2$O$_2$ were incubated in parallel to monitor changes in contractility over time. The contraction to 2 μM ACh was measured again at 30, 60, 90, 180, and 360 min after incubation with H$_2$O$_2$ and compared with the initial response. Five minutes before ACh, the bathing solution was replaced with warm Krebs solution so that ACh responses were recorded in the absence of H$_2$O$_2$. Because H$_2$O$_2$ may be degraded by muscle strips to an unknown and variable degree, we also tested an alternative protocol. The strips were continuously superfused with H$_2$O$_2$ at rates of 60, 200, and 600 nmol/min, and the contractility to 2 μM ACh was checked at the same intervals as given above. ACh was added 5 min after stopping H$_2$O$_2$ superfusion and replacing it with normal Krebs solution.

**Measurement of H$_2$O$_2$ in muscularis homogenates.** A modification of the method described by Ravindranath (35) was used. The method is based on the oxidation of 2′,7′-dichlorofluorescein (DCF) to dichlorofluorescein (DCF) by H$_2$O$_2$. Before oxidation, DCF diacetate (DCF-DA) is deacetylated by DCFH by intracellular esterases. Rats anesthetized with 50 mg/kg pentobarbital were perfused through the left ventricle with carbogented Krebs solution to remove vascular blood. A 3-cm-long segment of the distal colon was removed, and the mucosa was dissected as described above. The tissue was frozen immediately in liquid nitrogen and kept at –80°C for up to a week until processing. Before analysis, the tissue was quickly weighed and homogenized at 4°C in buffer (in mM: 120 KCl, 33 Na$_2$HPO$_4$, and 1 EDTA, pH 7.4) in a smooth glass homogenizer for 3 min. An aliquot was obtained to determine protein concentration (in triplicate) with the bicinchoninic acid method (Pierce, Rockford, IL). The homogenates were incubated at 37°C in the presence of 0.5 μM DCFH-DA (Sigma Chemical, St. Louis, MO). The incubation was stopped after 30 min by the addition of 4 vol of ice-cold buffer and subsequent centrifugation. The supernatant was separated for photon counting in an Aminco-Bowman spectrofluorometer (Spectronics, Rochester, NY). Peak wavelengths for excitation and emission were 500 and 520 nm, respectively. The contribution of nonoxidized DCFH-DA to the total signal was negligible. The autofluorescence of every sample was determined in parallel and subtracted. The excitation and emission spectra from DCFH-DA oxidized by the homogenates were identical to those from oxidized DCF obtained commercially. Commercial DCF was used to calibrate the measurements (expressed as nM oxidized DCF/mg protein).

**Myeloperoxidase assay and visual assessment of mucosal injury.** Myeloperoxidase (MPO) activity was measured by the protocol employed by Castro and Arntzen (6). The tissue was thawed and homogenized at 0.5 g/10 ml homogenization buffer (50 mM KH$_2$PO$_4$ and 0.5% hexadecyltrimethylammonium bromide, titrated with 0.1 M Na$_2$HPO$_4$ to pH 6). The homogenate was then immersed in liquid N$_2$, freeze thawed three times, and centrifuged at 2,000 g. The supernatant was used to assay for MPO activity.

The assay was performed in cuvettes containing 1 ml guaiacol (0.22 ml/100 ml H$_2$O), 2 ml phosphate buffer (0.01 M KH$_2$PO$_4$ titrated with 0.1 M Na$_2$HPO$_4$ to get pH 6.0), and 0.1 ml sample. The spectrophotometer was set to 0, and 5 μl H$_2$O$_2$ (0.44 μmol) were added to start the reaction. The optical density values were recorded and converted to MPO activity using the standard curve of horseradish peroxidase type II (Sigma Aldrich, St. Louis, MO). The protein in the supernatant was determined with Bio-Rad reagent (4). The calculated specific activity is expressed as myeloperoxidase activity per milligram of protein. One unit of MPO activity was defined as that degrading 1 μmol of H$_2$O$_2$ per minute at room temperature.

**Data analysis.** The contractile response of circular muscle strips to ACh was measured as the area under contractions for 2 min after the addition of ACh to the bath (expressed in N·s). The contractile area was normalized by the cross-sectional area, which was calculated as follows: weight in mg/(1.05 × length in mm). Multiple comparisons were performed by one-way ANOVA followed by the Student-Newmann-Keuls test. The comparisons between two means were performed by parametric Student’s t-test. All data populations were normally distributed. The curve fittings for all calibrations had the squared correlation coefficient as >0.99.
RESULTS

Gross morphology, clinical symptoms, and MPO activity in inflammation. All rats treated with DSS developed diarrhea by day 7, and their weight was 19 ± 6% less than that of their age-matched siblings. The entire colon and cecum contained liquid, bloody feces. Macroscopically, the mucosa was thicker and extensively ulcerated in the distal colon; the middle and proximal colon had fewer lesions. An enlargement of the submucosal layer throughout the colon was evident. The MPO activity was significantly increased in the distal and the proximal colon compared with age-matched normal rats (Table 1). The percent increase in MPO activity was greatest in the distal colon (~550% increase). The MPO activity in the middle colon of DSS-treated rats increased by about ~50%, but the difference did not reach statistical significance (Table 1). The MPO activity in the distal colon of HLA-B27 rats was also greater than that in Fisher 344 controls (0.32 ± 0.07 vs. 0.02 ± 0.006 U/mg protein, respectively; n = 6; P < 0.05).

Types of spontaneous colonic circular muscle contractions in normal, DSS-treated, and HLA-B27 rats. The muscle strips exhibited spontaneous rhythmic phasic contractions at a frequency similar to that of short spike bursts that are regulated by the slow waves (12). The frequency of phasic contractions did not differ in the proximal, middle, and distal colon strips (Table 2), but under identical experimental conditions, the mean amplitude in the middle colon was about one-seventh the mean amplitude of that in the proximal colon and in the distal colon it was about one-thirtyfifth of that in the proximal colon (Fig. 1). The distal colon muscle strips were mostly quiescent. In addition, the middle colon strips generated regular giant contractions (GCs) at a frequency of 0.3 ± 0.1 contractions/min. GCs were defined as contractions with duration >150% and amplitude >300% of the corresponding mean values of phasic contractions. The GCs were sometimes superimposed with phasic contractions, particularly in their falling phase (Fig. 1). The proximal and the distal colon strips rarely generated GCs. In muscle strips, both the phasic contractions and the GCs were insensitive to 1 μM atropine. This same dose of atropine completely blocked the response to 30 μM ACh.

The phasic contractions are regulated by slow waves whose pacemaker cells have been reported to reside at the submucosal border in the feline and canine colons (10, 11, 47). We sought to determine whether the pacemaker cells at the submucosal border also regulate GCs. The mucosa and submucosa were removed in 20 muscle strips from the middle colon. This completely blocked the spontaneous phasic contractions as has been reported in other species (10, 11, 47), but it had no effect on the frequency of GCs (Fig. 1B).

No differences were noted in the characteristics of spontaneous phasic contractions between strips from the normal and DSS-treated proximal, middle, and distal colons (Table 2). The frequency of spontaneous GCs in the middle colon was also not affected by inflammation.

We also compared the spontaneous contractions of the chronically inflamed distal colon of transgenic

### Table 1. MPO activity in proximal, middle and distal colon in normal and DSS-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.25 ± 0.02</td>
<td>0.16 ± 0.04</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>DSS treated</td>
<td>0.47 ± 0.04*</td>
<td>0.23 ± 0.05</td>
<td>0.72 ± 0.09*</td>
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</table>

Values are means ± SE. DSS, dextran sodium sulfate; MPO, myeloperoxidase. *P < 0.05 vs. control.

### Table 2. Frequency and amplitude of spontaneous contractions of colonic circular muscle in vitro in normal and DSS-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Frequency, contractions/min</th>
<th>Amplitude, mN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phasic contractions</td>
<td>Giant contractions</td>
</tr>
<tr>
<td>Normal colon</td>
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<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>6.3 ± 0.5</td>
<td>14 ± 0.4</td>
</tr>
<tr>
<td>Middle</td>
<td>6.2 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Distal</td>
<td>5.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>DSS-treated colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>5.8 ± 0.7</td>
<td>18 ± 0.7</td>
</tr>
<tr>
<td>Middle</td>
<td>6.6 ± 0.8</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Distal</td>
<td>6.4 ± 0.5</td>
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</table>

Values are means ± SE of 5 experiments.

Fig. 1. A: spontaneous contractions of the circular muscle in vitro in the different regions of the rat colon. Phasic contractions of similar frequency were observed throughout the colon, although with different amplitudes (see Table 2). In addition, the middle colon exhibited giant contractions (GCs). B: spontaneous contractions in mucosa and submucosa-free strips. Note that the spontaneous phasic contractions are absent.
HLA-B27 rats with those of age-matched control Fisher 344 rats. Circular muscle strips from the distal colon of Fisher 344 rats exhibited spontaneous phasic contractions (amplitude: 0.4 ± 0.2 mN; frequency: 9 ± 1.2 contractions/min; n = 5). They were not different from those seen in the distal colon of HLA-B27 rats (amplitude: 0.5 ± 0.2 mN; frequency: 10 ± 1.8 contractions/min; n = 5; P > 0.05). As with Sprague-Dawley rats, the circular muscle strips from the distal colons of Fisher 344 rats and HLA-B27 rats were usually quiescent and rarely exhibited spontaneous GCs.

ACh-induced contractions in normal, DSS-treated, and HLA-B27 rats. Low concentrations of ACh (up to 2 μM) increased the amplitude of ongoing phasic contractions in the distal rat colon (Fig. 2). Higher concentrations induced a tonic contraction superimposed with increased amplitude of phasic contractions. The phasic contractions were usually ablated during the peak of tonic contraction, but reappeared when the tone began to decrease from the maximum (Fig. 2). The contractile response, measured as the combined area under tonic and phasic contractions, was increased by ACh concentration dependently in strips from normal and DSS-treated rats, but the increase was significantly suppressed in the inflamed strips (~50% reduction in maximal response; n = 5; P < 0.01; Fig. 3).

To eliminate the potential mechanical constraint due to the thickened submucosa in inflamed colon strips, ACh responsiveness was tested in distal colon muscle strips in which the mucosa and submucosa were carefully removed. In addition, TTX (0.3 μM) and hexamethonium (0.1 mM) were included in these experiments to block any neural effects of ACh. The contractile response of circular muscle strips devoid of submucosa was also suppressed in DSS-treated rats compared with normal rats (maximal effect: 17.9 ± 4.2 vs. 42.1 ± 6.9 N·s·mm⁻²; n = 4; P < 0.05), indicating a defect in excitation-contraction coupling rather than a mechanical artifact. The mean cross-sectional areas of the strips devoid of submucosa from the two groups were not statistically different (0.21 ± 0.03 vs. 0.17 ± 0.02 mm² for inflamed vs. normal, respectively; P > 0.05), ruling out the presence of significant muscle hyperplasia of the muscularis in this model.

The contractile responsiveness to ACh was also significantly suppressed in HLA-B27 rats (Fig. 3). The maximal effective response was 32.1 ± 3.2 and 22.4 ± 2.9 N·s·mm⁻² in Fisher 344 control rats and HLA-B27 rats, respectively (n = 5, P < 0.05).

H₂O₂ production in DSS-inflamed and HLA-B27 rat colonic muscle. Muscle strip homogenates from the distal colons of normal, DSS-treated, and HLA-B27 rats generated fluorescence when incubated with the peroxide-sensitive compound DCFH-DA. The fluorescence increased linearly with time from 0 to 30 min. Autofluorescence from both normal and inflamed samples was always <5% of the signal. The fluorescent signal was almost abolished if 5,000 U/ml catalase was included in the incubation medium, in both normal (86 ± 5% inhibition) and DSS-treated rats (91 ± 7% inhibition).

Muscle homogenates from DSS-treated rat distal colons oxidized significantly more DCF (72 ± 3.0 nM DCF/mg protein) than those from normal distal colons (32 ± 11 nM DCF/mg protein; P < 0.05; n = 5; Fig. 4). Because catalase decomposes H₂O₂ very rapidly, it is possible that part of the H₂O₂ formed during the incubation was degraded by endogenous catalase present in the homogenates before it could oxidize DCFH, in which case H₂O₂ generation would have been underestimated in the above experiments. Therefore, we included the catalase inhibitor 3-amino-1,2,4-triazole (3-AT) during the incubation to avoid H₂O₂ decomposition.
by endogenous catalase. In the presence of 5 mM 3-AT, the oxidation of DCFH by inflamed muscle homogenates (241 ± 58 nM DCF/mg protein) was much more marked than that by the control muscle homogenates (27 ± 8 nM DCF/mg protein; P < 0.01; Fig. 4).

Generation of \( \text{H}_2\text{O}_2 \) in muscle homogenates from the distal colons of HLA-B27 rats produced a 1.7-fold increase in \( \text{H}_2\text{O}_2 \) production (118 ± 28 in HLA-B27 colon vs. 67.5 ± 15 in control colon). However, this difference was not statistically significant (n = 5, P = 0.149).

**Effects of exogenous \( \text{H}_2\text{O}_2 \) on normal colonic muscle contractility.** Incubation of muscle strips with 1 mM \( \text{H}_2\text{O}_2 \) suppressed the spontaneous contractions in the proximal, middle, and distal colon within 90 min in 69% of the strips (Fig. 5). The response of the strips to 2 \( \mu \text{M} \) ACh decreased in a time-dependent pattern when incubated with the above concentration of \( \text{H}_2\text{O}_2 \) (Fig. 6A). The suppression of the response to ACh by incubation with \( \text{H}_2\text{O}_2 \) was more marked in the distal than in the proximal or the middle colon (Fig. 6B). In muscle strips from the middle colon, the response to 2 \( \mu \text{M} \) ACh declined smoothly over time when incubated with 0.3 mM \( \text{H}_2\text{O}_2 \), and it decreased abruptly with 1 mM or higher concentrations of \( \text{H}_2\text{O}_2 \) (Fig. 6A). In contrast, the responsiveness of normal strips (used as time controls) to ACh was maintained or even slightly increased during the 360-min period (Fig. 6A). Additionally, in an attempt to simulate continuous \( \text{H}_2\text{O}_2 \) production as it may happen during an inflammatory episode, we superfused the strips with \( \text{H}_2\text{O}_2 \) at constant rates, rather than replacing \( \text{H}_2\text{O}_2 \) in the muscle bath at fixed intervals. At a superfusion rate of 60 nmol/min for up to 3 h, \( \text{H}_2\text{O}_2 \) did not alter the response to ACh (data not shown). However, at superfusion rates of 200 nmol/min and higher, \( \text{H}_2\text{O}_2 \) induced a time-dependent suppression of response to ACh, similar to that in the previous experiments.

After incubation and washout of \( \text{H}_2\text{O}_2 \) and a recovery period of 3 h, the spontaneous activity and the responsiveness to ACh reappeared in the middle colon (Fig. 7). The spontaneous contractions were primarily GCs...
without any superimposed or independent phasic contractions. The frequency of GCs in the postincubation period (0.8 ± 0.2 contractions/min) was significantly greater than that before incubation (0.3 ± 0.1 contractions/min; P < 0.05). The response to ACh showed partial recovery at this time (Fig. 7B).

To rule out the influence of inhibitory neurons and other submucosal elements in the inhibitory effect of H₂O₂, we repeated these experiments in strips without the mucosa and the submucosa and in the presence of 0.3 μM TTX or 0.1 mM N⁵-nitro-L-arginine (L-NNA). Incubation with H₂O₂ in these strips suppressed the contractile response to 2 μM ACh with a time course similar to that found in strips containing the mucosa and the submucosa (n = 3, data not shown).

Finally, we determined whether the suppression of contractility to ACh in the inflamed strips was due to the presence of H₂O₂ in the musculature at the time of the experiments or to a fundamental change in excitation-contraction coupling due to the long-term exposure of the muscle to H₂O₂. We found that the addition of 5,000 U/ml catalase to inflamed muscle strips did not immediately reverse the suppression of response to 100 μM ACh. The contractile response after catalase was 98 ± 4.3% of that in the untreated strips (n = 8, P > 0.05).

DISCUSSION

Types of spontaneous in vitro circular muscle contractions in rat colon. The circular muscle of the colon of higher species, such as dogs and humans, generates rhythmic phasic contractions, GMCs, and tone in the intact conscious state (39, 40). However, in muscle bath, the colons of these species generate only the phasic contractions and tone. Our present findings show that, unlike the higher species, muscle strips from the rat middle colon generate spontaneous GCs. In muscle bath, the propagation of GCs cannot be examined, but recent studies by Li et al. (25) show that the GCs do propagate in the intact rat colon and are therefore similar to the GMCs found in the dog and the human colon (2, 20, 30, 34, 44). We did not concurrently record myoelectric activity, but the duration and frequency of phasic contractions and GCs correspond to those of short and long spike bursts, respectively, as previously reported by Castex et al. (5).

Compared with the intact conscious state, a basic change in the generation of different types of spontaneous colon contractions seems to occur when the circular muscle strips are prepared for muscle bath. The intact rat colon in the conscious state generates GCs in the proximal, middle, and distal colon at a frequency of ~0.5–0.7 GCs/min (25). In addition, the rhythmic phasic contractions are present at about the same amplitude (but much smaller than that of GCs) and frequency (~12 contractions/min) in all three segments (25). In contrast, in muscle bath, the spontaneous GCs were noted predominantly in the middle colon and their frequency was about one-half of that seen in the intact conscious state (25). The frequency of rhythmic phasic contractions was also about one-half of that seen in the intact state. In addition, the amplitude of phasic contractions decreased dramatically from the proximal to the middle to the distal colon (~35-fold decrease in amplitude). The precise reasons for these changes from the intact conscious state to muscle strips are not known, but they are likely to be due to the effects of dissection and the nutritional environment of the muscle bath compared with the nutrition derived from regular blood flow. The muscle strips from all three regions of the colon were prepared in an identical manner.

Several studies (10, 11, 24, 47, 50) have reported that the pacemaker cells for the generation of slow waves in the colonic muscularis reside at the submucosal border. These cells have been identified as the interstitial cells of Cajal (ICC). Accordingly, the phasic contractions were absent in our muscle strips when the mucosa and the submucosa, along with the ICC layer, were removed. However, the GCs were not affected by the removal of the submucosal border containing the ICC, confirming that they are not regulated by slow waves or ICC (37). This finding also confirms that the GCs are distinct from the phasic contractions and, particularly, they are not due to tetany produced by phasic contractions.

The rat colon strips did not exhibit spontaneous variations in tone. Smaller doses of ACh primarily accentuated the amplitude of phasic contractions. Larger doses, however, increased the muscle tone, and the phasic contractions were superimposed on it. In the intact conscious state, the rat colon also does not show spontaneous variations in tone (25). The lower doses of
ACh may therefore be more physiological in reference to the generation of spontaneous colonic contractions. It has not been feasible to measure the concentration of ACh at the neuroeffector junction to determine the physiological range of neurotransmitter release. However, because the smaller doses of ACh in our experiments mimicked the generation of phasic contractions seen in intact rats, we used these concentrations (2 μM) to evaluate the effect of inflammation in muscle strips. Because the cellular mechanisms of initiation of tone, phasic contractions, and GCs differ (42, 43) and their motility functions are also markedly different, our findings suggest that the effects of pharmacological agents should be examined for each type of contraction separately.

Effects of inflammation on spontaneous and ACh-induced contractions in DSS-treated and HLA-B27 rats. The frequency of spontaneous in vitro phasic contractions was not affected by inflammation in the rat colon, which is similar to that reported for the canine colon (26). However, the response to ACh was significantly suppressed in the rat colon inflamed by DSS treatment. This is similar to the suppression of muscarinic response in muscle strips from the human ulcerative colitis colon and experimentally inflamed rabbit and dog colons (8, 26, 28, 48). The response to ACh was also suppressed in circular muscle strips from the colon of HLA-B27 rats, as previously reported by Venkova and Greenwood-Van Meerveld (52). Grossi et al. (14) found that the suppression of in vitro contractility of the circular muscle in the colon does not depend on the method of inducing inflammation. In the intact conscious state (27) and also in enzymatically single dispersed cells (45), the muscarinic response of circular muscle cells is suppressed in the inflamed colon.

The response to ACh was suppressed in muscle strips even when they were devoid of submucosa and ICC and the inhibitory neural input was blocked by TTX or L-NNA. Therefore, it seems that the suppression of contractility during inflammation is due to a defect in excitation-contraction coupling in smooth muscle cells. However, this does not preclude additional abnormalities due to neural dysfunction and damage to ICC. Other studies (26, 36) found that ICC processes are damaged but not totally absent in ulcerative colitis and in the inflamed canine colon.

Li et al. (25) reported recently that the spontaneous colonic phasic contractions and GMCS in intact conscious rats are blocked by atropine, whereas in vitro these contractions are insensitive to muscarinic receptor blockade. It therefore seems that the stimulus for the spontaneous contractions differs in vivo and in vitro. The stimulus for in vitro spontaneous contractions has not been identified, but it may be stretch. Li et al. (25) also found that the overall contractile activity is reduced in the inflamed rat colon. This is consistent with our findings that the response to ACh is suppressed in muscle strips. The lack of suppression of spontaneous phasic contractions in vitro may be because these contractions are independent of release of ACh and the signaling pathways for ACh and stretch-induced contractions may differ. However, in the intact state, a decrease in neurotransmitter release may also contribute to the suppression of overall contractile activity. Myers et al. (33) also found a decrease in rat circular muscle contractility in response to ACh in colonic inflammation induced by mucosal exposure to acetic acid.

H₂O₂ production in muscularis and its role in alteration of colon contractions in inflammation. Previous studies (13, 22, 46) have reported that H₂O₂ production is increased in the mucosa of patients with ulcerative colitis as well as in animal models of inflammation. Our findings show that H₂O₂ production is also increased in the muscularis of DSS-treated rats, which makes this ROS a potential candidate to contribute to some of the alterations in motility seen during inflammation. This role was supported by the finding that incubation of the normal muscle strips with H₂O₂ suppressed ACh-induced response similar to that seen in strips from the inflamed colon. Interestingly, after incubation with H₂O₂ and washout, the frequency of GCs was significantly increased at the same time that the spontaneous phasic contractions and the response to ACh were suppressed. Both of these alterations in motility patterns are seen in colonic inflammation in dogs and in patients with ulcerative colitis (7, 21, 44). It is unlikely, however, that the total effect of inflammation on motility patterns is due to a single inflammatory response mediator. Several cytokines and lipids, such as IL-1β and platelet-activating factor, are also increased in the muscularis during inflammation, and they may contribute to the motility abnormalities as well (18, 29, 31).

H₂O₂ production also increased about twofold in the HLA-B27 rats, but it did not reach statistical significance. The production of H₂O₂ in spontaneous inflammation may be less, but it may occur over a longer period of time. Our data with normal strips showed that the effect of H₂O₂ on circular muscle contractions depends on both the concentration of H₂O₂ as well as the duration of incubation.

Previous studies (16, 32, 51) investigating the effect of oxidative stress on gut smooth muscle examined the direct and immediate effect of addition of oxidants to the muscle bath. Our aim was to determine whether H₂O₂ alters the excitation-contraction coupling on its exposure to the smooth muscle cells. Therefore, in our experiments, H₂O₂ was washed out before the addition of ACh. We found that incubation with H₂O₂ time and concentration dependently suppressed the response to ACh. The suppression was, however, reversible over a period of a few hours. Our hypothesis that H₂O₂ causes a fundamental longer lasting change in excitation-contraction coupling was confirmed by the finding that the addition of catalase to inflamed muscle strips did not immediately reverse the suppression of contractility to ACh. Also, the addition of H₂O₂ to the muscle bath altered the excitation-contraction coupling for several hours after H₂O₂ was washed out. A similar
situation may exist for other inflammatory response mediators.

The exposure to \( \text{H}_2\text{O}_2 \) had a greater effect on the distal colon than on the proximal or the middle colon. This is in agreement with the reports (51) that the catalase activity decreases from the proximal to the middle colon. Therefore, the distal colon has less defense against the deleterious effects of \( \text{H}_2\text{O}_2 \). Nitric oxide, which has been proposed as both a protective and a deleterious molecule in inflammation, is also more abundant in the myenteric plexus of the proximal colon than that of the distal colon (49).

It should be noted, however, that \( \text{H}_2\text{O}_2 \) is only an intermediate, and not especially reactive, compound in the cascade of ROS formation that begins with the intermediate, and not especially reactive, compound in the formation of superoxide anion. It is therefore likely that the cytotoxic effects of \( \text{H}_2\text{O}_2 \) are produced by the breakdown products of \( \text{H}_2\text{O}_2 \), such as hydroxyl radical and hypochlorous acid, which are considerably more reactive.

Our findings show that the rat colon may be a useful model to study the in vitro cellular mechanisms of GCs. Particularly, the effects of genetic manipulation on the stimulation or suppression of GCs can be investigated in transgenic and gene knockout rodent models. So far, this is the only species reported that generates GCs in muscle bath. A decrease in the frequency of GCs has been associated with constipation (3) and an increased frequency with diarrhea (7, 21, 44).

Our findings also show that \( \text{H}_2\text{O}_2 \) production is increased in the muscularis during inflammation, which may be a contributing factor to the suppression of ACh-induced contractions seen in inflammation. In addition, incubation of normal muscle strips with \( \text{H}_2\text{O}_2 \) suppresses phasic contractions and generation of tone but increases the frequency of GCs. These effects are similar to those seen in patients with ulcerative colitis and in experimental models of inflammation (7, 21, 44). \( \text{H}_2\text{O}_2 \) may therefore be one of the inflammatory response mediators that contributes to motility defects and digestive dysfunction in acute and chronic inflammation of the colon.

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