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

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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 induce 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.

mac; protase-activated receptor; trypsin

PROTEASE-ACTIVATED RECEPTORS (PARs) are a novel sub-class of the seven-transmembrane-spanning, G protein-coupled receptors. Rather than being stimulated through ligand receptor occupancy, PARs are activated by a unique mechanism that involves recognition of the receptor by a protease, cleavage of the receptor at a specific enzymatic site located at the extracellular NH2 terminus, and finally exposure of a new NH2-terminal domain that acts as a “tethered ligand,” binding and activating the receptor itself (20, 32). Four PAR family members have been identified to date: PAR-1, PAR-3, and PAR-4, which are activated more specifically by thrombin, and PAR-2, which is activated by trypsin or human mast cell tryptase. PARs are expressed in a variety of tissues, including gastrointestinal (GI) tract, pancreas, kidney, liver, prostate, ovary, and eye, and have been found in epithelial cells, smooth muscle cells, fibroblasts, endothelial cells, T cell lines, neutrophils, tumor cells, and neurons (28).

PAR-2 is involved in a variety of physiological or pathophysiological activities in a number of organs, including the GI tract (32), cardiovascular and pulmonary systems (5, 26, 29), and skin (30). The prominent expression of PAR-2 along the entire GI length and the localization of PAR-2 in enterocytes, neuronal elements, and myocytes demonstrate the potential importance of PAR-2 in the regulation of GI function (18). PAR-2 agonists induce changes in intestinal epithelial cell transport in a number of species (9, 21). PAR-2 also participates in the regulation of GI motility; however, the results are inconsistent. Activation of PAR-2 results in contraction (16, 27) in the gastric body, alternating contraction and relaxation in the gastric fundus and taenia coli (6), and inhibition of spontaneous contractions in stomach and colon (7). More recently, in vivo GI transit in the mouse was promoted by both PAR-1 and PAR-2 by a mechanism involving L-type calcium channels (15).

The presence of PAR-2 on enteric neurons (8, 11) indicates that part of the effect of PAR-2 may be mediated by nerves. PAR-2-induced release of the proinflammatory sensory neuropeptides substance P and CGRP indicates a role for PAR in neurogenic inflammation (31, 33, 34). The observation that trypsin-induced release of substance P from sensory afferents mediates contraction of bronchial airways (3) suggests that substance P may be involved in the control of contractions in the GI tract. The purpose of this investigation was to determine 1) the effect of PAR-2 agonists on small intestinal smooth muscle contractility; 2) the contribution of enteric nerves to PAR-2-induced

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contractions; and 3) the contribution of neuropeptide 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 Nω-nitro-arginine (1–100 μM; to determine the contribution of nitric oxide), and the specific NK₁ antagonist SR-144998 (1 μM), the NK₂ 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 (100 μM). 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. Tissues were incubated with inhibitors or antagonists for 30 min before the application of agonists.

Desensitization and cross-desensitization of PARs and neuropeptidin receptors. To examine the effect of PAR receptor 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) washed, and subsequently 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 neuropeptide receptor desensitization on responses to PAR-2 agonists, strips were challenged repeatedly (2–3 times) with tachykinins, substance P (1 μM), or neuropeptide 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′-CACCAC-CTGTCACTGATGC CG-3′; antisense, 5′-CTCAGTAGAG-GTTTACAC-3′. 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′-TGACCTGATG-CATCCAGG-3′; antisense, 5′-TTCTCAGGGAGGAA-GAGGA-3′.

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 neuropeptide receptor antagonists SR-144998 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 by 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 intes-
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 (3.8 ± 0.4 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...
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). Contraction 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-

![Fig. 4. Effect of selective neurokinin receptor antagonists on trypsin or SLIGRL-induced contraction. Tissues were treated with SR-48968 (1 μM), SR-140333 (1 μM), or both for 30 min and then challenged with trypsin or SLIGRL. *P < 0.05 for the entire curve compared with vehicle (n ≥ 5 for each group).](http://ajpgi.physiology.org/)

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icant differences between the effects of the two tachykinins applied individually or in combination. In con-
trast, desensitization of PAR-2 receptors with repeated treatment of trypsin (1 μM) or SLIGRL (100 μM) did
not affect responses to substance P or NKA (Fig. 6).

Fig. 5. Effect of desensitization of neurokinin receptors by tachyki-
nin agonists on response to trypsin (A) or SLIGRL (B). Tissues were
repeatedly treated with substance P (SP; 1 μM), neurokinin A (NKA;
1 μM), or both until the tissue no longer responded to tachykinins
and then were challenged with trypsin (A; 0.1 μM) or SLIGRL (B; 50 μM). *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 μM). Following a washout for 30 min, strips were
challenged twice with trypsin (1 μM) or SLIGRL (100 μM) to desen-
sitize PAR-2 and were challenged with SP (A; 1 μM) or NKA (B; 1 μM) again. *P < 0.05 compared with the response to tachykinins
before desensitizing PAR-2 (n ≥ 5 for each group).

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, includ-
ing 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 ago-
nists is unclear, but resident inflammatory/immune
cells, such as mast cells, are likely candidates. The
present study demonstrates that in murine small in-
testine PAR agonists induce a contraction of longitudi-
nal 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 con-
tractility. Previous studies in smooth muscle showed
that effects of PAR-2 activation vary by region and
species and include relaxation, contraction, or a biphase
response of relaxation followed by contraction. In
the present study, PAR-2 agonists evoked a small re-
laxation followed by a concentration-dependent con-
traction 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

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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, abolished PAR-2 agonist-mediated release of CGRP and substance P (31). Capsaicin also completely abolished the PAR-2-mediated bronchus constriction (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-49686 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, SFLLRN 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 AY-PGKF 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 induce transient excitatory effects followed by a prolonged desensitization of these receptors that may be equally important.

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DISCLOSURES

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