Potent NK₁ antagonism by SR-140333 reduces rat colonic secretory response to immunocyte activation

DEREK MORIARTY,¹ NORMA SELVE,² ALAN W. BAIRD,¹,³ AND JON GOLDHILL²

¹Department of Veterinary Physiology and Biochemistry and ³Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland; and ²Department of Internal Medicine, Sanofi-Synthelabo, 92504 Rueil-Malmaison, France

Received 29 February 2000; accepted in final form 25 October 2000

Moriarty, Derek, Norma Selve, Alan W. Baird, and Jon Goldhill. Potent NK₁ antagonism by SR-140333 reduces rat colonic secretory response to immunocyte activation. Am J Physiol Cell Physiol 49: C852–C858, 2001.—The potent neurokinin receptor 1 (NK₁) antagonist SR-140333 has previously been shown to reduce castor oil-induced secretion in animal models. The importance of tachykinins in neuroimmune control of secretion and the effect of SR-140333 on key points in this pathway were elucidated in the present study to determine the type of intestinal dysfunction best targeted by this antagonist. Rat colonic secretion and substance P (SP) release were determined in vitro with the use of Ussing chamber and enzyme immunoassay techniques. NK₁ receptors played a secretory role as receptor agonists stimulated secretion and SR-140333 antagonized the response to SP response (pKᵦ = 9.2). Sensory fiber stimulation released SP and evoked a large secretion that was reduced by 69% in the presence of SR-140333 (10 nM). Likewise, mastocytes also released SP. The subsequent secretory response was reduced by 43% in the presence of SR-140333 (50 nM). SP was also released from granulocytes; however, this did not cause secretion. Functional NK₂ receptors were present in the colon as senktide stimulated secretion, an effect that was increased during stress. We conclude that NK₃ receptors may play a role in stress-related disorders, whereas NK₁ receptors are more important in mast cell/afferent-mediated secretion.

afferent; granulocyte; irritable bowel syndrome; inflammatory bowel disease; mast cell

THE TACHYKININS BELONG to a family of peptides including the products of two genes, the preprotachykinin (PPT) I gene, which produces substance P (SP) and neurokinin A (NKA) (33), and the PPT II gene, which produces neurokinin B (NKB) (24). These tachykinins preferentially bind to NK₁, NK₂, and NK₃ receptors, respectively. A wide range of synthetic agonists and antagonists exists for these receptor subtypes. Of these, Sar-SP (NKA) (13), β-Ala-NKA (39), and senktide (NKB) (26) are the most frequently used agonists. Of the antagonists, SR-140333 (NK₁) (15) and SR-48968 (NK₃) (28) have been well characterized. Tachykinin agonists are known to act as potent secretagogues in the small and large intestinal mucosa. In the guinea pig, ileal NK₁ activation and colonic NK₁ and NK₃ activation result in nonneural and cholinergic secretion (9, 22, 25, 35, 38). In the rat colon, stimulation of all three tachykinin receptor subtypes provokes neural and nonneural secretion (10).

Three common causes of diarrhea are allergy, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS), each involving different components of the intestinal tract. For example, allergy is generally accepted to have a strong dependence on mast cells. After exposure to antigen, IgE is expressed on the surface of mast cells, resulting in reexposure that causes IgE cross-linking, mediator release, and functional response. On the other hand, patients with IBD are in a chronic inflammatory state and subject to recurrent bouts of acute inflammation, characterized by a range of symptoms, including diarrhea, and initiated by various factors, including bacterial exposure (12). Disease flare-up is associated with a massive infiltration of granulocytes, in particular neutrophils. IBS is characterized by hyperalgesia and altered motility and epithelial ion transport (32, 34, 41). Unlike allergy and IBD, it is doubtful that IBS has a major inflammatory etiology. Instead, it is generally thought to involve a defect in sensory afferent processing.

Thus the pathophysiology of allergy, IBD, and IBS appears to involve mast cells, chronic and acute inflammatory cells, and sensory fibers, respectively. If any of these cell types are involved in tachykinergic-mediated secretion, tachykinin antagonists may play an important therapeutic role in treating associated diarrhea. The involvement of tachykinin signaling in the activation and interaction of these cell types should, therefore, be established to help determine which diarrheal conditions may be treated by tachykinin antagonists. It has previously been reported that NK₁ receptors are implicated in diarrhea after infection with Clostridium difficile (46), a model thought to involve mast cells. Further reports describe the widespread immunomodulatory activity of tachykinins and their receptors. Peptide and receptor are both overexpressed in IBD,

Address for reprint requests and other correspondence: J. Goldhill, LeadDiscovery, Unit 4, Quarry Farm, Bodiam, Robertsbridge, E. Sussex TN32 5RA, United Kingdom (E-mail: leaddisc@leaddiscovery.co.uk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
especially on mucosal monocytes (3, 6, 18, 19, 29, 36, 40), and consequently, SP release from inflammatory cells may perpetuate inflammation and/or provoke diarrhea in IBD as well as allergy. Unfortunately, there are no good models for IBS, and its etiology remains unclear. However, stress has been proposed to be a causative factor of this syndrome (30), which results in hypersecretion in humans. In animal models, tachykinins contribute to stress-induced motility changes (21) and could also mediate changes in epithelial function. If this results from altered afferent neural control, tachykinin receptors, which are found on nerve fibers, could represent a therapeutic target.

Unfortunately, mechanisms mediating the mucosal activity of the tachykinins have not been fully elucidated, and it remains difficult to adopt a rational approach to selecting clinical indications for tachykinin antagonists. In particular, it is not clear which population of nerves, when stimulated, evoke tachykinergic-mediated secretion. Here, we determine whether SP is released by electrical field stimulation (EFS) of secretomotor fibers or capsaicin stimulation of sensory afferent fibers. Furthermore, the sensitivity of the subsequent secretory response to tachykinin receptor antagonists was investigated to determine whether the SP is released in sufficient quantities to evoke a secretory response. Although the predominant source of the tachykinins is neural, SP has been identified in human eosinophils (1) and rat macrophages (23). It is not clear, however, whether granulocytes or mast cells release SP, and if they do, whether a secretory response ensues. Therefore, in addition to investigating tachykinin neurotransmission, an additional aim of the present study was to determine whether granulocytes or mast cells play a role in tachykinin-induced secretion. Finally, we used an animal model of IBS, wrap restraint stress, to investigate the role of altered tachykinergic control in this condition. Having identified the steps at which tachykinins may mediate secretion, we investigated their inhibition by antagonists, including SR-140333. The present study shows the vital role of NK1 receptors in mediating mast cell-induced secretion and the ability of SR-140333 to block this pathway, implying its therapeutic potential in secretory disorders characterized by mastocytosis.

**METHODS**

**Animals.** Male albino rats (250–500 g), allowed free access to standard rat chow and water, were used throughout this study. Animals were anesthetized using pentobarbital sodium (75 mg/kg), or they were stunned and decapitated and tissue was removed. All studies were performed under the rules set by the Declaration of Helsinki.

**Stress induction.** Animals were restrained by forepaw immobilization (17). This was performed over a 2-h period, starting at 9:30 AM, on three consecutive days. After the final period of stress, animals were anesthetized, and the most distal 10 cm of colon was removed for the preparation of epithelial sheets. Animals showed a consistent increase in the number of fecal pellets after periods of immobilization, indicating their stressed state.

**Colonic epithelial preparation.** The colonic epithelial preparation has previously been well documented (7). Briefly, the distal 5–10 cm of colon was removed, and the outer muscle layer was separated from the mucosa. The mucosa was opened along its antimesenteric surface, and the resultant epithelial preparation was mounted as a flat sheet between two Ussing chambers. Both the mucosal and serosal surfaces were circulated with Krebs buffer using a gas lift (95% O₂–5% CO₂; prehumidified by bubbling through distilled water) and maintained at 37 ± 1°C. Short-circuit current (SCC) generated by the epithelium was continuously monitored using an EVC4000 voltage clamp (World Precision Instruments). To do this, one voltage-sensing and one current-passing electrode were inserted into each half-chamber, and the electrodes were connected to the EVC4000 via a preamplifier. The voltage generated by the epithelium was continuously short circuited by passing current across the tissue with the current-passing electrodes. After a 30-min stabilization period, tachykinin agonists, N-formyl-methionyleucyl-phenylalanine (FMLP), anti-IgE, or capsaicin were added to the bathing solution. Agonist additions were made to the serosal solution, with the exception of capsaicin, which was administered to both serosal and mucosal solutions. Concentration-response curves were cumulative with 2-min intervals allowed between each addition. In studies performed to compare SP potency in healthy tissue against other agonists or against its potency in tissue from stressed animals, phosphoramidon (10 μM) was added to both the serosal and mucosal bathing solutions to control for peptidase activity. In nerve stimulation studies, two platinum electrodes were fixed to the wall of the mucosal chamber adjacent to the epithelium and attached to an isolated pulse stimulator (model 2100; A-M Systems). One-minute pulse trains (pulse width 1 ms; pulse strength 5 mamps) were delivered at 5-min intervals and frequencies of 1, 2, 5, 10, and 20 Hz. To study the effect of an antagonist on agonist and frequency-response curves, tissue preparations were incubated with mucosal and serosal vehicle or antagonist for 30 min. Paired tissues were used for agonist studies, whereas two consecutive frequency-response curves were constructed on individual preparations.

**Measurement of SP release.** The distal colon was opened along the mesenteric border and rinsed of its fecal contents. The smooth muscle layers were removed by blunt dissection, leaving a mucosal sheet consisting of epithelium and underlying lamina propria. Segments of mucosa (~1.5 × 0.5 cm) were placed in 12-well plates and allowed to equilibrate for 20 min in oxygenated Krebs-Henseleit solution at 37°C. Where appropriate, tissues were pretreated for 10 min with the neuronal blocker tetrodotoxin (TTX) before stimulation of tissues. Colonic mucosae were stimulated where appropriate for 10 min with EFS (1 ms, 7 Hz, 7 V) or capsaicin (50 μM) to activate enteric nerves and FMLP (50 μM) to activate granulocytes or mast cells, respectively. The peptidase inhibitors phosphoramidon (10 μM), leupeptin (10 μM), and captopril (10 μM) were used to reduce SP degradation. After a 10-min incubation, tissue bathing fluid solution was retrieved and snap frozen in liquid nitrogen for storage at −70°C. Colonic tissues were stored for protein determination.

SP levels in tissue supernatants were determined by a solid-phase ELISA (Caymen Chemicals). This kit is 100% specific for SP with only trace cross-reactivity with NKA or NKB. Protein levels were determined by the method of Bradford (5). Concentrations of SP were expressed as picograms per milligrams of protein.
Data handling. Data were continuously collected by an acquisition package that automatically determined SCC. The E$_{\text{max}}$ value was defined as the maximal measurable response over the range of concentrations or stimuli employed. To calculate pD$_2$ values, data were expressed as percent E$_{\text{max}}$ and plotted against log [agonist]. Sigmoid curve fitting was performed. Student’s t-test was performed to determine rank order potencies for agonists or to determine whether stress altered the response to agonists or EFS. To determine the effect of antagonists on agonist or EFS responsiveness, it was determined whether the antagonist significantly reduced the E$_{\text{max}}$ using a one-sample t-test, with comparisons made to a hypothetical mean of 100%. If E$_{\text{max}}$ values were unchanged, a Student’s paired t-test was used to compare pD$_2$ in the presence and absence of an antagonist. When values differed, the dose ratio for pairs of curves from control and antagonist-treated tissues was calculated and used to determine pK$_b$ values. When single agonist concentrations were used, significance of effect was determined using Student’s t-test. In all cases, P < 0.05 was considered significant. Values are given as means ± SE, with the number of replicants given representing the number of preparations. In some instances, multiple preparations were harvested from the same animal; however, each was incubated under separate conditions.

Drugs and solutions. The Krebs solution used for both contractile and epithelial transport studies was of the following composition (in mM): 118 NaCl, 4.7 KCl, 1.64 MgSO$_4$, 7H$_2$O, 1.18 KH$_2$PO$_4$, 11.5 glucose, 24.88 NaHCO$_3$, and 2.52 CaCl$_2$·2H$_2$O. Drugs used were purchased from Sigma unless specified otherwise and were as follows: anti-IgE (Nordic), NKA trifluoroacetic acid (TFA) salt and β-Ala$_6$-NKA$^{4–10}$ TFA salt (RB1), capsaicin, FMLP, [Sar$^9$, Met(O$_2$)$_{11}$]-SP, phosphoramidon, senktide [succinyl-(Asp$_6$, 6-Ala$_{11}$)-Ala-$N$-phenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane} and the NK2 antagonist SR-48968 {S-(1-[2–3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane}. SR-140333 {S–N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butylbenzamide}). Peptides were stored as a stock solution in 0.1 N acetic acid at −20°C. Stock concentrations of tachykinin antagonists and FMLP were dissolved in DMSO (100%). Capsaicin was dissolved in ethanol (100%), and anti-IgE was reconstituted in distilled water.

RESULTS

Agonist responsiveness. The two major peripheral tachykinins, SP and NKA, potent and concentration dependently increased colonic SCC (Fig. 1A). Secretion was also induced by synthetic agonists (Fig. 1B) with a rank order potency of senktide (NK$_3$) > Sar-SP (NK$_4$) > β-Ala-NKA. This corresponded to pD$_2$ values of 8.7 ± 0.1, 8.4 ± 0.1, and <7.0, respectively. Due to the low potency of β-Ala-NKA, it proved impossible to fit response curves to a sigmoidal model, and an exact pD$_2$ was not calculable. The maximal responsiveness to each of these agonists was, however, similar (33 ± 6, 43 ± 11, and 41 ± 11 μA/cm², respectively).

Antagonist sensitivity. The responses to SP and NKA were shifted to the right, respectively, by SR-140333 (pK$_b$ = 9.20 ± 0.31; Fig. 2A) and SR-48968 (pK$_b$ = 7.33 ± 0.46; Fig. 2B). This is the first time that the antisecretory potency of these nonpeptide antagonists has been reported, confirming that NK$_1$ and NK$_2$ subtypes are both functionally expressed in the rat colon and that they play a physiological role.

Tachykinin release. To add further evidence for a role of NK$_1$ and NK$_2$ receptors, we attempted to determine whether nerve stimulation resulted in the liberation of one of these ligands, SP. At 7 Hz, EFS increased SP release from 9 ± 2 to 15.3 ± 2 pg/mg protein (P < 0.05; n = 6). This was reduced to 10.7 ± 1 pg/mg protein by TTX (1 μM; n = 6; P > 0.05 compared with basal values), demonstrating the neural origin of SP. This also suggests that TTX-insensitive nerves do not liberate SP under the present conditions.

Secretomotor role of tachykinins. To determine whether tachykinin release is responsible for neurally mediated secretion, the effect of receptor antagonists on EFS stimulation of submucosal nerves was determined. Stimulation induced a frequency-dependent epithelial response. E$_{\text{max}}$ values were unaltered by both antagonists tested at concentrations found to be effective against exogenous agonists (Table 1), suggesting that secretomotor fibers do not release sufficient SP to increase secretory activity.

![Fig. 1. Response of rat colonic epithelium to the natural tachykinin agonists (A) substance P (SP, n = 14) and neurokinin A (NKA, n = 4) and the synthetic NK$_1$, NK$_2$, and NK$_3$ agonists (B) Sar-SP (n = 6), β-Ala-NKA (n = 6), and senktide (n = 12). Responses are given as mean change in short-circuit current (SCC) ± SE of the mean.](image)
The excitatory but not the inhibitory response to sensory stimulation. These data, along with the lack of effect of NK1 antagonism on the response to EFS, suggest that SP is released from afferent fibers.

Tachykinin control of colonic ion transport. Given the role of tachykinins as potent secretagogues, we tested the hypothesis that their control of the colonic epithelium may be altered in this model. Thus the effect of stress on the response to stimulation of either NK1 or NK3 receptors was investigated. The maximal response and sensitivity to Sar-SP was unaltered by pre-treatment with the NK1 antagonist SR-140333 (50 nM; n = 6). This effect was significantly (P < 0.05) reduced to 20.7 ± 3 μA/cm² by capsaicin pretreatment of 30 min with either vehicle or antagonist.

Sensory role of tachykinins. Capsaicin (50 μM) increased SP release from 8 ± 2 to 27.4 ± 4 pg/mg protein (P < 0.05; n = 6). This effect was significantly (P < 0.05) reduced to 20.7 ± 2 by 1 μM TTX (n = 6) but not abolished, suggesting that capsaicin can release SP from both neural and nonneural stores. Furthermore, capsaicin evoked a biphasic secretory response composed of a transient rise followed by a fall in SCC as previously described (47). The excitatory but not the inhibitory response was significantly reduced by NK1 antagonism. NK2 antagonism was without effect (Table 2). NK1 receptors, therefore, appear to mediate the response to sensory stimulation. These data, along with the lack of effect of NK1 antagonism on the

| Table 1. Effect of the NK1 antagonist SR-140333 and the NK2 antagonist SR-48968 on rat colonic short-circuit current response to electrical field stimulation |
|-----------------|-----------------|-----------------|
|                  | Control         | +SR-140333, 10 nM | +SR-140333, 10 nM |
| E_max, μA/cm²   | 77 ± 9          | 71 ± 8          | 106 ± 35        |
|                  | +SR-48968, 1 μM | +SR-48968, 1 μM |
|                 | 106 ± 25        | 106 ± 25        |

Values are means ± SE. Response curves were constructed over a range of frequencies, and from these, E_max values were calculated. Two successive curves were constructed for individual colonic mucosa preparations. The first series of responses was determined in the presence of vehicle, the second in the presence of antagonists. Contact times of 30 min were allowed in both instances. Data were compared to controls using Student’s paired t-tests with P < 0.05 considered different; n = 6.

| Table 2. Effect of the NK1 antagonist SR-140333 and the NK2 antagonist SR-48968 on rat colonic short-circuit current response to capsaicin |
|-----------------|-----------------|-----------------|
|                  | Control         | +SR-140333, 10 nM | +SR-48968, 1 μM |
| Stimulatory response, μA/cm² | 13 ± 3          | 4 ± 2          | 11 ± 5       |
| Inhibitory response, μA/cm²  | −27 ± 3         | −23 ± 2        | −87 ± 22     |

Values are means ± SE. Capsaicin (10 μM) was administered to the colonic mucosal bathing solution, inducing a biphasic response consisting of a stimulation followed by an inhibition of short-circuit current. Experiments were performed on paired preparations from adjacent areas of the colon; one preparation was incubated in the presence of antagonist and the other in vehicle. Maximal values for each phase of the response are given. Data were compared to controls using Student’s paired t-test; *P < 0.05; n = 6.

Tachykinin involvement in the response to immune activation. Eosinophils and macrophages have previously been shown to contain SP. Here we show that anti-IgE (1:250 dilution) stimulation of mast cells releases SP (9 ± 2 and 14 ± 2 pg/mg protein under control and anti-IgE-stimulated conditions; P < 0.05; n = 8). This was not affected by neuronal blockade (13.4 ± 2 ng/mg protein in the presence of 1 μM TTX; n = 8). Furthermore, anti-IgE increased SCC by 76 ± 7 μA/cm², a response significantly reduced to 43 ± 5 μA/cm² by SR-140333 (50 nM; n = 7), showing NK1 involvement. On the other hand, FMLP (50 μM), a bacterial wall product known to activate granulocytes, evoked an influx of SP from nonneural cells. Levels were increased from 9 ± 1 to 27 ± 10 pg/mg protein (P < 0.05; n = 7). Again, this was not significantly affected by neuronal blockade (20 ± 5 ng/mg protein in the presence of 1 μM TTX; n = 7). Like anti-IgE, FMLP also stimulates epithelial transport, increasing SCC by 25 ± 6 μA/cm²; however, this does not appear to be mediated via NK1 receptors, because SR-140333 was without significant effect (20 ± 6 μA/cm² in the presence of 50 nM SR-140333; n = 6).

Neuroimmune interaction. Both anti-IgE and capsaicin released SP and increased SCC via NK1 activation, and it therefore remains possible that these effects involve a common mechanism. This hypothesis is supported by subsequent studies in which we demonstrate that the SCC response to anti-IgE is reduced from 17.5 ± 4 to 9.5 ± 3 μA/cm² by capsaicin pretreatment (100 μM for 30 min; n = 5; P < 0.05).

Tachykinin involvement in stress-induced changes in secretion. Tachykinins have been implicated in motility disorders in an animal model of stress (21). Because tachykinins are also potent secretagogues, we tested the hypothesis that their control of the colonic epithelium may be altered in this model. Thus the effect of stress on the response to stimulation of either NK1 or NK3 receptors was investigated. The maximal response and sensitivity to Sar-SP was unaltered by
stress; however, the response to senktide was significantly increased (Table 3).

**DISCUSSION**

Tachykinins have previously been shown to be potent secretagogues in the rat colon (10). The present data extend this observation by confirming the presence of the different tachykinin receptor subtypes, by establishing their physiological role and by suggesting possible pathological implications. These studies were performed to determine which secretory disorders of the intestine may be treated by tachykinin antagonists such as SR-140333.

**SR-140333 and SR-48968 inhibit NK₁ and NK₂-mediated secretion.** As previously described (10), we have shown that the natural peptides SP and NKA, and their synthetic analogs, evoke a secretory response. The pD₂ values of the synthetic analogs, Sar-SP, β-Ala-NKA, and senktide were similar to those reported in pure NK₁, NK₂, and NK₃ receptor systems (13, 31, 39), suggesting the presence of each of these subtypes in the rat colonic epithelium. We next determined the effect of selective antagonists on the response to SP and NKA. SR-140333 antagonized the response to SP with a pKₓ, similar to reported values (15) in an NK₁ smooth muscle assay. SR-48968 antagonized the response to NKA with a pKₓ of 7.33. This is lower than the range (9.4–9.6) previously reported (14) in the rat, and the reason for this discrepancy remains unclear. These data confirm the presence of NK₁ and NK₂ receptors in the rat colonic mucosa (45). Because the response to SP and to NKA is blocked by NK₁ and NK₂ antagonists, these receptors may play a physiological role in the control of secretion. Moreover, these receptors may be important pathophysiologically as interleukin-1, castor oil, and *C. difficile*-induced secretion is mediated by NK₁ and/or NK₂ receptors (11, 16, 46). Our observation that both SR-140333 and SR-48968 block the response to tachykinin-induced secretion supports their potential therapeutic role. The NK₃ agonist senktide stimulated epithelial ion transport, even though there is little direct evidence for the preferred NK₃ agonist NKB being expressed in the rat periphery (44). For the moment, this receptor remains an orphan receptor in the intestinal tract.

**SR-140333 blocks sensory afferent-induced secretion.** For colonic NK₁- and NK₂-mediated responses to be considered (patho)physiological, it is important to demonstrate release of tachykinins. Data from the present study satisfy this criterion by showing that EFS of enteric nerves and capsaicin activation of sensory afferents both release SP. This is in agreement with observations from guinea pig ileum showing that nerve stimulation releases SP (20) and from rat showing that mRNA encoding for SP is localized to submucosal nerves (43). In the present study, we show that the secretory response to EFS was not blocked by NK₁ or NK₂ receptor antagonists at concentrations shown to be active against agonist-induced activity. In contrast, the response to capsaicin was abolished by SR-140333. This is similar to previously reported observations made in the guinea pig ileum using a different NK₁ antagonist, CP-99994 (27). SR-48968, on the other hand, had no effect on the SCC response to capsaicin. SP is, therefore, released from sensory rather than secretomotor nerves to activate NK₁ receptors. This is supported by findings that the response to SP is blocked by TTX (10) and that NK₁ receptors are expressed on cholinergic fibers (37). However, neuronal SP release appears to represent only a fraction of released SP because TTX was able to reduce capsaicin-induced SP release by only 35%, and, furthermore, the secretory response to capsaicin is largely TTX insensitive (47). This could be explained if capsaicin activates nerve terminals, TTX-insensitive nerves (2), or non-neuronal cells. We favor the third hypothesis because the response to EFS is fully blocked by TTX, and the amount of SP released by EFS is nearly identical to the TTX-sensitive component of the capsaicin response.

**SR-140333 blocked mast cell- but not granulocyte-induced secretion.** Food allergy and parasitic infection are both associated with mastocytosis, culminating in a close apposition of mast cells and sensory fibers (42). Here we show that mast cell stimulation by anti-IgE activates neural pain or motor pain pathways. The secretory response to mast cell-activated SP is mediated by NK₁ and/or NK₂ receptors (13, 31, 39). Our observation that mast cell stimulation by anti-IgE activates neural pain or motor pain pathways. The secretory response to mast cell-activated SP is mediated by NK₁ and/or NK₂ receptors (13, 31, 39).

<table>
<thead>
<tr>
<th>Table 3. Effect of stress on the response to SP, NKA, and senktide on rat colonic short-circuit current response</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Control, n = 14</td>
</tr>
<tr>
<td>Control, n = 12</td>
</tr>
<tr>
<td>pD₂</td>
</tr>
<tr>
<td>Eₘₐₓ, μA/cm²</td>
</tr>
</tbody>
</table>

Values are means ± SE. Colonic mucosal sheets were prepared from control animals and those that had been stressed for 2 h/day for 3 days before experimentation by forelimb restraint. Response curves were constructed over a range of concentrations, and from these, Eₘₐₓ and pD₂ values were calculated. Data were compared to controls using Student's paired t-tests with *P < 0.05 considered significant. SP, substance P; NKA, neurokinin A.
way may represent a target for anti-secretory treatments associated with mastocytosis. Targets could be the VR1 receptor or receptors binding released SP. The latter approach is vindicated by the observation that SR-140333 antagonism of the NK1 receptor reduces the response to mast cell stimulation. These data, therefore, suggest that SR-140333 could represent a treatment for mastocytic secretory disorders. The lack of effect of SR-48968 on the response to capsaicin suggests that NK2 receptors are less important in reducing mast cell-mediated secretion.

Granulocytes as a further source of SP. A further source of SP appears to be the granulocyte, because FMLP stimulation resulted in the release of SP. This was unaffected by TTX, and, therefore, is unlikely to be due to neural release. Instead, our findings are the first to report that intestinal granulocytes may release SP. In this respect, granulocytes are similar to human eosinophils (1) and rat macrophages (23). Whether this is relevant to chronic inflammatory diseases, such as IBD, is unclear because under the present conditions, we show that NK1 receptors are not involved in mediating the secretory response to granulocytic stimulation. We are unable to exclude the possibility that NK2 receptors mediate the secretory response to FMLP. It is curious that stimulation of both granulocytes and mast cells results in SP release and an increase in SCC, yet only the SCC response to mast cell stimulation is blocked by SR-140333. Mast cells have been shown by a number of authors to come into close apposition with afferent fibers (e.g., Ref. 42), explaining why mastocytic SP can evoke an NK1-mediated SCC response. Similar data, however, are not available for granulocytes, and it is not clear whether SP is released in close enough proximity to afferent fibers to cause their activation.

Role of tachykinins in IBS. A third clinical condition in which the tachykinins may play a role is IBS. In the absence of models for IBS, we determined the response to tachykinin agonists following stress, one of the putative contributory factors in the pathophysiology of IBS. We have previously demonstrated changes in 5-hydroxytryptamine receptor pharmacology in an animal model of stress (17). In the present study, we show that stress increased the sensitivity of the colonic epithelium to senktide. This effect was specific to the NK3 receptor, because the response to SP stimulation was unaltered. It was unlikely to be related to receptor expression, because the maximal response was unaltered. It was also unlikely to be related to changes in peptide degradation, because senktide is relatively insensitive to peptidase activity. Although the hypersensitivity described in the present study is relatively small, NKB levels are very low in the gastrointestinal tract. Thus a small change in sensitivity may bring these NKB secretions to a superthreshold concentration, and the observed hypersensitivity may be of biological importance. Further studies are required, however, to better understand this observation. It is generally accepted that stress plays a role in at least certain subsets of people suffering from IBS, and if this is the case, NK3 antagonists may help reduce some of the symptoms of this disorder. It should be noted, however, that stress is generally considered a poor model of IBS per se, and caution should be placed on drawing conclusions of relevance to the clinical stage.

In conclusion, we suggest that mast cells, and to a lesser extent, sensory nerves, release SP, provoking an NK1-mediated secretory response. NK2 receptors are present; however, their (patho)physiological role remains unclear. Although the low level of NKB in healthy intestine precludes conclusions regarding the physiological role of the NK3 receptor, the sensitivity of this receptor is altered after stress, suggesting that it may be relevant under certain pathophysiologic states. The role that NK1 receptors play in mediating the secretory response to mast cell stimulation suggests that antagonists capable of blocking this response, such as SR-140333, may have a therapeutic role in allergic disorders. Further in vivo studies are required that employ relevant disease models to investigate both mechanistic and drug efficacy issues in greater detail. However, the current findings explain the potent antisecretory activity of NK1 antagonism in animal models of diarrhea (46).

The authors thank Dominique Parisy for in vivo manipulations.

REFERENCES


