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Substance P in the Dorsal Motor Nucleus of the Vagus Evokes Gastric Motor Inhibition via Neurokinin 1 Receptor in Rat[1]

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ABSTRACT

Many gastrointestinal stimuli result in gastric fundic relaxation. This information is integrated at the interface of vagal afferents and efferents in the dorsal vagal complex. Substance P (SP) is present in this region, and the neurokinin$_1$ receptor (NK_1R) is highly expressed in preganglionic neurons of the dorsal motor nucleus of the vagus (DMN). However, its functional effects on vagal motor output to the stomach have not been investigated. Therefore, we determined the gastric motor effects of stereotaxic microinjection of SP and selective tachykinin receptor agents into the DMN of anesthetized rats. Dose-related decreases in intragastric pressure and antral motility were obtained on the microinjection of SP (135 and 405 pmol) into the DMN, without cardiovascular changes. Similar decreases in intragastric pressure were noted after the microinjection of [Sar^9,Met(O$_2$)^{11}]SP (NK_1R agonist; 135 pmol) but not senktide (NK_3R agonist; 135 pmol) or vehicle. The gastric motor inhibition evoked by SP (135 pmol) was attenuated by prior microinjection of 2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine (GR203040; 1 nmol; NK_1R antagonist). Vagotomy or hexamethonium (15 mg/kg i.v.) completely abolished the gastric relaxation evoked by SP (135 pmol) microinjected into the DMN. We conclude that SP acts on NK_1R preganglionic cholinergic vagal neurons in the DMN, which control enteric nonadrenergic noncholinergic motor inhibition of the fundus. The potential relevance is that an antiemetic site of action of NK_1R antagonists may be in the DMN to prevent excitation of neurons controlling fundic relaxation, which is an essential prodromal component of emesis.

Many stimuli to the gastrointestinal tract result in hormonal ("endoneurocrine") or neuronal feedback to other regions of the gut, and the vagus nerve is intimately involved in conveying this information to the upper gastrointestinal tract. Integration of "long-loop" vagal afferent-efferent pathways from the gut occurs in the dorsal vagal complex of the hindbrain medulla. This complex comprises the dorsal motor nucleus of the vagus (DMN), where preganglionic motor neurons innervating the gastrointestinal tract are located, and the nucleus tractus solitarius, where primary visceral afferents terminate. Preganglionic neurons in the DMN target the stomach (Shapiro and Miselis, 1985), and much progress has been made into the neurotransmitter candidates in this region, which can increase gastric motility and intragastric pressure (IgP). However, there is less information on receptor-mediated events at this site that result in gastric motor inhibition and fundic relaxation. This is surprising because fundic relaxation is a very important component of normal gastrointestinal function, such as during ingestion of food, during emesis, or in response to chemical signals arising from acid or fat in more distal regions of the gut.

One candidate neurotransmitter in the dorsal vagal complex that could mediate fundic relaxation is substance P (SP). The microinjection of SP into the nucleus tractus solitarius evokes gastric relaxation (Spencer and Talman, 1986). Moreover, SP is highly expressed in fibers in the dorsal vagal complex of all species studied, including humans (Fodor et al., 1994). SP has the highest affinity for the neurokinin$_1$ receptor (NK_1R), whereas neurokinins A and B (NK_A and NK_B) bind to NK_2R and NK_3R, respectively. In the dorsal vagal complex, the regional distribution of NK_1R immunocytochemical staining overlaps that of SP (Dixon et al., 1998). Interestingly, NK_1R is most highly expressed in neurons of the DMN, and this staining (Dixon et al., 1998), as well as SP binding (Manaker and Zucchi, 1993), is abolished by vagotomy. These data suggest that NK_1R is synthesized by vagal preganglionic motor neurons. In addition, ultrastructural studies have demonstrated that NK_1R is on the membrane surface of somatic and dendritic profiles of DMN neurons but never in axon terminals, axons, or glial processes (Baude and Shigemoto, 1998). Finally, the NK_1R is present in DMN neurons that project onto the greater curvature of the stom-

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ABBREVIATIONS: DMN, dorsal motor nucleus of the vagus; AUC, area under the curve; IgP, intragastric pressure; NANC, nonadrenergic noncholinergic; NO, nitric oxide; nAmb, nucleus ambiguus; NK_1R, neurokinin$_1$ receptor; SP, substance P.

ach (Ladic and Buchan, 1996). Together, these data imply that SP is intimately involved in controlling vagal output to the stomach via NK_1R on preganglionic motor neurons in the DMN. However, to our knowledge, the functional effects of SP or NKR ligands on vagal motor output in the DMN have not been investigated. Therefore, the first purpose of the present study was to determine the gastric motor effects of microinjection of SP and selective tachykinin receptor agonists into the DMN.

Our results demonstrated that SP inhibited gastric motor activity and evoked gastric relaxation via NK_1R in the DMN. Vagally evoked gastric relaxation is mediated via postganglionic/enteric nonadrenergic noncholinergic (NANC) inhibitory motor neurons. There are two possible vagal pathways leading to motor inhibition. One involves a vagal cholinergic preganglionic neuron synapsing, via nicotinic receptors, onto myenteric neurons, which then release nitric oxide (NO) (Desai et al., 1994; Meulemans et al., 1995; Takahashi and Owyang, 1995) and vasoactive intestinal polypeptide (Grundy et al., 1993; Takahashi and Owyang, 1995) to evoke smooth muscle relaxation. Gastric relaxation evoked by this pathway would be abolished by hexamethonium. The second possibility involves nitrergic vagal preganglionic neurons that innervate the gastrointestinal tract (Krowicki et al., 1997b), with a preference for the gastric fundus (Zheng et al., 1999). Excitation of these neurons evokes a gastric relaxation that is abolished by NO synthase inhibition but not by hexamethonium (Krowicki et al., 1999). Therefore, the second purpose of this study was to determine whether SP-evoked gastric relaxation can be abolished by hexamethonium and vagotomy. Preliminary reports of this study have been published elsewhere (Krowicki and Hornby, 1996, 1998).

Materials and Methods

Male Sprague-Dawley rats (200–390 g) from Charles River Laboratories (Wilmington, MA) were used in all experiments. The study was approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee.

The animals were initially anesthetized with a ketamine and xylazine mixture (36 and 3.6 mg/kg i.m., respectively), and separate indwelling cannulas were placed in the left femoral artery and vein. Then, α-chloralose (60–80 mg/kg) was administered i.v., and a tracheotomy was performed to connect the animal to the small animal respirator (Kent Scientific Corp., Litchfield, CT). A laparotomy was performed, and an intraluminal latex balloon was inserted into the stomach through an incision in the fundus for the recording of IgP. The imparting pressure within the intragastric balloon was maintained at approximately 5 cm H_2O before microinjection in all animals. A small strain gauge (Warren Research Products, Charleston, SC) was sutured onto the surface of the distal antral region for continuous recording of circular smooth muscle. In previous publications, we termed this the "pyloric region" but have revised our terminology to more accurately reflect the location of the strain gage. This is based on careful consideration of the complex organization of muscles in the pyloric region in dogs and the lack of information about this structure in rats, as well as the motility pattern that we obtain, which appears to be typical of circular muscle contractions of the terminal antrum. Rectal temperature was kept between 37.0° and 37.5°C by radiant heat.

Microinjection Technique. Animals were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), and the dorsal surface of the medulla and obex were exposed by an occipital craniotomy. Seven-barreled micropipettes (20- to 40-μm total external tip diameter), prepared from glass capillaries (Dagan Corp., Minneapolis, MN, or A-M Systems, Inc., Everett, WA), were attached with polyethylene tubing to a pneumatic pico-pump (model PV 830; World Precision Instruments, New Haven, CT). The micropipette tip was stereotaxically placed in the DMN (coordinates: 0.5–0.9 mm rostral to the obex and 0.4–0.5 mm below the surface of the obex, 0.5 mm lateral) and the nucleus ambiguus (nAmb; coordinates: 0.7 mm rostral to the obex and 1.3 mm below the surface of the obex, 1.5 mm lateral) according to the atlas of Paxinos and Watson (1986). Microinjections were delivered (at 30 psi) in a volume of 20 to 30 nl for 10 to 15 s.

Initially, L-glutamate (7.5 nmol) microinjection was used to precisely localize the DMN, where brief (<2 min) but marked increases in IgP and antral motility were obtained. Once the placement of the pipette tip in the DMN was ensured by the expected gastric motor responses, microinjections of SP and NKR-selective agents were made, after an interval of at least 15 min. This protocol has reliably resulted in microinjections in which the tip of the pipette is located within the DMN (Krowicki et al., 1997).

SP (Sigma Chemical Co., St. Louis, MO), [Sar^9,$Met(O_2)^{11}$]SP (NK_1R agonist; Research Biochemicals Inc., Natick, MA), senktide (NK_3R agonist; Research Biochemicals Inc.), and 2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine (GR203040, an NK_1R antagonist; Glaxo-Wellcome, Stevenage, UK) were dissolved in saline with 0.1% BSA and 2% ascorbic acid (as an antioxidant). At 15- to 30-min intervals, vehicle and SP were microinjected unilaterally into the DMN ($n = 6$) and nAmb ($n = 5$). A dose range of 35 to 405 pmol of SP was selected, and on the basis of these results, 135 pmol was selected as submaximum for the gastric relaxation response in the DMN. This dose was used to determine the receptor selectivity of the response. An electrophysiological study has demonstrated that SP and NK_1R agonists were equipotent to depolarize DMN neurons (Maubach and Jones, 1997). Therefore, NK agonists [Sar^9,$Met(O_2)^{11}$]SP and senktide were microinjected into the DMN at a dose of 135 pmol ($n = 5$). Vehicle and SP were microinjected into the DMN before and after microinjection of GR203040 (1 nmol; $n = 7$).

To determine the pathways responsible for NK-evoked gastric responses, SP was microinjected into the DMN at the maximal dose (405 pmol) before and after hexamethonium bromide (15 mg/kg i.v.; $n = 5$) or vagotomy ($n = 4$). Bilateral vagotomy was performed by avulsion during the experiment using a suture loosely looped around the vagi at midcervical level.

At the end of each experiment, 1% pontamine sky blue was microinjected into the DMN in a volume of 20 to 30 nl, and the animal was administered a bolus i.v. injection of pentobarbital sodium (60–80 mg/kg). Then, brains were removed, fixed in 4% paraformaldehyde, sectioned at 50 μm, and counterstained with neutral red. The placement of the microinjection tips in the DMN was subsequently confirmed histologically. Only the animals with appropriately placed microinjection sites were used for data analysis.

Peak decreases (nadir) in IgP were determined after microinjection and compared with preinjection levels. However, if no peak was noted, mean pressure was calculated over a 2-min period before and after microinjection. The area under the curve (AUC) (i.e., total decrease in IgP) after microinjection was calculated using a computer imaging program (Imaging Research Inc., St. Catherine's, Ontario, Canada). Minute Motility Index was calculated for antral motility for the 2-min period before and after microinjection (Ormsbee and Bass, 1976).

The differences between groups were assessed by one-way ANOVA followed by the Student-Newman-Keuls or Dunn's multiple comparisons test. Values of $P < .05$ were considered to be statistically significant.

Results

Figure 1 demonstrates the compiled data on microinjection of SP (35–405 pmol) into the DMN. There are significant

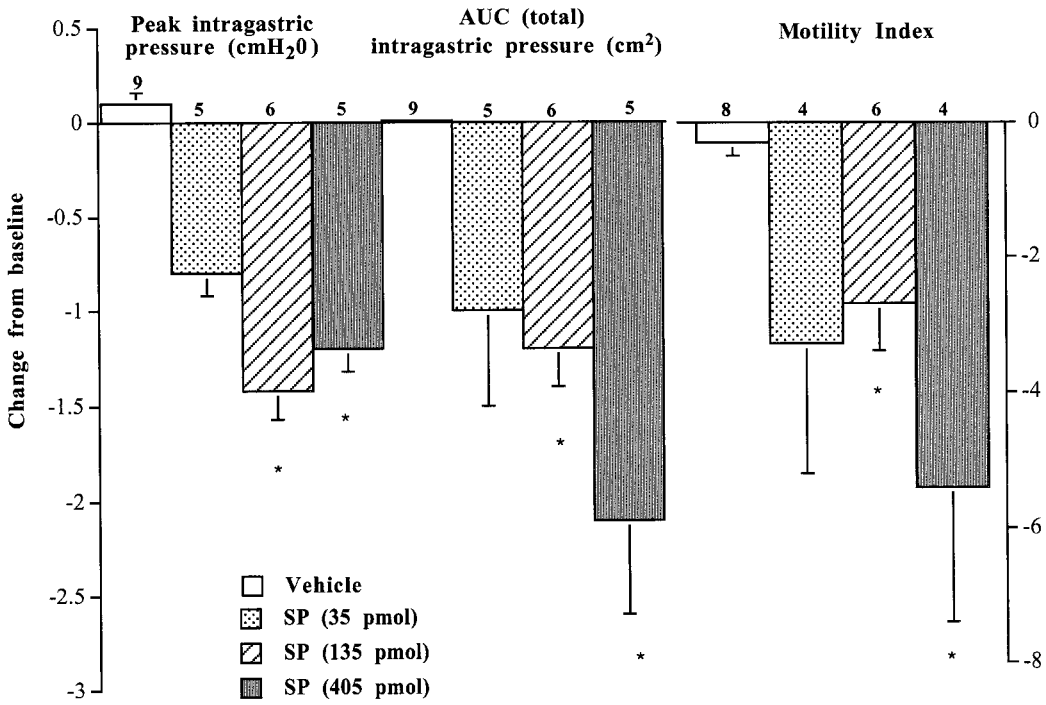

Fig. 1. Compiled data showing the decrease in IgP (both peak and total IgP) and antral motility on microinjection of vehicle and SP (35–405 pmol) into the DMN. *$P < .05$ compared with saline. Number above x-axis refers to n.

decreases in IgP (both peak and total AUCs) and antral motility at 135 and 405 pmol of SP (Fig. 1); however, there are no significant effects on mean arterial pressure or heart rate (Table 1). Figure 2 illustrates the location of the micropipette tips when 135 pmol of SP ($n = 6$) was microinjected in these experiments. Microinjections are generally placed on the ventral border of the DMN, rostral to the obex (according to Paxinos and Watson, 1986). The spread of the dye encompasses the DMN and part of the hypoglossal nucleus in some cases, but very little dye extended into the nucleus tractus solitarius. Figure 3 is a sample chart recording illustrating that, on microinjection of 135 pmol of SP into the DMN, there is a rapid decrease in IgP of approximately 2 cm H_2O, which returns to baseline within 6 min. Similarly, antral contractility is inhibited during this time, but there are no apparent changes in cardiovascular indices in this case.

In contrast to the situation for the DMN, microinjection of SP (35–405 pmol) into the nAmb significantly increased peak IgP, at doses of 135 and 405 pmol, and total IgP at a dose of 135 pmol (Table 2). The antral motor responses were variable and did not attain statistical significance overall. The location of the microinjection sites of the 135-pmol dose in these animals is generally in the region of the nAmb (Fig. 2). A

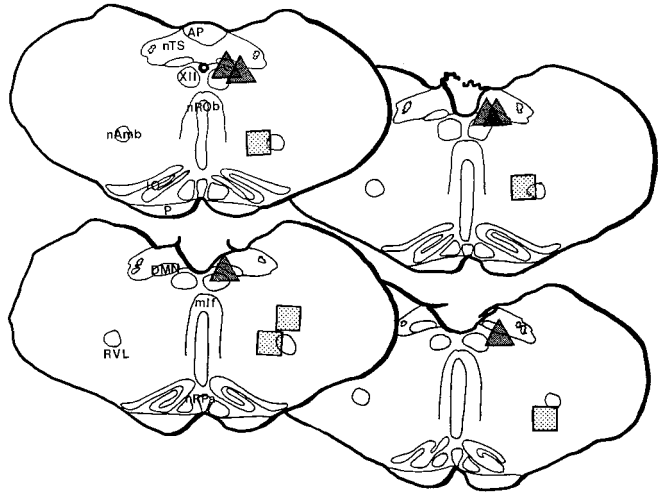

Fig. 2. Spatial dissection of the location of the tip of the micropipette for microinjections (135 pmol of SP) into the region of the DMN and nAmb. Sections were drawn by use of a drawing tube attached to a Nikon Labophot microscope and are arranged from most caudal (top left) to most rostral (bottom right). AP, area postrema; cc, central canal; IO, inferior olive; mlf, medial longitudinal fasciculus; nROb, nucleus raphe obscurus; nTS, nucleus of the solitary tract; P, pyramid; RVL, rostroventrolateral medulla; XII, hypoglossal nucleus.

TABLE 1

Effect of vehicle, SP (135 pmol), and GR203040 microinjected into the DMN on peak IgP response and total AUC as well as antral motility (MMI), mean arterial pressure (MAP), and heart rate (HR)

Values indicate the change from baseline.

Treatment	n	Peak IgP	Total IgP	MMI	MAP	HR
		cm	*cm²*		*mm Hg*	*beats/min*
Vehicle	7	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.2	0 ± 0	0 ± 0
SP	7	−1.4 ± 0.1*	−1.2 ± 0.3*	−5.3 ± 1.5*	−6 ± 8	−4 ± 14
GR203040	7	−0.5 ± 0.3	−0.2 ± 0.1	−0.7 ± 0.5	−1 ± 2	−4 ± 6
SP after GR203040	7	−0.9 ± 0.2*,**	−0.4 ± 0.2**	−2.6 ± 0.8*,**	−1 ± 1	7 ± 3

* $P < .05$ compared with vehicle.
** $P < .05$ compared with SP before GR203040.

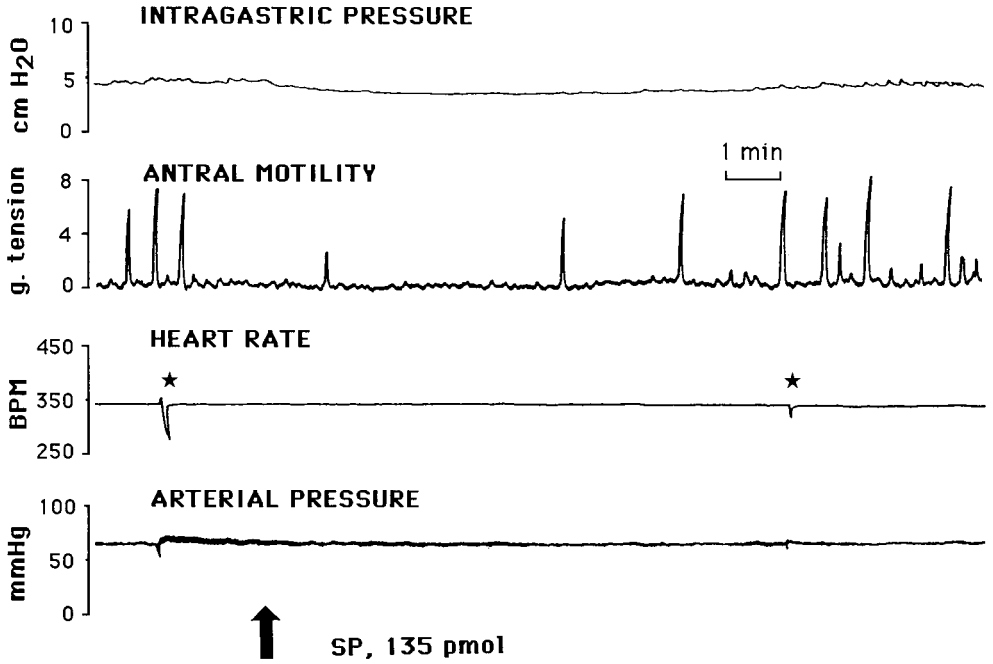

Fig. 3. Representative chart recording showing the effect of microinjection of 135 pmol of SP into the DMN on IgP, antral motility, heart rate, and blood pressure. *, cannula flushes that are unrelated to the microinjection.

TABLE 2

Effect of vehicle or SP (135–405 pmol) microinjected into the nucleus ambiguus on intragastric peak IgP response, total AUC IgP, and antral motility (MMI)

Values indicate the change from baseline.

Treatment	*n*	Peak IgP	Total IgP	MMI
		cm	*cm^2*	
Vehicle	7	0.0 ± 0.1	0.0 ± 0.0	0.2 ± 0.3
SP 35 pmol	4	1.2 ± 0.2	0.1 ± 0.0	0.7 ± 0.5
SP 135 pmol	5	3.2 ± 1.1*	0.7 ± 0.2*	2.0 ± 0.4
SP 405 pmol	4	1.7 ± 0.2*	0.4 ± 0.2	1.7 ± 1.1

* $P < .05$ compared with saline.

typical chart recording shows that SP at 135 pmol evokes a rapid transient increase in IgP that lasts for approximately 1.5 min (Fig. 4). In this particular instance, antral motility was also slightly increased. It appears that both motility and IgP exhibit a poststimulation rebound inhibition, but this was not quantified. There was a transient decrease in heart rate and a small increase in blood pressure in this animal.

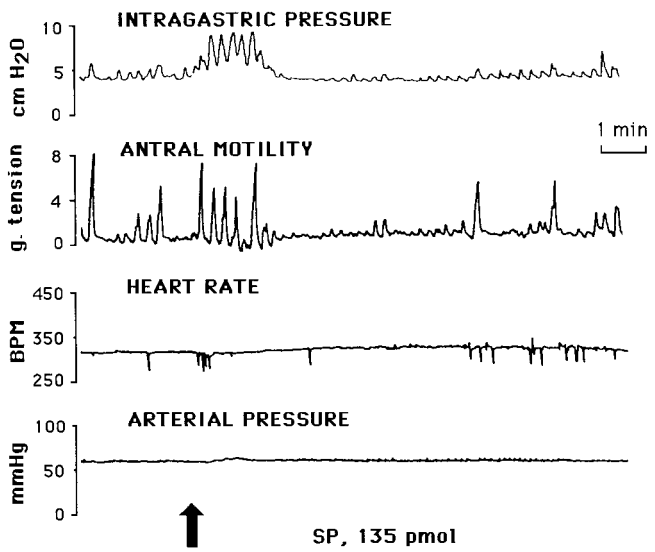

Fig. 4. Representative chart recording showing the effect of microinjection of 135 pmol of SP into the nAmb on IgP, antral motility, heart rate, and blood pressure.

Significant decreases in IgP (peak and total AUCs) and antral motility were observed after microinjection into the DMN of NK_1R agonist [Sar^9,Met(O$_2$)^{11}]SP but not after NK_3R agonist senktide (Fig. 5). A typical trace recording demonstrates that after the microinjection of [Sar^9,Met(O$_2$)^{11}]SP, there is a rapid decrease in baseline IgP and antral motility that is sustained for approximately 6 min (Fig. 6A). Microinjection of senktide has no apparent effects on gastric motor function (Fig. 6B). Microinjection into the DMN of an NK_1R antagonist, GR203040, alone did not significantly change gastric motor function, mean arterial pressure, or heart rate. However, this pretreatment attenuated the decrease in IgP evoked by microinjection of 135 pmol of SP into the DMN (Table 1).

The inhibition of gastric motor function evoked by microinjection of SP into the DMN was completely abolished by vagotomy (Table 3). Hexamethonium, administered at a dose of 15 mg/kg i.v. 10 min before microinjection of SP, abolished the expected decrease of total AUC IgP and inhibition of motor activity (Table 3). There still was a small but significant decrease in peak IgP compared with vehicle microinjec-

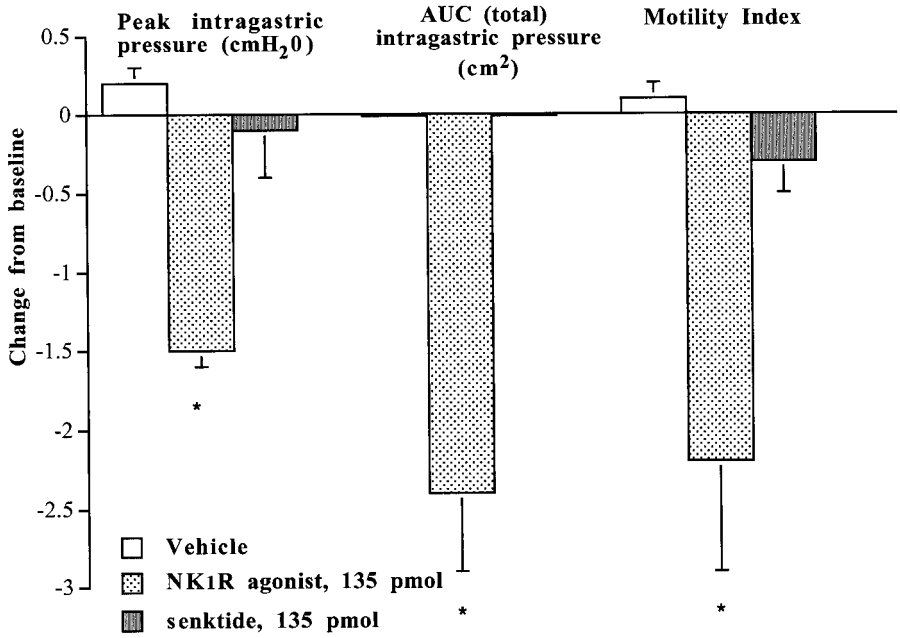

Fig. 5. Compiled data showing the decrease in IgP (both peak and total IgP) and antral motility on microinjection into the DMN of [Sar^9,Met(O$_2$)^{11}]SP, an NK_1R agonist (135 pmol), but not senktide, an NK_3R agonist (135 pmol). *$P < .05$ compared with saline.

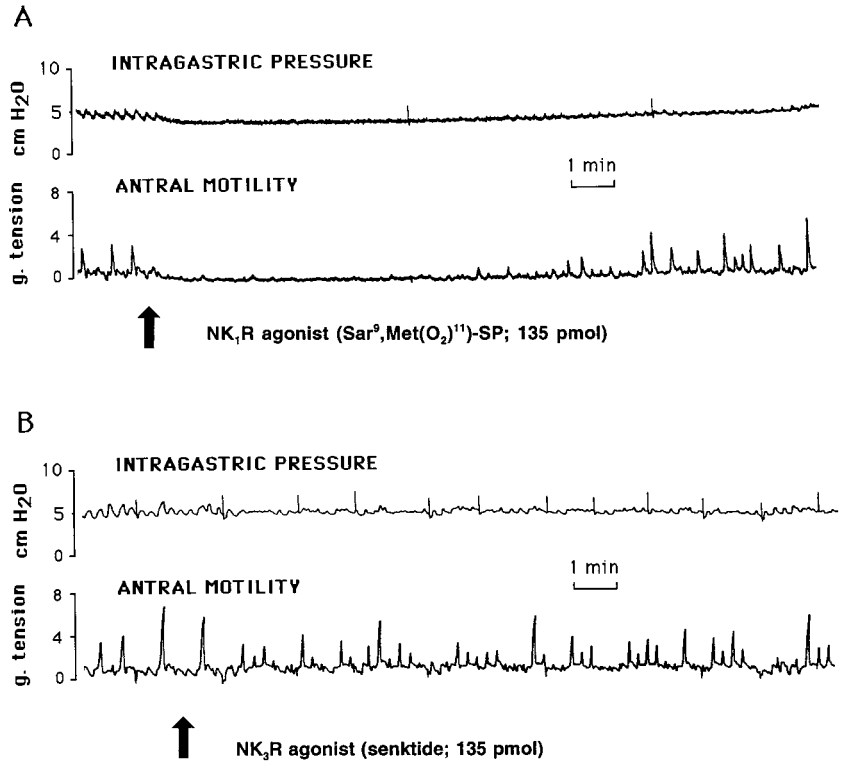

Fig. 6. Sample trace recording showing that the NK_1R agonist (A) but not the NK_3R agonist (B) decreases IgP and reduces antral motility.

tion, although this response is significantly attenuated compared with the effect of SP before hexamethonium (Table 3).

Discussion

The results of this study demonstrate for the first time that the functional sequelae of activation of NK_1R on vagal neurons in the DMN is a marked inhibition of IgP and gastric motility. This effect seems to be mediated through a cholinergic vagal pathway that involves a nicotinic receptor, presumably at the vagal-enteric NANC interface. Here, we discuss several technical and interpretative issues; these include the rationale for assuming that microinjections of SP and NK_1R agonist act primarily on neurons in the DMN and the receptor selectivity of the response. The implications of these data are discussed in terms of the role of SP in reflex

TABLE 3
Effect of vagotomy and hexamethonium (HEX; 15 mg/kg) on the inhibition of Peak IgP response, total AUC, and antral motility (MMI) evoked by SP (405 pmol) microinjected into the DMN
Values indicate the change from baseline.

Treatment	*n*	Peak IgP	Total IgP	MMI[a]	MAP	HR[a]
		cm	*cm^2*		*mm Hg*	*beats/min*
Vagotomy						
Vehicle	4	0.2 ± 0.1	0.0 ± 0.0	0.7 ± 0.4	0 ± 0	0 ± 0
SP before vagotomy	4	−1.3 ± 0.1*	−2.0 ± 0.6*	−2.8 ± 0.9*	4 ± 2	7 ± 7
SP after vagotomy	4	0.0 ± 0.0**	−0.0 ± 0.0**	0.3 ± 0.3**	4 ± 4	3 ± 3
HEX						
Vehicle	5	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2	1 ± 1	0 ± 0
SP before HEX	5	−1.4 ± 0.1*	−2.8 ± 0.3*	−5.1 ± 1.8*	−10 ± 10	−14 ± 13
SP after HEX	5	0.6 ± 0.1*,**	0.2 ± 0.1**	0.6 ± 0.9**	−6 ± 4	2 ± 2

[a] $n = 3$.
* $P < .05$ compared with vehicle.
** $P < .05$ SP before treatment versus SP after treatment.

control of gastric relaxation and the antiemetic site of action of NK$_1$R antagonists.

The conclusion that NK$_1$R-evoked gastric inhibition occurs via a direct action on vagal motor neurons is based on both anatomical and physiological data, as follows. First, the NK$_1$R is prevalent in preganglionic neurons of the DMN (Baude and Shigemoto, 1998; Dixon et al., 1998). Second, NK$_1$R is present in some (7%) neuronal cell bodies in the DMN that project onto the greater curvature of the stomach (Ladic and Buchan, 1996). These investigators did not look at the percentage of NK$_1$R-expressing cells that project to the fundus (a region where gastric relaxation is accomplished); it is possible that a higher percentage of NK$_1$R-expressing cells project to this region. Altogether, these data indicate that NK$_1$R is intimately involved in the control of vagal motor output to the stomach. However, NK$_1$R is also highly expressed in subnuclei of the nucleus tractus solitarius (Dixon et al., 1998), and SP is present within some vagal primary afferents (Sykes et al., 1994) and descending projections (Thor and Helke, 1989). In addition, SP evokes gastric relaxation when injected into the nucleus tractus solitarius (Spencer and Talman, 1986) and increases the firing rate of neurons in this nucleus in brain slice neonate preparations (Yuan and Lowell, 1997). Therefore, it could be argued that the gastric effects of SP and NK$_1$R agonist in the present study are due to actions on the neurons of the nucleus tractus solitarius and/or preganglionic neurons of the DMN. We counter this as follows. First, spatial dissection of the effective microinjection sites illustrates that the micropipette tips are located within or immediately below the DMN. Since we recognize that spread of the injectate to the nucleus tractus solitarius could still occur, there are two additional lines of evidence. We use L-glutamate microinjection to functionally locate the DMN before microinjection of test agents. Increased gastric motor activity is noted only on stimulation of DMN neurons (Ormsbee et al., 1984; Raybould et al., 1989; Sivarao et al., 1999), whereas decreased motor activity occurs after chemical stimulation of the nucleus tractus solitarius (Raybould et al., 1989). This protocol has been used in previous studies (Krowicki et al., 1997a; Sivarao et al., 1999) and allows us to be more confident that the functional site of microinjection is in the DMN. Finally, SP microinjected into the DMN had no effect on heart rate and blood pressure, whereas microinjection of SP (Spencer and Talman, 1986) and NK$_1$R agonist (Feldman, 1995) into the nucleus tractus solitarius decreases blood pressure. Feldman (1995) also noted that microinjections of SP and NK$_1$R agonist into DMN resulted in no significant cardiovascular changes. Thus, in our experiments, it is unlikely that the observed primary effects are due to a site of action on neurons of the nucleus tractus solitarius. Consequently, the results of the present study cannot address the mechanism of action by which SP in the nucleus tractus solitarius evoked gastric relaxation in the study of Spencer and Talman (1986).

We were initially surprised that SP and NK$_1$R activation in the DMN resulted in gastric relaxation. We wondered if this was a phenomenon of SP related to all vagal motor neurons, including those in the nAmb, which also innervate the stomach (Shapiro and Miselis, 1985). Agents applied to the nAmb evoke gastric motor responses (Garrick et al., 1989). In contrast to the case for the DMN, microinjection of SP into the nAmb significantly increased gastric motor activity. The functional significance of this effect is not clear. It has been noted that the application of NK$_1$R antagonists to the region of the subcompact nAmb in decerebrate dogs attenuates emetic responses (Fukuda et al., 1999), although we cannot predict how gastric contractility evoked at this site in rats relates to emetic responses.

The fact that opposite gastric motor sequelae results from SP microinjected into the DMN and the nAmb suggests that the ligand may be acting on different populations of neurons in these regions. This is because, in general, SP (Plata-Salaman et al., 1989) and NK$_1$R agonists (Martini-Luccarini et al., 1996) depolarize vagal motor neurons. Hyperpolarization occurred in fewer than 10% of DMN neurons in response to SP and never in response to NK$_1$R-selective agonists (Martini-Luccarini et al., 1996). Therefore, for SP and NK$_1$R in the DMN to evoke gastric relaxation, the most plausible explanation is that the receptor is activated on vagal pathways that control enteric inhibitory motor neurons.

The term NANC refers to the neurochemistry of the postganglionic/enteric nerves. The preganglionic neurons in this NANC pathway are cholinergic and act via nicotinic synapses at the ganglia to cause the release of NO (Desai et al., 1994; Meulemans et al., 1995; Takahashi and Owyang, 1995) and vasoactive intestinal polypeptide (Grundy et al., 1993; Takahashi and Owyang, 1995), which evoke smooth muscle relaxation. In addition to this NANC pathway, there are NO synthase-containing preganglionic neurons (Krowicki et al., 1997b) that project selectively to the fundus of the stomach (Zheng et al., 1999). Stimulation of these nitrergic neurons evokes gastric relaxation that is not abolished by hexame-

thonium (Krowicki et al., 1999). Therefore, we used hexamethonium and vagotomy to test whether gastric relaxation was a result of activation of NK_1R located on cholinergic or nitrergic neurons in the DMN that ultimately control enteric NANC inhibitory neurons. Both vagotomy and ganglionic blockade with hexamethonium largely abolished the gastric relaxation in response to SP microinjection in the DMN. This suggests that the NK_1R is on cholinergic neurons (which control NANC motor inhibitory pathways) rather than the hexamethonium-resistant pathway involving preganglionic nitrergic neurons. This also concurs with the anatomical observation that NK_1R was very rarely colocalized with NO synthase in neurons in the DMN (Dixon et al., 1998). We are able to conclude, therefore, that SP-evoked gastric relaxation is mediated primarily by classic vagal-enteric NANC pathways involving cholinergic preganglionic neurons. Because NK_1R is present only in a subpopulation of neurons in the DMN (Ladic and Buchan, 1998), this leads to the intriguing speculation that NK_1R may provide a marker for identifying cholinergic vagal neurons that control NANC inhibitory neurons. In addition, if NK_1R selectively activates neurons that initiate fundic relaxation, then reflexes, such esophageal or colonic distention-evoked gastric relaxation, may utilize the NK_1R at this site. Because reflex fundic relaxation could be accomplished by inhibition of vagal cholinergic excitatory output or excitation of vagal-enteric NANC inhibitory pathways to the fundus, these results imply that SP/NK_1R is a mechanism for selectively activating inhibitory vagal-enteric NANC inhibitory pathways.

In the DMN, SP and NK_1R agonists were equipotent to evoke similar depolarizing responses (Maubach and Jones, 1997). In the present study, similar doses of SP and NK_1R agonist in the DMN evoked comparable gastric motor inhibition. The response to SP was significantly attenuated by a prior microinjection of the selective NK_1R antagonist GR203040. Together, these data indicate that SP mediates its effects primarily on the NK_1R in the DMN. Gastric acid secretion is also inhibited by microinjection of SP or an NK_1R agonist into the dorsal vagal complex (Yang and Tache, 1997). It has also been reported that DMN neurons highly express NK_3R (Carpentier and Baude, 1996), and therefore we investigated the gastric motor effects of microinjection of the NK_3R agonist senktide. The fact that senktide microinjected into the DMN had no functional effect on IgP or motility in our experiments supports electrophysiological data that neurons in DMN were unaffected by both NKB, an NK_3R ligand (Martini-Luccarini et al., 1996; Maubach and Jones, 1997), and senktide (Maubach and Jones, 1997). Therefore, the functional significance of NK_3R in the DMN remains to be elucidated. Although NK_A has been reported to depolarize DMN neurons (Martini-Luccarini et al., 1996), these effects were not mimicked by a specific NK_2R agonist (Maubach and Jones, 1997). Therefore, we did not investigate any role of NK_2R drugs.

NK_1R antagonists have been shown to be antiemetic in pigs (Grelot et al., 1998) and in some studies in humans (Navari et al., 1999). The antiemetic properties of NK_1R antagonists are thought to be due to a site of action in the dorsal vagal complex (Watson et al., 1995; Tattersall et al., 1996; Rudd et al., 1999). Because fundic relaxation is a prodromal event essential for emesis, it is attractive to speculate that NK_1R antagonists inhibit emesis by blocking NK_1R on preganglionic neurons in the DMN. However, one study reported that an NK_1R antagonist, GR-205171, which abolished retching in response to vagal stimulation, apparently had no effect on corpus and antral relaxation (Furukawa et al., 1998). These investigators then concluded that GR-205171 acts on a vagal afferent pathway. However, the NK_1R antagonist did not affect the response of medial nucleus tractus solitarius neurons to vagal stimulation (Fukuda et al., 1998). Another NK_1R antagonist, CP-99,994, did not prevent loperamide-induced c-fos expression in the nucleus tractus solitarius, although retching and vomiting were abolished (Zaman et al., 2000). Thus, NK_1R antagonists are unlikely to prevent emesis at the level of the primary afferent inputs to the nucleus tractus solitarius. It was subsequently suggested that the site of the antiemetic action of NK_1R antagonists is in the central pattern generator for vomiting or in the pathway connecting the nucleus tractus solitarius to this region (Fukuda et al., 1999). In summary, the antiemetic site of action of NK_1R antagonists in the dorsal vagal complex is elusive and may involve multiple sites. The results of the present study suggest that the antiemetic action of NK_1R antagonists may be partially mediated in the DMN through the inhibition of preganglionic vagal cholinergic neurons that control enteric NANC inhibitory motor pathways to the fundus.

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References

Baude A and Shigemoto R (1998) Cellular and subcellular distribution of substance P receptor immunoreactivity in the dorsal vagal complex of the rat and cat: A light and electron microscope study. *J Comp Neurol* **402:**181–196.

Carpentier C and Baude A (1996) Immunocytochemical localisation of NK3 receptors in the dorsal vagal complex of rat. *Brain Res* **734:**327–331.

Desai KM, Warner TD, Bishop AE, Polak JM and Vane JR (1994) Nitric oxide, and not vasoactive intestinal peptide, as the main neurotransmitter of vagally induced relaxation of the guinea pig stomach. *Br J Pharmacol* **113:**1197–1202.

Dixon MK, Nathan NA and Hornby PJ (1998) Immunocytochemical distribution of neurokinin 1 receptor in rat dorsal vagal complex. *Peptides* **19:**913–923.

Feldman PD (1995) $Neurokinin_1$ receptor mediation of the vasodepressor effects of substance P in the nucleus of the tractus solitarius. *J Pharmacol Exp Ther* **273:**617–623.

Fodor M, Pammer C, Gorcs T and Palkovits M (1994) Neuropeptides in the human dorsal vagal complex: An immunohistochemical study. *J Chem Neuroanat* **7:**141–157.

Fukuda H, Koga T, Furukawa N, Nakamura E and Shiroshita Y (1998) The tachykinin NK1 receptor antagonist GR205171 prevents vagal stimulation-induced retching but not neuronal transmission from emetic vagal afferents to solitary nucleus neurons in dogs. *Brain Res* **802:**221–231.

Fukuda H, Nakamura E, Koga T, Furukawa N and Shiroshita Y (1999) The site of the antiemetic action of tachykinin NK1 receptor antagonists may exist in the medullary area adjacent to the semicompact part of the nucleus ambiguus. *Brain Res* **818:**439–449.

Furukawa N, Fukuda H, Hatano M, Koga T and Shiroshita Y (1998) A neurokinin-1 receptor antagonist reduced hypersalivation and gastric contractility related to emesis in dogs. *Am J Physiol* **275:**G1193–G1201.

Garrick T, Stephens RL, Ishikawa T, Sierra A, Avidan A, Weiner H and Taché Y (1989) Medullary sites for TRH analogue stimulation of gastric contractility in the rat. *Am J Physiol* **256:**G1011–G1015.

Grelot L, Dapzol J, Esteve E, Frugiere A, Bianchi AL, Sheldrick RL, Gardner CJ and Ward P (1998) Potent inhibition of both the acute and delayed emetic responses to cisplatin in piglets treated with GR205171, a novel highly selective tachykinin NK1 receptor antagonist. *Br J Pharmacol* **124:**1643–1650.

Grundy D, Gharib-Naseri MK and Hutson D (1993) Role of nitric oxide and vasoactive intestinal polypeptide in vagally mediated relaxation of the gastric corpus in the anaesthetized ferret. *J Auton Nerv Syst* **43:**241–246.

Krowicki ZK and Hornby PJ (1996) Gastric motor effects of substance P in the dorsal vagal complex and nucleus ambiguus of the rat. *Dig Dis Sci* **41:**1887.

Krowicki ZK and Hornby PJ (1998) Neurokinin 1 receptor activation in dorsal vagal complex evokes gastric motor inhibition. *Neurogastroenterol Motil* **10:**367.

Krowicki ZK, Nathan NA and Hornby PJ (1997a) Excitatory gastric motor and cardiovascular effects of endothelin-1 in the dorsal vagal complex are mediated through ET_A receptors. *J Pharmacol Exp Ther* **282:**535–542.

Krowicki ZK, Sharkey KA, Cardoza SV, Nathan NA and Hornby PJ (1997b) Distribution of nitric oxide (NO) synthase in rat dorsal vagal complex and effects of

microinjection of NO compounds upon gastric motor function. *J Comp Neurol* **377:**49–69.
Krowicki ZK, Sivarao DV, Abrahams TP and Hornby PJ (1999) Excitation of dorsal motor vagal neurons evokes non-nicotinic receptor mediated gastric relaxation. *J Auton Nerv Syst* **77:**83–89.
Ladic LA and Buchan AM (1996) Association of substance P and its receptor with efferent neurons projecting to the greater curvature of the rat stomach. *J Auton Nerv Syst* **58:**25–34.
Ladic LA and Buchan AM (1998) Three-dimensional spatial relationship of neuropeptides and receptors in the rat dorsal vagal complex. *Brain Res* **795:**312–324.
Manaker S and Zucchi PC (1993) Effects of vagotomy on neurotransmitter receptors in the rat dorsal vagal complex. *Neuroscience* **52:**427–441.
Martini-Luccarini F, Reynaud JC and Puizillout JJ (1996) Effects of tachykinins on identified dorsal vagal neurons: An electrophysiological study in vitro. *Neuroscience* **71:**119–131.
Maubach KA and Jones RS (1997) Electrophysiological characterisation of tachykinin receptors in the rat nucleus of the solitary tract and dorsal motor nucleus of the vagus in vitro. *Br J Pharmacol* **122:**1151–1159.
Meulemans AL, Eelen JG and Schuurkes JA (1995) NO mediates gastric relaxation after brief vagal stimulation in anesthetized dogs. *Am J Physiol* **269:**G255–G261.
Navari RM, Reinhardt RR, Gralla RJ, Kris MG, Hesketh PJ, Khojasteh A, Kindler H, Grote TH, Pendergrass K, Grunberg SM, Carides AD and Gertz BJ (1999) Reduction of cisplatin-induced emesis by a selective neurokinin-1-receptor antagonist: L-754,030 Antiemetic Trials Group. *N Engl J Med* **340:**190–195.
Ormsbee HSI, Barone FC, Lombardi DM and McCartney LC (1984) Effects of hindbrain L-glutamate acid application on gastrointestinal motor function in the cat, in *Gastrointestinal Motility: Proceedings of the Ninth International Symposium on Gastrointestinal Motility.* (Roman C ed) pp 583–591, MTP, Lancaster.
Ormsbee HSI and Bass P (1976) Gastroduodenal gradients in the dog after pyloroplasty. *Am J Physiol* **230:**389–397.
Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.
Plata-Salaman CR, Fukuda A, Minami T and Oomura Y (1989) Substance P effects on the dorsal motor nucleus of the vagus. *Brain Res Bull* **23:**149–153.
Raybould HE, Jakobsen LJ, Novin D and Taché Y (1989) TRH stimulation and L-glutamic acid inhibition of proximal gastric motor activity in the rat dorsal vagal complex. *Brain Res* **495:**319–328.
Rudd JA, Ngan MP and Wai MK (1999) Inhibition of emesis by tachykinin NK1 receptor antagonists in *Suncus murinus* (house musk shrew). *Eur J Pharmacol* **366:**243–252.
Shapiro RE and Miselis RR (1985) The central organization of the vagus nerve innervating the stomach of the rat. *J Comp Neurol* **238:**473–488.
Sivarao DV, Krowicki ZK, Abrahams TP and Hornby PJ (1999) Vagally regulated gastric motor function: Evidence for kainate and NMDA receptor mediation. *Eur J Pharmacol* **368:**173–182.
Spencer SE and Talman WT (1986) Central modulation of gastric pressure by substance P: A comparison with glutamate and acetylcholine. *Brain Res* **385:**371–374.
Sykes RM, Spyer KM and Izzo PN (1994) Central distribution of substance P, calcitonin gene-related peptide and 5-hydroxytryptamine in vagal sensory afferents in the rat dorsal medulla. *Neuroscience* **59:**195–210.
Takahashi T and Owyang C (1995) Vagal control of nitric oxide and vasoactive intestinal polypeptide release in the regulation of gastric relaxation in rat. *J Physiol (Lond)* **484:**481–492.
Tattersall FD, Rycroft W, Francis B, Pearce D, Merchant K, MacLeod AM, Ladduwahetty T, Keown L, Swain C, Baker R, Cascieri M, Ber E, Metzger J, MacIntyre DE, Hill RG and Hargreaves RJ (1996) Tachykinin NK1 receptor antagonists act centrally to inhibit emesis induced by the chemotherapeutic agent cisplatin in ferrets. *Neuropharmacology* **35:**1121–1129.
Thor KB and Helke CJ (1989) Serotonin and substance P colocalization in medullary projections to the nucleus tractus solitarius: Dual-colour immunohistochemistry combined with retrograde tracing. *J Chem Neuroanat* **2:**139–148.
Watson JW, Gonsalves SF, Fossa AA, McLean S, Seeger T, Obach S and Andrews PLR (1995) The anti-emetic effects of CP-99,994 in the ferret and the dog: Role of the NK1 receptor. *Br J Pharmacol* **115:**84–94.
Yang H and Tache Y (1997) Substance P in the dorsal vagal complex inhibits medullary TRH-induced gastric acid secretion in rats. *Am J Physiol* **272:**G987–G993.
Yuan CS and Lowell TK (1997) Gastric and brain stem, effects of substance P on nucleus tractus solitarius unitary responses. *Peptides* **18:**1169–1173.
Zaman S, Woods AJ, Watson JW, Reynolds DJ and Andrews PL (2000) The effect of the NK1 receptor antagonist CP-99,994 on emesis and c-fos protein induction by loperamide in the ferret. *Neuropharmacology* **39:**316–323.
Zheng ZL, Rogers RC and Travagli RA (1999) Selective gastric projections of nitric oxide synthase-containing vagal brainstem neurons. *Neuroscience* **90:**685–694.

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