NK2 Receptor-Mediated Spontaneous Phasic Contractions in Normal and Ulcerative Colitis Human Sigmoid Colon

Weibiao Cao, Karen M. Harnett, and Victor E. Pricolo

Departments of Medicine (W.C., K.M.H.) and Surgery (V.E.P.), Rhode Island Hospital and Brown Medical School, Providence, Rhode Island

Received October 12, 2005; accepted March 17, 2006

ABSTRACT

Human colonic circular muscle produces spontaneous phasic contractions that are reduced in ulcerative colitis. How the spontaneous phasic contractions develop and why they decrease in ulcerative colitis are not known. We found that spontaneous phasic contractions of normal sigmoid circular muscle strips were significantly reduced by 90-min incubation with tetrodotoxin (10⁻⁵ M), which blocked neurokinin A release in basal conditions and in response to electrical stimulation. In addition, spontaneous contraction of human sigmoid colon was significantly decreased by the NK2 receptor antagonists MEN10376 (Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Lys-NH₂) and NK2ra (Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂) but not by atropine or by the NK1 antagonist FK888 ([4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2H-benzimidazol-2-one (1:1) maleate), suggesting that spontaneous phasic contractions may be mediated by Ca²⁺ release from intracellular stores, and activation of calmodulin and protein kinase C. In ulcerative colitis patients, spontaneous phasic contractions are decreased, and this decrease may be in part due to overproduction of hydrogen peroxide affecting sigmoid circular muscle.

In vitro and in vivo studies have shown that colonic circular muscle produce spontaneous phasic contractions in rat, dog, and humans (Jouet et al., 1998; Gonzalez and Sarna, 2001). The amplitude and frequency of spontaneous phasic contractions may be different in different portions of colon (Gonzalez and Sarna, 2001). In ulcerative colitis, spontaneous contractions of human colon are reduced (Koch et al., 1988). How the spontaneous phasic contractions develop and why they decrease in ulcerative colitis, however, are not known.

We have previously examined normal motor function of human sigmoid circular muscle and have shown that neurokinin A may be an important excitatory neurotransmitter because contraction induced by electrical field (i.e., neural) stimulation (EFS) is abolished by an NK2 receptor antagonist and not affected by the NK1 receptor antagonist or by atropine (Cao et al., 2000). In addition, neurokinin A-induced contraction and calcium release from intracellular stores are reduced in human sigmoid circular muscle from patients with ulcerative colitis, indicating abnormal motor function. Disturbances of normal motor function may affect the defense of the gastrointestinal tract against noxious stimuli present in the lumen. For instance, motor dysfunction in the small intestine (Vantrappen et al., 1977) and opiate-induced suppression of migrating myoelectrical activity caused bacterial overgrowth, which disappeared once the normal motor pattern was restored (Scott and Cahall, 1982). Thus, disruption of normal motor function in ulcerative colon...
tis may lead to inappropriate growth of enteric flora, possibly worsening the inflammation.

We have also shown that levels of hydrogen peroxide (H₂O₂) in sigmoid circular muscle layer are increased in ulcerative colitis and that the H₂O₂ scavenger catalase restores the decreased Ca²⁺ signal and muscle cell shortening in response to neurokinin A (Cao et al., 2000), suggesting that H₂O₂ may contribute to the motor dysfunction in ulcerative colitis. Whether H₂O₂ also plays a role in the reduction of spontaneous phasic contractions is not known.

In this study, we examined the mechanisms of development of spontaneous phasic contractions in normal sigmoid colon and possible mechanisms of reduction of spontaneous phasic contractions in ulcerative colitis.

Materials and Methods

Tissue Specimens. Full-thickness 1- to 2-cm-long strips of sigmoid colon were obtained at the time of surgery from ulcerative colitis patients undergoing proctocolectomy for chronic active disease refractory to medical treatment (n = 10), e.g., prednisone, 5-amino-salicylic acid, or Imuran. Ulcerative colitis was diagnosed preoperatively and confirmed postoperatively. Pathological examination after surgery confirmed that these patients had active and moderate to severe inflammation in the inflamed colon, including the patients that had received medical treatment. Normal control specimens were taken from grossly and histologically normal margins of surgical resections from patients undergoing left colectomy for colon cancer (n = 13). These control patients had no previous history of a colonic motility disorder or evidence of diverticular disease. A full-thickness, circumferential strip of sigmoid colon (measuring approximately 1–2 cm in length) was excised at the most distal portion of the specimen. The strip of fresh tissue was then placed in a preoxygenated (95% O₂ and 5% CO₂) physiologic Krebs’ solution (116.6 mM NaCl, 21.9 mM NaHCO₃, 1.2 mM KH₂PO₄, 5.4 mM dextrose, 1.2 mM MgCl₂, 3.4 mM KCl, and 2.5 mM CaCl₂) and transported on ice to the laboratory. The Institutional Review Board at Rhode Island Hospital approved the experimental protocols described in this paper.

Preparation of Circular Muscle Strips. After the mucosa was removed by sharp dissection under a microscope, consecutive circular muscle strips (10 mm long, 2 mm wide) of normal and ulcerative colitis sigmoid colon were cut with razor blades held in a metal block 2 mm apart. The strips were mounted in separate 1-ml muscle chambers as described previously in detail (Cao et al., 2000). All muscle strips were initially stretched to 2.5 g of passive force. Both normal and ulcerative colitis muscle strips achieved optimum force development at this length. Muscle strips were equilibrated for an additional 30 min after continuous perfusion with oxygenated Krebs’ solution for 30 min. During the perfusion period, spontaneous phasic contractions developed gradually and stabilized after a 30-min period of equilibration.

Normal circular muscle strips were randomly divided into vehicle control or treatment group. In the treatment groups, tetrodotoxin (10⁻⁸ M), NK1 receptor antagonist FSK888 (N²-[3(R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-l-prolyl]-N-methyl-N-phenylmethyl-3-[2-naphthyl]-l-alaninamide; 10⁻⁶ M), NK2 receptor antagonists MEN10376 (Tyr-n-Trp-Val-n-Trp-n-Trp-Lys-NH₂; 10⁻⁶ to 10⁻⁵ M at a 30-min interval) and NK2ra (Bz-Ala-Ala-n-Trp-n-Pro-n-Pro-Nle-NH₂; 10⁻⁶ M), atropine (10⁻⁴ M), cyclopiazonic acid (10⁻⁴ M), thapsigargin (10⁻⁶ M), calmodulin inhibitor CGS9343B (1,3-dihydro-1-[1-[(4-methyl-4H)-6H-pyrrolo[1,2-b] [1,2-d]benzoxazepin-4-yl]methyl]-4-piperidinyl]-2H-benzimidazol-2-one (1:1 maleate; 10⁻⁶ M), or protein kinase C inhibitor chelerythrine (10⁻⁷ M) was used. Tetrodotoxin (10⁻⁸ M) was used in our experiments because this concentration of tetrodotoxin was required to abolish electrical field stimulation-induced contraction. The doses for other inhibitors are the maximally effective doses reported in our previous studies (Cao et al., 1999, 2000, 2004b).

The tracing graphs of spontaneous phasic contractions were continuously recorded. At the end of experiments, the length of the strips was measured in calcium-free medium with 10 mM EDTA, and the weight of the strips was also recorded. The area under the curve for a period of 5 min was measured by using NIH image software before and after adding agents. The amplitude of spontaneous phasic contraction was the difference between the basal level and the peak value. The data were normalized to the cross-sectional area of the strip (millimeters squared), which was calculated by the ratio of weight (milligrams) to length (millimeters) as described previously (Biancani et al., 1999).

Measurement of Neurokinin A Release. Sample preparation and neurokinin A radioimmunoassay were performed as described previously (Lindstrom and Andersson, 1997; Grider, 2003) with minor modification. After equilibration, the muscle strips were rinsed three times with fresh Krebs’ solution. One milliliter of fresh Krebs’ solution containing endopeptidase inhibitor phosphoramidon (10 µM), which is to minimize degradation of neurokinin A, was added to the muscle chamber. After 15-min incubation, the Krebs’ solution was collected to measure basal level of neurokinin A. After adding fresh Krebs’ solution with phosphoramidon, the muscle strips were stimulated with pulse trains of 100 V in amplitude, 10 s in duration, with a pulse duration of 0.5 ms at a frequency of 20 Hz, using a stimulator (Model S48; Grass Instruments, Quincy, MA) through platinum wire electrodes placed longitudinally on either side of the strips. The strips were stimulated five times at a 2-min interval within 15 min, and then the solution was collected to measure neurokinin A. When inhibitors were used, the above procedure was performed after incubation with tetrodotoxin, chelerythrine, CGS9343B, thapsigargin, or vehicle for 60 to 90 min. The collected samples were acidified with an equal volume of 1% trifluoroacetic acid and centrifuged at 12,000 g for 20 min at 4°C to remove interfering proteins. The supernatants were kept at −80°C for assay.

Neurokinin A in the supernatant was first purified by using a SEP-COLUMN containing 200 µg of C18 and then measured by using a radioimmunoassay kit from Bachem (Torrance, CA) according to the manufacturer’s instruction. In brief, samples or standards were mixed with neurokinin A antiserum and incubated overnight at 4°C. On day 2, 125I-Nle-neurokinin A was added to the above mixture and incubated for an additional 16 to 24 h at 4°C. On day 3, goat anti-rabbit IgG serum and normal rabbit serum were added and incubated at room temperature for 90 min. Finally, samples were centrifuged for 20 min at 1700 g, and the supernatants were aspirated. The radioactivity in the sediment was counted using a gamma counter and normalized to protein content in the strips. The cross-reactivity of the radioimmunoassay to kassinin, a dodecapeptide of amphibian origin, and neurokinin A is 100%, to neurokinin B 80%, and to substance P is less than 0.05%, with a detection range 1 to 128 pg/tube. Neurokinin B has not yet been identified in the human gut (Holzer and Holzer-Petsche, 1997).

Protein Measurement. The amount of protein was determined by colorimetric analysis (Bio-Rad protein assay; Bio-Rad Laboratories, Richmond, CA) according to the method of Bradford (1976).

Drugs and Chemicals. Chelerythrine was purchased from Calbiochem (San Diego, CA); thapsigargin and cyclopiazonic acid were from Molecular Probes (Eugene, OR); NK2ra, SEP-COLUMN, and neurokinin A RIA kit were from Bachem; FSK888 was from RBI (Natick, MA); MEN10376 was from Biomol (Plymouth Meeting, PA); CGS9343B was a gift from Dr. Richard A. Lovell of Ciba-Geigy (Summit, NJ); and neurokinin A, trifluoroacetic acid, tetrodotoxin, atropine, catalase, and other reagents were purchased from Sigma (St. Louis, MO).

Statistical Analysis. All data are expressed as mean ± S.E.M. Statistical differences between means were determined by Student’s t test. Differences between multiple groups were tested using anal-
ysis of variance (ANOVA) and tested for significance using Fisher’s protected least significant difference test.

Results

NK2 Receptors and Spontaneous Phasic Contractions. To test whether neurotransmitters contribute to spontaneous phasic contractions in human sigmoid colon, normal circular muscle strips were incubated with $10^{-5}\text{ M}$ tetrodotoxin, which blocks axonal action potential transmission and neurotransmitter release from nerve terminals. After administration of tetrodotoxin, spontaneous phasic contractions increased at first and then decreased gradually and reached their lowest levels 1.5 h after administration. Figure 1 shows that tetrodotoxin significantly reduced the amplitude of the spontaneous phasic contractions, indicating that excitatory neurotransmitters may be involved in their maintenance. Tetrodotoxin did not affect neurokinin A-induced contraction (data not shown), demonstrating that muscle strips were not damaged by tetrodotoxin.

To determine which excitatory neurotransmitter(s) contributes to maintenance of spontaneous phasic contractions, different antagonists were used. Spontaneous phasic contractions were significantly decreased by the NK2 receptor antagonists MEN10376 (Fig. 2A) and NK2ra (McElroy et al., 1992) but not by atropine or by the NK1 receptor antagonist FK888 (Fig. 2B).

To examine whether neurokinin A is an excitatory neurotransmitter in normal sigmoid colon, we measured neurokinin A release after electrical field stimulation. In basal condition, normal sigmoid circular muscle released $13 \pm 2.5$ pg of neurokinin A/g protein/min. Electrical stimulation significantly increased the neurokinin A level to $148.8 \pm 29.3$ pg/g protein/min (Fig. 3A) and caused contraction (Cao et al., 2000). Treatment with tetrodotoxin ($10^{-5}\text{ M}$) for 90 min significantly decreased basal neurokinin A release (Fig. 3B) as well as EFS-induced neurokinin A release (Fig. 3C).

Signaling in Spontaneous Phasic Contractions. We have shown previously that neurokinin A-induced contraction is mediated by activation of NK2 receptors, calcium release from intracellular stores, and activation of calmodulin and protein kinase C. Therefore, we examined the role of calcium release of intracellular calcium stores and the role of calmodulin and protein kinase C in spontaneous phasic contractions.

Spontaneous phasic contractions of normal human sigmoid colon were almost abolished by cyclopiazonic acid (Fig. 4A) and thapsigargin (Fig. 4B), both of which are Ca$^{2+}$-ATPase inhibitors and can deplete intracellular Ca$^{2+}$ stores. Spontaneous phasic contractions were also significantly decreased by the calmodulin inhibitor CGS9343B and by the protein kinase C inhibitor chelerythrine (Fig. 5). In normal sigmoid circular muscle strips, treatment with thapsigargin, chelerythrine, or CGS9343B for 60 min had no effect on basal neurokinin A release (Fig. 6).
Reduced Spontaneous Phasic Contractions in Ulcerative Colitis. We have shown that neurokinin A and neurally induced contraction was reduced in ulcerative colitis. Likewise, spontaneous phasic contractions were also significantly decreased in patients with ulcerative colitis (n = 4 patients, 16 strips, P < 0.0001, unpaired Student’s t test; Fig. 7), compared with normal colon (n = 4 patients, 17 strips).

To determine whether the reduction in spontaneous phasic contractions in ulcerative colitis is due to a decrease in release of excitatory neurotransmitter, neurokinin A release was measured. In the basal condition, ulcerative colitis sigmoid circular muscle released 12.3 ± 7.4 pg of neurokinin A/g protein/min (n = 4). Electrical stimulation significantly increased the neurokinin A level to 150.4 ± 33.4 pg/g protein/min (n = 4). Neurokinin A release at basal condition and in sigmoid circular muscle strips, electrical field stimulation significantly increased neurokinin A release, indicating that neurokinin A is an excitatory neurotransmitter in sigmoid colon. Treatment with tetrodotoxin (TTX; 10⁻⁶ M) for 90 min significantly decreased basal neurokinin A release (B) as well as EFS-induced neurokinin A release (C), which was calculated from the difference between the neurokinin A levels at basal condition and after EFS stimulation. *, P < 0.02; **, P < 0.01; Student’s t test, n = 4. (Please note differences in scale.)

Fig. 6. In normal sigmoid circular muscle strips, treatment with thapsigargin (10⁻⁶ M), chelerythrine (10⁻⁵ M), or CGS9343B (10⁻⁵ M) for 60 min had no effect on basal neurokinin A release (n = 4), suggesting that these inhibitors may not inhibit neurokinin A release.
response to EFS had no difference between normal and ulcerative colitis colon (Fig. 8). Next, we examined the role of H$_2$O$_2$ in reduction of spontaneous phasic contractions in ulcerative colitis. In ulcerative colitis sigmoid muscle strips, the H$_2$O$_2$ scavenger catalase significantly restored the reduced spontaneous phasic contractions, compared with control (Fig. 9, A–C). The effect of 100 U/ml catalase was not statistically different from 600 U/ml, suggesting that 100 U/ml catalase may be the maximally effective dose. Conversely, in normal muscle strips, catalase slightly reduced spontaneous phasic contractions (Fig. 9, B and C), although the difference was not statistically significant.

**Discussion**

**NK2 Receptors Mediate Spontaneous Phasic Contractions.** The mechanisms of spontaneous phasic contractions in human sigmoid colon are not fully understood. We found that spontaneous phasic contractions in human sigmoid colon increased at first, perhaps because of blockade of inhibitory neurotransmitter release (Bossone et al., 2001), then significantly decreased in response to tetrodotoxin, suggesting that both inhibitory and excitatory neurotransmitters may be involved in the regulation of spontaneous phasic contractions of human sigmoid colon. Tetrodotoxin is a reversible and selective blocker of sodium channels and blocks propagation of impulses in excitable membranes (Catterall, 1980), thereby blocking the release of neurotransmitters. We also found that spontaneous phasic contractions were significantly reduced by two NK2 receptor antagonists, MEN10376 and NK2ra, but not by the NK1 receptor antagonist FK888 or by the muscarinic receptor antagonist atropine. The finding that spontaneous phasic contractions were not affected by atropine is consistent with similar findings in rat colon (Gonzalez and Sarna, 2001). Both MEN10376 and NK2ra are highly potent and selective NK2 antagonists (McElroy et al., 1992). FK888 is a potent and selective NK1 antagonist, which is active both in vitro and in vivo. FK888 has at least 10,000 times higher affinity for NK1 receptor in the guinea pig ileum compared with rat NK2 and NK3 receptors (Fujii et al., 1992). We have also confirmed that FK888 is a selective antagonist for the NK1 receptor and that NK2ra is a selective antagonist for the NK2 receptor in human sigmoid circular smooth muscle (Cao et al., 2000). Therefore, our data suggest that spontaneous phasic contractions in human sigmoid colon may be mediated at least in part by NK2 receptors.

Tachykinin receptors (both NK1 and NK2) are present in the muscle layers of the human colon (Gates et al., 1988). Neurokinin A binding sites are thought to reflect NK2 receptors, whereas substance P binding sites reflect NK1 receptors. The muscle layers of the human colon have specific binding sites for both neurokinin A and substance P as shown by autoradiography (Goode et al., 2000; Renzi et al., 2000). We have shown previously that the NK2 agonist (β-AI$_n$)-neurokinin A (4–10) is approximately 100 times more potent than the NK1 agonist GR73632 and substance P in causing contraction of human sigmoid colon and that substance P-induced contraction is significantly reduced by NK2 receptor antagonist (Cao et al., 2000), which has been recently confirmed by others (Liu et al., 2002), suggesting that NK2 receptors may play a dominant role in tachykinin-induced contraction in human sigmoid colon.

In the present study, we report that electrical field stimu-
have shown previously that neurokinin A-induced contraction in human sigmoid colon (Cao et al., 2000). NK2 receptors mediate electrical field stimulation-induced sigmoid colon. These data support our previous finding that neurokinin A is an excitatory neurotransmitter mediating spontaneous phasic contractions of normal sigmoid colon. Both cyclopiazonic acid and thapsigargin cause depletion of intracellular calcium stores by specifically inhibiting sarcoplasmic reticulum Ca2+-ATPase (Demaurex et al., 1992; Davidson and Varhol, 1995). In addition, spontaneous phasic contractions may also depend on activation of both calmodulin and protein kinase C since spontaneous phasic contractions were significantly decreased by the calmodulin inhibitor CGS93943B (Norman et al., 1987) and by the protein kinase C inhibitor chelerythrine (Herbert et al., 1990) (Fig. 5). The finding that spontaneous contractions are mediated by the same intracellular pathway that is activated by neurokinin A is consistent with neurokinin A being the mediator of spontaneous contractions. Thapsigargin, chelerythrine, and CGS93943B did not affect basal neurokinin A release (Fig. 6), indicating that the reduction in spontaneous phasic contractions induced by these inhibitors is not due to inhibition of neurokinin A release.

In normal sigmoid circular muscle, neurokinin A-induced contraction is mediated by activation of Gq-linked NK2 receptors (Cao et al., 2000). Gq is known to activate phosphoinositide-specific phospholipase C (Taylor et al., 1991). Therefore, it is possible that, in normal sigmoid circular muscle, neurokinin A activates Gq protein via the NK2 receptor and stimulates phosphoinositide-specific phospholipase C, resulting in the formation of inositol 1,4,5-trisphosphate, which causes a sufficiently high calcium release from intracellular stores to activate a calmodulin-dependent pathway, and in concurrent formation of diacylglycerol, which activates a protein kinase C-dependent pathway.

**Reduced Spontaneous Phasic Contractions in Ulcerative Colitis.** Abnormal colonic motor function occurs in patients with ulcerative colitis (Koch et al., 1988; Tomita et al., 1998; Al-Saffar and Hellstrom, 2001) and in animal models of colitis (Sethi and Sarna, 1991; Lu et al., 1997; Tsukamoto et al., 1997; Hosseini et al., 1999; Shi and Sarna, 2000; Gonzalez and Sarna, 2001). We have shown that neurokinin A and EFS-induced contraction were reduced in ulcerative colitis (Sethi and Sarna, 1991; Lu et al., 1997; Tsukamoto et al., 1997; Hosseini et al., 1999; Shi and Sarna, 2000; Gonzalez and Sarna, 2001). We have shown that neurokinin A basal release and release in response to electrical stimulation are reduced in ulcerative colitis. Similarly spontaneous phasic contractions were significantly decreased in patients with ulcerative colitis, indicating ulcerative colitis-associated motor dysfunction. The mechanisms of reduction in spontaneous phasic contractions in ulcerative colitis are not known. Since neurokinin A basal release and release in response to electrical stimulation are similar in ulcerative colitis sigmoid colon and normal colon (Fig. 8), our data suggest that reduction in spontaneous phasic contractions in ulcerative colitis is not due to inhibition of neurokinin A release but may be due to decreased muscle contractility (Cao et al., 2004a,b).

The reduction in spontaneous contractions in ulcerative colitis was significantly reversed by the H2O2 scavenger catalase (Fig. 9). We have shown previously that H2O2 levels are increased in sigmoid circular muscle layer as well as in isolated intact sigmoid muscle cells from patients with ulcerative colitis and that the increased levels of H2O2 in ulcerative colitis sigmoid muscle strips, the H2O2 scavenger catalase (100 and 600 U/ml) significantly increased neurokinin A release and that these data indicate that the increased levels of H2O2 in ulcerative colitis may contribute to the reduced spontaneous phasic contractions in ulcerative colitis. Reduced Spontaneous Phasic Contractions in Ulcerative Colitis was significantly decreased by the calmodulin inhibitor CGS93943B (Norman et al., 1987) and by the protein kinase C inhibitor chelerythrine (Herbert et al., 1990) (Fig. 5). The finding that spontaneous contractions are mediated by the same intracellular pathway that is activated by neurokinin A is consistent with neurokinin A being the mediator of spontaneous contractions. Thapsigargin, chelerythrine, and CGS93943B did not affect basal neurokinin A release (Fig. 6), indicating that the reduction in spontaneous phasic contractions induced by these inhibitors is not due to inhibition of neurokinin A release.

In normal sigmoid circular muscle, neurokinin A-induced contraction is mediated by activation of Gq-linked NK2 receptors (Cao et al., 2000). Gq is known to activate phosphoinositide-specific phospholipase C (Taylor et al., 1991). Therefore, it is possible that, in normal sigmoid circular muscle, neurokinin A activates Gq protein via the NK2 receptor and stimulates phosphoinositide-specific phospholipase C, resulting in the formation of inositol 1,4,5-trisphosphate, which causes a sufficiently high calcium release from intracellular stores to activate a calmodulin-dependent pathway, and in concurrent formation of diacylglycerol, which activates a protein kinase C-dependent pathway.

**Reduced Spontaneous Phasic Contractions in Ulcerative Colitis.** Abnormal colonic motor function occurs in patients with ulcerative colitis (Koch et al., 1988; Tomita et al., 1998; Al-Saffar and Hellstrom, 2001) and in animal models of colitis (Sethi and Sarna, 1991; Lu et al., 1997; Tsukamoto et al., 1997; Hosseini et al., 1999; Shi and Sarna, 2000; Gonzalez and Sarna, 2001). We have shown that neurokinin A and EFS-induced contraction were reduced in ulcerative colitis. Similarly spontaneous phasic contractions were significantly decreased in patients with ulcerative colitis, indicating ulcerative colitis-associated motor dysfunction. The mechanisms of reduction in spontaneous phasic contractions in ulcerative colitis are not known. Since neurokinin A basal release and release in response to electrical stimulation are similar in ulcerative colitis sigmoid colon and normal colon (Fig. 8), our data suggest that reduction in spontaneous phasic contractions in ulcerative colitis is not due to inhibition of neurokinin A release but may be due to decreased muscle contractility (Cao et al., 2004a,b).

The reduction in spontaneous contractions in ulcerative colitis was significantly reversed by the H2O2 scavenger catalase (Fig. 9). We have shown previously that H2O2 levels are increased in sigmoid circular muscle layer as well as in isolated intact sigmoid muscle cells from patients with ulcerative colitis and that the increased levels of H2O2 in ulcerative colitis sigmoid muscle strips, the H2O2 scavenger catalase (100 and 600 U/ml) significantly increased neurokinin A release and that these data indicate that the increased levels of H2O2 in ulcerative colitis may contribute to the reduced spontaneous phasic contractions in ulcerative colitis. Reduced Spontaneous Phasic Contractions in Ulcerative Colitis was significantly decreased by the calmodulin inhibitor CGS93943B (Norman et al., 1987) and by the protein kinase C inhibitor chelerythrine (Herbert et al., 1990) (Fig. 5). The finding that spontaneous contractions are mediated by the same intracellular pathway that is activated by neurokinin A is consistent with neurokinin A being the mediator of spontaneous contractions. Thapsigargin, chelerythrine, and CGS93943B did not affect basal neurokinin A release (Fig. 6), indicating that the reduction in spontaneous phasic contractions induced by these inhibitors is not due to inhibition of neurokinin A release.

In normal sigmoid circular muscle, neurokinin A-induced contraction is mediated by activation of Gq-linked NK2 receptors (Cao et al., 2000). Gq is known to activate phosphoinositide-specific phospholipase C (Taylor et al., 1991). Therefore, it is possible that, in normal sigmoid circular muscle, neurokinin A activates Gq protein via the NK2 receptor and stimulates phosphoinositide-specific phospholipase C, resulting in the formation of inositol 1,4,5-trisphosphate, which causes a sufficiently high calcium release from intracellular stores to activate a calmodulin-dependent pathway, and in concurrent formation of diacylglycerol, which activates a protein kinase C-dependent pathway.

**Reduced Spontaneous Phasic Contractions in Ulcerative Colitis.** Abnormal colonic motor function occurs in patients with ulcerative colitis (Koch et al., 1988; Tomita et al., 1998; Al-Saffar and Hellstrom, 2001) and in animal models of colitis (Sethi and Sarna, 1991; Lu et al., 1997; Tsukamoto et al., 1997; Hosseini et al., 1999; Shi and Sarna, 2000; Gonzalez and Sarna, 2001). We have shown that neurokinin A and EFS-induced contraction were reduced in ulcerative colitis. Similarly spontaneous phasic contractions were significantly decreased in patients with ulcerative colitis, indicating ulcerative colitis-associated motor dysfunction. The mechanisms of reduction in spontaneous phasic contractions in ulcerative colitis are not known. Since neurokinin A basal release and release in response to electrical stimulation are similar in ulcerative colitis sigmoid colon and normal colon (Fig. 8), our data suggest that reduction in spontaneous phasic contractions in ulcerative colitis is not due to inhibition of neurokinin A release but may be due to decreased muscle contractility (Cao et al., 2004a,b).

The reduction in spontaneous contractions in ulcerative colitis was significantly reversed by the H2O2 scavenger catalase (Fig. 9). We have shown previously that H2O2 levels are increased in sigmoid circular muscle layer as well as in isolated intact sigmoid muscle cells from patients with ulcerative colitis and that the increased levels of H2O2 in ulcerative colitis sigmoid muscle strips, the H2O2 scavenger catalase (100 and 600 U/ml) significantly increased neurokinin A release and that these data indicate that the increased levels of H2O2 in ulcerative colitis may contribute to the reduced spontaneous phasic contractions in ulcerative colitis.
Spontaneous Contractions and Ulcerative Colitis

Spontaneous contractions are significantly decreased by catalase. In addition, H$_2$O$_2$ significantly inhibits neurokinin A-induced contraction and calcium signal in normal sigmoid muscle cells (Cao et al., 2004a). Therefore, the data suggest that H$_2$O$_2$ may at least in part contribute to the reduction in spontaneous phasic contractions in ulcerative colitis. Our data cannot exclude that other mediators, e.g., interleukin 1β, might also contribute to the reduction of spontaneous contractions. H$_2$O$_2$ has been shown to consistently depress the Ca$^{2+}$-ATPase responsible for uptake of Ca$^{2+}$ into the endoplasmic reticulum (Rowe et al., 1983; Grover and Samson, 1988; Grover et al., 1992, 1995; Lee and Okabe, 1995) and cause release of Ca$^{2+}$-stores through both ryanodine- and IP$_3$-sensitive Ca$^{2+}$ release channels (Kourie, 1998), thereby depleting intracellular Ca$^{2+}$ stores and inhibiting neurokinin A-induced contraction (Cao et al., 2004a). In normal muscle strips, catalase slightly reduced spontaneous phasic contractions, suggesting that a certain level of reactive oxygen species may contribute to maintenance of spontaneous phasic contractions. It has been shown that reactive oxygen species, generated by NADPH oxidase, contribute to the maintenance of tonic contraction in gallbladder (Cong et al., 2005).

We conclude that spontaneous phasic contractions of human sigmoid circular smooth muscle may be mediated by activation of NK2 receptors, calcium release from intracellular stores, and activation of calmodulin and protein kinase C. In patients with ulcerative colitis, spontaneous phasic contractions are decreased, and this decrease may be in part due to overproduction of H$_2$O$_2$ in sigmoid muscle, resulting in depletion of intracellular calcium stores (Cao et al., 2004a) that support neurokinin A-induced contraction.

References


Biancani P, Zabinski M, Kerstein M, and Behar J (1982) Lower esophageal sphincter relaxations in response to overproduction of H$_2$O$_2$ in sigmoid circular muscle, resulting in depletion of intracellular calcium stores (Cao et al., 2004a) that support neurokinin A-induced contraction.


Address correspondence to Dr. Weibiao Cao, Department of Medicine, Brown Medical School and Rhode Island Hospital, 55 Claverick Street, Room 337, Providence, RI 02903. E-mail: weibiao_cao@brown.edu