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Interleukin 1β-Induced Production of H_2O_2 Contributes to Reduced Sigmoid Colonic Circular Smooth Muscle Contractility in Ulcerative Colitis

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ABSTRACT

We have shown that neurokinin A-induced contraction of human sigmoid circular muscle (HSCM) is reduced in patients with ulcerative colitis and that interleukin (IL)-1β may play a role in this change. We now examine changes in the signal transduction pathway mediating neurokinin A-induced contraction of HSCM and explore the role of IL-1β and of H_2O_2 in these changes. In Fura 2-AM-loaded ulcerative colitis HSCM cells, neurokinin A- and caffeine-induced peak Ca^{2+} increase and cell shortening were significantly reduced. In normal cells, neurokinin A-induced contraction was decreased by protein kinase C inhibitor chelerythrine and by calmodulin inhibitor CGS9343B [1,3-dihydro-1-[1-[(4-methyl-4*H*,6*H*-pyrrolo[1,2-*a*][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2*H*-benzimidazol-2-one (1:1) maleate]. In ulcerative colitis muscle cells, contraction was inhibited only by chelerythrine but not by CGS9343B. IL-1β treatment of normal HSCM strips and cells reproduced the changes observed in ulcerative colitis. IL-1β-induced reduction in caffeine-induced peak Ca^{2+} increase and contraction was reversed by catalase, suggesting a role of H_2O_2. IL-1β-induced H_2O_2 production was inhibited by mitogen-activated protein kinase (MAPK) kinase inhibitor PD98059 (2′-amino-3′-methoxyflavone) and by cytosolic phospholipase A2 (cPLA$_2$) inhibitor AACOCF3 (arachidonyltrifluoromethyl ketone), but neither by p38 MAPK inhibitor SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1*H*-imidazole] nor by nuclear factor-κB (NF-κB) inhibitory peptide NF-κB SN50 (H-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Val-Gln-Arg-Lys-Arg-Gln-Lys-Leu-Met-Pro-OH). IL-1β significantly increased the phosphorylation of extracellular signal-regulated kinase 1 (ERK1)/ERK2 MAPKs and cPLA$_2$ and IL-1β-induced cPLA$_2$ phosphorylation was blocked by PD98059. We conclude that Ca^{2+} stores of HSCM cells may be reduced in ulcerative colitis and that the signal transduction pathway of neurokinin A-induced contraction switches from calmodulin- and protein kinase C-dependent in normal cells to protein kinase C-dependent in ulcerative colitis cells. IL-1β reproduces these changes, possibly by production of H_2O_2 via sequential activation of MAPKs (ERK1/ERK2) and cPLA$_2$.

Ulcerative colitis is a chronic inflammatory condition that affects the large bowel, most commonly the rectosigmoid area. Abnormalities of colonic circular smooth muscle function have been demonstrated in patients with ulcerative colitis (Koch et al., 1988). Major symptoms of ulcerative colitis are diarrhea, constipation, and crampy abdominal pain, all of which may be related to smooth muscle dysfunction.

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 is abolished by an NK-2 receptor antagonist, and not affected by NK-1 receptor antagonist or by atropine (Cao et al., 2000), but the mechanism of motor dysfunction in ulcerative colitis is not fully understood.

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ABBREVIATIONS: NK, neurokinin; IL, interleukin; IL-1ra, IL-1 receptor antagonist; CGS9343B, 1,3-dihydro-1-[1-[(4-methyl-4*H*,6*H*-pyrrolo[1,2-*a*][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2*H*-benzimidazol-2-one (1:1) maleate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PD98059, 2′-amino-3′-methoxyflavone; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1*H*-imidazole; cPLA$_2$, cytosolic phospholipase A2; AACOCF3, arachidonyltrifluoromethyl ketone; NF-κB, nuclear factor-κB; SN50, H-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Val-Gln-Arg-Lys-Arg-Gln-Lys-Leu-Met-Pro-OH; ERK, extracellular signal-regulated kinase; ANOVA, analysis of variance.

Ulcerative colitis may depend on an inappropriate activation of the mucosal immune system initiated by normal luminal flora or by their products. Genetic factors determine differential susceptibility to the development of the disease and proinflammatory cytokines [tumor necrosis factor and interleukin (IL)-1 and 6] potentiate inflammatory processes that eventually cause many of the clinical manifestations (Podolsky, 2002).

IL-1α and IL-1β are major proinflammatory cytokines involved in the inflammatory cascade. Enhanced production of IL-1 has been demonstrated in the colonic mucosa from ulcerative colitis patients (Fiocchi, 1998) and mononuclear cells isolated from ulcerative colitis colonic mucosa (Mahida et al., 1989). Tissue levels of IL-1β correlate with disease activity and the ratio of the endogenous IL-1 receptor antagonist (IL-1ra) to IL-1 (IL-1ra/IL-1) shows a close correlation with inflammation (Nishiyama et al., 1994; Casini-Raggi et al., 1995). Carriage of the allele 2 of the IL-1ra gene is related to decreased production of total IL-1ra protein, and its presence is associated with increased incidence and severity of ulcerative colitis (Tountas et al., 1999). These data support a possible role of IL-1β in the pathogenesis of ulcerative colitis. This notion is further supported by the data obtained from animal models showing that administration of IL-1ra attenuates rabbit immune complex colitis (Cominelli et al., 1992) and reduction of IL-1ra levels in gene knockout mice (Hirsch et al., 1996) and by antibody neutralization in rabbits (Ferretti et al., 1994) increases the susceptibility to the induction of experimental colitis.

IL-1β is produced mainly by macrophages, located near the lamina propria beneath epithelial cells in normal mucosa and located throughout the lamina propria of inflamed mucosa in ulcerative colitis. Macrophages are normally present in the intestinal muscle layers (Mikkelsen and Rumessen, 1992), are among the earliest cells to become activated after mucosal inflammation (Collins, 1996) and may directly influence muscle cells. Smooth muscle cells, in addition to being targets of cytokines, may also be a source of inflammatory mediators. Expression of IL-1β mRNA is significantly increased in the colonic muscle of rats with acetic acid-induced colitis (Khan and al-Awadi, 1997). Activation of IL-1 receptor by IL-1β during inflammation may result in the release of IL-6 (Khan et al., 1995).

We have previously found that neurokinin A-induced contraction and calcium release from intracellular Ca^{2+} stores are reduced in human sigmoid circular muscle from patients with ulcerative colitis and that IL-1β may play a role in these changes via production of H_2O_2 (Vrees et al., 2002). In this study, we examined the changes in the signal transduction pathway of neurokinin A-induced contraction in ulcerative colitis and explored the role of IL-1β and of IL-1β-induced production of H_2O_2, which may be responsible for the abnormalities, in motor function observed in this condition.

Materials and Methods

Tissue Specimens. Full thickness 1- to 2-cm-long strips of sigmoid colon were obtained at the time of surgery from patients undergoing proctocolectomy for ulcerative colitis (n = 16). Ulcerative colitis was diagnosed preoperatively and confirmed postoperatively. Normal control specimens were taken from grossly and histologically normal margins of surgical resections from patients undergoing left colectomy for colon cancer (n = 17). These patients had no previous history of 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% O_2 and 5% CO_2) physiological Krebs' solution (116.6 mM NaCl, 21.9 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 5.4 mM dextrose, 1.2 mM $MgCl_2$, 3.4 mM KCl, and 2.5 mM $CaCl_2$) and transported on ice to the laboratory. The Institutional Review Board at Rhode Island Hospital approved the experimental protocols described in this article.

Preparation of Circular Muscle Strips. After the mucosa was removed by sharp dissection under a microscope, consecutive circular muscle strips (10 mm in length, 2 mm in width) 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 in detail previously (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 additional 30 min after continuous perfusion with oxygenated Krebs' solution for 30 min. During the perfusion period, spontaneous contractions developed gradually and stabilized after a 30-min period of equilibration. Then a cumulative dose-response to neurokinin A was obtained. For the IL-1β treatment, normal circular muscle strips were randomly divided into control or IL-1β group, and a cumulative dose-response to neurokinin A was obtained after normal strips were incubated with IL-1β (200 units/ml) or vehicles (Krebs' solution containing 1.5% albumin) for 2 h. Contractile forces in response to neurokinin A were measured in grams above the peak value of basal spontaneous contraction.

Isolation of Smooth Muscle Cells. Sigmoid circular muscle was cut into thin muscle strips (approximately 1 mm in width) and digested in HEPES-buffered solution to obtain isolated smooth muscle cells, as described previously (Cao et al., 2000). The collagenase solution contained 0.5 mg/ml collagenase Sigma type F, 1 mg/ml papain, 1 mg/ml bovine serum albumin, 1 mM $CaCl_2$, 0.25 mM EDTA, 10 mM glucose, 10 mM HEPES (sodium salt), 4 mM KCl, 125 mM NaCl, 1 mM $MgCl_2$, and 10 mM taurine. The solution was oxygenated (100% O_2) at 31°C and pH adjusted to 7.2. The tissue was put into cold enzyme solution at 4°C for 14 h, warmed up at room temperature for 30 min, and incubated in water bath at 31°C for an additional 30 min. At the end of the digestion period, the tissue was rinsed with collagenase-free HEPES buffered solution to remove any trace of collagenase. Collagenase-free HEPES buffered solution (pH 7.4) contained 112.5 mM NaCl, 3.1 mM KCl, 2.0 mM KH_2PO_4, 10.8 mM glucose, 24.0 mM HEPES (sodium salt), 1.9 mM $CaCl_2$, 0.6 mM $MgCl_2$, 0.3 mg/ml basal medium Eagle amino acid supplement, and 0.08 mg/ml soybean trypsin inhibitor. Gentle agitation was used to release single cells.

Agonist-Induced Contraction of Isolated Muscle Cells. Isolated cell contraction was induced by exposure to neurokinin A (10^{-13}–10^{-9} M) for 30 s and to thapsigargin (3 μM) for different times (from 15 s to 20 min). Thapsigargin, which can release Ca^{2+} from the intracellular Ca^{2+} stores by inhibiting Ca^{2+} ATPase, was used to test the integrity of the calcium stores. To examine the role of protein kinase C and calmodulin in neurokinin A-induced contraction, muscle cells were incubated with the protein kinase C inhibitor chelerythrine (10^{-6} M) or the calmodulin inhibitor CGS9343B [1,3-dihydro-1-[1-[(4-methyl-4*H*,6*H*-pyrrolo[1,2-*a*][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2*H*-benzimidazol-2-one (1:1) maleate] (10^{-5} M) for 5 min before adding neurokinin A. To test the role of IL-1β in normal muscle cell contraction, normal muscle cells were incubated with IL-1β 100 units/ml for 2 h at 37°C before stimulation with agonist. To test whether IL-1ra can restore contraction of sigmoid muscle cells from patients with ulcerative colitis, ulcerative colitis cells were incubated with IL-1ra (20 ng/ml) for 2 h. When the

H_2O_2 scavenger catalase was used, the cells were incubated with this enzyme (78 units/ml) for 50 min before stimulation with agonists.

After exposure to agonists, the cells were fixed in acrolein at a 1% final concentration. Cell length was measured with a phase-contrast microscope (Carl Zeiss, Jena, Germany) and a closed circuit television camera (Panasonic, Seacaucus, NJ) connected to a Macintosh Computer with NIH Image software. In Figs. 7 and 10, the average length of 30 cells, measured in the absence of agonists, was taken as the "control" length and compared with length measured after addition of test agents. Shortening was defined as percentage of decrease in average length after agonists compared with the control length.

Cytosolic Calcium Measurements. Freshly isolated cells were loaded with Fura 2-AM 1.25 μM for 40 min and placed in a 5-ml chamber mounted on the stage of an inverted microscope (Carl Zeiss). The cells were allowed to settle onto a coverslip at the bottom of the chamber. Neurokinin A (1 μM) or caffeine (20 mM) was applied directly to the cells using a pressure ejection micropipette system. Caffeine directly releases calcium from intracellular stores through activation of ryanodine-sensitive channels (Pessah et al., 1987; Flynn et al., 2001). Neurokinin A (1 μM) is a maximally effective dose in our setup because 1 μM neurokinin A and 10^{-5} M neurokinin A caused the same cell shortening and peak Ca^{2+} increase (data not shown). Because the tip of the glass pipette is very small, only a very small volume of solution was ejected from the pipette and, through the bathing solution, reached the cells. Thus, the concentration of the agonists reaching the cells was much lower than in the micropipette. For instance, to cause maximal cell shortening, 10^{-9} M neurokinin A was used for cell suspensions and 1 μM neurokinin A was used in a puffing pipette. The concentrations of neurokinin A in these two preparations were 1000 times different (Cao et al., 2004). The bathing solution is the same as the collagenase free HEPES-buffered solution. The Ca^{2+}-free medium is the HEPES-buffered solution without $CaCl_2$, but with 200 μM BAPTA, which completely blocked KCl-induced Ca^{2+} influx (Cao et al., 2004). When the Ca^{2+}-free medium was used, bathing solution was changed twice with Ca^{2+}-free medium after the cells had settled to the bottom of the chamber. Solutions in the pressure ejection micropipettes were identical to the bathing solutions except the agonists.

Ca^{2+} measurements were obtained using a modified dual excitation wavelength imaging system (IonOptix Corp., Milton, MA) as described previously (Cao et al., 2000). Ratiometric images were masked in the region outside the borders of the cell because low photon counts give unreliable ratios near the edges. We developed a method for generating an adaptive mask that follows the borders of the cell as Ca^{2+} changes and as the cell contracts. A pseudoisobestic image (i.e., an image insensitive to Ca^{2+} changes) was formed in computer memory from a weighted sum of the images generated by 340-nm excitation and 380-nm excitation. This image was then thresholded, i.e., values below a selected level were considered to be outside the cell and assigned a value of 0. For each ratiometric image, the outline of the cell was determined and the generated mask was applied to the ratiometric image. This method allows the simultaneous imaging of the changes in Ca^{2+} and in cell length. Our algorithm has been incorporated into the IonOptix software. This algorithm calculates the conversion of the ratios of fluorescence elicited by 340-nm excitation to 380-nm excitation to Ca^{2+} concentrations using techniques described in detail by Grynkiewicz et al. (1985). Peak Ca^{2+} increase was defined as the difference between the peak value and the basal value. After the experiment the cell images were copied into Microsoft Powerpoint file, which was converted into JPG file. The cell length was then measured by using NIH Image software. We found that agonists firstly caused Ca^{2+} increase and then cell shortening. The maximal cell shortening was reached about 45 s after applying agonists. Therefore, in Figs. 4C, 5, 8, and 9B, cell shortening was defined as percentage of decrease in average length 45 s after agonists compared with the initial length of the same cell before using agonists.

Measurement of H_2O_2 and Protein Content. Sigmoid circular smooth muscle strips were incubated with Krebs' solution (control) or IL-1β (200 units/ml) for 2 h in the absence or presence of mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor PD98059 (2′-amino-3′-methoxyflavone) (10^{-5} M), p38 MAPK inhibitor SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole] (10^{-5} M), cytosolic phospholipase A_2 ($cPLA_2$) inhibitor AACOCF3 (arachidonyltrifluoromethyl ketone) (10^{-5} M), or NF-κB inhibitory peptide NF-κB SN50 (H-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Val-Gln-Arg-Lys-Arg-Gln-Lys-Leu-Met-Pro-OH) (10^{-5} M) (Lin et al., 1995), which is membrane-permeable. NF-κB SN50 contains the nuclear localization sequence of the transcription factor NF-κB p50, which inhibits translocation of the NF-κB active complex into the nucleus, and the hydrophobic region (h-region) of the signal peptide of Kaposi fibroblast growth factor, which confers cell permeability. After treatment, muscles were frozen and kept in liquid nitrogen.

Sigmoid circular smooth muscles were homogenized in phosphate-buffered saline buffer. An aliquot of homogenate was taken for protein measurement. The homogenate was centrifuged at 36,000*g* for 15 min at 4°C in a J2–21 centrifuge with a fixed-angle JA-20 rotor (Beckman Coulter Inc., Palo Alto, CA) and the supernatant collected. H_2O_2 content was measured by BIOXYTECH H_2O_2-560 quantitative hydrogen peroxide assay kit (OXIS Research, Inc., Portland, OR).

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

Western Blot. Sigmoid circular muscle was homogenized in Triton X lysis buffer containing 50 mM Tris·HCl (pH 7.5), 100 mM NaCl, 50 mM NaF, 5 mM EDTA, 1% (v/v) Triton X, 40 mM β-glycerolphosphate, 40 mM *p*-nitrophenylphosphate, 200 μM sodium orthovanadate, 100 μM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 μg/ml aprotinin. The suspension was centrifuged at 15,000*g* for 5 min, and the protein concentration in the supernatant was determined. Western blot was done as described previously (Cao et al., 2003). Briefly, after these supernatants were subjected to SDS-PAGE, the separated proteins are electrophoretically transferred to a nitrocellulose membrane at 30 V overnight. The nitrocellulose membranes are blocked in 5% nonfat dry milk and then incubated with anti-phosphorylated ERK1/ERK2 MAPK antibody (1:5000) or anti-phosphorylated $cPLA_2$ antibody (1:1000) for 1 h (ERK1/ERK2 MAPK) or overnight ($cPLA_2$), followed by 60-min incubation in horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences Inc., Piscataway, NJ). Detection is achieved with an enhanced chemiluminescence agent (Amersham Biosciences Inc.).

After detecting the phosphorylated MAPKs (ERK1/ERK2) or $cPLA_2$, the membranes were incubated in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, and 62.6 mM Tris·HCl, pH 6.7) at 50°C for 30 min, washed three times (10 min each), and then reprobed by using anti-ERK2 antibody (1:500) or anti $cPLA_2$ antibody (1:1000), respectively.

Drugs and Chemicals. PD98059, SB203580, AACOCF3, and NF-κB SN50 were purchased from Calbiochem (San Diego, CA); soybean trypsin inhibitor was purchased from Worthington Biochemicals (Freehold, NJ); Fura 2-AM and BAPTA were purchased from Molecular Probes (Eugene, OR); anti-phosphorylated ERK1/ERK2 MAPK antibody was purchased from Promega (Madison, WI); anti-$cPLA_2$ and anti-phosphorylated $cPLA_2$ antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA); anti-ERK2 MAPK antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); CGS9343B was a gift from Dr. Richard A. Lovell of Ciba-Geigy (Summit, NJ); and neurokinin A, caffeine, catalase, collagenase type F, papain, BME amino acid supplement, ethylene glycol-bis(B-aminoethyl ether)-*N*,*N*,*N*,*N*,-tetraacetic acid, HEPES, and other reagents were purchased from Sigma-Aldrich.

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 analysis of variance (ANOVA) for repeated measures and tested for significance using Fisher's protected least significant difference test.

Results

Decreased Human Sigmoid Circular Smooth Muscle Contraction in Ulcerative Colitis. Consistent with previously reported data (Cao et al., 2000), the endogenous neurotransmitter neurokinin A caused dose-dependent contraction of normal human sigmoid circular muscle strips. In strips from patients with ulcerative colitis neurokinin A-induced contraction was significantly reduced compared with normal muscle strips (Fig. 1; $P < 0.0001$, ANOVA). Some of these data have been reported previously (Vrees et al., 2002) and are shown here for the readers' convenience.

Reduced Ca^{2+} Release in Ulcerative Colitis. We have previously reported that, in normal sigmoid circular muscle neurokinin A-induced contraction is mediated by release of Ca^{2+} from intracellular stores, because the contraction and the peak Ca^{2+} increase induced by neurokinin A are maintained even when the cells are kept in Ca^{2+}-free medium (Cao et al., 2000). We have also shown that thapsigargin-induced contraction was reduced in sigmoid circular muscle cells from patients with ulcerative colitis (Vrees et al., 2002) and that neurokinin A-induced peak Ca^{2+} increase and cell shortening in Ca^{2+}-free medium with 200 μM BAPTA were significantly lower in ulcerative colitis cells than in normal cells (Cao et al., 2004) (Fig. 2). These data suggest that in ulcerative colitis releasable Ca^{2+} in intracellular stores may be reduced, possibly contributing to the observed motor dysfunction. A figure similar to Fig. 2 has been reported previously (Cao et al., 2004). It is shown here for the readers' convenience.

To further confirm that Ca^{2+} release from intracellular Ca^{2+} stores is reduced in ulcerative colitis, we examined caffeine-induced contraction and peak Ca^{2+} increase. In Fura 2-AM-loaded normal sigmoid circular muscle cells the caffeine-induced peak Ca^{2+} increase was the same in normal Ca^{2+} medium and in Ca^{2+}-free medium (Fig. 3). Therefore, caffeine-induced peak Ca^{2+} increase in normal Ca^{2+} medium may reflect the levels of Ca^{2+} release from intracellular stores.

In Fura 2-AM-loaded cells from normal and ulcerative colitis colons, both peak Ca^{2+} increase and cell shortening induced by caffeine were significantly lower in ulcerative colitis than in normal cells (Fig. 4; $P < 0.001$, unpaired *t* test): in normal cells, 20 mM caffeine caused a 482.8 ± 47.3 nM peak Ca^{2+} increase and 18.8 ± 2.5% cell shortening and in ulcerative colitis cells, 20 mM caffeine caused a 255.7 ± 34.8 nM peak Ca^{2+} increase and 5.7 ± 1.4% cell shortening.

These data indicate that caffeine-induced Ca^{2+} release from Ca^{2+} stores is reduced in ulcerative colitis, indicating that inflammation may affect mechanisms responsible for Ca^{2+} uptake into or release from intracellular stores.

Protein Kinase C and Calmodulin in Neurokinin A-Induced Contraction. In normal sigmoid circular muscle cells, neurokinin A-induced contraction was blocked by the calmodulin inhibitor CGS9343B ($P < 0.0001$, unpaired *t* test) (Norman et al., 1987) and by the protein kinase C inhibitor chelerythrine (Fig. 5A; $P < 0.001$, unpaired *t* test) (Herbert et al., 1990), suggesting that neurokinin A activates both calmodulin- and protein kinase C-dependent pathways in normal cells. In ulcerative colitis cells, however, neurokinin A-induced contraction was blocked by chelerythrine ($P < 0.001$, unpaired *t* test), but not by CGS9343B (Fig. 5B), suggesting that the contractile intracellular signal transduction pathway of neurokinin A changed from calmodulin- and protein kinase C-dependent in normal cells to protein kinase C-dependent only in ulcerative colitis.

Role of Interleukin 1β in Motor Dysfunction in Ulcerative Colitis. Elevated levels of IL-1β have been demonstrated in whole colonic mucosa of patients with ulcerative colitis (Fiocchi, 1998) as well as in their sigmoid circular muscle layer (Vrees et al., 2002).

To test whether IL-1β reproduces the changes observed in ulcerative colitis, we treated normal sigmoid circular muscle

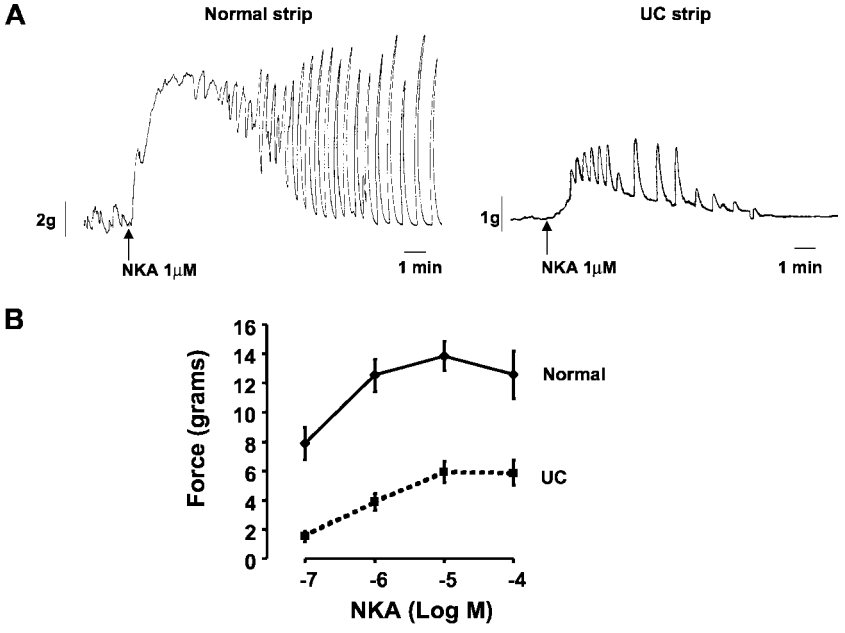

Fig. 1. Neurokinin A-induced contraction of sigmoid circular muscle strips from normal and ulcerative colitis colons. A, in a typical normal muscle strip, 1 μM neurokinin A caused 10 g of maximal contraction, whereas in an ulcerative colitis strip 1 μM neurokinin A only caused 2.4 g of maximal contraction. B, neurokinin A caused dose-dependent contraction of normal human sigmoid circular muscle strips. In strips from patients with ulcerative colitis, neurokinin A-induced contraction was significantly reduced compared with normal muscle strips ($P < 0.0001$, ANOVA). Values are mean ± S.E.M. of 5 to 17 strips of five patients. Some of these data have been reported previously (Vrees et al., 2002) and are shown here for the readers' convenience.

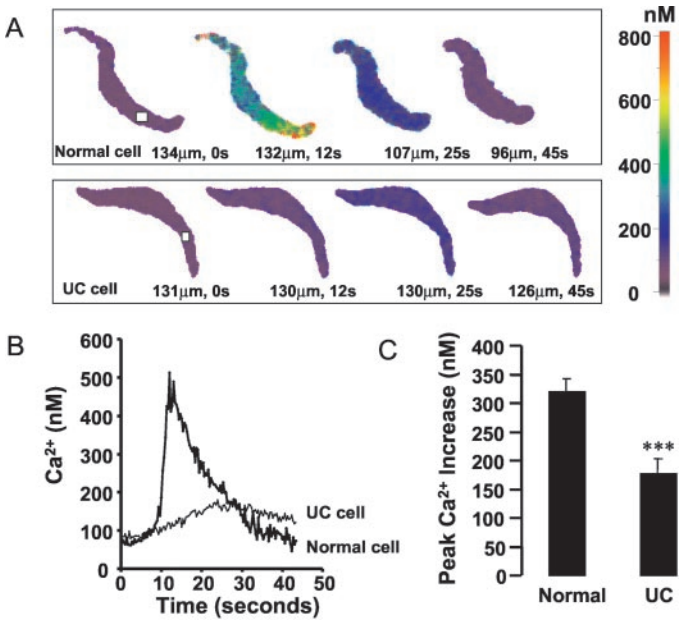

Fig. 2. Sequential shortening and Ca^{2+} levels of a typical normal and ulcerative colitis cell in Ca^{2+}-free medium after applying neurokinin A (1 μM). A, pseudocolor ratiometric images of Fura 2-AM fluorescence (see *Materials and Methods*) indicate the change in cytosolic Ca^{2+} and track the shape of the cell as it contracts. The numbers below the images indicate the cell length and the point in time (seconds) when the images were acquired. The images of intracellular Ca^{2+} were filtered with a two-point digital median filter. Maximum shortening occurred approximately 45 s after neurokinin A. Neurokinin A application caused transient increase in cytosolic Ca^{2+} concentration and subsequent contraction of normal sigmoid circular smooth muscle cell. Both shortening and Ca^{2+} increase were significantly lower in ulcerative colitis than in normal cell. B, Ca^{2+} concentration, as a function of time, in a small window shown in the image of each cell. C, summarized data showed that neurokinin A-induced peak Ca^{2+} increase was significantly lower in ulcerative colitis (n = 35 cells, 5 patients) than in normal cells (n = 14 cells, 3 patients). ***, $P < 0.0001$, unpaired t test. A figure similar to Fig. 2 has been reported previously (Cao et al., 2004). It is shown here for the readers' convenience.

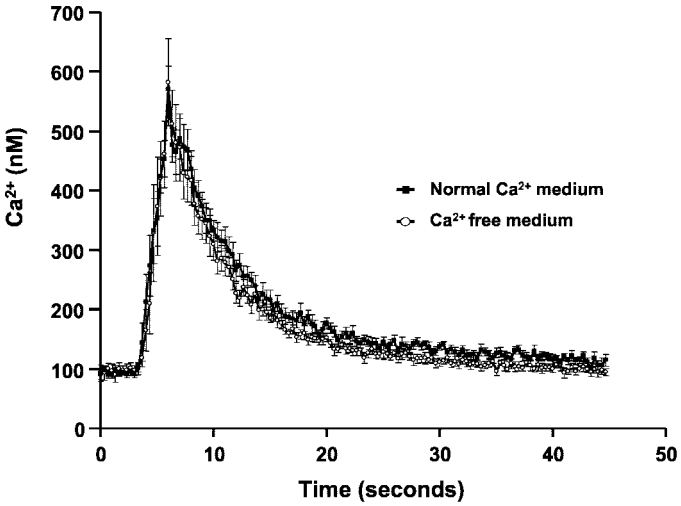

Fig. 3. Caffeine-induced Ca^{2+} change in Fura 2-AM-loaded normal sigmoid circular muscle cells. Caffeine-induced peak Ca^{2+} increase was the same in normal Ca^{2+} medium (n = 3 patients, 17 cells) and in Ca^{2+}-free medium (n = 2 patients, 9 cells). Therefore, caffeine-induced peak Ca^{2+} increase in normal Ca^{2+} medium may reflect the levels of Ca^{2+} release from intracellular stores.

strips with IL-1β (200 units/ml for 2 h). Consistent with data reported previously (Vrees et al., 2002), IL-1β treatment significantly reduced neurokinin A-induced contraction of muscle strips at all neurokinin A concentrations tested (Fig. 6). We also treated intact normal sigmoid muscle cells with IL-1β (100 units/ml, 2 h). The unstimulated cell length of normal muscle cells is 96.1 $\pm$ 11 μm (three patients, 90 cells). Similarly to ulcerative colitis, IL-1β treatment of normal cells caused a reduction in neurokinin A-induced cell shortening from 23.2 $\pm$ 1.7% in untreated cells to 14.1 $\pm$ 0.5% in IL-1β-treated cells (Fig. 7). In these cells, neurokinin A-induced contraction was blocked by chelerythrine ($P < 0.01$, ANOVA) but not by CGS9343B (Fig. 7), demonstrating that IL-1β mimics the changes observed in ulcerative colitis.

Because neurokinin A may be a physiological mediator of contraction in the human sigmoid colonic circular muscle (Cao et al., 2000), these results suggest that IL-1β may induce motor dysfunction in the human sigmoid colon by reducing contraction in response to neurokinin A.

To examine whether damage to the mechanisms of Ca^{2+} release from intracellular Ca^{2+} stores may play a role in IL-1β-induced motor dysfunction, we examined caffeine-induced contraction and peak Ca^{2+} release in normal cells exposed to IL-1β (100 units/ml, 2 h). The caffeine-induced peak Ca^{2+} increase was reduced from 482.8 $\pm$ 47.3 nM Ca^{2} in untreated cells to 281 $\pm$ 46.6 nM Ca^{2+} in IL-1β-treated cells (Fig. 8; $P < 0.01$, unpaired t test). Similarly, caffeine-induced shortening decreased from 18.8 $\pm$ 2.5% in untreated cells to 8.8 $\pm$ 2% in IL-1β-treated cells (Fig. 8; $P < 0.01$, unpaired t test). The reduction in peak Ca^{2+} increase and in contraction were reversed by the H_2O_2 scavenger catalase (Fig. 8). In Fura 2-AM-loaded normal cells directly applying IL-1β (1000 U/ml) or catalase (780 U/ml) onto cells did not cause any change in cytosolic calcium within the time frame (60 s) of the experiment (data not shown).

These data suggest that the reduction in contraction induced by IL-1β may be related either to reduction of releasable Ca^{2+} in intracellular Ca^{2+} stores or to the reduction of the size of the stores, or both, and that the effect of IL-1β may be mediated by IL-1β-induced production of H_2O_2.

To confirm that IL-1β plays a role in motor dysfunction in ulcerative colitis we examined caffeine-induced contraction and peak Ca^{2+} increase of ulcerative colitis muscle cells in the presence of an IL-1ra. Two-hour incubation of ulcerative colitis sigmoid circular muscle cells with IL-1ra (20 ng/ml), however, did not increase caffeine-induced cell shortening and peak Ca^{2+} increase (Fig. 9). The lack of effect of the IL-1ra may be due to IL-1β-induced production of other inflammatory mediators, such as H_2O_2, which are still present in the muscle layer after IL-1β's effect is blocked and which play a role in motor dysfunction.

Role of H_2O_2 in IL-1β-Induced Motor Dysfunction. To confirm a possible role of H_2O_2 in IL-1β-induced damage to Ca^{2+} storage/release mechanisms, we tested the H_2O_2 scavenger catalase in thapsigargin-induced contraction.

In normal muscle cells, IL-1β caused a reduction in thapsigargin-induced contraction (Fig. 10), similarly to the reduction in caffeine-induced contraction (Fig. 8B). Contraction returned almost to normal in muscle cells pretreated with catalase (78 units/ml, 50 min), confirming that H_2O_2 is involved in IL-1β-induced depletion of intracellular Ca^{2+} stores (Fig. 10).

To further confirm a role of H_2O_2 in IL-1β-induced motor

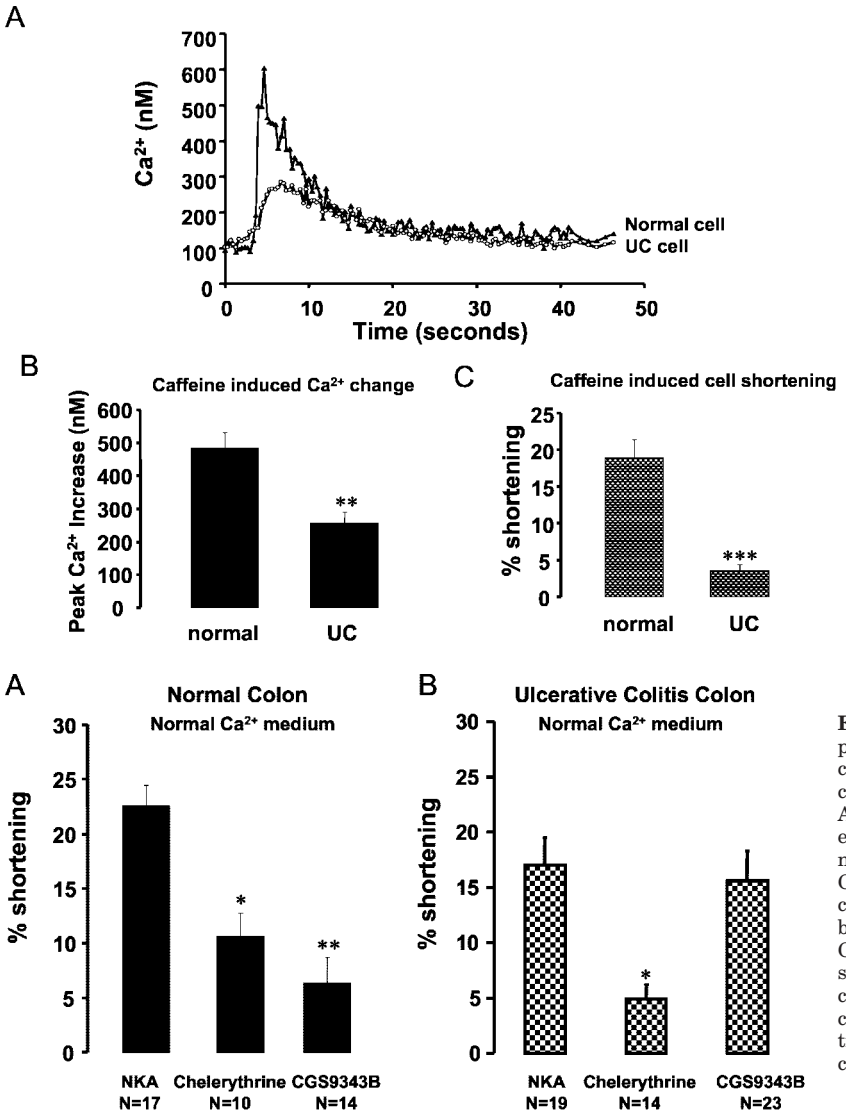

Fig. 4. Caffeine-induced contraction and peak Ca^{2+} change in Fura 2-AM-loaded sigmoid circular muscle cells from normal and ulcerative colitis colons. A, average Ca^{2+} concentration, as a function of time, in a typical sigmoid circular smooth muscle cell from a normal and an ulcerative colitis colon. Both peak Ca^{2+} increase (B) and cell shortening (C) were significantly lower in ulcerative colitis (n = 22 cells, 3 patients) than in normal cells (n = 16 cells, 3 patients). **, $P < 0.001$; ***, $P < 0.0001$, unpaired t test.

Fig. 5. Neurokinin A-induced contraction is mediated by protein kinase C and calmodulin in normal sigmoid muscle cells and by protein kinase C alone in ulcerative colitis cells. A, in normal sigmoid circular muscle cells, neurokinin A-induced contraction was blocked by CGS9343B and chelerythrine ($P < 0.001$, unpaired t test), suggesting that neurokinin A activates both calmodulin and protein kinase C-dependent pathways in normal cells. B, in ulcerative colitis cells, neurokinin A-induced contraction was blocked by chelerythrine ($P < 0.001$, unpaired t test) but not by CGS9343B, suggesting that the contractile intracellular signal transduction pathway of neurokinin A changed from calmodulin- and protein kinase C-dependent in normal cells to protein kinase C-dependent only in ulcerative colitis. Values are mean ± S.E.M. of three patients. The total cell number for each group is shown in the figure.

dysfunction, we measured production of H_2O_2 in normal sigmoid circular muscles in response to IL-1β treatment (200 units/ml, 2 h) (Fig. 11). IL-1β significantly increased the production of hydrogen peroxide from 0.12 ± 0.04 nmol/mg protein in untreated tissue to 0.29 ± 0.04 nmol/mg protein in IL-1β-treated muscle (n = 3, $p < 0.0001$, ANOVA). IL-1β-induced production of H_2O_2 was significantly blocked by the MEK inhibitor PD98059 (10^{-5} M, $P < 0.01$, ANOVA) (Alessi et al., 1995) and by the $cPLA_2$ inhibitor AACOCF3 (10^{-5} M, $P < 0.01$, ANOVA) (Street et al., 1993), but neither by the p38 MAP kinase inhibitor SB203580 (10^{-5} M) (Cuenda et al., 1995) nor by the cell-permeable NF-κB inhibitory peptide NF-κB SN50 (10^{-5} M) (Lin et al., 1995) (Fig. 11). Because 2-h treatment with IL-1β produced a significant inhibition of muscle contractility and significant stimulation of H_2O_2 production, we examined the phosphorylation of ERK1/ERK2 MAPKs and cPLA2 at 2 h after IL-1β treatment. We found that IL-1β significantly increased the phosphorylation of ERK1/ERK2 MAPKs (Fig. 12) and of cPLA2 (Fig. 13), suggesting that IL-1β may activate MAPK and $cPLA_2$. IL-1β-induced ERK1/ERK2 MAPK phosphorylation was blocked by PD98059, but not by AACOCF3 (Fig. 12), suggesting that MAPK phosphorylation may not depend on activation of

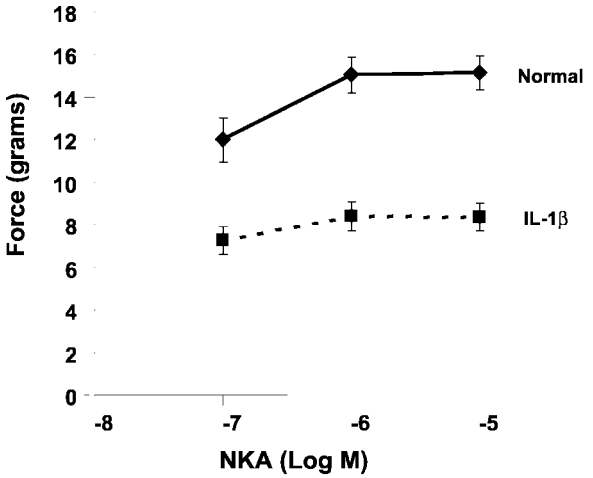

Fig. 6. Neurokinin A-induced contraction is reduced when normal sigmoid circular muscle strips are incubated in IL-1β. IL-1β treatment (200 units/ml for 2 h) significantly reduced neurokinin A-induced contraction of muscle strips at all neurokinin A concentrations tested ($P < 0.01$, ANOVA), suggesting that IL-1β reproduces the changes in motor function observed in ulcerative colitis. Values are mean ± S.E.M. of three patients with two strips measured for each patient.

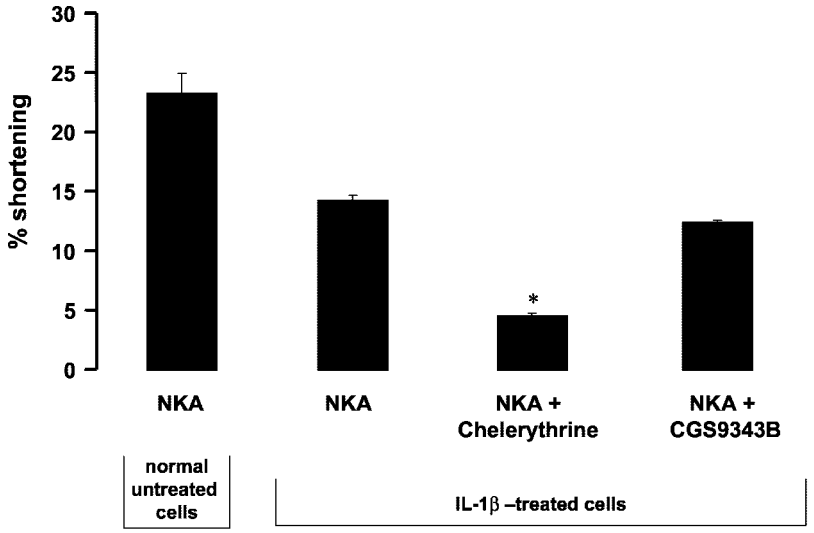

Fig. 7. Neurokinin A-induced contraction of IL-1β-treated cells is mediated by protein kinase C. Sigmoid muscle cells treated with IL-1β (100 units/ml for 2 h) were contracted with neurokinin A (10^{-8} M) alone or after 10 min incubation in the protein kinase C inhibitor chelerythrine (10^{-6} M) or the calmodulin inhibitor CGS 9343B (10^{-5} M). In these cells, neurokinin A-induced contraction was blocked by chelerythrine ($P < 0.01$, ANOVA) but not by CGS9343B, demonstrating that IL-1β mimics the changes observed in ulcerative colitis. Values are mean ± S.E.M. of nine patients with 30 cells measured for each patient.

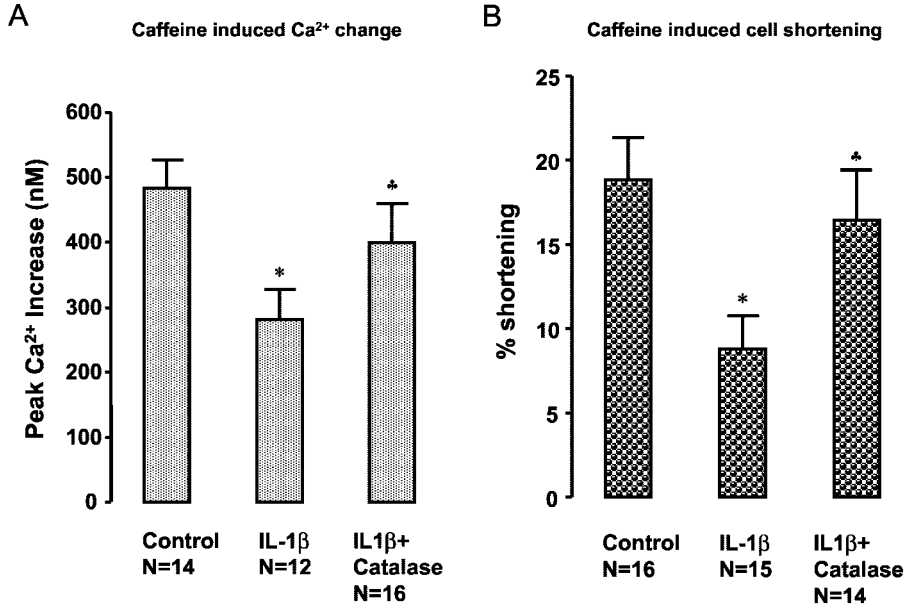

Fig. 8. Caffeine-induced contraction and peak Ca^{2+} increase in Fura 2-AM-loaded sigmoid circular muscle from normal cells (control) and cells treated with IL-1β (100 units/ml for 2 h) in normal Ca^{2+} medium. Both peak Ca^{2+} increase (A) and cell shortening (B) were significantly lower in IL-1β-treated cells than in control cells. The reduction in peak Ca^{2+} increase and contraction was reversed by the H_2O_2 scavenger catalase (78 units/ml, 50 min), suggesting that the effect of IL-1β may be mediated by IL-1β-induced production of H_2O_2. *, $P < 0.01$, unpaired t test, compared with control group; ♣, $P < 0.05$, unpaired t test, compared with IL-1β group. Values are mean ± S.E.M. of 12 to 16 cells of three patients.

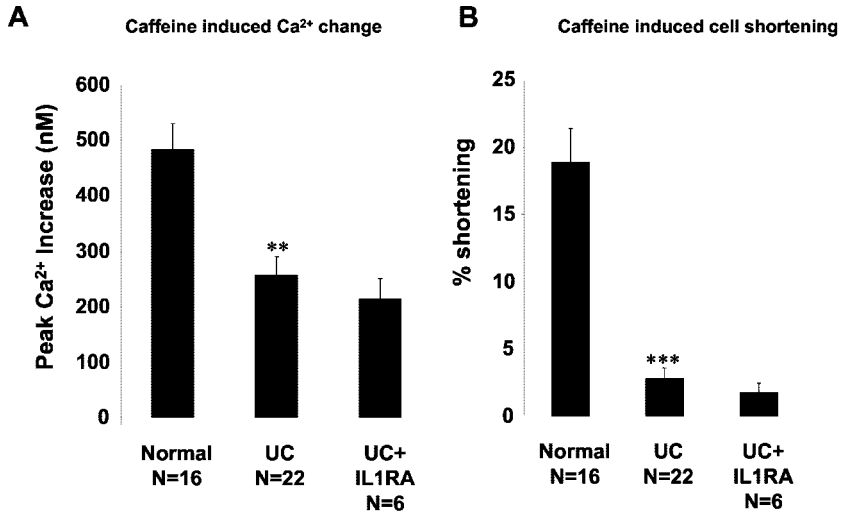

Fig. 9. Caffeine-induced contraction and peak Ca^{2+} increase in Fura 2-AM-loaded sigmoid circular muscle from normal cells (control) and ulcerative colitis cells with or without treatment with IL-1 receptor antagonist (IL-1ra, 20 ng/ml for 2 h). Both peak Ca^{2+} increase (A) and cell shortening (B) were significantly lower in ulcerative colitis cells than in normal cells. Two-hour incubation of ulcerative colitis muscle cells with IL-1ra (20 ng/ml) did not affect caffeine-induced cell shortening and peak Ca^{2+} increase. The lack of effect of the IL-1ra may be due to IL-1β-induced production of other inflammatory mediators, such as H_2O_2, which are still present in the muscle layer after IL-1β's effect was blocked and which play a role in motor dysfunction. **, $P < 0.001$; ***, $P < 0.0001$, unpaired t test, compared with control group. Values are mean ± S.E.M. of 6 to 22 cells of three patients.

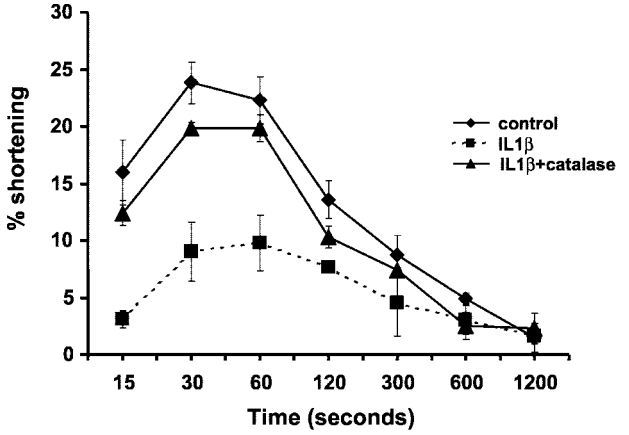

Fig. 10. Normal sigmoid circular smooth muscle cell contraction was induced by exposure to thapsigargin (3 μM) for the indicated times (from 15 s to 20 min). Thapsigargin releases Ca^{2+} from the intracellular Ca^{2+} stores by inhibiting Ca^{2+} ATPase. Two-hour incubation of normal sigmoid muscle cells with IL-1β (100 units/ml) significantly reduced thapsigargin-induced contraction ($P < 0.001$, ANOVA, $n = 3$ patients). Contraction returned to almost normal in muscle cells pretreated with catalase (78 units/ml, 50 min) ($P < 0.001$, ANOVA, $n = 3$ patients), confirming that H_2O_2 is involved in IL-1β-induced depletion of intracellular Ca^{2+} stores.

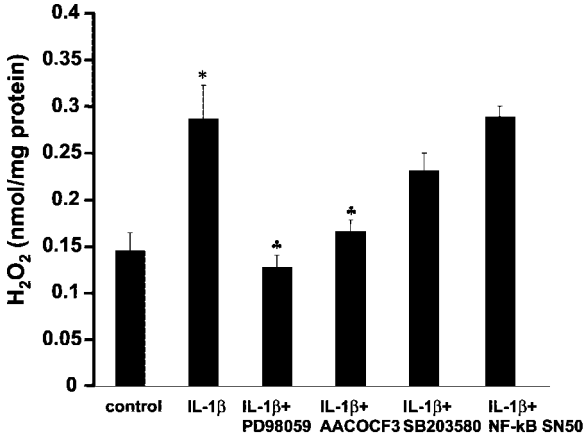

Fig. 11. IL-1β stimulates H_2O_2 production through a MAP kinase (ERK1/ERK2)- and cPLA2-dependent signaling pathway in normal sigmoid circular muscle. Treatment with IL-1β (200 units/ml, 2 h) significantly increased the production of H_2O_2. The increased H_2O_2 production was significantly reduced by MAPK kinase inhibitor PD98059 and cPLA2 inhibitor AACOCF3 but neither by p38 MAPK inhibitor SB203580 nor NF-κB inhibitory peptide NF-κB SN50. These data confirm IL-1β-induced production of H_2O_2 and demonstrate that H_2O_2 production depends on activation of MAP kinase (ERK1/ERK2) and $cPLA_2$. Values are mean ± S.E.M. of three patients. *, $P < 0.0001$, ANOVA, compared with control group. ♣, $P < 0.01$, ANOVA, compared with IL-1β group.

$cPLA_2$. The same concentration of PD98059 that effectively blocked ERK1/ERK2 MAPK activation blocked IL-1β-induced $cPLA_2$ phosphorylation, suggesting that IL-1β-induced cPLA2 phosphorylation may depend on activation of ERK1/ERK2 MAPKs.

These results confirm that IL-1β causes production of H_2O_2 and demonstrate that H_2O_2 production depends on sequential activation of MAP kinases (ERK1/ERK2) and $cPLA_2$.

Discussion

Motor Dysfunction in Ulcerative Colitis. Contraction of human sigmoid circular muscle strips in response to neurokinin A (Fig. 1) was significantly reduced in patients with

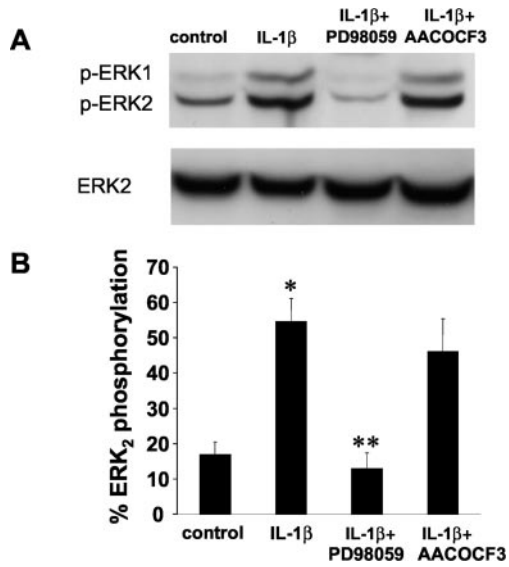

Fig. 12. IL-1β significantly increased the phosphorylation of ERK1/ERK2 MAPKs. This increased phosphorylation was not blocked by $cPLA_2$ inhibitor AACOCF3, suggesting that the phosphorylation of MAPKs may not depend on activation of $cPLA_2$. PD98059 abolished IL-1β-induced phosphorylation of MAPKs, confirming that PD98059 effectively inhibited the activation of MAPKs. A, typical Western blot of phosphorylated ERK1/ERK2 in sigmoid circular smooth muscle after IL-1β (200 units/ml, 2 h) treatment. The supernatant of tissue homogenate was probed with anti-active [phosphorylated (p)] MAPK antibody (top) or anti-ERK2 antibody (bottom). Bottom shows total ERK2 and demonstrates equal protein loading between lanes. B, average ERK2 kinase activation (i.e., phosphorylation) in response to IL-1β (200 units/ml, 2 h) treatment in the absence or presence of the MEK inhibitor PD98059 (10^{-5} M) or cPLA2 inhibitor AACOCF3. Values are means ± S.E. of three patients. *, $P < 0.02$, ANOVA, compared with control; **, $P < 0.01$, ANOVA, compared with IL-1β group.

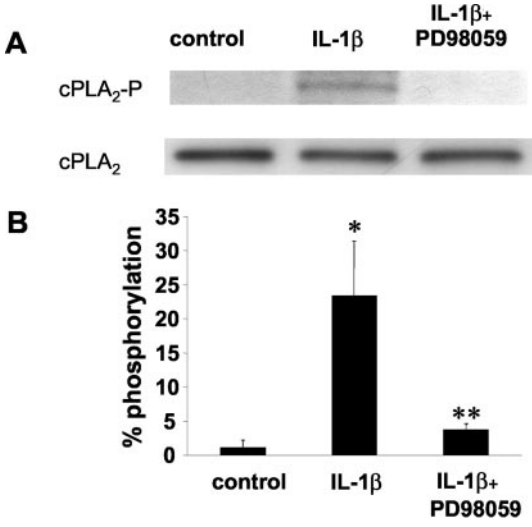

Fig. 13. IL-1β significantly increased the phosphorylation of $cPLA_2$. This increased phosphorylation was blocked by MEK inhibitor PD98059, suggesting that the phosphorylation of cPLA2 may depend on activation of ERK1/ERK2 MAPKs. A, typical Western blot of phosphorylated cPLA2 in sigmoid circular smooth muscle after IL-1β (200 units/ml, 2 h) treatment. The supernatant of tissue homogenate was probed with anti-phosphorylated (p) cPLA2 antibody (top) or anti-cPLA2 antibody (bottom). Bottom shows total cPLA2 and demonstrates equal protein loading between lanes. B, average cPLA2 phosphorylation in response to IL-1β (200 units/ml, 2 h) treatment in the absence or presence of the MEK inhibitor PD98059 (10^{-5} M). Values are means ± S.E. of three patients. *, $P < 0.05$, ANOVA, compared with control; **, $P < 0.05$, ANOVA, compared with IL-1β group.

ulcerative colitis, compared with normal controls. The data are consistent with our previous findings (Vrees et al., 2002) and with other reports (Koch et al., 1988; Shi and Sarna, 2000; Vrees et al., 2002).

Our data suggest that this reduced contractility of sigmoid muscle may result from impairment of Ca^{2+} release from the calcium storage sites. We have previously shown that in sigmoid circular smooth muscle cells from patients with ulcerative colitis thapsigargin-induced contraction (Vrees et al., 2002) and neurokinin A-induced peak calcium increase in calcium-free medium (Fig. 2) (Cao et al., 2004) were significantly reduced. To confirm that Ca^{2+} release mechanisms are impaired in ulcerative colitis, we contracted cells with caffeine, an agonist that directly releases calcium from intracellular stores through activation of ryanodine-sensitive channels (Pessah et al., 1987; Flynn et al., 2001). We found that in normal cells caffeine-induced peak Ca^{2+} increase was the same in normal Ca^{2+} medium and in Ca^{2+}-free medium. Therefore, caffeine-induced peak Ca^{2+} increase in normal Ca^{2+} medium may reflect the levels of Ca^{2+} release from intracellular stores. However, our data cannot completely rule out the possible involvement of capacitative Ca^{2+} entry invoked by caffeine-induced depletion of Ca^{2+} stores after the peak because cytosolic Ca^{2+} concentrations after the peak were slightly lower in Ca^{2+}-free medium than in normal Ca^{2+} medium (Fig. 3). It has been reported that in rat cardiomyocytes capacitative Ca^{2+} entry occurs after the peak of angiotension II-induced Ca^{2+} increase (Hunton et al., 2004). For this reason, we only reported the peak Ca^{2+} increase, but not the Ca^{2+} change over 45 s after puffing caffeine.

Although caffeine caused higher peak Ca^{2+} increase than neurokinin A, caffeine induced less cell shortening than neurokinin A. This difference may be due to differences between the contractile signal transduction pathways activated by caffeine and those activated by neurokinin A. For instance, neurokinin A activates G protein-coupled receptors that may magnify the signal, whereas caffeine causes release of calcium by directly activating ryanodine receptors, without activating G proteins.

In ulcerative colitis cells caffeine-induced peak Ca^{2+} increase was significantly reduced, suggesting that caffeine-induced Ca^{2+} release from Ca^{2+} stores may be impaired in ulcerative colitis. Our data differ from findings in circular muscle cells from the right colon in a canine model of colitis (Shi and Sarna, 2000) where intracellular Ca^{2+} release was not affected. This difference may be due to different species and/or differences in the parts of the colon that were used in these experiments. Anatomy (Cohn and Birnbaum, 1999), physiological functions (Raybould and Pandol, 1999), and luminal environment are different between right and sigmoid colon. Therefore, right and sigmoid colon may be differently affected by inflammation.

Because neurokinin A plays an important role in mediating contraction of sigmoid circular smooth muscle, we examined whether ulcerative colitis alters the signal transduction pathway mediating signaling in neurokinin A-induced contraction. We found that neurokinin A-induced contraction in normal cells depends both on calmodulin and on protein kinase C-mediated mechanisms because it was blocked by both chelerythrine, and by CGS9343B. In contrast neurokinin A-induced contraction in ulcerative colitis cells was blocked only by chelerythrine, suggesting that ulcerative colitis causes a change in the signal transduction pathway of neurokinin A-induced contraction from calmodulin- and protein kinase C-dependent in normal cells to only protein kinase C-dependent in ulcerative colitis cells. These data are consistent with previous findings in a cat model of experimental esophagitis (Sohn et al., 1997).

In sigmoid circular muscle, neurokinin A-induced contraction is mediated by activation of Gq-linked NK-2 receptors and release of calcium from intracellular stores (Cao et al., 2000). Gq is known to activate phosphoinositide-specific phospholipase C (Taylor et al., 1991). Therefore, it is possible that in normal cells neurokinin A activates Gq protein via the NK-2 receptor and stimulates phosphoinositide-specific phospholipase C resulting in formation of inositol 1,4,5-triphosphate, which causes a sufficiently high calcium release from intracellular stores to activate a calmodulin-dependent pathway, and in concurrent formation of diacylgycerol, which activates a protein kinase C-dependent pathway. In ulcerative colitis, however, the calmodulin-dependent pathway may be inhibited by inflammatory mediators and/or neurokinin A may not release enough calcium from the sarcoplasmic reticulum Ca^{2+} stores to activate this pathway.

IL-1β Mimics the Changes in Ulcerative Colitis. We have previously reported increased IL-1β content of human sigmoid circular muscle in patients with ulcerative colitis and that IL-1β treatment of normal sigmoid muscle cells reduced neurokinin A-induced contraction (Vrees et al., 2002). In the present study, we demonstrate that IL-1β treatment of normal sigmoid circular muscle mimics the changes in motor function observed in ulcerative colitis because 1) neurokinin A-induced contraction of normal sigmoid circular muscle strips was reduced after IL-1β treatment (Fig. 6); 2) IL-1β treatment significantly reduced caffeine-induced peak Ca^{2+} increase by 42% (Fig. 8A), a change similar to the reduction in peak Ca^{2+} increase observed in ulcerative colitis cells (47% reduction; Fig. 4B); and 3) IL-1β treatment caused a switch in the signal transduction pathway of neurokinin A-induced contraction from calmodulin- and protein kinase C-dependent pathway to a protein kinase C-dependent pathway (Fig. 7). These data suggest that elevated levels of IL-1β may play a role in the motor dysfunction observed in ulcerative colitis.

To confirm that IL-1β plays a role in motor dysfunction in ulcerative colitis, we examined caffeine-induced contraction and peak Ca^{2+} increase of ulcerative colitis muscle cells in the presence of IL-1ra. Two-hour incubation of ulcerative colitis muscle cells with IL-1ra did not recover the reduction in caffeine-induced cell shortening and peak Ca^{2+} increase (Fig. 9). The lack of effect of the IL-1ra may be due to IL-1β-induced production of other inflammatory mediators, such as H_2O_2, which may still be present in the muscle layer after IL-1β is blocked and which may play a role in motor dysfunction. A role of H_2O_2 in ulcerative colitis is supported by the finding that, in ulcerative colitis cells, removal of H_2O_2 by catalase restores motor function and agonist-induced Ca^{2+} release from stores (Cao et al., 2004).

Role of H_2O_2 in IL-1β-Induced Motor Dysfunction. Because the isolated sigmoid circular smooth muscle cells from patients with ulcerative colitis contain excess H_2O_2 (Cao et al., 2004) and the reduced contraction and release of intracellular Ca^{2+} in ulcerative colitis can be recovered by

catalase (Cao et al., 2004) and not by IL-1ra, we tested the role of H_2O_2 in IL-1β-induced motor dysfunction.

After muscle cells exposed to IL-1β had been treated with catalase, IL-1β-induced reduction in contraction in response to both thapsigargin (Fig. 10) and caffeine (Fig. 8) were almost completely restored. The caffeine-induced Ca^{2+} release (Fig. 8) was also restored by catalase. In addition, IL-1β treatment significantly increased the production of H_2O_2 in sigmoid circular muscles.

These data suggest that H_2O_2 is involved in IL-1β-induced motor dysfunction and in the reduction of Ca^{2+} release from intracellular stores. It has been reported that IL-1 may cause Ca^{2+} release from intracellular stores through cell-matrix interactions in fibroblasts spread on surfaces coated with fibronectin (Wang et al., 2003). In isolated normal sigmoid muscle cells, however, we did not find that IL-1β caused cytosolic Ca^{2+} increase under our experimental conditions. In addition, interleukin 1β has been reported to modulate neurons as a result of activation of a nonselective cationic conductance (Desson and Ferguson, 2003). Whether this mechanism is also involved in IL-1β-induced sigmoid motor function needs to be further explored.

We also found that IL-1β-induced production of H_2O_2 is mediated through sequential activation of MAPKs (ERK1/ERK2) and $cPLA_2$, but not through activation of p38 MAPK and NF-κB because 1) H_2O_2 production was significantly blocked by PD98059 and by AACOCF3, but neither by SB203580 nor by NF-κB SN50; 2) IL-1β significantly increased the phosphorylation of ERK1/ERK2 MAPKs and $cPLA_2$; and 3) IL-1β-induced phosphorylation of $cPLA_2$ was blocked by PD98059. The link between ERK1/ERK2 MAP kinases and cPLA2 is consistent with the data on acetylcholine-induced contraction of rabbit intestinal smooth muscle (Zhou et al., 2003).

These data are in agreement with IL-1β-induced activation of MAPKs (Hungness et al., 2002; Parker et al., 2002) and $cPLA_2$ (Beasley, 1999; Higashi et al., 2000) in variety of cells. Our data suggest that H_2O_2 production induced by short-period incubation with IL-1β does not depend on activation of NF-κB, even though NF-κB may be activated by IL-1β (Janssen-Heininger et al., 2000; Ganster et al., 2001). It is possible that NF-κB may take a much longer time to up-regulate enzymes, e.g., NADPH oxidase, increasing H_2O_2 production. The possibility that NF-κB may be involved in H_2O_2 production induced by long-period (e.g., 24-h) treatment with IL-1β needs to be further explored.

In conclusion, the inflammation-associated changes occurring in ulcerative colitis reduced sigmoid circular muscle contractility and Ca^{2+} release from intracellular Ca^{2+} stores and caused a switch in the signal transduction pathway of neurokinin A-induced contraction from calmodulin- and protein kinase C-dependent in normal cells to protein kinase C-dependent in ulcerative colitis cells. The changes in motor function and Ca^{2+} release in ulcerative colitis may be mediated by elevated levels of IL-1β, which may induce production of H_2O_2 by sequential activation of ERK1/ERK2 MAP kinases and $cPLA_2$.

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