PAR-2 agonists induce contraction of murine small intestine through neurokinin receptors

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Nutritional Requirements and Function Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville 20705; and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

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Zhao, Aiping, and Terez Shea-Donohue. PAR-2 agonists induce contraction of murine small intestine through neurokinin receptors. Am J Physiol Gastrointest Liver Physiol 285: G696–G703, 2003. First published June 11, 2003; 10.1152/ajpgi.00064.2003.—Protease-activated receptor-2 (PAR-2) is a G protein-coupled receptor and is expressed throughout the gut. It is well known that PAR-2 participates in the regulation of gastrointestinal motility; however, the results are inconsistent. The present study investigated the effect and mechanism of PAR-2 activation on murine small intestinal smooth muscle function in vitro. Both trypsin and PAR-2-activating peptide SLIGRL induced a small relaxation followed by a concentration-dependent contraction. The sensitivity to trypsin was greater than that to SLIGRL (EC50 = 0.03 vs. 40 μM), but maximal responses were similar (12.3 ± 1.6 vs. 13.7 ± 1.3 N/cm²). Trypsin-evoked contraction (1 μM) exhibited a rapid desensitization, whereas the desensitization of response to SLIGRL was less even at high concentration (50 μM). Atropine had no effect on PAR-2 agonist-induced contractions. In contrast, TTX and capsaicin significantly attenuated those contractions, implicating a neurogenic mechanism that may involve capsaicin-sensitive sensory nerves. Furthermore, contractions induced by trypsin and SLIGRL were reduced by neurokinin receptor NK₁ antagonist SR-140333 or NK₂ antagonist SR-48968 alone or were further reduced by combined application of SR-140333 and SR-48968, indicating the involvement of neurokinin receptors. In addition, desensitizing neurokinin receptors with substance P and/or neurokinin A decreased the PAR-2 agonist-evoked contraction. We concluded that PAR-2 agonists induced a contraction of murine intestinal smooth muscle that was mediated by nerves. The excitatory effect is also dependent on sensory neural pathways and requires both NK₁ and NK₂ receptors.

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contractions; and 3) the contribution of neurokinin receptors NK1 and NK2 to PAR-2-induced contractions.

MATERIALS AND METHODS

In vitro contractility. Segments of jejunum (1 cm) were taken from female 8- to 12-wk-old BALB/c mice (National Cancer Institute, Frederick, MD); their intestinal contents were flushed off, and segments were suspended longitudinally in individual 8-ml organ baths and maintained in oxygenated Krebs’ solution at 37°C. One end of the tissue was attached to an isometric tension transducer (Model FT03; Grass Medical Instruments Quincy, MA) and the other to the bottom of the bath. Tissues were stretched to a load of 9.9 mN, because preliminary experiments showed that this load stretched tissues to their optimal length for active contraction (12). Tissues were then allowed to equilibrate for at least 30 min in Krebs’ buffer solution, and the bath solution was replaced every 10 min throughout each study. Tension was recorded by using a Grass model 79 polygraph (Grass Medical Instruments) and was expressed as force per cross-sectional area (37).

After equilibration for 30 min, tissues were challenged with the PAR-2 agonists trypsin (1 nM–1 μM) or activating peptide SLIGRL (1–100 μM). Responses were performed in the presence or absence of the neurotoxin tetrodotoxin (TTX; 2 μM), the muscarinic receptor antagonist atropine (2 μM), the sensory neural inhibitor capsaicin (10 μM), the nitric oxide synthase inhibitor L-NAME (10 μM), the NK1 receptor antagonist SR-140333 (1 μM), the NK2 receptor antagonist SR-48968 (1 μM), or a combination of the NK1 and NK2 antagonists. In addition, the responses were determined to PAR-2 reverse peptide LRGILS (100 μM) and to trypsin in the presence of soybean trypsin inhibitor (10 μg/ml). PAR-2 agonists were applied only once to each tissue preparation because of the desensitization of the receptor (2), except in those experiments that specifically address receptor desensitization.

Desensitization and cross-desensitization of PARs and neurokinin receptors. To examine the effect of PAR desensitization or cross-desensitization, strips were first challenged with trypsin or the PAR-activating peptides SFLLRN (PAR-1/PAR-2), SLIGRL (PAR-2), or AYPGKF (PAR-4) (100 μM) and challenged with trypsin (1 μM) 10 min later. Alternately, strips were challenged with SLIGRL (100 μM) or trypsin (1 μM), washed, and challenged with SLIGRL (100 μM) 10 min later. Boiled trypsin (1 μM, 100°C for 5 min) or PAR-2 reverse peptide LRGILS (100 μM) were used as controls for the PAR-2 agonists in the desensitization experiments. To evaluate the effect of neurokinin receptor desensitization on responses to PAR-2 agonists, strips were challenged repeatedly (2–3 times) with tachykinins, substance P (1 μM), or neurokinin A (NKA; 1 μM) alone or in combination without washing until responses exhibited tachyphylaxis. These desensitized strips then were challenged with PAR-2 agonists.

Alternately, to examine whether desensitization of PAR-2 affected responses to tachykinins, strips were exposed first to substance P or NKA. Following a washout for 30 min, a period allowing full recovery of the response, strips were challenged twice with trypsin (1 μM) or SLIGRL (100 μM) to desensitize PAR-2 and were subsequently challenged with substance P or NKA again. Responses of substance P and NKA were compared before and after PAR-2 agonist treatment. Preliminary experiments showed that 30 min allowed for full recovery of responses to substance P and NKA. At the beginning and end of each experiment, all strips were challenged with carbachol to assess the integrity of the smooth muscle.

RT-PCR. Small intestine from mouse was flushed with ice-cold saline and placed in RNa Later (Ambion, Austin, TX) immediately to stabilize the RNA. Total RNA was extracted by using RNAzol isolation reagent (Ambion). One microgram of total RNA was reverse transcribed by using a first-strand cDNA synthesis kit (MBI Fermentas, Hanover, MD). The resultant cDNA was diluted fivefold for PCR. Oligonucleotide primers to mouse PAR-2 were as follows: sense, 5‘-CACCACCTGTCGACGTGCTC‘; antisense, 5‘-CTCAGTAGAGGGTTTTAACACG‘. PCR was performed for 35 cycles with the temperature profile 94°C, 1 min; 54°C, 1 min; and 72°C, 1 min, using PCR Master Mix (Promega, Madison, WI). The amplified PCR products were analyzed by 1% agarose gel electrophoresis and ethidium bromide staining. The integrity of cDNA samples used was confirmed by amplifying β-actin by using the following primers: sense, 5‘-TGTACOTGACCATCCAGGCT‘; antisense, 5‘-TTCTCCAGGGGGAAGAGAGGA‘.

Solutions and drugs. Krebs’ buffer contained (in mM) 118.5 NaCl, 4.75 KCl, 2.54 CaCl2, 1.19 MgSO4, 25 NaHCO3, 1.19 NaH2PO4, and 11 glucose. All drugs were obtained from Sigma (St. Louis, MO) unless otherwise stated. Stock solutions were prepared as follows: trypsin (1 mM), soybean trypsin inhibitor (200 mg/ml), substance P, and NKA (0.1 mM) were each dissolved in distilled water; SLIGRL, SFLLRN, AYPGKF, and LRGILS (10 mM each; Bachem Bioscience, King of Prussia, PA or synthesized by University Biomedical Instrumentation Center) were dissolved in 10% DMSO; specific neurokinin receptor antagonists SR-140333 and SR-48968 (1 mM; generous gifts from Sanofi-Synthelabo, Paris, France) were dissolved in DMSO and stored at −70°C in aliquots; TTX (1 mM) was dissolved in citrate buffer; capsaicin (10 mM) was dissolved in ethanol and stored at 4°C. On the day of the experiment, appropriate dilutions of trypsin, SLIGRL, SFLLRN, AYPGKF, LRGILS, substance P, and NKA were made using distilled water.

Data analysis. Appropriate vehicle and time- and age-matched controls were performed for each group; however, there were no significant differences among control groups and therefore only one control group is shown for comparison. Statistical analysis was performed using one-way ANOVA to compare the responses after receptor desensitization. Concentration-dependent responses were compared using MANOVA (Systat 5.2), with post hoc analysis for multiple comparisons. P < 0.05 was considered significant. Agonist responses were fitted to a sigmoid curve (Graphpad), and EC50 values (with 95% confidence limits) were used to assess changes in sensitivity of response.

Experiments and animal care were conducted in compliance with guidelines outlined in the Guide for the Care and Use of Laboratory Animals (Institute of Animal Research Council), and all procedures were approved by our institutional animal care and use committee.

RESULTS

RT-PCR detection of PAR-2 mRNA in murine intestine. Expression of mRNA for PAR-2 was examined by using RT-PCR. RT-PCR yielded the expected amplification product of 600 bp for PAR-2 (Fig. 1), confirming the presence of PAR-2 in mouse small intestine.

Responses to PAR-2 agonists. Trypsin evoked sustained, concentration-dependent contractions of intest-
tinal longitudinal smooth muscle preparations (Fig. 2). Preincubation with soybean trypsin inhibitor (10 mg/ml, 30 min) completely blocked all effects of trypsin (data not shown). In ~50% of the muscle strips, trypsin also induced a small relaxation before the contraction that was independent of the agonist concentration and was unrelated to the amplitude of the subsequent contraction. In addition, this inhibitory response was unaffected by pretreatment with TTX (1 µM) or the nitric oxide synthase inhibitor l-NNA (100 µM), indicating a direct effect on smooth muscle that is not mediated by nitric oxide. The specific PAR-2 agonist, SLIGRL, induced a transient relaxation in only ~20% of the strips, but the amplitude of the contraction was similar to that of trypsin (Fig. 2). The sensitivity of the response to trypsin was greater than that to SLIGRL (EC50 = 0.03 vs. 40 µM), but maximal responses were similar (12.3 ± 1.6 vs. 13.7 ± 1.3 N/cm²). The PAR-2 reverse peptide LRGILS (200 µM) had no effect (data not shown).

Desensitization and cross-desensitization of the effect of PAR-2 agonists. Trypsin-evoked contractions (1 µM) exhibited a rapid desensitization to a repeat challenge (Fig. 3A), and strips remained unresponsive to trypsin even 2 h later. Desensitization of the response to SLIGRL was less than that to trypsin because, even at higher concentrations, second exposure to SLIGRL (100 µM) still evoked ~50% of the response to the initial exposure (6.4 ± 1.4 vs. 13.7 ± 1.3 N/cm²; P < 0.05; Fig. 3B). Cross-desensitization experiments with PAR-2 agonists trypsin and SLIGRL showed that first challenging the tissue with trypsin (1 µM) markedly reduced (~70%) the response to SLIGRL (100 µM; 3.8 ± 0.4 vs. 13.7 ± 1.3 N/cm²; P < 0.05; Fig. 3C). In contrast, if the tissue was challenged first with

Fig. 1. Expression of protease-activated receptor (PAR)-2 mRNA in murine small intestine. Total RNA was isolated from small intestine by RNAwiz and subjected to RT-PCR using primers for PAR-2 and β-actin. Deduced amplification products for PAR-2 and β-actin were 600 and 300 bp, respectively.

Fig. 2. Concentration-dependent contraction induced by trypsin and SLIGRL and decreased by tetrodotoxin (TTX) and capsaicin in intestinal smooth muscle. Tissues were challenged with trypsin or SLIGRL in the presence of vehicle, TTX (1 µM), or capsaicin (10 µM). *P < 0.05 for the entire curve compared with vehicle (n ≥ 5 for each group).

Fig. 3. Desensitization and cross-desensitization of PAR-2 agonist-evoked contraction. Intestinal strips were first challenged with trypsin (1 µM) or SLIGRL (100 µM), washed out, and then were challenged a second time with PAR-2 agonists 10 min later. Prior treatment with trypsin abolished the response to second challenge of trypsin (A) or markedly decreased the response to SLIGRL (C). Prior treatment with SLIGRL markedly decreased the response to second challenge of SLIGRL (B) or slightly decreased to the response to trypsin (D). Tracings are representative of at least 5 individual experiments.
SLIGRL (100 μM), there was a smaller, but significant, decrease in the response to trypsin (1 μM; 9.0 ± 0.7 vs. 12.3 ± 1.6 N/cm²; P < 0.05; Fig. 3D). Contractions to the PAR-1/PAR-2 agonist SFLLRN (maximal response, 15.9 ± 1.3 N/cm²) were similar to those of SLIGRL. SFLLRN also partially desensitized the response to a second exposure to SFLLRN (6.5 ± 0.8 vs. 15.9 ± 1.3 N/cm²; P < 0.05) but had no effect on responses to trypsin (data not shown). Similarly, prior exposure to SLIGRL did not alter responses to SFLLRN (data not shown). Although SFLLRN is reported to have some action on PAR-2 (17), our data do not support a major effect of SFLLRN on PAR-2 in the murine small intestine and are consistent with previous results in rat colon (22). The PAR-4-activating peptide AYPGKF had no effect on murine small intestinal contractility in vitro. Repeated treatment of strips with AYPGKF did not desensitize the response to a subsequent challenge with trypsin (data not shown). In control experiments for desensitization, separate strips were used to test the effect of boiled trypsin or LRGILS on responses to PAR-2 agonists. Neither boiled trypsin (1 μM) nor PAR-2 reverse peptide LRGILS (100 μM) evoked any response on tissue strips nor did they desensitize responses to the PAR-2 agonists trypsin (1 μM) or SLIGRL (100 μM) (data not shown). Desensitization to PAR agonists did not affect responses to other excitatory neurotransmitters such as acetylcholine, substance P, or carbachol (data not shown).

Neurogenic mechanism of PAR-2 agonist-induced contraction. To determine the contribution of enteric nerves on the response to PAR-2 agonists, responses were compared in the presence and absence of the neurotoxin TTX. TTX decreased the amplitude of spontaneous contractions and significantly inhibited contractions to trypsin and SLIGRL (Fig. 2), indicating that the response to PAR-2 agonists was dependent on nerves. To determine the contribution of cholinergic pathways, responses to PAR-2 agonists were compared in the presence and absence of the muscarinic receptor antagonist atropine (2 μM). Atropine also reduced the amplitude of spontaneous contractions in intestinal smooth muscle but had no effect on PAR-2 agonist-induced contractions (data not shown), suggesting a lack of muscarinic cholinergic control.

To assess the role of sensory nerves in PAR-2 agonist-evoked contraction in longitudinal smooth muscle of small intestine, strips were treated with capsaicin to deplete the sensory neuropeptides and then challenged with PAR-2 agonists. As shown previously (1), capsaicin elicited an initial contraction followed by a decrease in the amplitude of the spontaneous contractions. After continuous exposure to capsaicin (10 μM for 30 min), contractions induced by PAR-2 agonists trypsin and SLIGRL were decreased significantly (Fig. 2), implicating capsaicin-sensitive sensory nerves.

Involvement of neurokinin receptors in PAR-2-evoked contraction. Sensory nerves release tachykinins, substance P, and NKA, which bind preferentially to NK1 and NK2 receptors, respectively, leading to contraction of intestinal smooth muscle (13). To examine whether neurokinin receptors participate in PAR-2 agonist-induced contractions, muscle strips were treated with specific neurokinin receptor antagonists SR-140333 (NK1; 1 μM) and SR-48968 (NK2; 1 μM) alone or in combination. Incubation with SR-140333 or SR-48968 alone decreased the amplitude of the spontaneous contractions, and in combination they abolished spontaneous contractions (data not shown). Exposure to either antagonist alone inhibited contractions evoked by trypsin or SLIGRL (Fig. 4), and these contractions were decreased further in the presence of both antagonists, indicating an additive effect.

Similar to other G protein-coupled receptors, responses to tachykinins binding to neurokinin receptors undergo rapid desensitization (13). To examine whether desensitization of neurokinin receptors affect the responses to PAR-2 agonists, strips were challenged repeatedly with substance P (1 μM) or NKA (1 μM) alone or in combination and then were challenged with PAR-2 agonists. Contractions in response to trypsin (0.1 μM) or SLIGRL (50 μM) were decreased by pretreatment of tachykinins, substance P, and NKA alone or in combination (Fig. 5). There were no signif-
significant differences between the effects of the two tachykinins applied individually or in combination. In contrast, desensitization of PAR-2 receptors with repeated treatment of trypsin (1 M) or SLIGRL (100 M) did not affect the responses to substance P or NKA (Fig. 6).

**DISCUSSION**

Serine proteases are present in significant quantities in the lumen of the small intestine, and their presence is linked to a number of pathological processes, including inflammation (4). The increasing numbers of cells identified within the intestinal wall that express PARs suggest that the proteases may also act as signaling molecules and play a role in the regulation of intestinal function. The precise source of endogenous PAR agonists is unclear, but resident inflammatory/immune cells, such as mast cells, are likely candidates. The present study demonstrates that in murine small intestine PAR agonists induce a contraction of longitudinal smooth muscle that was mediated, in part, by enteric nerves. Furthermore, the mechanism of the PAR-2-induced contractions involves sensory nerves and neurokinin receptors.

PAR-1 and PAR-2 are expressed on smooth muscle and appear to modulate intestinal smooth muscle contractility. Previous studies in smooth muscle showed that effects of PAR-2 activation vary by region and species and include relaxation, contraction, or a biphasic response of relaxation followed by contraction. In the present study, PAR-2 agonists evoked a small relaxation followed by a concentration-dependent contraction in murine small intestinal longitudinal smooth muscle. In addition, PAR-2 agonists induced a transient relaxation in some, but not all, muscle strips that was insensitive to TTX or L-NNA, suggesting a lack of involvement of nerves or nitric oxide. The lack

**Fig. 5.** Effect of desensitization of neurokinin receptors by tachykinin agonists on response to trypsin (A) or SLIGRL (B). Tissues were repeatedly treated with substance P (SP; 1 M), neurokinin A (NKA; 1 M), or both until the tissue no longer responded to tachykinins and then were challenged with trypsin (A; 0.1 M) or SLIGRL (B; 50 M). *P < 0.05 compared with the responses to PAR-2 agonists without predesensitizing neurokinin receptors (n = 5 for each group).

**Fig. 6.** Desensitization of PAR-2 receptor by PAR-2 agonists did not affect the responses to SP (A) or NKA (B). Tissues were exposed first to tachykinins (1 M). Following a washout for 30 min, strips were challenged twice with trypsin (1 M) or SLIGRL (100 M) to desensitize PAR-2 and were challenged with SP (A; 1 M) or NKA (B; 1 M) again. *P < 0.05 compared with the response to tachykinins before desensitizing PAR-2 (n = 5 for each group).
of involvement of nitric oxide differs from studies in vascular smooth muscle in which thrombin and trypsin induced only nitric oxide-dependent relaxations (24). These differences can be explained, in part, by reports that vascular smooth muscle relaxation is attributed to PAR-2 on endothelium (14), whereas vascular contraction is mediated by PAR-1 located on smooth muscle (24). The concentration-dependent contraction, however, is consistent with studies in rats in which the contraction was mediated by activation of phospholipase C (23).

Previous studies in rat small intestine showed that PAR-2 is expressed not only on smooth muscle cells (6, 7) but also on enteric neurons in the small intestine (18). In the present study, a significant portion of the effects of PAR-2 on smooth muscle contractility was dependent on enteric nerves. The contraction was inhibited by TTX but not by atropine, implying a role for noncholinergic excitatory nerves. Others have reported (19) that activation of PARs on myenteric neurons in the guinea pig ileum elicits a prolonged depolarization and excitation. Immunoreactive PAR-1 and PAR-2 could be detected in approximately two-thirds of the neurons from the myenteric plexus of guinea pig small intestine in primary culture, and RT-PCR further confirmed the expression of PAR-1 and PAR-2 in the myenteric plexus (11). Of interest is that ~42% of the guinea pig intestinal neurons labeled had S-type electrophysiology, and these exhibited little tachyphylaxis to trypsin and were immunoreactive for nitric oxide. The remainder of the neurons demonstrated AH-type electrophysiology and desensitized to the repeated application of proteases. In the current study, trypsin also induced contractions that were TTX sensitive and were followed by a prolonged tachyphylaxis, suggesting that the excitation involved AH-type neurons.

PAR-2 is also coexpressed with substance P in dorsal root ganglia neurons, and PAR-2 agonists stimulate the release of both substance P and CGRP from C fibers in peripheral tissues and in the spinal cord in a calcium-dependent manner (31). An interesting observation was that a large proportion of neurons that expressed substance P or vasoactive intestinal peptide also expressed PAR-1 and PAR-2 (8). Capsaicin, an agent known to deplete functional pools of neuropeptides in primary sensory nerves, abolishes PAR-2 agonist-mediated release of CGRP and substance P (31). Capsaicin also completely abolished the PAR-2-mediated bronchus contraction (3) and even reversed the protection exerted by PAR-2 agonists on trinitrobenzene sulfonic acid-induced colitis in mice (10). Our data confirmed that capsaicin initially induced a small contraction that is considered to be the result of tachykinin release from sensory neurons (13). Repeated application of capsaicin failed to induce further contractions, indicating desensitization of the local sensory nerves. Under these conditions, capsaicin markedly decreased the trypsin- and SLIGRL-induced contractions in intestinal smooth muscle of mice, strongly suggesting involvement of the capsaicin-sensitive sensory neurons. Both primary spinal afferent neurons (extrinsic) and intrinsic enteric neurons are present in small intestine and release tachykinins on stimulation. Capsaicin, which is believed to only affect extrinsic primary afferent but not intrinsic enteric neurons (13), significantly inhibited the PAR-2 agonist-evoked contraction, suggesting that PAR-2 agonist-induced release of tachykinins is primarily from extrinsic primary afferent neurons.

A further insight into the mechanism of the PAR-2 effects on small intestine smooth muscle is shown by the finding that NK1 antagonist SR-140333 and NK2 antagonist SR-48968 both inhibited the PAR-2-induced contraction, implying the involvement of neurokinin receptors. Trypsin and SLIGRL, however, do not appear to directly activate neurokinin receptors, because depletion of sensory neuropeptides with repeated exposure to capsaicin also markedly reduced the ability of PAR-2 agonists to evoke contractile responses. Thus these data show that PAR-2 agonists induced a contraction in small intestine of mice by stimulating the release of tachykinins from capsaicin-sensitive sensory neurons, which in turn bind to neurokinin receptors.

Neurokinin receptors, as well as PARs, belong to the seven-transmembrane-spanning, G protein-coupled receptors. Cellular responses to agonists acting at G protein-coupled receptors are rapidly attenuated in the continuous presence of agonist and desensitize to repeated application of agonist. In our studies, PAR-2-mediated intestinal contractions undergo a rapid desensitization, as reported previously (2, 7, 31). The initial challenge with trypsin or SLIGRL abolished or attenuated the response to a subsequent application. This raises an important concept in the functional role of PAR-2 activation in diseases such as inflammation in that impact of chronic elevation of proteases is likely to be linked equally to the prolonged desensitization of the PAR-2 as to the transient effects of activation of PAR-2. Differences between trypsin- and SLIGRL-induced desensitization can be explained by the fact that trypsin, but not SLIGRL, cleaves PAR-2, which ensures that a single receptor molecule, once cleaved, cannot respond again to trypsin. The mechanisms for the PAR-2 desensitization are proposed to involve the irreversible cleavage of the receptor by trypsin, protein kinase C-mediated termination of signaling, and PAR-2 targeting to lysosomes (2). Cross-desensitization experiments showed that trypsin was more effective in desensitizing responses to SLIGRL than SLIGRL was in reducing responses to trypsin. These data confirm that at least a portion of the intestinal smooth muscle response to trypsin is through PAR-2 activation and are consistent with differences between the desensitization to proteases vs. PAR agonists in enteric neurons (11). In addition to PAR-2, trypsin also activates other PAR family members, including PAR-1, 3, and 4, that are present in the gut (32, 35, 36). In our experiments, SFLRN activation of PAR-1 induced a contraction that was similar in amplitude to that in response to SLIGRL and did not desensitize responses to trypsin. Therefore, we conclude that PAR-1 is not the primary mediator of trypsin-evoked contraction of...
murine intestinal smooth muscle. This conclusion is supported by a recent report showing that trypsin cleaves, but does not activate, PAR-1 in vascular endothelial cells (25). The PAR-4-activating peptide AYPGKF did not affect the small intestinal contractility or desensitize responses to trypsin, suggesting that PAR-4 is not involved in trypsin-evoked contractions.

Because the PAR-2-induced contractions were dependent on neurokinin receptors, we also examined whether the desensitization of tachykinin responses affects the PAR-2-evoked contraction. Our experiments showed that desensitization of response to substance P and NKA attenuated the response to PAR-2 agonists. Alternatively, desensitization of PAR-2 response had no effect on responses to substance P or NKA. The results further confirm that binding to neurokinin receptors is a downstream event from PAR-2 activation.

In conclusion, PAR-2 agonists induced a contraction of murine intestinal smooth muscle that was mediated by nerves, was dependent on sensory neural pathways, and required both NK1 and NK2 receptors. The similarity of responses between trypsin and the PAR-2 agonist suggest that trypsin acts primarily at PAR-2. We hypothesized that activation of PAR-2 present in capsaicin-sensitive neurons triggers the release of tachykinins such as substance P and NKA, which in turn activate the NK1/NK2 receptor located on smooth muscle and enteric nerves. The pronounced tachyphylaxis to the endogenous PAR-2 ligand suggests that chronic elevation of protease levels in disease will modulate cholinergic neural control of mouse small intestinal longitudinal muscle. Am J Physiol Gastrointest Liver Physiol 272: G1135–G1140, 1997.

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DISCLOSURES

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