Cellular pathways mediating tachykinin-evoked secretomotor responses in guinea pig ileum

W. MacNaughton, B. Moore, and S. Vanner. Cellular pathways mediating tachykinin-evoked secretomotor responses in guinea pig ileum. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1127–G1134, 1997.—This study characterized tachykinin-evoked secretomotor responses in vitro in submucosal and mucosal-submucosal preparations of the guinea pig ileum using combined intracellular and Ussing chamber recording techniques. Superfusion of endogenous tachykinins substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) depolarized single submucosal neurons and evoked increases in short-circuit current (Isc) responses in Ussing chamber preparations. The NK1-receptor agonist [Sar9, Met(O2)11]SP blocked 50% of secretomotor responses, whereas the NK2-receptor agonist [Ala4, β-Ala8]NKA-(4–10) had no effect on membrane potential. [Sar3, Met(O2)11]SP and [Ala4, β-Ala8]NKA-(4–10) had no effect on membrane potential. [Sar3, Met(O2)11]SP and [Ala4, β-Ala8]NKA-(4–10) had no effect on membrane potential.

Recent studies have shown that these fibers are capsaicin-sensitive neurons projecting to the submucosal plexus (7). SP immunoreactive enteric sensory nerves (5, 7) have shown that electrical stimulation of axonal projections from the myenteric plexus evokes a slow excitatory postsynaptic potential (4, 19). Consequently, although SP has been shown to evoke important secretomotor responses in the small intestine, further study is needed to clarify the role of other endogenous tachykinin and the receptor subtype(s) that mediate tachykinin-evoked secretion.

The recent development of more selective tachykinin agonists and antagonists has enabled a more detailed characterization of tachykinin-evoked responses (20, 31). These pharmacological tools also provide an opportunity to examine cellular sources of endogenous tachykinins. Within the intestine, neurons are the principal source of tachykinins (11). Immunohistochemical studies employing antibodies directed against SP or a related tachykinin have demonstrated the presence of immunoreactivity in both intrinsic enteric and extrinsic sensory nerves (5, 7). SP-immunoreactive enteric neurons have been localized within the myenteric plexus with axonal projections to submucosal neurons in the submucosal plexus (5). A role for SP as a putative neurotransmitter has been further supported by electrophysiological studies in the submucosal plexus that have shown that electrical stimulation of axonal projections in fiber tracts, which include projections from the myenteric plexus, evokes a slow excitatory postsynaptic potential (EPSP), and the exogenous application of SP can mimic the slow EPSP (23, 24). SP immunoreactivity is also found in nerve terminals of extrinsic sensory nerves projecting to the submucosal plexus (7).

Recent studies have shown that these fibers are capsaicin sensitive (8, 27) and can be selectively activated by SP at NK2 receptors. Within the enteric nervous system both NK1 and NK2 receptors have been identified (1, 11, 15, 19, 21, 26). These receptor subtypes are not uniformly distributed, however, and appear to be confined to specific organs and subsets of neurons within enteric ganglia (1, 15, 19, 21). In the submucosal plexus, the study of tachykinin-evoked secretion has focused predominantly on the actions of SP (4, 10, 13, 19, 23), but a systematic study of the tachykinin family and their receptors has not been conducted. It has been suggested that the NK3 receptor mediates intestinal ion transport because the actions of SP were partially blocked by the NK3-receptor antagonist CP-96,345 (19). Interpretation of these findings, however, has since proven problematic because micromolar concentrations of this antagonist are now recognized to result in nonselective inhibition, including blockade of NK3 receptors (11, 25). Furthermore, in other studies, activation of submucosal secretomotor neurons by SP was not inhibited by the SP analog [d-Arg1, d-Pro2, d-Trp7,9, Leu11]SP (10). However, peptide analog antagonists have also been proven to lack specificity and potency (10, 11, 20, 28, 31). Consequently, although SP has been shown to evoke important secretomotor responses in the small intestine, further study is needed to clarify the role of other endogenous tachykinins and the receptor subtype(s) that mediate tachykinin-evoked secretion.
this neurochemical probe. Capsaicin-evoked stimulation of these nerves results in local release of neurotransmitter(s) from nerve terminals within the submucosal plexus, which in turn activates submucosal secretomotor neurons (27). These fibers have been shown to release SP or a related tachykinin in some neuronal circuits, but in others calcitonin gene-related peptide (CGRP) appears to be the principal neurotransmitter (8). Together, these data imply that both intrinsic and extrinsic nerves may activate this submucosal secretomotor pathway, but direct evidence remains to be shown.

The aim of this study was to first systematically characterize the actions of endogenous tachykinins that evoke intestinal ion secretion and to establish the neurokinin receptor subtypes that mediate these actions. Subsequent studies examined potential sources of endogenous tachykinin(s) that activate the defined neurokinin receptors in this secretomotor reflex, using selective neurokinin antagonists. A combined approach of intracellular recording and Ussing chamber studies was used to enable cellular properties to be studied in a functionally defined secretomotor pathway.

MATERIALS AND METHODS

Experiments were conducted on in vitro submucosal and mucosal-submucosal preparations from the guinea pig ileum using intracellular and Ussing chamber recording techniques. Approval of the Animal Care Committees of Queen’s University and the University of Ottawa was obtained.

Intracellular Recording

Guinea pigs (125–175 g) were rendered immediately unconscious by a blow to the head and were killed by decapitation. In vitro submucosal preparations were dissected from the ileum, as previously described (22, 26). Preparations were continuously perfused in a 0.5-ml organ bath with a physiological saline solution containing (in mM) 126 NaCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.5 CaCl2, 5 KCl, 25 NaHCO3, and 11 glucose gassed with 95% O2-5% CO2 and maintained at 35–36°C. All drugs were added to the bath by superfusion. 

Recordings were obtained from submucosal neurons with intracellular electrodes filled with 2 M KCl (60–120 MΩ), as previously described (22). Membrane potential was measured with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Fiber tracts were stimulated with small bipolar tungsten electrodes (20 Hz, pulse duration 0.7 ms, train duration 400–500 ms).

Ussing Chamber Recordings

Guinea pigs (200–250 g) were killed by lethal injection of pentobarbital sodium, and in vitro mucosal-submucosal preparations were dissected from the ileum as previously described (27). Preparations were mounted in standard Ussing chambers and bathed with a physiological saline solution containing (in mM) 115 NaCl, 2 KH2PO4, 2.4 MgCl2, 1.3 CaCl2, 8 KCl, and 25 NaHCO3. The mucosal buffer contained 10 mM mannitol and the serosal buffer contained 10 mM glucose. Solutions were gassed with 95% O2-5% CO2 and maintained at 37°C. All drugs were added to the serosal side of the tissue after a 20-min equilibration period.

Tissue responses were recorded by clamping the potential difference across the tissue to 0 mV by applying a short-circuit current (Isc) with a voltage-clamp apparatus (DCV-1000, World Precision Instruments, Sarasota, FL). Isc was recorded as an indicator of net active electrolyte transport across the tissue. Electrical field stimulation (EFS; 100 V, pulse duration 500 µs, 25 Hz, 3 s) was delivered with a dual-impedance stimulator (Harvard Instruments). Baseline Isc was reestablished between different applications of EFS or drugs.

Drugs

The following drugs were used: SP, NKA, NKB, [Sar9, Met(O2)11]SP, [Ala5, β-Ala5]NKA-4(—10), and senktide (from Bachem); CP-99,994 and CP-96,345 (gifts from Pfizer); SR-142801 (a gift from Sanofi Recherche); tetrodotoxin (TTX), capsaicin, 5-hydroxytryptamine (5-HT), atropine, and histamine (from Sigma); thiopental and LY-53857 (Research Biochemicals); BRL-43694 (a gift from SmithKline Beecham); and SDZ-205-557 (a gift from Sandoz). Capsaicin was dissolved in Tween-80, alcohol, and saline (10:10:80, vol/vol/vol). SR-142801 was dissolved in a 100% ethanol 10 mM stock solution. [Ala5, β-Ala5]NKA-4(—10) was dissolved in 10% acetic acid stock solution, and NKA was dissolved in 2.5% acetic acid in Ussing chamber studies. All other peptides were dissolved in distilled water. Vehicle effects were negligible and any minimal effects on Isc were subtracted from agonist-evoked changes in Isc in Ussing chamber studies.

Statistics

Data are expressed as means ± SE. Comparisons between matched pairs of tissues in Ussing chamber studies were made using Student’s t-test for paired data and repeated-measures analysis of variance where appropriate. An associated P value of <0.05 was considered to be significant.

RESULTS

Tachykinin-Evoked Depolarizations of Submucosal Neurons

Intracellular recordings were obtained from submucosal neurons (n = 75) that had resting membrane potentials ranging from −48 to −65 mV. Fiber tract stimulation elicited slow excitatory postsynaptic potentials (EPSPs; >5 mV) in all neurons. Stable impalements were typically obtained for >1 h, enabling multiple agonists to be examined on a single neuron. Agonists were applied in a noncumulative fashion, and 10-min washouts were allowed between applications to prevent desensitization.

Effects of SP, NKA, and NKB on submucosal neurons. Superfusion of SP [1–100 nM; 50% effective concentration (EC50 = 6 nM)] depolarized all neurons (Fig. 1; n = 4) and evoked action potential discharge, as previously described (16, 22, 24). NKA (30–300 nM; EC50 = 80 nM) and NKB (30–300 nM; EC50 = 90 nM) superfusion also depolarized submucosal neurons and activated action potential discharge (Fig. 1; n = 4).

Effects of selective tachykinin agonists on submucosal neurons. All neurons examined (n = 35) were depolarized by superfusion of the NK1 agonist [Sar9, Met(O2)11]SP (Fig. 2). Noncumulative applications (n = 4) evoked concentration-dependent depolarizations (1–50 nM; EC50 = 2 nM). Depolarizations were associated with an increase in membrane resistance (mean = 50%, n = 7) and typically evoked action potential discharge (Fig. 2).
with bursts of fast EPSPs. The NK3 agonist senktide (3–100 nM; EC50 = 520 nM) depolarized 25 of 55 cells examined (Fig. 2). Senktide (30 nM) increased membrane resistance (mean = 47%, n = 3). Maximal effective concentrations (EC100) (30–100 nM) evoked mean maximal depolarization of 8 mV with a range of 5–16 mV. The effects of both senktide and the NK1 agonist [Sar9,Met(O2)11]SP were examined in 21 cells. In 11 cells, the NK1 agonist (30 nM) depolarized the neuron (mean = 12 mV), but senktide (30–100 nM) had no effect on membrane potential. The NK2 agonist [Ala5,β-Ala8]NKA-(4–10) (100–300 nM; n = 4) had no effect on membrane potential. The effects of both senktide (100 nM) and [Sar9,Met(O2)11]SP (30 nM) were also examined in the presence and absence of TTX to determine whether the depolarizations resulted solely from activation of receptors on the postsynaptic membrane. TTX (1 µM) blocked all synaptic potentials evoked by electrical stimulation of fiber tracts (n = 8). Compared with controls, TTX had no effect on depolarizations evoked by [Sar9,Met(O2)11]SP (3.7 ± 2.6 vs. 15.0 ± 2.9 mV, respectively, n = 3) or senktide (5.7 ± 0.5 vs. 5.3 ± 0.3 mV, respectively, n = 3).

Effects of NK1 and NK3 antagonists on tachykinin-evoked depolarizations. CP-99,994 (30 nM) completely inhibited maximal depolarizations evoked by SP (30 nM) and caused a parallel shift to the right in the concentration-response curve (Fig. 3A). Maximal depolarizations evoked by [Sar9,Met(O2)11]SP (30 nM) were also completely blocked by CP-99,994 (30 nM; n = 3) (Fig. 3B), but depolarizations evoked by senktide (30 nM; n = 3; mean depolarization 8 vs. 7 mV, respectively) were unaffected. Senktide-evoked depolarizations (30–100 nM) were completely blocked by the selective NK3 antagonist SR-142801 (100 nM; n = 3) (Fig. 3B). SP-evoked depolarizations (10 nM) were unaffected by the NK1 antagonist (100 nM; mean depolarization 10 vs. 9 mV, respectively; n = 3).

**Tachykinin-Evoked Secretomotor Responses**

The effects of tachykinins on Isc recorded from submucosal-mucosal preparations in Ussing chambers were examined to extend the findings of the intracellular recording studies to a functionally defined set of neurons. Previous studies (19) have shown that SP-evoked increases in Isc result almost entirely from activation of submucosal secretomotor neurons. These secretomotor neurons consist of two populations, cholinergic and noncholinergic neurons.

![Fig. 1. Endogenous tachykinins substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) depolarize submucosal neurons. A: representative intracellular recordings from single submucosal neurons comparing responses to superfusion of SP, NKA, and NKB. Resting membrane potential (Vm) of each cell was (left to right) −63, −63, and −59 mV, respectively. B: summary of concentration-response for SP, NKA, and NKB. Values are means ± SE for ≥4 cells.](http://ajpgi.physiology.org/)

![Fig. 2. NK1- and NK3-receptor agonists depolarize submucosal neurons. A: representative traces of intracellular recordings from single submucosal neurons comparing responses to superfusion of NK1 agonist [Sar9,Met(O2)11]SP, NK2 agonist [Ala5,β-Ala8]NKA-(4–10), and NK3 agonist senktide. The NK1 agonist (30 nM) evokes a large depolarization and discharge of action potentials. The NK2 agonist has no effect on membrane potential. Resting Vm of each cell was (left to right) −63, −55, and −60 mV, respectively. B: summary of concentration-response of NK receptor agonists. Values are means ± SE of ≥4 recordings.](http://ajpgi.physiology.org/)
Effect of NK_1-receptor antagonist CP-99,994 and NK_3-receptor antagonist SR-142801 on agonist-evoked depolarizations of submucosal neurons. A: CP-99,994 (30 nM) causes parallel shift to right in SP dose-response curve. Values are means ± SE of ≥3 cells. B: representative recordings from single submucosal neurons show that CP-99,994 (30 nM) and SR-142801 (100 nM) block NK_1- and NK_3-agonist-evoked responses, respectively. In top 3 traces (resting V_m = −52 mV), NK_1 agonist [Sar^9,Met(O_2)^11]SP is superfused for duration of black bar. Depolarization evoked by NK_1 agonist (top left) is completely blocked when agonist is reapplied in presence of CP-99,994 (top middle). After 10-min washout, reaplication of agonist evokes response similar to control (top right). In bottom 3 traces (resting V_m = −56 mV), NK_3 agonist senktide (bottom left) is completely blocked when agonist is reapplied with SR-142801 (bottom middle). After prolonged 45-min washout, reaplication of senktide evokes only a minimal response (bottom right).

SP-, NKA-, and NKB-evoked changes in I_{sc}. SP (1–100 nM; n = 5) evoked concentration-dependent changes in I_{sc} (Fig. 4), as previously reported (10, 18, 19). NKA (1–100 nM; n = 5) and NKB (1–100 nM; n = 5) also caused a concentration-dependent increase in I_{sc}. Repeat application of SP and NKA up to 100 nM did not desensitize, but reaplication of NKB (10–100 nM) resulted in a significant attenuation of the response.

Changes in I_{sc} evoked by selective activation of tachykinin receptors. The NK_1 agonist [Sar^9,Met(O_2)^11]SP (n = 5) and the NK_3 agonist senktide (0.1–100 nM; n = 5) evoked concentration-dependent increases in I_{sc} (Fig. 5). The NK_2 agonist [Ala^5,β-Ala^8]NKA-(4–10) (10–100 nM) did not significantly alter I_{sc} compared with baseline (Fig. 5). The NK_1 antagonist CP-99,994 (50 nM) completely blocked maximal I_{sc} responses evoked by [Sar^9,Met(O_2)^11]SP (30 nM; n = 4) but had no effect on I_{sc} responses evoked by senktide (1 nM, mean I_{sc} = 88 ± 31 vs. 54 ± 11 µA/cm^2, respectively) compared with control responses (Fig. 5B). Maximal senktide-evoked changes in I_{sc} (100 nM) were completely blocked by the NK_3 antagonist SR-142801 (100 nM), but this antagonist had no effect on submaximal responses evoked by [Sar^9,Met(O_2)^11]SP compared with controls (10 nM; 114 ± 29 vs. 84 ± 24 µA/cm^2, respectively, n = 5) (Fig. 5B). EFS-evoked changes in I_{sc} were not altered by CP-99,994 (50 nM; n = 5) or SR-142801 (100 nM; n = 6).

Effect of TTX on tachykinin-evoked changes in I_{sc}. The effects of TTX on tachykinin-evoked increases in I_{sc} were examined to determine whether these increases resulted from activation of receptors on submucosal secretomotor neurons (TTX sensitive) and/or the enterocyte (TTX insensitive). Pretreatment with TTX (1 µM) inhibited mean NKA-evoked I_{sc} by 91% (100 nM; n = 8) and [Sar^9,Met(O_2)^11]SP-evoked I_{sc} by 91% (100 nM; n = 7) and completely blocked I_{sc} responses evoked by SP (100 nM; n = 3), senktide (100 nM; n = 5), and NKB (100 nM; n = 5) (Fig. 6). EFS-evoked changes in I_{sc} were completely blocked by TTX (1 µM), but responses to carbachol (500 nM) alone were not significantly different from those obtained in the presence of TTX (P = 0.093).

Effect of NK_1 and NK_3 Antagonists on Intrinsic and Extrinsic Nerve-Evoked Responses

Capsaicin-sensitive extrinsic sensory nerves. Our previous studies have demonstrated that capsaicin-sensitive nerves evoke a biphasic change in I_{sc} in submucosal-mucosal preparations (27). In these studies, 200 nM capsaicin was shown to selectively activate extrinsic sensory nerves. These nerves release neurotransmitter(s) from nerve terminals within the submucosal
plexus, which in turn activates submucosal secretomotor neurons. In the present study, the possibility that these neurotransmitters might include one or more tachykinins acting at either NK1 and/or NK3 receptors was examined. Capsaicin (200 nM) evoked typical biphasic increases in $I_{sc}$ (mean phase 1 response 41 ± 6 µA/cm², mean phase 2 response 82 ± 18 µA/cm²; n = 10), as previously described (27). The larger phase 2 responses were inhibited 53% ($P < 0.05; n = 5$) by the nonpeptide NK1 antagonist CP-99,994 (50 nM) compared with controls (Fig. 7). Phase 1 responses were unaffected. The nonpeptide NK1 antagonist CP-96,345 (200 nM) also significantly reduced (71%) the second phase of the response (107 ± 20 vs. 31 ± 10 µA/cm², control vs. CP-96,345 treated, respectively, $P < 0.01; n = 5$) but had no effect on the first phase. Responses to EFS (84 ± 18 vs. 72 ± 12 µA/cm², control vs. drug, respectively) and carbachol (1 µM) (130 ± 31 vs. 178 ± 29 µA/cm², control vs. drug, respectively) were not affected by either NK1 antagonist. In contrast, the NK3 antagonist SR-124801 (100 nM), which completely blocked $I_{sc}$ responses evoked by the NK3 agonist senktide (see above), had no effect on capsaicin-evoked changes in $I_{sc}$ (mean phase 1 $I_{sc} = 59 ± 10$ vs. 42 ± 6 µA/cm², $P = 0.116$, control vs. antagonist; mean phase 2 $I_{sc} = 106 ± 15$ vs. 119 ± 26 µA/cm², control vs. antagonist, respectively; n = 6) (Fig. 7A).

Previous studies suggest that, in some tissues, SP or a related tachykinin degranulates mast cells through a receptor-independent mechanism (11). Mast cell mediators histamine and 5-HT evoke a secretory response through activation of submucosal secretomotor neurons (2, 30). Consequently, we examined the possibility that the capsaicin-evoked increase in $I_{sc}$ may in part be mediated by tachykinin-evoked mast cell degranulation. Histamine (1 µM–1 mM; EC100 = 100 µM, n = 6) and 5-HT (0.1–100 µM; EC100 = 100 µM; n = 6) evoked a dose-dependent increase in $I_{sc}$, as previously described (4, 9). Antagonist concentrations were derived from our studies of the H1 antagonist pyrilamine (50 nM), which completely blocked histamine (10 µM)-evoked changes in $I_{sc}$ (n = 5), and from previous studies of 5-HT secretomotor responses (9). In the present study, antagonists to H1 (pyrilamine, 50 nM; n = 8), H2 (cimetidine, 10 µM; n = 7), and H3 (thioperamide, 300 µM) were used. Bar graphs show means ± SE of 5 preparations. **$P < 0.01$.

Fig. 5. Tachykinin-evoked increases in $I_{sc}$ are mediated by NK1 and NK3 receptors. A: NK1 agonist [Sar9-Met(O2)11]SP and NK3 agonist senktide evoke a concentration-dependent increase in $I_{sc}$. NK2 agonist [Ala5, β-Ala8]NKA-(4—10) has virtually no effect. Values are means ± SE of 5 preparations. B: comparison of NK agonist and electrical field stimulation (EFS)-evoked increases in $I_{sc}$ in presence (filled bars) and absence (open bars) of NK1 antagonist CP-99,994 (CP; 100 nM) and NK3 antagonist SR-124801 (SR; 100 nM). CP-99,994 (100 nM) completely blocks increases in $I_{sc}$ evoked by [Sar9-Met(O2)11]SP (30 nM) but has no effect on responses evoked by EFS. Similarly, SR-124801 (100 nM) completely blocks senktide (100 nM)-evoked increases in $I_{sc}$ but has no effect on EFS-evoked responses. Bars represent means ± SE of 5 preparations. **$P < 0.01$.

Fig. 6. NK1- and NK3-receptor-mediated increases in $I_{sc}$ result predominantly from activation of receptors located on submucosal secretomotor neurons. Left to right: SP, NKA, NKB, NK1 agonist, NK3 agonist, carbachol, and EFS responses were compared in presence and absence (control) of tetrodotoxin (TTX). TTX (1 µM) blocks neurally evoked secretion (EFS) but has no effect on carbachol responses mediated by muscarinic receptors directly on the enterocyte. Bars represent means ± SE of 5 experiments. **$P < 0.01$. 
CGRP-(8—37) (2 µM) have no effect on either phase of capsaicin—
comparison of effect of NK1 (top traces) and NK3 antagonists (bottom traces) on slow excitatory postsynaptic potentials (EPSPs) evoked by fiber tract stimulation of axons of intrinsic enteric neurons. Neither antagonist has any effect on amplitude or time course of slow EPSP compared with control. Resting \( V_m \) values were \(-55 \) (top traces) and \(-52 \) mV (bottom traces).

CGRP is a putative neurotransmitter released from capsaicin-sensitive nerves in some neural circuits (8, 26). In the present study, superfusion of human CGRP-II (200 nM – 2 µM) had no effect on membrane potential in intracellular recording studies (mean change in membrane potential compared with control responses 0.5 ± 0.4 mV; \( n = 7 \)), and CGRP-II (100 nM) did not alter \( I_{sc} \) (mean change in \( I_{sc} \) compared with control responses 0.0 ± 0 µA/cm²; \( n = 3 \)) in Ussing chamber studies. Pretreatment with supramaximal concentrations (26) of the CGRP antagonist CGRP-(8—37) (2 µM) did not affect responses to capsaicin (200 nM), EFS, or carbachol (1 µM) (\( n = 5 \) for each group).

Intrinsic enteric neurons. The effect of the NK₁ and NK₃ antagonists on nerve-evoked slow EPSPs was examined to determine if the release of tachykinins acting at these receptors mediated this synaptic potential. Previous studies have shown that slow EPSPs in the submucosal plexus are resistant to cholinergic blockade (22, 23). These studies were confined to cells in which the nerve-evoked slow EPSP was reproducible in amplitude and duration in control experiments. Slow EPSPs were not inhibited (Fig. 7B) by CP-99,994 (100 nM, \( n = 7 \); control mean amplitude 11 ± 2 mV and mean half-duration 6 ± 0.06 s vs. CP-99,994 mean amplitude 12 ± 2 mV and mean half-duration 7 ± 0.4 s) or SR-142801 (100 nM, \( n = 6 \); control mean amplitude 11 ± 2 mV and mean half-duration 7 ± 0.6 s vs. SR-142801 mean amplitude 11 ± 2 mV and mean half-duration 7 ± 0.9 s).

DISCUSSION

This study characterized tachykinin responses in a defined neural secretory pathway in the submucosal plexus. This pathway consists of cholinergic and noncholinergic secretomotor neurons that innervate mucosal enterocytes in the small intestine (4). Activation of this pathway stimulates chloride secretion from intestinal enterocytes, which results in the concomitant passive movement of water into the lumen. In the present study, the results of intracellular recording from single submucosal neurons demonstrate that both NK₁ and NK₃ receptors are found on submucosal neurons. Ussing chamber studies provided direct evidence that these receptors are located on submucosal secretomotor neurons, which in turn stimulate mucosal enterocytes. Capsaicin-sensitive nerves release endogenous tachykinins, which activate NK₁ receptors in this pathway as well as other as yet unidentified neurotransmitter(s).

Systematic studies were conducted to identify NK₁- and NK₃-receptor subtypes and to localize these receptors to submucosal secretomotor neurons. In these studies, the NK₁ agonist [Sar⁹,Met(O₂)₁¹]SP and the NK₃ agonist senktide activated single submucosal neurons during intracellular recording. Senktide, however, did not depolarize all neurons examined, which may suggest that NK₃ receptors are not homogeneously distributed among submucosal neurons and/or that the receptor density varies between individual neurons. Nonetheless, both NK₁ and NK₃ agonists evoked large secretory responses in Ussing chamber studies, demonstrating that both receptors mediate functional responses. The selectivity of these agonists was demonstrated in both intracellular and Ussing chamber studies in which NK₁ and NK₃ agonist responses were selec-
In the present study, capsaicin-evoked changes in extrinsic nerve fibers, which innervate both cholinergic the submucosal plexus, capsaicin selectively activates the predominant neurotransmitter (8). In some tissues, but in other neural circuits CGRP appears to play little role. The possibility that intrinsic enteric neurons and extrinsic capsaicin-sensitive nerves release tachykinins, which could activate the NK1 and NK3 receptors described in this study (4, 16, 21, 24). The extrinsic capsaicin-sensitive nerves are known to release SP or a related tachykinin in some tissues, but in other neural circuits CGRP appears to be the predominant neurotransmitter (8). In the submucosal plexus, capsaicin selectively activates extrinsic nerve fibers, which innervate both cholineric and noncholineric submucosal secretomotor neurons (27). In the present study, capsaicin-evoked changes in $I_{\text{sc}}$ were significantly blocked by NK1 antagonists but not by CGRP antagonists. These data demonstrate that capsaicin-sensitive nerves release tachykinin(s), which activates this neural secretatory pathway, and that CGRP appears to play little role.

There is considerable indirect evidence to suggest that both intrinsic enteric neurons and extrinsic capsaicin-sensitive nerves release tachykinins, which could activate the NK1 and NK3 receptors described in this study (4, 16, 21, 24). The extrinsic capsaicin-sensitive nerves are known to release SP or a related tachykinin in some tissues, but in other neural circuits CGRP appears to be the predominant neurotransmitter (8). In the submucosal plexus, capsaicin selectively activates extrinsic nerve fibers, which innervate both cholineric and noncholineric submucosal secretomotor neurons (27). In the present study, capsaicin-evoked changes in $I_{\text{sc}}$ were significantly blocked by NK1 antagonists but not by CGRP antagonists. These data demonstrate that capsaicin-sensitive nerves release tachykinin(s), which activates this neural secretory pathway, and that CGRP appears to play little role. The possibility that intrinsic neurons also released endogenous tachykinins was examined by studying the effects of the tachykinin antagonist CP-99,994 on the slow EPSP. Previous studies (22, 23) have shown that exogenous application of SP can mimic the time course and conductance changes characteristic of the slow EPSP. Furthermore, immunohistochemical studies have demonstrated that SP-immunoreactive fibers project from the myenteric plexus to the submucosal plexus (5) and that after selective lesioning studies (3), which destroyed fibers containing other putative neurotransmitters such as 5-HT, somatostatin, and vasoactive intestinal peptide, but not SP, the electrically evoked slow EPSP appeared unchanged. Despite these suggestive data, the present study failed to find evidence that the slow EPSP was mediated by the release of tachykinins acting at either the NK1 or NK3 receptor. These data may imply that tachykinins are not the predominant mediator of the slow EPSP but do not preclude that tachykinins mediate some EPSPs in the submucosal plexus. Indeed, it has been suggested (23, 24) that it is unlikely that the slow EPSP results from the release of neurotransmitter from one or a few discrete fibers, but rather results from the release of neurotransmitter from multiple fibers containing a number of different neuropeptides. Therefore, the removal of one peptide input might not be expected to markedly alter the amplitude or time course of the nerve-evoked slow EPSP (23, 24).

The possibility that tachykinin-evoked secretion mediated by capsaicin-sensitive nerves involved, at least in part, a mast cell-dependent pathway was also examined in this study. Nerve-mast cell interactions have been widely reported (2, 4). Capsaicin-sensitive nerves are thought to be one of the principal neural networks innervating the mast cell, leading to release of histamine and possibly other mediators such as 5-HT, prostaglandins, and bradykinin. In studies in which SP has been shown to degranulate mast cells, this action appears to involve a receptor-independent mechanism (30). Therefore, in the present study, evidence for a role of a mast cell-dependent pathway was sought using antagonists of the putative mast cell mediators, histamine and 5-HT. However, these antagonists had no effect on the capsaicin-evoked response, supporting the findings of previous studies, which show that exogenous application of SP does not appear to degranulate mast cells in this tissue (10, 19), and extending this observation to include other related tachykinins or putative neurotransmitters of the capsaicin-evoked response. Further studies are needed to determine whether this finding also applies to inflamed tissue because many studies in which nerve-mast cell connections are described (14, 29) involve allergic or inflammation models.

Activation of the NK1 and NK3 receptors described in this study could play a significant role in modulating the homeostatic regulation of water and electrolyte transport in the intestine. In addition to this physiological role, there is considerable evidence that tachykinins play multiple roles in the expression of the inflammatory response. These actions may reflect exaggerated actions of tachykinins because there is evidence that during inflammation the expression and release of tachykinins from capsaicin-sensitive nerves is increased and upregulation of tachykinin receptors may occur (6, 12). Not surprisingly, tachykinin antagonists have shown considerable potential in the treatment of inflammatory responses (31). Diarrhea is a prominent feature of intestinal inflammatory responses, and, consequently, the NK1 and NK3 receptors described in this study could provide targets for altering this pathophysiological response.

This work was supported by the Medical Research Council of Canada (MRCC; S. Vanner) and the Crohn’s and Colitis Foundation of Canada (W. MacNaughton). B. Moore is a recipient of the MRC-Canadian Association of Gastroenterology-Janssen Research Fellowship.
REFERENCES


