Acute experimental colitis decreases colonic circular smooth muscle contractility in rats

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Myers, Brian S., John S. Martin, Daniel T. Dempsey, Henry P. Parkman, Rebecca M. Thomas, and James P. Ryan. Acute experimental colitis decreases colonic circular smooth muscle contractility in rats. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G928–G936, 1997.—Distal colitis decreases the contractility of the underlying circular smooth muscle. We examined how time after injury and lesion severity contribute to the decreased contractility and how colitis alters the calcium-handling properties of the affected muscle. Distal colitis was induced in rats by intrarectal administration of 4% acetic acid. Contractile responses to acetylcholine, increased extracellular potassium, and the G protein activator NaF were determined for circular muscle strips from sham control and colitic rats at days 1, 2, 3, 7, and 14 postenemas. Acetylcholine stimulation of tissues from day 3 colitic rats was performed in a zero calcium buffer, in the presence of nifedipine, and after depletion of intracellular stores of calcium. The colitis was graded macroscopically as mild, moderate, or severe. Regardless of agonist, maximal decrease in force developed 2 to 3 days posttreatment, followed by a gradual return to control by day 14. The inhibitory effect of colitis on contractility increased with increasing severity of inflammation. Limiting extracellular calcium influx had a greater inhibitory effect on tissues from colitic rats; intracellular calcium depletion had a greater inhibitory effect on tissues from control animals. The data suggest that both lesion severity and time after injury affect the contractile response of circular smooth muscle from the inflamed area. Impaired utilization of intracellular calcium may contribute to the decreased contractility.

Patient with inflammatory bowel disease have altered bowel habits, usually diarrhea. In addition to changes in mucosal secretory and absorptive functions, alterations in colonic motility also may contribute to the increased urgency and frequency of defecation in patients with ulcerative colitis (17, 23–26, 30). Clinical studies suggest that active colitis is accompanied by a decrease in contractile activity in the inflamed area. The reduced colonic segmentation is believed to decrease the segmental to and from movements of the colonic contents and accentuate diarrhea (23, 25, 30). The mechanisms underlying the colonic dysmotility are unclear but may involve changes in colonic smooth muscle contractility (6, 13, 15, 31, 29, 33), enteric neurotransmission (14, 20), or afferent sensory input from the bowel wall (23).

In vitro studies using colonic muscle strips obtained from patients with ulcerative colitis and from animal models of colitis support the idea that colitis is accompanied by a decrease in the contractility of smooth muscle from the inflamed area (6, 13, 15, 29, 33). The decreased contractility develops independent of the manner in which the colitis is induced and is mediated at a receptor-independent site on the smooth muscle. Several questions remain with respect to the relationship between colitis and altered colonic smooth muscle contractility. For example, is the decreased contractility influenced by the duration of the inflammatory process? How does the severity of the inflammation impact the contractile response of the muscle? How does colitis affect the contribution of intracellular calcium stores to the contraction?

The aims of this study were to address these questions. Using an acetic acid model of distal colitis in rats (13, 18, 34), we determined 1) the temporal relationship between acute mucosal inflammation and the altered contractility of the underlying circular smooth muscle, 2) the relationship between the severity of colitis and the resultant decrease in smooth muscle active force development, and 3) the effect of colitis on extracellular and intracellular calcium utilization in acetylcholine (ACh)-induced contraction of circular smooth muscle from the distal colon.

MATERIALS AND METHODS

Animal model. Male Sprague-Dawley rats (250–300 g) were purchased from Ace Animals (Boyertown, PA) and were housed in a restricted-access room with controlled temperature (23°C) and light-dark cycles (12:12 h). Standard rodent food and tap water were provided ad libitum. The study was approved by the Institutional Animal Care and Use Committee, and all experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Induction of colitis. Distal colitis was induced by intracolic instillation of acetic acid. This model has been used extensively to investigate the pathogenesis of the acute phase of inflammation (9, 10, 18, 34). After an overnight fast, each rat was lightly anesthetized with isoflurane, and a polyethylene cannula (PE-240) was inserted into the lumen of the colon via the anus. The cannula was advanced so that the tip was 8 cm proximal to the anus. Initially, each rat received a 1-ml saline (0.9%) flush followed by manual palpation of the abdomen to remove any fecal matter. Next, 1 ml of acetic acid (4% vol/vol in 0.9% NaCl) was slowly infused into the distal colon, and the rat was maintained in a head-down position for 30 s to limit expulsion of the solution. Finally, each rat received a 1-ml colonic wash containing phosphate-buffered saline (pH 7.4). Control animals were treated identically except that, instead of 4% acetic acid, they received a 1-ml normal saline infusion. Control animals were studied 48 h post saline injections; colitic rats were studied 1, 2, 3, 7, and 14 postacetic acid instillation.
Assessment of injury. Each animal was anesthetized, and the entire colon was removed and placed in a petri dish containing chilled (5°C) N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) buffer solution. The colon was opened along the antimesenteric border from the anus to the cecocolonic junction and was rinsed with cold buffer to remove all fecal matter. The severity of the inflammation was graded using a modification of the gross mucosal inflammation scoring system described by Bell et al. (1). A score of zero represents an area with no macroscopic evidence of inflammation. A score of one to two defines an area with hypertumescence and petechia without ulceration (mild inflammation). A score of three to five means an ulcer was present with inflammatory changes (moderate inflammation). A score of six and above indicates severe inflammation with an ulcer >2 cm. Details of the grading system are presented in Table 1.

Samples of control and macroscopically inflamed colons were excised and immediately immersed in neutral buffered Formalin. The samples, prepared by cutting the tissues in cross section, were processed by routine techniques (American Histolabs, Gaithersburg, MD) for subsequent histological examination.

Response to agonist stimulation. After visual examination of the colon for determination of the inflammation index and after removal of tissue for histological analysis, the remaining distal colon was pinned mucosal side up in a dissecting dish filled with oxygenated (100% O₂) HEPES buffer solution of the following composition (in mM): 5.0 HEPES, 137.5 NaCl, 5.0 KCl, 1.0 MgCl₂, 2.5 CaCl₂, and 10 glucose at pH 7.4. With the aid of a stereomicroscope, full-thickness muscle strips (2 mm x 10 mm) were prepared by careful separation of the mucosa from the underlying muscle layers. The microscope permitted clear visualization of the muscle bundles and ensured that the long axis of the muscle strip was parallel to the circular muscle layer.

Muscle strips were mounted in individual 10-ml tissue baths containing warm (37°C) oxygenated (100% O₂) HEPES buffer solution and attached to an isometric force transducer (Grass, FT03; Quincy, MA). After a 1-h equilibration period, muscle strips were stretched in 0.5-mm increments and were stimulated with 100 µM ACh until the length at which maximal active force develops (L₀) was achieved. All experiments were carried out with the muscle strips set to L₀. A permanent record of the data was obtained using a Grass 7D polygraph (Quincy, MA).

For the dose-response experiments, muscle strips from control and experimental animals (days 1, 2, 3, 7, and 14) were stimulated with ACh (10⁻⁸ to 10⁻⁴ M) and KCl (10⁻⁸ to 80 mM). When KCl was used as the agonist, the NaCl concentration of the buffer solution was adjusted accordingly to maintain osmolality. In some of the experiments, the neurotoxin tetrodotoxin (1 µM) was added to the buffer solution to determine the myogenic versus the neurogenic components of the contractile responses.

Muscle strips from sham control and colitic (days 2, 3, and 14) rats also were stimulated with 10 mM NaF, a direct activator of G proteins (4, 12). The studies involving NaF were carried out to further test the hypothesis that colitis-induced decreases in colonic smooth muscle contractility are due to an alteration in the excitation-contraction-coupling process at a site distal to receptor occupancy.

Zero extracellular calcium experiments. In a separate series of experiments, muscle strips from control and colitic rats (day 3) were stimulated with 100 µM ACh in normal calcium-containing buffer solution and in buffer containing no added calcium and 1 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N’-tetraacetic acid (EGTA). Tissues were stimulated 30 s after exposure to the zero calcium buffer solution. For the purpose of data analysis, the magnitude of the contractile response in the zero calcium buffer was expressed as the percent decrease in the response obtained in the calcium-containing buffer solution (22, 27).

Nifedipine experiments. Manipulation of extracellular calcium influx into the colonic smooth muscle also was achieved by incubating the tissues for 5 min in normal buffer solution containing the calcium channel blocker nifedipine (0.1 µM) before ACh (100 µM) stimulation. The contractile response in the presence of nifedipine was expressed as the percent decrease of the control response recorded immediately before exposure to the calcium channel blocker (22, 27).

Intracellular calcium depletion experiments. Depletion of ACh-sensitive intracellular calcium stores was achieved using a previously described protocol (22, 27). Briefly, muscle strips were contracted for 20 min in a 60 mM KCl normal calcium buffer solution followed by relaxation in a 60 mM KCl zero calcium-EGTA buffer solution. The muscle strips were stimulated repeatedly with 100 µM ACh until no contractions were evident. This was followed by immediate exposure of the muscle strips to normal buffer solution containing 100 µM ACh. The agonist and buffer were added simultaneously to prevent calcium loading by the sarcoplasmic reticulum and other intracellular stores (16). The contractile response achieved after stimulation of the calcium-depleted muscle was expressed as the percent decrease from the response measured in normal tissues.

Upon completion of each experiment, the length and wet weight of each muscle strip were used to calculate tissue cross-sectional area as described previously (5). All contractions were normalized for tissue cross-sectional area, and the data were reported as stress in kilograms per square centimeter. Because of a concern that edema associated with the inflammatory process might affect the use of tissue wet weight in the normalization of the data as stress, muscle strips were selected at random and were desiccated to obtain the dry weights of the tissues. The percent decrease in tissue wet weight did not differ significantly among the animal groups: control (80.4 ± 3.7%), day 1 (78.8 ± 3.4%), day 2 (76.7 ± 3.9%), day 3 (79.4 ± 4.4%), day 7 (81.2 ± 4.3%), and day 14 (79.3 ± 3.7%). This suggests that normalization of

### Table 1. Gross mucosal inflammation scoring index

<table>
<thead>
<tr>
<th>Score</th>
<th>Grouping</th>
<th>Macroscopic Appearance</th>
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<tbody>
<tr>
<td>0</td>
<td>No inflammation</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild inflammation</td>
<td>No ulcer; mild petechia/hypervascularity</td>
</tr>
<tr>
<td>2</td>
<td>Mild inflammation</td>
<td>No ulcer; moderate petechia/hypervascularity</td>
</tr>
<tr>
<td>3</td>
<td>Moderate inflammation</td>
<td>Ulcer &lt;1 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>4</td>
<td>Moderate inflammation</td>
<td>Ulcer ≥1 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>5</td>
<td>Moderate inflammation</td>
<td>Same as above at 2 or more sites</td>
</tr>
<tr>
<td>6</td>
<td>Severe inflammation</td>
<td>Ulcer 3 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>7</td>
<td>Severe inflammation</td>
<td>Ulcer 4 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>8</td>
<td>Severe inflammation</td>
<td>Ulcer 5 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>9</td>
<td>Severe inflammation</td>
<td>Ulcer 6 cm with petechia/hypervascularity</td>
</tr>
<tr>
<td>10</td>
<td>Severe inflammation</td>
<td>Ulcer &gt;6 cm with petechia/hypervascularity</td>
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See Fig. 1, A and F, for details.
force to tissue cross-sectional areas using tissue wet weight is valid for both the control and colitic animals.

Data analysis. The data were analyzed with respect to the effects of time after acetic acid instillation and lesion severity on colonic smooth muscle contractility. Statistical analysis involved one-way analysis of variance and the Student's t-test for unpaired observations. Differences were considered statistically significant when P was <0.05.

RESULTS

General observations. Intrarectal administration of 4% acetic acid in the distal colon of rats evoked an inflammatory response in all experimental animals (n = 39). The distal colons of rats killed 24 h postenemas (n = 4) showed mild macroscopic inflammation (grade 1). Diarrhea, defined as the presence of watery stools and wet tail, was not seen in any animal 24 h postacetic acid enema. Seventy-five percent of the animals killed 2 or 3 days postenemas presented with diarrhea. All rats studied for longer periods of time developed diarrhea by 4 days posttreatment. The diarrhea was self-limiting and was not observed in any animal by 7 days postenemas. The presence of diarrhea did not have a significant effect on body weight. Rats killed 48 h (n = 13) and 72 h (n = 13) after acetic acid enemas evidenced a broad range of inflammation (grades 1-8). No diarrhea or macroscopic evidence of inflammation was found in the sham control animals (n = 13).

Figure 1 presents the histology of the distal colon in normal (A) and colitic (B-F) rats. Tissue from colitic rats 1 day postacetic acid treatment (Fig. 1B) showed diffuse reduction in goblet cells, patchy surface erosion, and loss of the upper half of crypt epithelium. Mucosal...
hemorrhage with a mild mixed inflammatory infiltrate was present in the lamina propria. Submucosal expansion was present with edema and a mild mixed inflammatory infiltrate. Minimal focal splaying of muscle fibers was present in the muscularis propria. At 2 days posttreatment (Fig. 1C), there was a patchy loss of the entire thickness of the crypt epithelium, with a moderate mixed inflammatory infiltrate in the submucosa extending into the muscularis propria. The muscularis propria also showed edema and splaying of the muscle fibers. By day 3 posttreatment (Fig. 1D), there was mucosal ulceration with granulation tissue. The muscularis propria continued to show edema, splaying of the muscle fibers, and an inflammatory infiltrate. On day 7 (Fig. 1E), there was extensive mucosal ulceration but with adjacent reepithelialization and restoration of a normal complement of goblet cells. The muscle underlying the ulceration continued to show some edema, splaying of the muscle fibers, and inflammation, but the adjacent muscle appeared unremarkable. By day 14 (Fig. 1F), there was ongoing repair of the ulceration with fibrosis of the lamina propria and submucosa and reepithelialization. Hemosiderin deposition with a focal mixed inflammatory infiltrate was present in the mucosa and submucosa. A mild focal inflammatory infiltrate was seen in the muscularis propria, which was otherwise unremarkable.

Effect of time postacetic acid treatment on contractility. Figure 2 shows the ACh dose-response data obtained from sham control and colitic rats 1, 2, 3, and 7 days postacetic acid enemas. In all groups, ACh stimulation produced a dose-dependent increase in force with the maximal response developed to 100 µM ACh. Note that the rightward shift in the dose-response curves evident at days 2 and 3 postenemas shows a return toward control data at day 7. The dose-response data for tissues from animals 14 days postenemas were identical to the control data (data not shown).

The relationship between the time postacetic acid enema and the contractile response to 100 µM ACh is presented in Fig. 3A. Colonic smooth muscle contractility was significantly decreased by 2 days posttreatment (P < 0.01), remained decreased at 3 days posttreatment (P < 0.01), and was followed by a gradual return to control values by 14 days postenemas. The tension generated by the tissues from colitic animals was 88 ± 7, 54 ± 6, 48 ± 7, 63 ± 8, and 95 ± 6% of the sham control response at 1, 2, 3, 7, and 14 days, respectively.

Figure 4 shows the KCl dose-response data obtained from sham control and colitic rats 1, 2, 3, and 7 days postacetic acid treatment. In all animal groups, the magnitude of the contractile response was dose dependent, with the maximal response developed to 60 mM KCl. Note that the rightward shift in the dose-response curve evident at days 2 and 3 shows a return toward control data at day 7. The dose-response data for tissues from animals 14 days postenemas were identical to the control data (data not shown).
Figure 3B depicts the relationship between time postacetic acid treatment and the contractile response to 60 mM KCl stimulation. As with ACh stimulation, colonic smooth muscle contractility was significantly decreased \((P < 0.01)\) at 2 days posttreatment, remained decreased at 3 days posttreatment \((P < 0.01)\), and returned to control values by 14 days postenemas. The active tension generated by tissues from inflamed colons was 89 ± 65, 64 ± 67, 71 ± 78, 85 ± 68, and 111 ± 9% of sham control at 1, 2, 3, 7, and 14 days, respectively.

NaF (10 mM), a direct stimulant of G proteins, also evoked contractions in tissues from control and colitic rats. The contractile response of tissues from day 2 \((1.25 ± 0.11 \text{ kg/cm}^2)\) and day 3 \((1.15 ± 0.10 \text{ kg/cm}^2)\) colitic rats was significantly less \((P < 0.01)\) than the contractile response of tissues from control rats \((2.27 ± 0.19 \text{ kg/cm}^2)\). The contractile response of tissues from colitic rats killed 2 wk postenemas \((2.15 ± 0.15 \text{ kg/cm}^2)\) did not differ significantly \((P > 0.05)\) from control data. The results are shown in Fig. 5.

Effect of lesion severity on contractility. Using the gross mucosal inflammation index described previously, we determined the relationship between the severity of the lesion and the resultant decrease in colonic smooth muscle contractility. None of the colons removed from sham control animals evidenced any gross mucosal injury and were assigned a mucosal inflammation index score of zero (no injury). Each one of the animals treated with acetic acid presented with some degree of colonic inflammation. The inflammation was graded as mild (score of 1–2), moderate (score of 3–5), or severe (score of 6 and above), depending on the mucosal inflammation index score. Figure 6, A and B, presents the relationship between gross mucosal injury score and the contractile response to ACh and KCl stimulation. Even in the absence of ulcers (mild inflammation), the contractile response of the inflamed colon to both ACh and KCl was significantly reduced compared with the data from control rats. Development of a mild inflammatory reaction (ulcers <2 cm) was associated with an additional significant decrease in force development. The progression of the inflammatory process from moderate to severe (ulcers >2 cm) also was characterized by a further significant inhibitory effect on the contractile response of the colonic smooth muscle. Thus both the severity of the inflammatory process and the duration of the inflammation affect contractility.

Contractile response in zero extracellular calcium. Incubation of colonic smooth muscle from the distal colon of sham control and colitic rats (day 3) in a zero calcium-EGTA buffer solution significantly decreased the contractile response to 100 µM ACh compared with the contractile response in normal calcium-containing buffer (Fig. 7A). Incubation in zero calcium buffer had a significantly greater inhibitory effect on tissues from colitic rats \((77 ± 3\%\) compared with sham control \((63 ± 4\%; P < 0.05)\). The absolute force generated in the zero calcium buffer was significantly less for tissues from...
Colitic rats (0.37 ± 0.10 kg/cm²) compared with control (1.20 ± 0.15 kg/cm²; P < 0.01). Response to nifedipine. The calcium channel blocker nifedipine (0.1 µM) also decreased ACh-induced contractions of colonic smooth muscle from sham control and colitic rats (day 3; Fig. 7). Similar to incubation in zero calcium, nifedipine had a significantly greater inhibitory effect on the contractile response of tissues from colitic rats (35 ± 4%) compared with sham control (20 ± 5%; P < 0.01). The absolute force developed in the presence of nifedipine was significantly less for tissues from colitic rats (1.04 ± 0.23 kg/cm²) compared with control (2.61 ± 0.21 kg/cm²; P < 0.01).

Contractile response after intracellular calcium depletion. Depletion of intracellular calcium stores significantly decreased the contractile response to ACh in both the control and colitic (day 3) rats (Fig. 8). In contrast to results of the protocols designed to manipulate the availability of extracellular calcium to the contractile process, depletion of intracellular calcium pools had a significantly greater inhibitory effect on tissues from control rats (33 ± 5%) compared with day 3 colitic animals (18 ± 4%; P < 0.05). After depletion of the intracellular calcium stores, the magnitude of the contraction did not differ significantly between sham control (2.18 ± 0.26 kg/cm²) and colitic rats (1.95 ± 0.27 kg/cm²; P > 0.05).

DISCUSSION

In humans (17, 23–26, 30, 31) and experimental animals (6, 13, 15, 21, 29, 33), colitis is accompanied by alterations in the in vivo and in vitro motility of smooth muscle from the inflamed area. Because different methods have been used to produce experimental colitis and because the extent of injury varies with the type and concentration of agent used, it can be difficult to compare results between studies. In the present study, we used acetic acid to produce a mild acute mucosal inflammation in the distal colon of rats. This model is commonly used to investigate the pathogenesis of inflammatory bowel disease (13, 18, 21, 25) and has been shown to have effects similar to more immunologically based models of colitis (8, 9, 32, 34). The purpose of our study was to determine how the decreased contractility of circular smooth muscle from the inflamed area is influenced by the time after injury and the severity of the inflammation. In addition, we examined how inflam-
Inflammation affects the calcium-handling properties of the smooth muscle, specifically the involvement of intracellular calcium stores.

Our finding of a decreased contractile response to ACh and KCl stimulation in tissues from colitic rats supports the findings of others (6, 13, 15, 29, 33) that colitis alters contractility at a postreceptor site. To further test this hypothesis, tissues were stimulated with NaF, a direct activator of G proteins (4, 12). Activation of membrane-bound G proteins leads to the generation of intracellular messengers that couple receptor activation to the final response of the cell. NaF-induced contractions were significantly reduced in tissues from colitic rats compared with control, further suggesting that colitis alters smooth muscle contractility by disturbing elements in the signal transduction pathway distal to receptor activation of the G proteins. Possibilities include alterations in the properties of the G proteins, changes in the structural or biochemical properties of the contractile proteins, and/or changes in the role of extracellular and intracellular calcium to the contractile event.

The results suggest that colitis affects the contractile response to ACh by decreasing the involvement of intracellular calcium. Incubation of tissues in a calcium-free EGTA buffer solution had a significantly greater inhibitory effect on the contractile response of tissues from colitic rats. The absolute force generated in the zero calcium buffer was less in tissue from colitic rats compared with control. Because it is generally accepted that a contraction that develops in the absence of extracellular calcium reflects the utilization of intracellular calcium stores (16, 22), our data can be explained by proposing that colitis decreases the contribution of intracellular calcium to the contraction. A reduced contribution of intracellular calcium in tissues from colitic rats would explain the smaller contractile response that develops in the absence of extracellular calcium and the apparent increased dependence on extracellular calcium.

The calcium channel blocker nifedipine also decreased ACh-induced contraction of colonic smooth muscle from sham control and colitic rats. Similar to the effect of incubating the tissues in a zero calcium buffer, limiting calcium influx through voltage-dependent L-type calcium channels had a greater inhibitory effect on the contractile response of tissues from colitic rats. These data can be explained by assuming that colitis decreases the contribution of the intracellular calcium stores to the contraction.

To further test the hypothesis that colitis decreases the contribution of intracellular calcium stores to the contractile event in colonic smooth muscle, we stimulated tissues from control and colitic rats with ACh after depletion of intracellular calcium stores. We have used this protocol previously to define the role of extracellular calcium in ACh-mediated gallbladder and gastric smooth muscle contraction (22, 27). Depletion of intracellular calcium stores significantly decreased the contractile response to ACh in both the control and colitic rats.

However, depletion of intracellular calcium had a significantly greater inhibitory effect on the contractile response of tissue from control rats. After depletion of intracellular calcium, the absolute magnitude of the contractile response to ACh was similar in both animal groups. The contraction that develops in the absence of intracellular calcium stores represents the contribution of extracellular calcium influx to the contractile event (27). Thus the finding of comparable contractions in tissues depleted of intracellular calcium suggests that extracellular calcium utilization is unaffected by colitis and further supports the hypothesis that colitis-associated decreases in colonic circular smooth muscle contractility reflect a decrease in the utilization of intracellular calcium stores.

The finding that inflammation alters the calcium-handling properties of the underlying smooth muscle is similar to data reported for other regions of the bowel (3, 11). Using a nematode model of inflammation, Fox-Robichaud and Collins (11) concluded that the increased contractility of longitudinal muscle from the infected rat jejunum involves both an increased dependence on extracellular calcium and a decreased dependence on intracellular stores of calcium. Biancani et al. (3) reported that the inhibitory effect of acid-induced inflammation on ACh-mediated contraction of the feline lower esophageal sphincter may involve alterations in the mechanisms responsible for the release of intracellular calcium. It seems reasonable to speculate that, although differences may exist with respect to site or response to inflammation, the change in contractility involves alterations in intracellular calcium utilization.
The decreased contractile response of tissues from inflamed colons cannot be accounted for solely on the basis of decreased utilization of intracellular calcium. In our study, colitis also impaired the contractile response to KCl stimulation. KCl initiates smooth muscle contraction via membrane depolarization and activation of calcium influx through voltage-activated channels on the plasmalemma; intracellular calcium mobilization is not believed to play an important role (27). Thus steps in the excitation-contraction-coupling in addition to intracellular calcium mobilization also must be impaired. Xie et al. (33) have suggested that alterations in actin and myosin interaction beyond the initial step of myosin light-chain phosphorylation might be responsible for the decreased contractile response of colonic smooth muscle from rabbits with experimental colitis.

In contrast to the rapid changes in electrolyte transport and mucosal permeability reported to occur with colitis (1, 32), we did not observe a significant decrease in contractility until 2 days postenemas. The maximal decrease in contractility occurred 2–3 days postinjury and was followed by a gradual return to control values by day 14. This pattern parallels the histological changes reported by us and others (10) and suggests that mucosal injury and the subsequent development of an acute inflammatory process, rather than a nonspecific, toxic effect of acetic acid on the smooth muscle, are responsible for the development of impaired smooth muscle contractility. The simultaneous return toward normal histology and control levels of active force developed postenemas lends additional support to this idea.

Our finding of a close temporal association between colitis and altered contractility differs from the findings of other investigators who used higher concentrations of acetic acid as the inflammatory agent (18, 28). MacPherson and Pfeiffer (18) used a minimum of a 10% acetic acid solution to produce experimental colitis in rats and reported gross mucosal and histopathological changes lasting up to 60 days posttreatment. Sethi and Sarna (28) infused a 75% acetic acid solution into the proximal colon of dogs and observed changes in the duration of the contractile state, the cycle length of colonic migrating motor complexes, and the incidence of giant migrating contractions that lasted for the 3-wk duration of the experiment. Altered motility was evident even though the animals were asymptomatic and the mucosa appeared normal at colonoscopy. The differences between our data and the data of other investigators may reflect the different concentrations of acetic acid used to induce injury. It is reasonable to propose that higher concentrations of acid would produce more severe and prolonged effects compared with the 4% solution used in our study. In addition, the differences between our data and that of Sethi and Sarna (28) may relate to the parameters used to assess colonic motility. It is possible that in vivo measures of motility such as the pattern or duration of contractions might show different temporal relationships than contractility changes studied in an in vitro model.

Others have reported that inflammation affects bowel motility by altering enteric neurotransmission and inflammatory mediator production at the site of inflammation (14, 20, 31). Systemic factors also may contribute to altered motility not only within the inflamed region of the bowel but also at noninflamed sites (14, 19). Although our study did not address the mechanism(s) by which distal colitis affects the contractility of the underlying circular smooth muscle, we recently reported that acetic acid-induced distal colitis in rats alters the in vitro and in vivo motility of the noninflamed proximal colon (21). This suggests that systemic factors are involved in the colon's response to inflammation. It does not, however, rule out local changes in nerve and muscle function as an explanation for altered motility within the inflamed colon.

Our data also suggest that the contractility of the underlying circular smooth muscle is affected by the severity of the inflammatory process, as defined by macroscopic determination of a gross mucosal injury score. Even in the absence of ulcers (mild inflammation), active force development was significantly reduced compared with the sham control. The subsequent development of ulcers of <2 cm (moderate inflammation) was associated with a further significant decrease in active force development. Progression to a severe inflammation was characterized by an additional decrease in contractility. Because all grades of mucosal inflammation were present at 2, 3, or 7 days postacetic acid enemas, both the severity and the temporal effects of the inflammation may contribute to the reduced force development.

It is possible that the correlation between severity of inflammation and decreased motility reflects disruption of the integrity of the muscularis. It is generally reported that acetic acid-induced colitis is limited primarily to injury of the mucosa and submucosa (10, 18). Our histological data support this finding. However, one cannot rule out that ultrastructural examination of the muscularis would reveal changes in intracellular membrane integrity. Biancani et al. (2) have shown that, despite the lack of demonstrable muscle damage by light microscopy, acid-induced esophagitis in cats is accompanied by changes in mitochondrial and sarcoplasmic reticulum membrane integrity.

Our finding of a correlation between lesion severity and decreased contractility of the underlying circular smooth muscle differs from the observation of Grossi et al. (13). Two points should be kept in mind when considering the reasons for the differences. First, different measures were used to assess the severity of the inflammation. We used a gross macroscopic evaluation of the colonic lesions to determine the extent of the inflammatory process. In contrast, Grossi et al. (13) determined myeloperoxidase activity as an index of inflammation. Second, Grossi et al. (13) examined the contractile response of smooth muscle from the longitudinal muscle layer. It is possible, as has been suggested by other investigators (7, 11), that the response to inflammation of smooth muscle from the circular and longitudinal muscle layers may be different.
In summary, our study shows that acute colonic mucosal inflammation causes a marked decrease in the contractility of the underlying circular smooth muscle. The level of impaired contractility is related to both the duration and the severity of the inflammatory process. The decreased contractile response to multiple agonists suggests that impairment in contractility lies past the level of the cell membrane receptor and most likely involves multiple steps in the excitation-contraction-coupling pathway, including decreasing the utilization of intracellular calcium stores.

This was supported in part by a grant from the Crohn’s and Colitis Foundation of America, Inc.

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Received 24 October 1996; accepted in final form 7 July 1997.

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