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Effects of substance P on identified neurons of the rat dorsal motor nucleus of the vagus

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Abstract

Previous evidence suggests that substance P (SP) activates subpopulations of neurons within the dorsal motor nucleus of the vagus (DMV). In this study we aimed at identifying these subpopulations in relation to their gastrointestinal projection organs or vagal branches and characterizing pharmacologically the SP response. Using whole cell patch-clamp recordings from identified gastrointestinal-projecting vagal motoneurons, we found that SP induced an inward current in all neuronal groups except for cecum-projecting cells. The lowest percentage of SP-responding neurons was found in fundus-projecting cells, where SP also had a concentration-response curve that was shifted to the left ($P < 0.05$). Independently from the projections, the SP response was reduced by sendide and MEN 10,376 and mimicked by a combination of [Sar⁹-Met(O₂)¹¹]SP and α -neurokinin. SP and α -neurokinin also increased the frequency, but not the amplitude, of postsynaptic currents. In conclusion, we demonstrated that SP induces both pre- and postsynaptic effects on DMV neurons via activation of neurokinin NK₁ and NK₂ receptors. The magnitude of the SP response was correlated to the peripheral target organ.

Keywords

neurokinin; electrophysiology; brain stem; gastrointestinal tract

Preganglionic parasympathetic motor neurons located in the dorsal motor nucleus of the vagus (DMV) innervate the subdiaphragmatic viscera (7). The DMV has a columnar organization in which the various subdiaphragmatic vagal branches are arranged in a mediolateral fashion (6). The lateral tip of the DMV has been shown to contain neurons that project to the intestine via the celiac branches of the vagus (3,30). The medial portions of the DMV contain preganglionic neurons that project to the gastric regions and to the proximal duodenum via the gastric branches of the vagus (3).

The DMV receives input from various central nervous system centers including the nucleus of the tractus solitarius (NTS) and the medullary raphe nuclei. Substance P (SP), along with serotonin and thyrotropin-releasing hormone, are contained within projections from the raphe nuclei to the DMV (14). SP-immunoreactive (SP-IR) fibers have been observed throughout both the NTS and the DMV (5). SP is a member of the neurokinin family of peptides and binds preferentially to the neurokinin 1 (NK₁) receptor, whereas neurokinin A

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and neurokinin B bind to the neurokinin 2 (NK₂) and 3 (NK₃) receptors, respectively (9). The DMV has been shown to contain all three neurokinin receptors (25). In the DMV, SP-IR-positive nerve fibers surround immunoreactive NK₁ receptor-positive (NK1R-IR) cell bodies. The NK1R-IR cell bodies are restricted to a subpopulation of gastric projection neurons (20,21). Most NK1R-IR neurons are found to be located rostral to the obex (20,21) and in the lateral half of the DMV (2). From the nodose ganglia, SP-IR-positive vagal afferents convey sensory information from the pyloric region of the stomach (10,22,36). Vagotomy has been shown to reduce the level of SP binding in the DMV but not the NTS (24), which suggests that SP receptors are localized on preganglionic motor neurons that project to the subdiaphragmatic viscera (36).

Microinjection of SP in the DMV (33) and nucleus raphe obscurus (nROb) decreased tonic gastric pressure and gastric phasic activity (18), possibly via a centrally mediated nitric oxide mechanism (18). Recent in vivo studies have suggested that the decrease in gastric tone and motility obtained on microinjection of SP in the DMV is mediated via activation of NK₁ receptors only (19). Conversely, in vitro electrophysiological studies have reported that subpopulations of DMV neurons can be excited by both NK₁ and NK₂ receptor activation (26). Given that the DMV neurons project to both subdiaphragmatic viscera and to other brain stem areas, we questioned whether the in vivo vs. in vitro pharmacological observations were due to nonselective sampling of DMV neurons in the electrophysiological preparations.

We have recently developed a technique that allows us to selectively label the peripheral gastrointestinal projections of DMV neurons and perform electrophysiological recordings on the identified neurons (4). Thus the aims of this study were 1) to identify the subpopulations of gastrointestinal-projecting DMV neurons responsive to SP, 2) to investigate whether the response to SP was correlated to a specific target organ or a particular vagal branch, and 3) to characterize pharmacologically the SP-induced response.

METHODS

Retrograde tracing

In accordance with veterinary guidelines, 12-day-old Sprague-Dawley rats of either sex were anesthetized with a 6% solution of 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane) bubbled with air (400–600 ml/min). During surgery, the head of the rat was placed in a custom-made anesthetic chamber through which the halothane mixture was administered. The depth of anesthesia (foot pinch withdrawal reflex) was monitored throughout surgery. The abdominal and thoracic areas were shaved and cleaned with 70% ethanol, and a laparotomy was performed. Crystals of the retrograde tracer 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were applied to the major curvature of the gastric fundus and corpus, antrum/pylorus, or intestinal duodenum (antimesenteric border at the level of the bifurcation of the hepatic and pancreaticoduodenal arteries) or cecum (at the level of the ileocecal junction). Additionally, in some rats, DiI crystals were applied to the anterior gastric branch of the vagus (AGB). To restrict the dye to the application site, DiI was fixed in place with a fast-hardening epoxy compound. The epoxy compound was allowed to dry (3–5 min) before the area was examined visually to ensure that the dye was restricted to the organ. The DiI-treated area was then washed with warm sterile saline solution, the excess solution was blotted with cotton tips, and the wound was sutured with 5-0 silk. The rat was allowed to recover under a heat lamp until normal activity had returned. Then the animal was returned to the home cage for 10–15 days. In six experiments in which DiI was applied to the cecum, the right cervical and subdiaphragmatic anterior branch of the vagus were cut 3 or 7 days before experimentation. In an additional four experiments in which DiI was applied to the duodenum, the subdiaphragmatic AGB was cut

at the time of surgery and neurons on the left side of the brain slice were examined for response to SP. Lastly, nine experiments were conducted 10–14 days after the placement of DiI crystals on the AGB itself.

Electrophysiology

The method used for the tissue slice preparation has already been described (4,34). Briefly, the rat was placed in a transparent chamber and anesthetized with a mixture of halothane and air (see *Retrograde tracing*). When a deep level of anesthesia was induced (see *Retrograde tracing*), the rat was killed by severing the major blood vessels in the chest in accordance with veterinary guidelines. The brain stem was removed and placed in oxygenated physiological Krebs solution at 4°C (see *Solution composition*). With the use of a vibratome, five to six coronal slices (200- μ m thick) containing the DMV were cut. The slices were incubated for at least 1 h in oxygenated physiological Krebs solution at $35 \pm 1^\circ\text{C}$ until use. A single brain slice was retained by a nylon mesh in a custom-made perfusion chamber (volume 500 μ l). The chamber was warmed and maintained at 35°C while oxygenated physiological Krebs' solution was perfused at a rate of 2.5 ml/min.

Before electrophysiological recordings, retrogradely labeled DMV neurons were identified using a Nikon E600-FS microscope equipped with TRITC epifluorescent filters. Carbocyanine dyes (such as DiI) do not cause adverse effects with the brief illuminations used for neuronal identification (4,12,28). Once a labeled cell was identified, the neuron's location was confirmed under bright-field illumination using DIC (Nomarski) optics.

Whole cell recordings were performed with patch pipettes (5–8 M Ω) filled with potassium gluconate intracellular solution (see *Solution composition*) by using a single-electrode voltage-clamp amplifier (Axoclamp 2B; Axon Instruments, Foster City, CA). All experiments were conducted in voltage-clamp configuration at a holding potential of -50 mV. All recordings were made from DMV neurons that were identified as labeled by the presence of DiI (4). Data were filtered at 2 kHz, digitized via a Digidata 1200C interface (Axon), and acquired and stored on a PC using pClamp8 software (Axon). Only those recordings having a series resistance (i.e., pipette + access resistance) <15 M Ω were used. Correction was made for the tip potential. Whole cell data were analyzed with pClamp8 software, and miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs) were analyzed with the Mini Analysis program (Synaptosoft, Leonia, NJ).

Drugs were applied to the bath via a series of manually operated valves, and superfusion was continued until a plateau effect was observed. To determine whether or not a given neuron would respond to SP, all experiments began with a 1 μM SP superfusion. When experiments were performed to determine concentration-response curves, the subsequent superfusion of different concentrations of SP was randomized and a washout period of at least 15 min between drug treatments was allowed.

Statistical analysis

Results are expressed as means $\pm$ SE. The SP concentration that produced the one-half maximum drug response (EC_{50}) was estimated using a third-order polynomial regression (31). Group differences in the EC_{50} were evaluated by an ANOVA by using Statistica (Statsoft, Tulsa, OK), with the projection region being the independent variable and the EC_{50} values being the dependent variable. Group differences in the frequency of the response to SP per projection were determined by χ^2 analysis.

The relationship between the SP concentration and the neuronal response was evaluated by regression analysis, with the dependent variable being the SP-mediated inward current and 1) the log of the SP concentration and 2) the different projection neurons within the DMV

[using dummy variables to define cell types (17)] being the independent variables. The relationship between the SP maximum current ($I_{\max}$) and the DMV projection was evaluated by ANOVA, with the dependent variable being the log of the SP-mediated inward current and the independent variable being the different projection neurons within the DMV. Significance was defined as $P < 0.05$.

Solution composition

Krebs solution was composed of (in mM): 126 NaCl, 25 NaHCO₃, 2.5 KCl, 1.2 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, and 11 dextrose, maintained at pH 7.4 by bubbling with O₂-CO₂ (95%-5%). Intracellular solution was composed of (in mM): 128 K-gluconate, 10 KCl, 0.3 CaCl₂, 1 MgCl₂, 10 HEPES, 1 EGTA, 2 ATP, and 0.25 GTP, adjusted to pH 7.35 with KOH.

Drugs and chemicals

DiI [DiIC₁₈(3)] was purchased from Molecular Probes (Eugene, OR); SP, [Sar⁹-Met(O₂)¹¹]SP (SM-SP), α -neurokinin (α NK), and SP fragment [Tyr^{6,D}-Phe^{7,D}-His⁹]-Fragment 6–11 (sendide) were purchased from Sigma (St. Louis, MO), and neurokinin fragment [Tyr^{5,D}-Trp^{6,8,9},Lys¹⁰]-Fragment 4–10 (MEN 10,376) was purchased from Peninsula Labs (Belmont, CA).

RESULTS

Results were acquired from a total of 322 neurons projecting to the various areas of the gastrointestinal tract (see Table 1). The basic characteristics of DMV neurons were similar to those previously reported by this laboratory (4). Briefly, gastric-projecting neurons could be differentiated from intestinal neurons on the basis of a smaller, shorter afterhyperpolarization following a single action potential, a narrower action potential width, and faster and steeper frequency response to current injection (data not shown). Lastly, the gastric-projecting neurons were located in the medial DMV, whereas the intestinal-projecting neurons were located in more lateral portions of the DMV.

SP induced an inward current in identified subpopulations of DMV neurons

DMV neurons were classified as SP responders if perfusion with 1 μ M SP induced an inward current of at least 17.5 pA in amplitude that recovered to baseline levels on washout. In responsive neurons, SP induced a concentration-dependent inward current in distinct subpopulations of DMV neurons (Fig. 1). There were differences with regard to the number of SP responders among the various gastrointestinal projections. Most significant was the fact that none of the cecum-projecting neurons responded to SP (i.e., 0 out of 27); additionally, there were fewer fundusprojecting neurons that responded to SP than either corpus-, antrum/pylorus-, or duodenum-projecting neurons ($P < 0.05$, χ^2 ; $n = 274$; Table 1).

To ascertain whether the SP-induced current was due to activation of postsynaptic receptors only (on the DMV neuronal membrane), we measured the amplitude of the 1 μ M SP-induced inward current in the presence of the synaptic transmission blocker tetrodotoxin (TTX; 0.3 μ M). In seven neurons (4 gastric and 3 intestinal), perfusion with SP induced a 69 ± 10.3 -pA inward current that recovered to baseline on washout. Following a 15-min superfusion with TTX, reperfusion with SP in the presence of TTX induced a 52 ± 9.1 -pA inward current (i.e., 75.9% of control values; $P < 0.05$). There was no difference in the TTX-mediated reduction in the SP response between gastric and intestinal neurons ($P > 0.05$). It should be noted that in two gastric neurons TTX was without effect. The reduction of the SP current by TTX was reversible on washout (not shown). These results suggest that the SP-mediated inward current is, at least in part, indirect in nature.

To test whether the SP-induced current was determined by the end organ or the distinct vagal branch, we conducted the following experiments. First, the retrograde tracer DiI was applied to the AGB [which projects to the stomach and the proximal duodenum (6)]. Ten to fourteen days later, identified DMV neurons were challenged with SP (1 μ M). Four out of nine (i.e., 44%) labeled AGB neurons responded to SP with an inward current. The frequency of AGB responders was not different from the pooled responses of individual groups (Table 1; $P > 0.05$). The magnitude of the 1 μ M SP response in the pooled sample of DMV neurons was similar to the magnitude of the response in labeled AGB neurons ($P > 0.05$; 65 ± 2.8 pA in the pooled sample and 85 ± 22.8 pA in labeled AGB neurons). Second, the retrograde tracer DiI was applied to the duodenum [which is innervated by both gastric and celiac branches of the vagus (6)] and the AGB was sectioned. Ten to fourteen days later, recordings were made from labeled neurons located in the left DMV only. These identified DMV neurons would, therefore, represent motor neurons that project to the duodenum via the accessory celiac branch only (6). All of the tested neurons (i.e., 6 out of 6) responded to SP with an inward current.

To test whether the lack of response to SP of cecum-projecting cells was due to below-detection levels of receptors, we performed a right cervical and a left subdiaphragmatic vagotomy three to seven days after we applied the retrograde tracer DiI to the cecum. The sectioning of afferent nerve projections is a procedure that has been shown to induce receptor upregulation (8,11,16). However, when labeled cecum-projecting neurons were challenged with SP after vagotomy, no response to SP was obtained ($n = 6$).

Pharmacology of the response to SP

Regression analysis revealed a concentration-dependent effect in responsive DMV neurons. The concentration-response curves were parallel with the fundus curve shifted to the left (Fig. 2; $P < 0.05$).

The analysis of the $I_{\max}$ showed that the fundus had a significantly smaller $I_{\max}$ than the other responsive DMV projections ($P < 0.05$; Fig. 2). The mean $I_{\max}$ for the pooled projections was 62 ± 2.7 pA ($n = 184$). The analysis of the EC_{50} estimated from the concentration-response curves showed the EC_{50} to be similar among all subpopulations of DMV neurons ($P > 0.05$; Fig. 2). The mean EC_{50} for the pooled projections was 260 ± 30 nM ($n = 26$).

SP induced an inward current via NK₁ and NK₂ receptors

In a subpopulation of DMV neurons, SP (1 μ M) induced a 78 ± 10.5 -pA inward current. Following 20-min pretreatment with the NK₁ receptor antagonist sendide (1 μ M), SP induced a 36 ± 9.9 -pA inward current ($P < 0.05$; $n = 11$; Fig. 3). In detail, gastric-projecting neurons responded to SP with a 85 ± 19 -pA inward current in control and a 53 ± 14 -pA current following sendide pretreatment ($P < 0.05$; $n = 6$). In intestine-projecting neurons, the response to SP was 70 ± 6.6 pA in control and 15 ± 5.7 pA following sendide pretreatment ($P < 0.05$; $n = 5$; Fig. 4). Thus sendide decreased the SP-induced current to a greater degree in intestine- vs. gastric-projecting neurons. ($P < 0.05$).

In another group of neurons (3 gastric and 4 intestinal), SP (1 μ M) induced a 79 ± 20.7 -pA inward current. Following 20 min pretreatment with the NK₂ receptor antagonist MEN 10,376 (1 μ M), SP induced a 28 ± 5.9 -pA inward current ($P < 0.05$; $n = 7$). No differences in the amount of inhibition by MEN 10,376 were observed between gastric and intestinal neurons ($P > 0.05$; Figs. 3 and 4).

Perfusion with the selective NK₁ receptor agonist SM-SP (0.1–3 μ M) induced an inward current with a calculated $I_{\max}$ near 3 μ M ($n = 33$; Fig. 5). In a pooled sample of gastric- and

intestine-projecting neurons, SM-SP (3 μ M) induced an inward current that was normalized to $79 \pm 7.6\%$ of the magnitude of the 1 μ M SP-induced current (55 ± 5.9 pA for SP vs. 42 ± 5.4 pA for SM-SP; $P < 0.05$; $n = 20$). In gastric-projecting neurons, the amplitude of the SM-SP-induced current was significantly different from the amplitude of the SP-induced current ($P < 0.05$; 50 ± 6.0 for SP vs. 31 ± 3.6 pA in SM-SP; Fig. 6). In intestine-projecting neurons, however, the response to SM-SP was similar to the response to SP ($P > 0.05$; 61 ± 10.2 pA for SP vs. 53 ± 9.1 pA for SM-SP; Fig. 6).

In 17 DMV neurons (8 gastric- and 9 intestine-projecting neurons), perfusion with the selective NK₂ receptor agonist α NK (0.3–10 μ M) induced an inward current with a calculated $I_{\max}$ close to 3 μ M ($n = 31$). In this pooled sample, perfusion with α NK (3 μ M) induced an inward current that was $60 \pm 7.0\%$ of the magnitude of the 1 μ M SP response (77 ± 9.5 pA for SP vs. 46 ± 9.3 pA for α NK; $P < 0.05$; $n = 17$; Fig. 5). In gastric-projecting neurons the response to 3 μ M α NK was $48 \pm 10.6\%$ of the SP response, and in intestinal-projecting neurons it was $70 \pm 8.3\%$ of the SP response. No differences between gastric- and intestinal-projecting neurons were observed ($P > 0.05$; $n = 17$; Fig. 6).

In a separate group of neurons, when the NK₁ agonist SM-SP (3 μ M) and the NK₂ agonist α NK (3 μ M) were perfused together, DMV neurons responded with an inward current that was similar in magnitude to that induced by 1 μ M SP ($86 \pm 12.9\%$ of the SP response, i.e., 83 ± 15.0 pA for SP vs. 71 ± 20.3 pA for SM-SP and α NK combined; $P > 0.05$; $n = 5$; Fig. 5).

The selectivity of NK₁ and NK₂ receptor agonists and antagonists were then cross-analyzed. In 5 DMV neurons, the NK₂ receptor antagonist MEN 10,376 (1 μ M) did not significantly reduce the current induced by the NK₁ receptor agonist SM-SP (3 μ M), (32 ± 6.9 pA for SM-SP and 24 ± 7.73 for SM-SP + MEN 10,376; $P > 0.05$; not shown). Conversely, in 7 DMV neurons (3 gastric and 4 intestinal), MEN 10,376 (1 μ M) significantly reduced the current induced by the NK₂ agonist α NK (3 μ M) by 59% (57 ± 17.3 pA for α NK and 21 ± 6.5 for α NK + MEN 10,376; $P < 0.05$; not shown).

In three DMV neurons, pretreatment with the NK₁ receptor antagonist sendide had no effect on the α NK-induced inward current ($P > 0.05$; not shown). In two other neurons, pretreatment with sendide reduced the current induced by the NK₁ receptor agonist SM-SP (3 μ M) by 49% (100 and 45 pA for SM-SP vs. 35 and 30 pA for SM-SP + sendide, respectively; data not shown).

SP activated presynaptic receptors

In 34 out of 301 neurons in which spontaneous synaptic activity could be measured (27 gastric and 7 intestinal), SP induced an inward current that was accompanied by an increase in the frequency of mEPSCs (with an amplitude of at least 25 pA; Fig. 7). In detail, SP increased the frequency of mEPSCs from 2.97 ± 1.4 Hz in control to 6.0 ± 2.0 Hz in the presence of SP ($P < 0.05$; Fig. 7). SP did not increase the amplitude (40 ± 4.1 pA in control and 40 ± 1.2 pA in the presence of SP; Fig. 7). There was no difference in the increased frequency between gastric and intestinal neurons (gastric, 1.6 ± 0.24 Hz control and 3.7 ± 0.8 Hz in SP; intestinal, 8.3 ± 6.9 Hz and in control and 15.1 ± 8.9 Hz in SP; $P > 0.05$). Similarly, in nine neurons, SP significantly increased the frequency of mIPSCs from 1.0 ± 0.2 Hz in control to 7.6 ± 2.1 Hz in the presence of SP ($P < 0.05$; Fig. 7). SP did not affect the amplitude of mIPSCs (57 ± 16.3 in control and 38 ± 0.9 pA in the presence of SP; $P > 0.05$; Fig. 7). In two neurons SP induced an increase in the frequency of mIPSCs without evoking any inward current (0.6 Hz in control and 8.8 Hz in the presence of SP; data not shown).

In three neurons the NK₂ receptor agonist α NK (3 μ M) increased the frequency of mEPSCs from 0.6 ± 0.3 Hz in control to 3.6 ± 1.7 Hz in the presence of α NK ($P > 0.05$; data not shown). In three additional neurons, α NK increased the frequency of mIPSCs from 0.2 ± 0.1 Hz in control to 9.6 ± 6.4 Hz in the presence of α NK ($P > 0.05$; Fig. 7). Like SP, α NK did not affect the amplitude of either mEPSCs (31 ± 4.0 pA in control and 40 ± 3.6 pA in α NK) or mIPSCs (21 ± 410.6 pA in control and 44 ± 8.3 pA in α NK).

DISCUSSION

In this study we have shown that 1) SP induced an inward current in discrete subpopulations of gastrointestinal-projecting DMV neurons and, furthermore, that the incidence and concentration dependence of the SP-induced current was dependent on the specific gastrointestinal target organ but not the vagal projection branch and that 2) the excitatory effects of SP were mediated by activation of NK₁ and NK₂ receptors located both on the postsynaptic DMV neuron and on presynaptic nerve terminals.

The response to SP is organotropically organized

The frequency and concentration dependency of the SP-induced inward current was contingent on the peripheral target organ of the DMV. In fact, among gastrointestinal-projecting DMV neurons, the lowest frequency of response was observed in fundus-projecting neurons and the highest frequency of response was observed in the duodenum-projecting neurons. Conversely, none of the cecum-projecting neurons responded to SP. The lack of response from cecum-projecting neurons was unexpected given that NK1R-IR fibers in rat and cat were shown to be preferentially located in the lateral tips of the DMV (2), the source of neurons that project to the cecum (1,3).

The lack of response to SP by cecum-projecting neurons was more likely to be due to the absence of SP receptors on those neurons than a below-detection level of receptors. We surmised that if SP receptors were present on the nonresponsive cecum neurons, they would have responded to SP if the condition of receptor upregulation had taken place. However, the sectioning of the right cervical and subdiaphragmatic vagus failed to unmask a SP-mediated response in identified cecum-projecting motor neurons, suggesting a complete lack of SP receptors on these neurons.

In addition, given that DMV neurons project to the cecum via the celiac branches, whereas the DMV neurons project to the duodenum via both the celiac and gastric vagal branches (1,3), we sought to determine if the duodenum- and cecum-projecting neurons that did not respond to SP were common to the celiac branches. Following the sectioning of the AGB, experiments were conducted on identified DMV neurons that projected to the duodenum. Recordings were made only from labeled DMV neurons that were localized to the left side of the brain [from which both the anterior gastric branch and the accessory celiac branch originate (6)]. This localized group of identified DMV neurons, therefore, represented motor neurons that projected to the duodenum via the accessory celiac branch of the vagus only (6). Under these conditions, if duodenal neurons failed to respond to SP, then one could reasonably conclude that nonresponding neurons projecting to the duodenum and cecum were common to the accessory celiac nerve. This would argue that the SP-mediated response was determined not by the target organ but rather by the vagal projection branch. Instead, our results showed that the duodenum neurons that project via the accessory celiac branch responded to SP with an inward current, suggesting that the response to SP was determined by the target organ rather than by a specific vagal branch. Additionally, our results suggested that the laterally positioned intestinal neurons that responded to SP project to the duodenum instead of the cecum.

In this study, fundus-projecting DMV neurons had the lowest frequency of response to SP along with a concentration-response curve that was shifted to the left compared with duodenum- and antrum/pylorus-projecting DMV motoneurons. The low EC₅₀ value suggests that the fundus projections are the most sensitive of these projections to the effect of SP and that SP may have a greater affinity for receptors localized on these neurons.

NK₁ and NK₂ receptors mediate the postsynaptic response to SP

The SP-induced inward current was mediated by activation of both the NK₁ and NK₂ receptors. Our evidence is the following: 1) pretreatment with the NK₁ receptor antagonist sendide or the NK₂ receptor antagonist MEN 10,376 inhibited the SP-induced current; 2) perfusion with either the NK₁ receptor agonist SM-SP or the NK₂ receptor agonist α NK partially mimicked the SP-induced current; and, 3) perfusion with a combination of SM-SP and α NK reproduced the SP-induced current.

Interestingly, differences between gastric- and intestine-projecting neurons were observed when NK₁ receptor agonists or antagonists were used. In both cases, intestine-projecting neurons seemed to be more sensitive than gastric-projecting neurons to NK₁-selective drugs. Conversely, no differences were observed between gastric- and intestine-projecting neurons when either the SP-induced inward current was attenuated by the NK₂ receptor antagonist MEN 10,376 or when the SP response was mimicked by the NK₂ receptor agonist α NK.

The effects of the NK₁ receptor agonist SM-SP and the NK₂ receptor agonist α NK were concentration dependent. SM-SP, however, was more efficacious than α NK in mimicking the SP-induced current. In fact, SM-SP induced a current that was ~80% of the SP-induced current, whereas α NK induced a current that was ~60% of the SP-induced current. Given that their summation was larger than unity, we sought to determine whether this was due to a nonselective interaction with neurokinin receptors other than the ones that the agonists were supposed to interact with. Indeed, we observed that the NK₁ receptor antagonist sendide blocked a significant portion of the NK₁ receptor agonist-mediated (i.e., SM-SP) inward current, whereas it failed to block any of the NK₂ receptor agonist-mediated (i.e., α NK) inward current. Conversely, the NK₂ receptor antagonist MEN 10,376 partially blocked some of the NK₁ receptor agonist-mediated inward current, whereas it blocked most of the NK₂ receptor agonist-mediated inward current. These data would then imply that a portion of the NK₁ receptor agonist-induced (i.e., SM-SP) current is, at least in the DMV, mediated by neurokinin receptors other than NK₁, possibly NK₂ receptors. Results from our study are in agreement with previous electrophysiological studies in the DMV suggesting that the SP-induced inward current was mediated by both NK₁ and NK₂ receptors (26), although conflicting results were obtained (23,27,32).

Presynaptic effects of SP

Our evidence for an effect of SP on presynaptic sites is the following: 1) SP increased the frequency, but not the amplitude, of both mEPSCs and mIPSCs, probably via activation of NK₁ receptors; 2) α NK increased the frequency, but not the amplitude, of both mEPSCs and mIPSCs, probably via activation of NK₂ receptors; and, 3) in some neurons, pretreatment with the synaptic blocker TTX reduced the SP-mediated inward current. SP has been shown to excite NTS neurons through activation of the NK₁ receptor (13,27). We thus postulate that the observed SP- and α NK-induced increase in mEPSCs resulted from the presynaptic release of the excitatory amino acid transmitter glutamate and the increase in mIPSCs from the presynaptic release of GABA onto DMV neurons (34,35).

Physiological significance

In a recent *in vivo* study, Krowicki and Hornby (19) showed that microinjection of SP or the NK₁ receptor agonist SM-SP into the DMV decreased intragastric pressure and antral motility in the rat, a response attenuated by pretreatment with the NK₁ receptor antagonist GR-203040. These authors suggested that SP activates NK₁ receptors located on preganglionic cholinergic DMV neurons that control enteric nonadrenergic, noncholinergic motoneurons involved in the receptive relaxation reflex (19). Additionally, these authors demonstrated that microinjection of SP into the nROb of the rat decreased the intragastric pressure and gastric motility, which was dependent on an intact vagal pathway (19). These same authors then showed that the microinjection of SP into the nROb decreased intragastric pressure via a nitric oxide-dependent mechanism in the DMV (19).

Our study has shown that the response to SP in fundus-projecting neurons is less frequently encountered, has the lowest $I_{\max}$, and a leftward-shifted concentration response curve relative to the other SP-responsive neurons (cecum excluded); furthermore, Krowicki and Hornby (19) have shown the effect of SP on gastric motility to be a robust phenomenon of physiological significance. Together, this information suggests that the central control of the fundus by preganglionic motor neurons in the DMV is mediated by a small, discrete population of neurons.

A physiological role for SP in the DMV is supported by observation that SP receptor immunoreactivity is present in the raphe nuclei (29) and the DMV (20,21), that SP-IR fibers have been shown to be present in the nROb (15) and the NTS (5), and that vagal afferents in the nodose ganglion are SP-IR-positive (10,22,36). Together, this would suggest that SP-containing pathways from both the periphery and central nervous system converge in the DMV to control gastric motility.

In conclusion, we have shown that SP modulates the activity of a known subpopulation of DMV neurons. Significant differences were found between the five peripheral projections studied. Induction of the postsynaptic inward current by SP was correlated to the peripheral organ rather than the vagal branch used. SP activated both NK₁ and NK₂ receptors located on the membrane of DMV neurons projecting to the gastric fundus, corpus, antrum/pylorus, and duodenum, and SP acted at NK₁ and NK₂ receptors located presynaptically within the dorsal vagal complex (NTS and DMV) to increase synaptic transmission to gastrointestinal-projecting DMV neurons.

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Fig. 1.

Substance P (SP) induced a concentration-dependent current. *Left*: representative traces from a duodenum-projecting neuron showing that SP produced a concentration-dependent inward current. SP was superfused until an effect plateau was observed. The break in the current trace indicates an ~5-min washout period. The horizontal line indicates the period of the SP superfusion. Holding potential was -50 mV. *Right*: concentration-response curve for the SP-induced inward current. Data from gastric- and intestine-projecting neurons were pooled. Each point represents data from 9–31 neurons. Each neuron was measured at a minimum of 3 different concentrations.

Fig. 2.

The SP-induced response is dependent on the dorsal motor nucleus of the vagus (DMV) projection. *Top*: regression analysis of the linear portion of the concentration-response curve to SP (0.1–1 μM). A/P, antrum/pylorus. **P* < 0.05 vs. fundus. *Middle*: maximum current evoked by SP (*I*_{max}) was smaller in fundus-projecting neurons. **P* < 0.05 vs. other groups. *Bottom*: half-maximal amplitude of the SP-induced current (EC₅₀) was similar for all gastrointestinal projections except the cecum, which did not respond to SP.

Fig. 3.

SP-induced inward current was mediated by activation of neurokinin receptors NK₁ and NK₂: antagonist studies. **A:** representative traces from a gastric-projecting neuron showing attenuation of the SP-induced current by pretreatment with the NK₁ receptor antagonist sendide. Perfusion with 1 μ M SP induced an inward current. Following washout of SP, sendide (1 μ M) was superfused for 20 min. Reperfusion with SP in the presence of sendide yielded an attenuated current response. Holding potential = -50 mV. **Bottom:** representative traces from a different gastric-projecting neuron showing attenuation of the SP-induced current by pretreatment with the NK₂ receptor antagonist MEN 10,376. Perfusion with 1 μ M SP induced an inward current. Following washout of SP, MEN 10,376 (1 μ M) was superfused for 15 min before reperfusion with SP. Notice that MEN 10,376 pretreatment attenuated the SP-induced current response. // indicates 5-min break in trace. Holding potential = -50 mV.

Fig. 4.

Summary of the antagonism of the SP-mediated inward current. *A*: sendide significantly attenuated the SP-induced inward current in both gastric- and intestine-projecting neurons. Sendide, however, was more effective in attenuating the SP-induced current in intestine-projecting neurons. *B*: in intestinal neurons only, MEN 10,376 significantly attenuated the SP-induced inward current. * $P < 0.05$ vs. SP alone; ** $P < 0.05$ vs. intestine-projecting neurons.

Fig. 5.

SP-induced inward current was mediated by activation of NK₁ and NK₂ receptors: agonist studies. *Top*: representative traces from a gastric-projecting neuron showing the effect of perfusion with the NK₁ receptor agonist [Sar⁹-Met(O₂)¹¹]SP (SM-SP; 3 μM). SP was perfused for the time indicated by the horizontal bar. Following a 20-min washout, perfusion with SM-SP mimicked the SP-induced current. Holding potential = -50 mV. *Middle*: representative traces from a different gastric-projecting neuron showing that perfusion with the NK₂ receptor agonist α-neurokinin (αNK; 3 μM) induced an inward current of lesser magnitude than the current induced by SP (1 μM). Holding potential = -50 mV. *Bottom*:

when SM-SP (3 μ M) and α NK (3 μ M) were perfused together, the resulting inward current was of equal magnitude to the SP- (1 μ M) mediated response. Holding potential = -50 mV.

Fig. 6.

Summary of the agonist studies. *A*: in intestinal neurons, the inward current induced by the NK₁ receptor agonist SM-SP (3 μ M) was of similar magnitude compared with the 1 μ M SP-induced current. Conversely, in gastric neurons, the NK₁ receptor agonist-induced current was significantly smaller than the SP-induced current. * P < 0.05 vs. SP in gastric neurons. *Bottom*: in both gastric and intestinal neurons, the magnitude of the current induced by the NK₂ receptor agonist α NK (3 μ M) was significantly smaller than the magnitude of the current induced by SP. There was no difference between gastric and intestinal neurons.

Fig. 7.

SP increased the frequency of postsynaptic currents. *Top left:* representative trace from an intestine-projecting neuron showing that the superfusion with 1 μ M SP induced a 100-pA inward current accompanied by an increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs). *Top middle:* recording from the same neuron showing that superfusion with 3 μ M α NK induced a 90-pA inward current accompanied by an increase in the frequency of miniature inhibitory postsynaptic potentials (mIPSCs). *Top right:* Washout. Holding potential = -50 mV. Breaks in record indicate a 15-min period between the 1st and 2nd traces and a 6-min break between the 2nd and 3rd traces. *Middle left:* expanded detail of the above SP trace showing mEPSCs before (a) and after (b) SP perfusion. *Middle right:* expanded detail of the above α NK trace showing the absence of mIPSCs before (c) and the presence of mIPSCs after (d) α NK perfusion. Holding potential = -50 mV. *Bottom:* computer-generated graphics from the same neuron as above showing that SP induced an increase in the frequency (left; $P < 0.05$) but not in the amplitude (right) of mEPSCs.

Table 1

Summary of responsive cells

Projections	Total, N	Responders		Nonresponders	
		N	%	N	%
Fundus *	40	22	55	18	45
Corpus	74	48	65	26	35
Antrum/pylorus	69	46	67	23	33
Duodenum	91	68	75	23	25
Cecum	27	0	0	27	100
AGB	9	4	44	5	66
Duodenum via accessory celiac branch	6	6	100	0	0
Cecum-bilateral vagotomy	6	0	0	6	100
Total	322	194		128	

AGB, anterior gastric branch of the vagus.

* $P < 0.05$ fundus vs. corpus, antrum/pylorus, and duodenum by χ^2 .