Differential changes in ACh-, motilin-, substance P-, and K⁺-induced contractility in rabbit colitis

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Differential changes in ACh-, motilin-, substance P-, and K⁺-induced contractility in rabbit colitis. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G61–G68, 1999.—To test the hypothesis that the changes in intestinal contractility, which accompany inflammation of the gut, are agonist specific, we compared the response of inflamed strips to substance P (SP), motilin, ACh, and K⁺ as a function of time. In parallel experiments, changes in the general mechanical properties (passive tension, optimal stretch) of the colitic tissue were evaluated. Colitis was induced by trinitrobenzenesulfonic acid, and rabbits were killed after 1, 2, 3, 5, or 8 days. Passive tension was increased starting from day 2 until day 8, and maximal active tension (T_{max}) was generated at less stretch from day 5. A 50% decrease in T_{max} was observed for ACh and K⁺ between days 2 and 3 and for motilin and SP between days 3 and 5. For all compounds, T_{max} returned to normal after 8 days. The pEC_{50} value (negative logarithm of the concentration that induces 50% of the maximal contractile activity) for ACh was increased from day 3 until day 8 and for SP at day 3, whereas for motilin it was decreased at day 1. The changes in passive tension and optimal stretch indicate generalized structural alterations of smooth muscle tissue. However, the different time profiles of the changes in active tension and contractile potency for different contractile agents suggest that inflammation specifically affects receptor-mediated mechanisms.

It is now well established that intestinal inflammation is associated with disturbed motility. However, it remains unclear whether inflammation causes nonspecific generalized damage to the neuromuscular apparatus or whether specific contractile mechanisms are affected.

Altered contractility has been demonstrated in muscle tissue resected from patients with inflammatory bowel disease but was attributed to various mechanisms. Snape et al. (17, 18) reported a significant reduction in the development of maximal tension by circular muscle from ulcerative colitis patients, which appeared to be agonist nonspecific, whereas Vermillion et al. (20) demonstrated agonist-dependent changes of the contractility of muscle from the small intestine of Crohn’s disease patients, which were also different between circular and longitudinal muscle. It may also be noted that another study found no difference between patients and controls (11).

Conflicting data were also obtained in animal models of intestinal inflammation. One study, in rabbits, failed to find changes in the muscle layer (15), but all the others noted either increased or decreased contractility. A receptor-independent decrease in tension development was reported in longitudinal strips from rats with colitis induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS), acetic acid, Trichinella spiralis larvae, or intraperitoneal injection of mitomycin c (10). The increased responsiveness of jejunal longitudinal muscle in Trichinella-infected rats (19) and the decreased contractility in jejunal circular muscle from nematode-infected rats (6) were also found to be pharmacologically nonselective. However, another study using TNBS-induced ileitis in guinea pigs found that nonreceptor-mediated contraction is not modified by inflammation, whereas receptor-mediated contractions are differentially altered in the longitudinal and circular layers (13).

The above-mentioned studies mainly compared the effects of cholinergic agents, adrenergic agents, or histamine with KCl-mediated responses at one particular timepoint, eventually in both circular and longitudinal muscle, and used the KCl response to classify observed changes as due to nonreceptor or receptor-mediated mechanisms. Such an approach overlooks the possibility that both mechanisms may play a role. We hypothesized that if receptor-mediated mechanisms would be involved the time course of inflammatory-induced changes would differ for different agonists. We therefore decided to compare the responses to different agonists as a function of time in a model of rabbit TNBS colitis. Because few studies have determined the effect of inflammation on the general mechanical properties of smooth muscle tissue, this effect was studied in parallel. We selected four stimuli: substance P (SP), motilin, ACh, and KCl. ACh is the classical excitatory neurotransmitter in the gut, whereas with KCl receptor-independent mechanisms can be monitored. SP was chosen because it may act as a mediator of neurogenic inflammation during inflammatory bowel disease, and motilin was chosen because it is an important endocrine regulator of gastrointestinal motility. Furthermore, motilin’s levels have been shown to be increased in patients with Crohn’s disease and ulcerative colitis (1). The model of rabbit colitis was selected because the pharmacological responses of rabbit colonic smooth muscle to inflammatory mediators closely resemble those of the human colon (14). The rabbit is also the best model to study the effect of motilin in vitro because...
only in this species have motilin receptors been demonstrated in the colon (7).

MATERIALS AND METHODS

Induction of Colitis

Colitis was induced according to the method described by Percy et al. (16). New Zealand White rabbits of either sex (2.5–3 kg) were anesthetized, and a Foley catheter was then inserted ~15 cm into the colon and inflated with 3 ml of air. Gentle withdrawal of the catheter caused fecal pellets to be expelled by muscle action. A dialysis bag (7.5 mm diameter, Spectrum Medical Industries, Houston, TX) filled with 135 mg/kg TNBS (Fluka, Buchs, Switzerland) in 50% ethanol was inserted into the distal colon for 1 h and then removed. Rabbits were killed by a blow to the neck at 1, 2, 3, 5, or 8 days after the induction of colitis. The distal colon was removed and rinsed with 0.9% NaCl. All procedures were approved by the local animal care and use committee.

Histological Evaluation of Colitis

Selected segments of distal colon taken at 5 cm from the rectum (part I) and 25 cm from the proximal colon (part II) were snap-frozen in 2-methylbutane at ~30°C. Cryostat sections (5 µm) were stained with hematoxylin and eosin and scored in a blinded fashion for structural and inflammatory changes. Scores ranged from 0 (normal) to 3 and were determined for 1) surface epithelial cell integrity, 2) mucosal architecture (parallel course of crypts, distance between crypts, relation of the crypt base to the muscularis mucosa), 3) thickness of the muscularis mucosa, 4) presence of edema in submucosa and muscularis propria, 5) segmentation (formation of a discontinuous layer, with islands of smooth muscle cells isolated from neighboring cells), and 6) thickening of the muscularis propria. The inflammatory infiltrate was scored according to its intensity and distribution, limited to the upper part or lower part of the mucosa, and checked for presence in the muscularis propria.

Myeloperoxidase Activity

Myeloperoxidase (MPO), a marker for tissue neutrophil content, was measured in mucosal sections taken 5 cm from the rectum using the procedure described by Bradley et al. (2).

Contraction Studies

Approximately 8 cm from the rectum, a piece of colon of 5 cm was removed. Circular strips, freed from mucosa, of 0.2 × 2.5 cm were cut and suspended along their circular axis in a tissue bath filled with HEPES buffer (pH 7.4), and the response of the strips was measured either isotonically or isometrically.

Isotonic measurements. Strips were mounted with a preload of 1 g. After a stabilization period, cumulative dose-response curves (10−8 to 10−4 M) toward ACh were established. After a washout period, the response of strips to increasing concentrations of motilin (10−9 to 10−4 M) and SP (10−10 to 10−6 M) was measured. Results were expressed relative to a supramaximal dose of ACh (10−4 M) added at the end of the dose-response curves. The contractions were measured using HP 7DCDT-1000 transducers from Hewlett-Packard (Palo Alto, CA) with a displacement transducer control unit obtained from J Hansen Scientific Instrument Division (Beers, Belgium) and recorded on multirecorder MC6601 (Watanabe Instruments, Tokyo, Japan) and on a Pentium computer with a DI-200 PGL ADC card and using the WINDAQ/200 acquisition software (DATAQ Instruments, Akron, OH). Calculations were performed with the WINDAQ/EX playback software. Values of negative logarithm of the concentration that induces 50% of the maximal contractile activity (pEC50) were derived from the concentration-response curves by linear interpolation.

Isometric measurements. After an equilibration period, length-tension relationships of the strips were established. Transducers (Harvard Apparatus, Edenbridge, UK) were raised via a micrometer system to a point such that the tissues were held rigidly but no tension was recorded by the system. The length of the strip under these conditions was designated as the initial length. Muscle strips were then stretched in 5% increments of initial length to a maximum stretch of 100% initial length. The passive tension that developed in response to stretch was measured for each increment in length. A contraction was then elicited by adding maximally active doses, as determined in a previous study (7), of ACh (10−6 M), motilin (10−6 M), SP (10−7 M), or K+ (150 mM) to the bathing medium. This contraction above the passive tension was considered the active tension. Optimal stretch (L0) was defined as that degree of stretch that gave the maximum response to a contractile agent. The signals were recorded on a BD112 recorder (Kipp and Zonen) and on a Pentium computer using the same data acquisition system as described above. The magnitude of the tension was expressed in grams and normalized for cross-sectional area of the strip using the following equation: cross-section (mm²) = tissue wet weight (mg)/[tissue length (mm) × density (mg/mm²)]. The density of smooth muscle was assumed to be 1.05 mg/mm².

Statistical Analysis

Data are presented as means ± SE. Time-dependent changes in contractility were compared using one-way ANOVA. Specific comparisons were made by calculating appropriate t-test values. Significance was accepted at the 5% level.

RESULTS

Evaluation of Colitis

Appearance of colitis, TNBS-treated rabbits lost weight after the induction of inflammation. At day 8, they had lost 375 ± 50 g, ~13% of their initial weight, whereas control rabbits showed a gain of 70 ± 20 g during the same period. The cross-sectional area of the distal colon gradually increased from 30.7 ± 3.4 mm² (control) to 65.3 ± 3.7 mm² (day 3) and persisted until day 8 (62.4 ± 7.2 mm²). The data are illustrated in Fig. 1. A similar increase in cross-sectional area was observed in the second, more proximal, part of the distal colon (data not shown).

Histology. Tissue sections, stained with hematoxylin-eosin, from the colon of control rabbits and TNBS-treated rabbits obtained 5 days after the induction of inflammation are shown in Figs. 2 and 3. The wall of the colon of the TNBS-treated rabbit is diffusely thickened with loss of mucosal haustration and folding. There is evidence of submucosal edema and distortion of the mucosal architecture. Crypts are branched and no longer follow a parallel course. The distance between the crypt base and muscularis mucosae is increased, and the intercryptal distance is highly variable. The
lamina propria cellular infiltrate is increased in intensity, mainly basal in location, and mixed in composition (with eosinophils, neutrophils, and mononuclear cells). Vasodilatation, thickening of the muscularis mucosae, and segmentation of the circular muscle layer are also clearly seen. Changes in histological features after the induction of the inflammation, as reflected in the scores of the parameters that were observed, are summarized for several time points in Tables 1 and 2. Desquamation of epithelial cells started at day 1 and lasted until day 5, leaving a general defect in the structure of the mucosa. Reepithelialization was observed from day 3 on. Complete healing was observed after 8 days. There was a marked increase in edema in the submucosa and within the muscularis propria from day 1 until day 5 after the induction of inflammation. This was accompanied by an unraveling or segmentation of the circular muscle layer. A thickening of the muscularis mucosae and the muscularis propria became apparent at day 5 postinjury and was most pronounced for the circular and longitudinal muscle layers after 8 days. The intensity of the inflammatory infiltrate increased toward a maximum 5 days after the induction of the injury and was still elevated at day 8. The composition of the infiltrate was mononuclear during the first 3 days and then consisted of a mixed population of polymorphonuclear granulocytes at days 5 and 8. The distribution of the inflammatory cells was limited to the mucosa during the first 3 days, with an increased intensity in the basal part compared with the top part. In addition, a few inflammatory cells were observed in the muscularis propria at days 5 and 8 after the induction of inflammation. This histological profile in the proximal part of the distal colon (part II) was similar to the distal part of the distal colon (part I), confirming the uniformity of the inflammation.

MPO activity. Colonic mucosal MPO activity in healthy controls was $0.233 \pm 0.096 \text{ U} \cdot \text{mg wet wt}^{-1} \cdot \text{min}^{-1}$. Intracolonic administration of TNBS resulted in a time-dependent increase in MPO activity (Fig. 4). At day 8, MPO activity was still 3.2-fold higher than in controls.

Contractile Response of Colitic Strips

Passive tension. In control rabbits, passive tension at 70% stretch amounted to $1.86 \pm 0.82 \text{ g/mm}^2$ and was not significantly ($P = 0.49$) different from the tension at day 1 ($1.89 \pm 1.39 \text{ g/mm}^2$). Passive tension at 70% stretch started to increase at day 2 to $13.09 \pm 2.39 \text{ g/mm}^2$ ($P < 0.0005$) and remained unchanged for the rest of the observation period (day 3: $11.29 \pm 6.87 \text{ g/mm}^2$, day 5: $19.54 \pm 4.32 \text{ g/mm}^2$, day 8: $14.98 \pm 8.20 \text{ g/mm}^2$).

Length-tension relationships to ACh and motilin. Inflammation markedly affected the length-tension relationship for the response to ACh $(10^{-4} \text{ M})$ and motilin $(10^{-7} \text{ M})$, and changes gradually developed during the observation period. As an example, Fig. 5 shows the results obtained with ACh in a control rabbit and in a rabbit on day 5. In both cases, the response to ACh increased to a maximum with increasing stretch (maximal active tension reached at $L_o$) and declined thereafter. However, in inflamed strips, maximum active tension was lower (more than 60%), and less stretch was...
needed to reach it. $L_0$, expressed as the percent increase of the initial muscle length, was decreased from 63% (control) to 40% (inflamed).

All data for the different time periods are summarized in Table 3. Tension developed by motilin in control strips was lower ($8.80 \pm 0.62$ g/mm²) than the response induced by ACh ($12.85 \pm 1.03$ g/mm²). For both compounds, there was a time-dependent decrease in maximum active tension. Also, the $L_0$ required to induce maximum active tension was significantly ($P < 0.05$) reduced by 30% starting from day 5 for both compounds.

To obtain more information on changes in the mechanical properties of inflamed strips, changes in the relative positions of the passive and active length-tension curves were determined. Therefore, plots were made of the tension, expressed relative to the maximum active tension (%maximum active tension), vs. length normalized to $L_0$ ($L/L_0$), and the intersection between active and passive tension on these plots was determined. This point depends on the ratio between connective and contractile tissue in the muscle. An example is shown in Fig. 6 for a control rabbit and a colitis rabbit on day 5. The active tension data were generated using ACh but did not differ significantly from those obtained using motilin. It can be seen that in inflamed strips the passive tension curve is shifted to the left relative to the active tension curve. In control strips, passive tension is low at $L_0$, whereas in the inflamed strips it is already markedly increased at this point, such that the passive tension curve intersects with the active tension curve just before $L_0$ instead of 1.2 times after $L_0$. This suggests that inflamed strips have a higher collagen content relative to actomyosin content than control strips. The data, summarized in Table 3, show that the intersection point differs significantly ($P < 0.05$) from the control value starting from day 3.

Comparison of maximum active tension induced by ACh, motilin, SP, and $K^+$. Figure 7 compares the maximum active tension generated by inflamed strips in response to ACh, SP, motilin, and KCl at different

Table 1. Scoring of inflammation-induced alterations of the mucosa and submucosa-muscularis propria in the distal and proximal parts of the distal colon

<table>
<thead>
<tr>
<th></th>
<th>Mucosa</th>
<th>Submucosa-Muscularis Propria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epithelial cell integrity</td>
<td>Crypt appearance</td>
</tr>
<tr>
<td>Part I</td>
<td>Part II</td>
<td>Part I</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.33</td>
<td>0.67</td>
</tr>
<tr>
<td>Day 3</td>
<td>2.00</td>
<td>1.67</td>
</tr>
<tr>
<td>Day 5</td>
<td>1.25</td>
<td>2.00</td>
</tr>
<tr>
<td>Day 8</td>
<td>0.00</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Normal score is 0, and inflamed score is 3. Part I, distal part of the distal colon; part II, proximal part of the distal colon. Musc. mucosae, muscularis mucosae.
days after the induction of inflammation. For all agents, inflammation resulted in a decrease of the maximum active tension, but the magnitude of the decrease and its time course were not identical. Maximum active tension induced by ACh and KCl decreased in parallel and was already decreased by 50% between day 2 and day 3. The response to motilin and SP decreased more slowly and was only decreased by 50% between day 3 and day 5. Eight days after the induction of inflammation, maximum active tension returned to its normal value with all contractile agents used.

Contractile potency of inflamed strips to ACh, motilin, and SP. The contractile potency of ACh, motilin, and SP was determined from dose-response curves under isotonic conditions. Figure 8 shows those toward ACh obtained 1, 2, 3, 5, and 8 days after TNBS treatment. The pEC50 values deduced from these curves indicate an increase from 5.87 (day 3) to 6.60 (day 6) and a decrease for motilin at 6.74 (day 8). All data are summarized in Table 4. There is a significant increase of the pEC50 for SP at day 3 (from 8.23 ± 0.18 to 8.72 ± 0.09) and a decrease for motilin at day 1 (from 8.13 ± 0.13 to 7.78 ± 0.01).

Fig. 4. Myeloperoxidase (MPO) activity measured in samples from mucosa of control rabbits (n = 8) and from rabbits at day 1 (n = 4), day 3 (n = 4), day 5 (n = 6), and day 8 (n = 3) after the induction of inflammation. MPO activity is expressed in U · mg wet wt−1 · min−1, and results are expressed as means ± SE. *P < 0.05 vs. controls.

Fig. 5. Example of the effect of stretch on active tension generated by ACh (10−4 M) in muscle from a control rabbit (●) and a rabbit 5 days after TNBS treatment (○). Tension is expressed in grams per cross-sectional area (g/mm²).

DISCUSSION

This study demonstrates that TNBS-induced colitis in the rabbit changes the contractile response of circular colonic smooth muscle tissue, but the extent of the change and its time course depends on the stimulus. Moreover, the time courses of mucosal damage and of changes in the passive tension and in the L0 to induce maximal tension differ as well. Our results demonstrate that, although TNBS-induced colitis in the rabbit changes the general mechanical properties of the smooth muscle, TNBS-induced colitis also has specific effects on receptor-mediated pathways.

The mechanical properties of smooth muscle depend on the amount of elastic components and connective tissue of the muscle relative to the contractile component.

Table 3. Comparison of length-tension relationship of colitic strips to ACh and motilin at different time points after the induction of colitis

<table>
<thead>
<tr>
<th>Time after the induction of colitis (days)</th>
<th>MPO activity (U/mg wet weight·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>0.7 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. L0, optimal stretch; *P < 0.05 vs. control; †P < 0.01; ‡P < 0.001; §P < 0.0001.

Table 2. Scoring of inflammation-induced alterations in cellular infiltrate according to intensity and distribution in the distal and proximal parts of the distal colon

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Mucosa</th>
<th>Muscularis propria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I</td>
<td>Part II</td>
<td>Part I</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.50</td>
<td>1.67</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.33</td>
<td>1.33</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.33</td>
<td>2.33</td>
</tr>
<tr>
<td>Day 5</td>
<td>2.25</td>
<td>2.75</td>
</tr>
<tr>
<td>Day 8</td>
<td>1.50</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Normal score is 0, and inflamed score is 3.
nents of the preparation. It has previously been shown that muscles with a high passive tension at lengths below $L_0$ have a greater connective tissue content than muscles with a low passive tension (9). Therefore, our data indicate that inflammation increases connective tissue content. However, the increase in edema, as reflected in the histological analysis and in the increased cross-sectional area, could also contribute to the increased passive tension. Edema, eventually accompanied by a change in connective tissue content, may reduce contact between neighboring smooth muscle cells and in this way change the contractile properties of the tissue.

A decrease in maximal tension has also been observed in a rabbit model of Formalin-immune complex colitis (4) and in patients with ulcerative colitis (18). In the rabbit model, a decrease in maximal tension was accompanied by a decreased membrane potential, which could not be ascribed to a change in the $Na^+\text{-}K^+$-ATPase and was therefore suggested to be due to altered intracellular $Ca^{2+}$ fluxes. However, another study found that the decreased force development in this model was due to an abnormal rate of actin myosin cross-bridge cycling (21). It remains to be investigated whether similar changes are involved in rabbit TNBS colitis.

![Fig. 6](image)

Fig. 6. Example of the relationship between active ($\bullet$) and passive ($\square$) tension in muscle from a control rabbit (A) and a rabbit 5 days after induction of inflammation (B). Data were generated using ACh as the stimulus for active tension. Results are expressed as a percentage of the maximum active tension, and stretch is normalized toward optimal stretch ($L_0$). Note shift of the passive tension curve relative to the active tension curve to the left in colitic strips.

![Fig. 7](image)

Fig. 7. Maximum active tension generated by inflamed colonic strips at different time points (days 1, 2, 3, 5, and 8) after the induction of inflammation in response to ACh ($10^{-4}$ M) and KCl (140 mM) (A) and motilin ($10^{-7}$ M) and substance P (SP; $10^{-7}$) (B). Results are means ± SE of at least 4 determinations.

![Table 4](image)

Table 4. $pEC_{50}$ values calculated from contraction dose-response curves of inflamed rabbit colonic strips treated with different contractile agents as a function of time

<table>
<thead>
<tr>
<th>Time after the induction of colitis (days)</th>
<th>Motilin</th>
<th>SP</th>
<th>ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>8.13±0.13</td>
<td>8.23±0.18</td>
<td>5.87±0.15</td>
</tr>
<tr>
<td>Day 1</td>
<td>7.78±0.01*</td>
<td>8.12±0.12</td>
<td>5.97±0.07</td>
</tr>
<tr>
<td>Day 2</td>
<td>8.16±0.14</td>
<td>8.31±0.29</td>
<td>6.03±0.13</td>
</tr>
<tr>
<td>Day 3</td>
<td>8.22±0.27</td>
<td>8.72±0.09*</td>
<td>6.45±0.09*</td>
</tr>
<tr>
<td>Day 5</td>
<td>8.13±0.29</td>
<td>8.25±0.17</td>
<td>6.60±0.08*</td>
</tr>
<tr>
<td>Day 8</td>
<td>8.19±0.06</td>
<td>7.61±0.31</td>
<td>6.74±0.23*</td>
</tr>
</tbody>
</table>

Values are means ± SE of at least 3 determinations. SP, substance P. *P < 0.05, †P < 0.01.
The most interesting finding of our study is that the decrease in maximum active tension development is time and agonist dependent, which leads to the conclusion that the effects of inflammation are not limited to generalized damage of the contractile apparatus or generalized disturbance of postreceptor events. Indeed, although the decrease in response to ACh parallels the decrease in response to KCl, suggesting that events at the receptor level are not important, the decrease of the response to SP and motilin develops more slowly. This decrease may reflect a decreased number of receptors. In fact, for motilin, we have shown that colitis in rabbits is accompanied by a downregulation of motilin receptors (8).

In addition, temporal changes were also observed in the pEC$_{50}$ values for the respective contractile agents studied, suggesting that inflammation also affects either the affinity of the agonist for its receptor or the conformational changes involved in the interaction between the receptor and its effector system. The fact that the time course for the changes in pEC$_{50}$ values differs for the contractile agents studied again supports the hypothesis that specific receptor-dependent mechanisms are affected. A practical consequence is that when different studies are compared care should be taken to take into account the stimulus and the time point at which effects were evaluated.

Together with the observation that plasma motilin levels are increased during inflammation (1), our study for the first time also emphasizes a role for motilin during inflammation. Also, SP is actively involved in the disturbed motility effects. Although previous studies have shown that SP receptor binding sites are upregulated in the small arterioles and venules of the intestine in patients with inflammatory bowel disease (12), our study indicates that the contractile response to SP is decreased. A downregulation of SP receptors at the smooth muscle level remains therefore to be investigated.

The present study shows that the different parameters that determine the contractile activity follow different time courses. The decrease in active tension induced by all contractile agents studied is maximal at day 5 and normalized at day 8, whereas the increase in passive tension is already maximal at day 2 and remains increased for the rest of the observation period. In contrast, changes in L$_o$ become only significant from day 5 on. The above-mentioned alterations cannot be simply correlated to histological changes. For instance, at day 8, the architecture of the mucosa is normal, although the cellular infiltrate and MPO levels are still increased. Inflammatory mediators still present at that time may mediate the changes in passive tension and L$_o$ but not the changes in active tension. From a therapeutic point of view, these observations suggest that anti-inflammatory substances should be evaluated on all parameters that determine the contractile activity induced by several agonists.

Cominelli et al. (5) have shown the ability of the interleukin-1 receptor antagonist to suppress inflammation, tissue damage, and eicosanoid production in the rabbit model of Formalin-immune complex colitis in a dose-dependent manner. In the same model, it was shown that administration of a novel inhibitor of proinflammatory cytokine synthesis, CGP-47969A, reduces inflammation and tissue damage (3). Furthermore, it has been demonstrated that in vivo production of leukotrienes B$_4$ and C$_4$ correlates with indications of inflammation in rabbit colitis (22). Together, these studies suggest that proinflammatory cytokines and eicosanoids are involved in the inflammatory process in rabbit colitis. It remains to be investigated whether they also trigger the changes in smooth muscle contractility and mechanical properties that were observed in the present study.

In conclusion, our results suggest that the changes in contractility observed in TNBS-induced colitis in the rabbit are not only due to nontspecific smooth muscle damage but also to disturbances of specific neuropeptide-mediated receptor mechanisms. Motilin and SP are actively involved in the changed motility effects induced by inflammation.

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