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Modulation of gastric contractions in response to tachykinins and bethanechol by extrinsic nerves

Ulrike Holzer-Petsche

Department of Experimental and Clinical Pharmacology, University of Graz, Universitätsplatz 4, A-8010 Graz, Austria

1 Extrinsic reflexes elicited by changes in gastric wall tension play an important role in regulating gastric tone. The present study investigated whether such reflexes modulate gastric contractions induced by close arterially administered neurokinin A (NKA), substance P (SP), SP-methylester and bethanechol in anaesthetized rats.

2 Reflex pathways were acutely interrupted by either subdiaphragmatic vagotomy or prevertebral ganglionectomy. C-fibre afferent nerve activity was abolished by pretreating rats with capsaicin 10 to 16 days before the experiments.

3 The order of potency in inducing gastric contractions was NKA > SP > bethanechol. SP-methylester was markedly less effective than SP and its effects did not fit sigmoid dose-response curves (DRCs). The maximal responses to NKA, SP, and bethanechol were similar, whilst the DRC for SP was significantly flatter than those for NKA or bethanechol. Pretreatment of the rats with the peptidase inhibitors phosphoramidon or captopril did not increase the contractile response to SP.

4 Prevertebral ganglionectomy had no significant effect on the DRCs for SP and NKA, whereas vagotomy shifted the DRCs for all three test substances to the left.

5 Capsaicin pretreatment did not change the DRC for NKA in rats with intact vagus but shifted that for bethanechol to the left. The leftward shift of the DRC for NKA caused by vagotomy was prevented in capsaicin-pretreated rats whereas the vagotomy-induced shift of the DRC for bethanechol remained unaltered. The shift of the DRC for SP seen in response to vagotomy was only slightly reduced by capsaicin pretreatment.

6 These data may be interpreted as demonstrating two neuronal mechanisms for modulating drug-induced gastric contractions. First, the contractions themselves activate a vago-vagal negative feedback involving capsaicin-sensitive afferents. Second, NKA, and to a lesser degree SP, seem to induce a non-vagal non-splanchnic mechanism which via capsaicin-sensitive afferent neurones reinforces tachykinin-induced gastric contractions.

Keywords: substance P; neurokinin A; bethanechol; gastric motility; afferent; vagus

Introduction

Reflex nerve activity plays a major role in regulating gastric tone. Probably the most important of these reflexes is the so-called adaptive relaxation, i.e. gastric relaxation initiated by gastric distension, involving afferent and efferent branches in the vagus nerves (Abrahamsson & Jansson, 1973). Relaxant responses are also evoked by selective distension of the antrum, one component being a vago-vagal reflex, the other a spinal one (Abrahamsson, 1973a,b). Such a reflex was also elicited by close arterial injection of acetylcholine to the antrum (Abrahamsson, 1973b). Antral contractions in response to distension of the antrum or corpus have also been described (Andrews *et al.*, 1980; Grundy *et al.*, 1986).

In view of the importance of such extrinsic reflexes, the possibility was envisaged that gastric contractions brought about by exogenously administered motor stimulants might be modulated by similar mechanisms. The tachykinins neurokinin A (NKA) and substance P (SP) were chosen as test substances since they have been shown to be involved in the regulation of gastrointestinal motility (see Barthó & Holzer, 1985; Holzer-Petsche *et al.*, 1987; Koelbel *et al.*, 1988; Allescher *et al.*, 1989). NKA contracts the rat stomach through receptors on the smooth muscle, whereas SP acts in part via cholinergic neurones (Holzer-Petsche *et al.*, 1987). In order to gain information as to the type of tachykinin receptor involved, the NK_1 receptor agonist, SP-methylester (Watson *et al.*, 1983) was administered. Furthermore, bethanechol, a muscarinic agonist mimicking the action of acetylcholine on smooth muscle (see Holzer-Petsche *et al.*, 1987) was also tested. Extrinsic modulation of the effects of these substances could be effected in two ways: either by a tonic influence from the autonomic nervous system or by nervous reflexes involving afferent and efferent neurones. Since gastric afferents mediating various reflexes have been shown to be sensitive to the sensory neurotoxin capsaicin (McCann *et al.*, 1988; Raybould & Taché, 1988; 1989), this latter possibility was investigated by the use of the neurotoxic action of capsaicin on sensory neurones.

A possible reflex regulation might not only be elicited by the motor effect of, but also by direct chemical stimulation of afferent nerve endings by, the tachykinins (see Lew & Longhurst, 1986; Maggi *et al.*, 1986; Cervero & Sharkey, 1988). To differentiate between these possibilities, experiments were performed with bethanechol, since muscarinic agonists have been shown not to stimulate visceral or cutaneous afferent nerve endings (Armett & Ritchie, 1961; Juan, 1982). Metabolic mechanisms in the blood might also influence the drug on its way to the target organ. SP has been shown to be eliminated from rat blood much faster than NKA (Holzer-Petsche *et al.*, 1988). To test whether the rapid breakdown of SP might account for the lower potency of SP compared with NKA (Holzer-Petsche *et al.*, 1987), SP was tested in rats pretreated with the peptidase inhibitors phosphoramidon or captopril (see Bunnett, 1987).

Methods

Animals

Sprague-Dawley rats (Institut für Versuchstierkunde, Himberg, Austria) of either sex weighing 200–250 g were used. Before the experiments the rats were fasted overnight but had free access to water.

Experimental procedure

The rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and the trachea was cannulated to keep a patent airway. Blood pressure was monitored from a carotid artery. The oesophagus was ligated in the neck. In the experiments with ganglionectomized rats, the abdominal aorta was cannulated so that the tip of the catheter lay at the branching of the coeliac artery. Through this cannula, 154 mM NaCl was continuously infused to the stomach at a rate of 60 μl min^{-1}. For drug administration the infusion was changed from saline to the appropriate drug solution for 3 min. Sequential dose-response curves for SP and NKA were constructed in the same rat with intervals of at least 20 min between consecutive additions. In the experiments with vagotomized or capsaicin-pretreated rats, a retrograde catheter was inserted into the splenic artery for close arterial drug injection as a bolus of 100 μl. In this instance, only one substance was tested in each rat, with intervals of at least 20 min between increasing doses.

Gastric motility was measured as intragastric pressure by means of a pressure transducer attached to a cannula placed into the stomach through an incision in the duodenum and fixed by a ligation around the pylorus. The signals were recorded on a pen recorder. Fifteen minutes after the end of the operation, the stomach was rapidly filled with 3 ml saline and drug administration was begun 20 min later.

In order to eliminate possible reflex loops, two types of lesions were performed immediately before the experiments: for subdiaphragmatic vagotomy, all nerve fibres along the distal oesophagus were severed under the microscope. The reflex pathway via the splanchnic nerves was interrupted by blunt removal of the coeliac-superior mesenteric ganglion complex ('ganglionectomy'). Control animals were sham-operated.

Pretreatment with peptidase inhibitors

In order to inhibit peptidases possibly involved in the breakdown of SP, captopril was administered i.v. at a dose of 10 μmol kg^{-1} 2 min (Shore *et al.*, 1988) before SP or, as after 2 min no effect on blood pressure was observed, 30 min before SP. Phosphoramidon was administered i.v. at 2 or 20 μmol kg^{-1} 2 min before SP (Thompson & Sheppard, 1988). Since i.v. administration of the inhibitors was ineffective, both inhibitors were also administered close arterially into the splenic artery (0.5 μmol captopril or 1 μmol phosphoramidon) 30 s before the i.a. test dose of SP, in order to achieve the same site and time of action of the peptidase inhibitors as of SP.

Capsaicin pretreatment

In order to eliminate the function of small diameter afferent nerve fibres, adult rats were treated s.c. with 125 mg kg^{-1} (400 μmol kg^{-1}) capsaicin divided into three doses of 25 and 50 mg kg^{-1} on the first, and 50 mg kg^{-1} on the second day 10 to 16 days before the experiment according to the protocol of Gamse *et al.* (1981). For the part of the study testing the effect of vagotomy, all control rats were treated with equal volumes of the vehicle consisting of 10% ethanol and 10% Tween 80 in 154 mM NaCl (v/v). All injections were performed under ether anaesthesia.

Evaluation of results

Andrews & Lawes (1985) reported that gastric motor responses to extrinsic neural stimuli depended on the basal tone. The peak of the contractions, however, corresponded to the strength of the stimulus. Therefore the peak pressure reached during substance infusion, or within 1 min after the injection, was measured.

Response maxima, ED_{50}s, and slope factors were calculated for individual DRCs by fitting the data to sigmoid curves by use of the software ALLFIT (for description see De Lean *et al.*, 1978). All results are represented as mean ± s.e.mean. Since tests for normal distribution are not reliable with low n, statistical comparisons were made by the Kruskal-Wallis H-test (Sachs, 1982). A value of $P < 0.05$ was considered statistically significant.

Substances

SP, SP-methylester, and NKA were obtained from Cambridge Research Biochemicals (Cambridge, UK), bethanechol-Cl from Schuchardt (München, Germany), and phosphoramidon from Peninsula (St. Helens, UK). Captopril was kindly supplied by Squibb-von Heyden (Vienna, Austria). Stock solutions of the substances (1 mM for the peptides) as well as dilutions were made with 154 mM NaCl.

Results

During filling of the stomach with 3 ml saline, intragastric pressure increased sharply and then decreased again over several minutes to a level slightly higher than in the empty stomach reflecting adaptive relaxation. This rise in intragastric pressure was significantly greater in vagotomized rats than in rats with intact vagus or in ganglionectomized rats indicating impairment of adaptive relaxation (Table 1). Capsaicin pretreatment without vagotomy also caused a slight but significant augmentation of this rise in intragastric pressure indicating minor inhibition of adaptive relaxation. Conversely, vagotomy in capsaicin-pretreated rats produced a smaller rise in the intragastric pressure than vagotomy in vehicle controls (Table 1).

Retrograde injections of SP, NKA, or bethanechol into the splenic artery led to rapid and transient gastric contractions. After higher doses of NKA and bethanechol these tonic responses were followed by phasic activity lasting up to 10 min (cf. Holzer-Petsche *et al.*, 1987). The order of potency was NKA > SP > bethanechol (Table 2). There was no significant difference between the response maxima of SP, NKA, and bethanechol in control rats. The DRC for SP was significantly flatter than those for NKA and bethanechol (Table 2). SP-methylester in a dose range of 0.15–50 nmol induced only minor tonic gastric contractions up to 2.2 mN, which did not reasonably fit to sigmoid curves.

Captopril, whether injected into the splenic artery or i.v. (each $n = 4$) did not change the gastric motor response to a test dose of 0.5 nmol SP, although 30 min after i.v. captopril, mean blood pressure was significantly lowered from 98 ± 3 to 74 ± 2 mmHg. Phosphoramidon close arterially or i.v. (each $n = 3$) had no effect on either the gastric motor response to 0.5 nmol SP or on blood pressure.

Acute removal of the coeliac-superior mesenteric ganglion complex did not significantly influence gastric contractions in response to intraarterial infusions of NKA or SP into the coeliac artery (Figure 1). The calculated ED_{50}s were 0.22 ± 0.06 nmol for NKA and 15.8 ± 4.6 nmol for SP in control rats (each $n = 4$), 0.20 ± 0.03 nmol for NKA and

Table 1 Increase in baseline pressure (in kPa) 5 min after instillation of 3 ml saline into the stomach

Treatment	*Increase* (kPa)
Controls (untreated)	0.20 ± 0.08 (4)
Ganglionectomy	0.19 ± 0.03 (5)
Controls (vehicle-treated)	0.20 ± 0.02 (18)
Vagotomy (vehicle-treated)	0.63 ± 0.04 (17)[a]
Capsaicin	0.26 ± 0.02 (16)[c]
Capsaicin plus vagotomy	0.51 ± 0.03 (17)[a,b]

Values are means ± s.e.mean, (n).
[a] $P < 0.001$ vs. controls and capsaicin; [b] $P < 0.05$ vs. vagotomy; [c] $P < 0.05$ vs. controls and $P < 0.001$ vs. vagotomy and capsaicin plus vagotomy (Kruskal-Wallis H-test).

Table 2 Response maxima, ED_{50}s, and slope factors for the contractile effects of neurokinin A (NKA), substance P (SP) and bethanechol in control rats (treated with the vehicle for capsaicin) as obtained by fitting the experimental values to sigmoid curves by ALLFIT

	Maximum (kPa)	ED_{50} (nmol)	*Slope factor*[1]
NKA	4.66 ± 0.57	1.11 ± 0.47[a]	−0.96 ± 0.10
SP	5.64 ± 0.79	9.99 ± 3.62[a]	−0.48 ± 0.08[b]
Bethanechol	4.87 ± 0.23	39.4 ± 4.9	−0.92 ± 0.23

Common response minima were assumed.
Values are means ± s.e.mean, (n = 5–6).
[a] $P < 0.001$ vs. other two compounds; [b] $P < 0.05$ vs. NKA and bethanechol (Kruskal-Wallis H-test).
[1] 'The slope factor corresponds to the slope of the logit-log plot when the dose is portrayed in terms of natural logarithms' (DeLean *et al.*, 1978). Due to the assignment of the variables in the four-parameter logistic equation used by ALLFIT the slope factor is negative when the curve rises.

15.3 ± 0.6 nmol for SP in ganglionectomized rats (each n = 5). The slope factors were −0.59 ± 0.05 (n = 4) for NKA in controls, −0.80 ± 0.25 (n = 5) for NKA in ganglionectomized rats; −0.43 ± 0.15 (n = 4) for SP in controls, −0.46 ± 0.33 for SP (n = 5) in ganglionectomized rats (no statistically significant difference).

In vehicle-treated rats, the DRCs for NKA, SP, and bethanechol were shifted to the left by acute subdiaphragmatic vagotomy (Figure 2 and Table 3). Capsaicin pretreatment had no significant effect on the gastric responses to exogenous NKA or SP as long as the vagus nerves were intact. The DRC to bethanechol, however, was shifted to the left by capsaicin pretreatment. In rats pretreated with capsaicin, vagotomy induced only a minor shift to the left of the DRC for NKA (Figure 2a). The DRCs for bethanechol in vagotomized rats were identical whether the rats had been pretreated with capsaicin or its vehicle (Figure 2c). The leftward shift of the DRC for SP observed after vagotomy was reduced by capsaicin pretreatment (Figure 2b). Analysis of the DRCs by ALLFIT indicated no significant changes of the response minima, maxima, or slope factors.

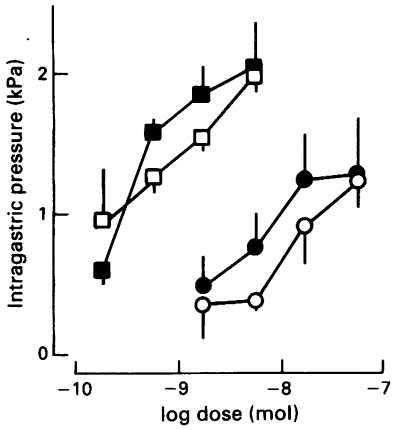

Figure 1 Effect of acute removal of the coeliac-superior mesenteric ganglion complex on gastric contractions induced by a 3 minute-infusion of substance P (○, ●) or neurokinin A (□, ■) into the coeliac artery; (○, □): controls; (●, ■): ganglionectomy. Values are means with s.e.mean shown by vertical lines; n = 4–5.

Table 3 Effect of various pretreatments on the relative potencies of neurokinin A (NKA), substance P (SP) and bethanechol as calculated from the ED_{50}s

	NKA	*SP*	*Bethanechol*
Vehicle	1 (1.11 nmol)	1 (9.99 nmol)	1 (39.4 nmol)[a]
Vehicle + vagotomy	17.5[a]	9.34[b]	12.0
Capsaicin	0.34	2.39	2.86
Capsaicin + vagotomy	2.30	7.62	11.3

[a] $P < 0.05$ vs. other three treatments.
[b] $P < 0.05$ vs. capsaicin and vehicle (Kruskal-Wallis H-test).

Discussion

The order of potency of the test substances for inducing gastric contractions in control rats confirms previous observations (Holzer-Petsche *et al.*, 1987). While the maximal responses to NKA, SP, and bethanechol were equal, the slope of the DRC for SP was smaller than those for the other two substances. The minor effect of SP-methylester indicates that NK_1 receptors contribute little to the gastric contraction induced by SP. SP itself is less specific for NK_1 receptors than SP-methylester, as higher doses also activate NK_2 receptors (Cascieri *et al.*, 1985), which is reflected by the greater ED_{50} of SP than NKA in control rats. Thus, the DRC for SP may represent activation of a mixed receptor population, a further indication of which is the smaller slope of the DRC for SP compared with NKA or bethanechol.

It has frequently been reported that neutral endopeptidase ('enkephalinase', EC 3.4.24.11) or peptidyl dipeptidase A ('angiotensin-converting enzyme', EC 3.4.15.1) is involved in the breakdown of SP (see Bunnett, 1987). Phosphoramidon, an inhibitor of neutral endopeptidase, impairs the breakdown of SP in the ferret small intestine (Djokic *et al.*, 1989), and peptidyl dipeptidase A appears to be involved in the breakdown of SP in the rat stomach wall as demonstrated by the use of captopril as inhibitor (Orloff *et al.*, 1986). Neither of the two inhibitors, however, had any effect on the gastric motor response to SP indicating that the faster breakdown of SP compared with NKA cannot be made responsible for the lesser potency of SP after close arterial injection.

Coeliac ganglionectomy did not increase gastric responses to NKA or SP. In contrast, pretreatment of the rats with guanethidine shifted the DRCs to both NKA and SP to the left, which was interpreted as abolition of an adrenergic inhibitory tone (Holzer-Petsche *et al.*, 1987). If such an adrenergic tone were exerted exclusively by splanchnic fibres, one would

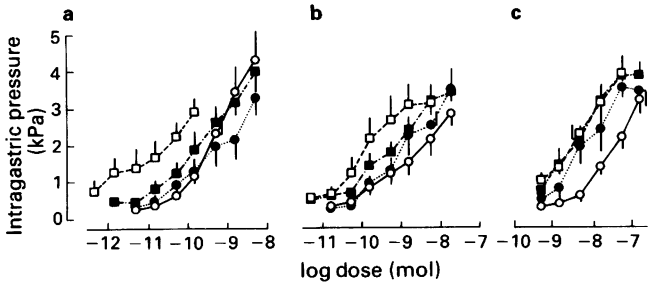

Figure 2 Dose-response curves for the gastric motor effects of neurokinin A (a), substance P (b), and bethanechol (c) after various pretreatments: (○) controls (vehicle-treated); (□) vagotomy (vehicle-treated); (●) capsaicin pretreatment; (■) capsaicin plus vagotomy. Values are means with s.e.mean shown by vertical lines; n = 5–6.

expect ganglionectomy to mimic guanethidine in stimulating the gastric responses to the tachykinins. The lack of effect of ganglionectomy, however, cannot be explained as being due to incomplete removal of the ganglia, since Kirchgessner & Gershon (1989) reported that the vagus nerve of rats contained postganglionic sympathetic axons from the superior cervical ganglion. Thus, sympathetic inhibition is not totally abolished after coeliac ganglionectomy but may be prevented by chemical sympathectomy.

Vagogomy significantly increased the potency of NKA, SP, and bethanechol in contracting the rat stomach. This suggests that an inhibitory influence is exerted via the vagus nerves, which, in rats with intact vagus, partly counteracts the evoked contractions. Vagal efferent discharge is modulated by changes in gastric wall tension, be they contraction, distension or compression of the stomach wall (Davison & Grundy, 1978). Thus it is feasible that every contraction of the stomach elicits a negative feedback via the vagus, which in turn counteracts the contraction. In the whole stomach, the measured change in gastric tension would therefore be the resultant of the contraction elicited by the agonist and the reflex relaxation. The increased contraction of the stomach in response to i.a. administered agents observed after vagotomy may thus be explained by the destruction of such a vagal negative feedback mechanism.

SP has been described as stimulating visceral afferents (Lew & Longhurst, 1986), and Maggi *et al.* (1986) proposed that, in the rat bladder, SP-E (NK_2) receptors (where NKA is more potent than SP) might be located on afferent nerve endings. In contrast, Cervero & Sharkey (1988) concluded from their experiments on afferents of rat ileum that the afferent stimulation by SP might be indirectly mediated through the peptide-induced contractions of the smooth muscle. To differentiate between these possibilities, bethanechol was also administered since electrophysiological experiments had demonstrated that this muscarinic agonist did not stimulate visceral or cutaneous afferent nerve fibres (Armett & Ritchie, 1961; Juan, 1982). The potentiation of bethanechol-induced gastric contractions by vagotomy suggests that the postulated negative feedback was initiated by the gastric contractions rather than by chemical stimulation of afferent nerve endings.

A tonic inhibition of the stomach via the vagus nerve has also to be considered as opposed to a reflex relaxation. However, since capsaicin pretreatment shifted the DRC for bethanechol to the left it seems that abolition of afferent nerve activity rather than of tonic efferent inhibition is responsible for the increased response to bethanechol after vagotomy. From the shift to the left of the DRCs for bethanechol after ablation of capsaicin-sensitive afferents it must be concluded that at least part of the mechanoreceptors in the gastric wall are sensitive to capsaicin and that these capsaicin-sensitive afferents are involved in the vago-vagal inhibition. This observation is paralleled by the slightly but significantly greater rise in baseline pressure after initial instillation of saline into the stomach of capsaicin-pretreated rats.

Unlike the DRCs for bethanechol in capsaicin-pretreated rats, which mimicked those after vagotomy in vehicle-treated rats, the DRCs for NKA in capsaicin-pretreated rats were similar to those in control rats whether or not the vagus nerves were intact. Explanation of this finding is possible by assuming that NKA initiates a positive feedback which uses capsaicin-sensitive structures and which does not require an intact vagus nerve. The negative feedback (via the vagus) and the positive feedback (via capsaicin-sensitive afferents) balance each other so that abolition of both regulatory mechanisms at the same time (as in capsaicin-pretreated rats with vagotomy) does not shift the DRC for NKA from its position in control rats.

The localization of the capsaicin-sensitive endings stimulated chemically by NKA cannot be deduced from the present experiments. If the positive feedback required impulse conduction through extrinsic centres, these afferents could not run in the vagus, because then they would also be destroyed by vagotomy in vehicle-treated rats. If the positive feedback used impulse conduction via the splanchnic nerves through the coeliac ganglion, it would be abolished after prevertebral ganglionectomy. Another mechanism to be considered for the processing of the positive feedback is a local one within the gastric wall. In this case the afferent nerve endings stimulated by NKA would mediate the positive feedback by means of an axon or enteric reflex. Neuhuber (1987) described afferent nerve endings in the proximal stomach of the rat which showed presynaptic structures in contact with intrinsic neurones and which might be the anatomical correlate of such an intramural reflex. Abolition of an intramural reflex involving capsaicin-sensitive afferents might explain the smaller increase in intragastric pressure after filling the stomach in the 'capsaicin plus vagotomy' group as compared to vehicle-treated rats with vagotomy (Table 1). If capsaicin-sensitive fibres are stimulated by changes in gastric tension, this stimulus might lead to a local positive feedback similar to the one postulated to be evoked by NKA. Such an intramural reflex would not be affected by acute vagotomy, since this procedure does not immediately impair the function of the peripheral endings of the cut nerve.

The shift of the DRCs for SP by the various treatments was less pronounced than for NKA. Since, however, SP acts only partly via muscular tachykinin receptors but also via cholinergic interneurones through atropine-sensitive receptors (Holzer-Petsche *et al.*, 1987), the mechanism of action of SP can be understood to comprise the actions of both NKA and bethanechol.

The present results show that extrinsic nerves are able to influence contractions induced by gastric motor stimulants. Vago-vagal inhibition of gastric motility is not only elicited by gastric distension but seems also to play a role in modulating drug-induced contractions. Capsaicin-sensitive afferents take part in mediating this modulation. Furthermore, there is indirect evidence for tachykinin receptors (presumably NK_2) on capsaicin-sensitive afferent nerve endings.

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References

ABRAHAMSSON, H. (1973a). Studies on the inhibitory nervous control of gastric motility. *Acta Physiol. Scand.*, Suppl. **390**, 1–38.

ABRAHAMSSON, H. (1973b). Vagal relaxation of the stomach induced from the gastric antrum. *Acta Physiol. Scand.*, **89**, 406–414.

ABRAHAMSSON, H. & JANSSON, G. (1973). Vago-vagal gastro-gastric relaxation in the cat. *Acta Physiol. Scand.*, **88**, 289–295.

ALLESCHER, H.-D., KOSTOLANSKA, F., TOUGAS, G., FOX, J.E.T., REGOLI, D., DRAPEAU, G. & DANIEL, E.E. (1989). The actions of neurokinins and substance P in canine pylorus, antrum and duodenum. *Peptides*, **10**, 671–680.

ANDREWS, P.L.R., GRUNDY, D. & SCRATCHERD, T. (1980). Reflex excitation of antral motility induced by gastric distension in the ferret. *J. Physiol.*, **298**, 79–84.

ANDREWS, P.L.R. & LAWES, I.N.C. (1985). Gastric tone modifies the responses to extrinsic neural stimuli in the anaesthetized ferret. *J. Physiol.*, **366**, 1–16.

ARMETT, C.J. & RITCHIE, J.M. (1961). The action of acetylcholine and some related substances on conduction in mammalian non-myelinated nerve fibres. *J. Physiol.*, **155**, 372–384.

BARTHÓ, L. & HOLZER, P. (1985). Search for a physiological role of substance P in gastrointestinal motility. *Neuroscience*, **16**, 1–32.

BUNNETT, N.W. (1987). Postsecretory metabolism of peptides. *Am. Rev. Resp. Dis.*, **136**, S27–S34.

CASCIERI, M.A., CHICCHI, G.G. & LIANG, T. (1985). Demonstration of two distinct tachykinin receptors in rat brain cortex. *J. Biol. Chem.*, **260**, 1501–1507.

CERVERO, F. & SHARKEY, K.A. (1988). An electrophysiological and anatomical study of intestinal afferent fibres in the rat. *J. Physiol.*, **401**, 381–397.

DAVISON, J.S. & GRUNDY, D. (1978). Modulation of single vagal efferent fibre discharge by gastrointestinal afferents in the rat. *J. Physiol.*, **284**, 69–82.

DE LEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97–E102.

DJOKIC, D.J., SEKIZAWA, K., BORSON, D.B. & NADEL, J.A. (1989). Neutral endopeptidase inhibitors potentiate substance P-induced contraction in gut smooth muscle. *Am. J. Physiol.*, **256**, G39–G43.

GAMSE, R., LEEMAN, S.E., HOLTZER, P. & LEMBECK, F. (1981). Differential effects of capsaicin on the content of somatostatin, substance P, and neurotensin in the nervous system of the rat. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **317**, 140–148.

GRUNDY, D., HUTSON, D. & SCRATCHERD, T. (1986). A permissive role for the vagus nerves in the genesis of antro-antral reflexes in the anaesthetized ferret. *J. Physiol.*, **381**, 377–384.

HOLZER-PETSCHE, U., LEMBECK, F. & SEITZ, H. (1987). Contractile effects of substance P and neurokinin A on the rat stomach in vivo and in vitro. *Br. J. Pharmacol.*, **90**, 273–279.

HOLZER-PETSCHE, U., SARIA, A., RUPITZ, M., SCHULIGOI, R. & LEMBECK, F. (1988). Degradation of substance P, neurokinin A, and calcitonin gene-related peptide in rat plasma in vivo. *Regul. Pept.*, **22**, 89.

JUAN, H. (1982). Nicotinic nociceptors in perivascular sensory nerve endings. *Pain*, **12**, 259–264.

KIRCHGESSNER, A.L. & GERSHON, M.D. (1989). Identification of vagal efferent fibers and putative target neurons in the enteric nervous system of the rat. *J. Comp. Neurol.*, **285**, 38–53.

KOELBEL, C.B., MAYER, E.A., VAN DEVENTER, G., SNAPE, JR, W.J. & PATEL, A. (1988). Characterization of the effects of neurokinins on canine antral muscle. *Am. J. Physiol.*, **255**, G779–G786.

LEW, W.Y.W. & LONGHURST, J.C. (1986). Substance P, 5-hydroxytryptamine, and bradykinin stimulate abdominal visceral afferents. *Am. J. Physiol.*, **250**, R465–R473.

MAGGI, C.A., SANTICIOLI, P., GIULIANI, S., REGOLI, D. & MELI, A. (1986). Activation of micturition reflex by substance-P and substance-K: indirect evidence for the existence of multiple tachykinin receptors in the rat urinary bladder. *J. Pharmacol. Exp. Ther.*, **238**, 259–266.

MCCANN, M.J., VERBALIS, J.G. & STRICKER, E.M. (1988). Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats. *J. Auton. Nerv. Syst.*, **23**, 265–272.

NEUHUBER, W.L. (1987). Sensory vagal innervation of the rat esophagus and cardia: a light and electron microscopic anterograde tracing study. *J. Auton. Nerv. Syst.*, **20**, 243–255.

ORLOFF, M.S., TURNER, A.J. & BUNNETT, N.W. (1986). Catabolism of substance P and neurotensin in the rat stomach wall. *Regul. Pept.*, **14**, 21–31.

RAYBOULD, H.E. & TACHÉ, Y. (1988). Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. *Am. J. Physiol.*, **255**, G242–G246.

RAYBOULD, H.E. & TACHÉ, Y. (1989). Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anaesthetized rats. *Eur. J. Pharmacol.*, **167**, 237–243.

SACHS, L. (1982). *Applied Statistics. A Handbook of Techniques.* New York: Springer.

SHORE, S.A., STIMLER-GERARD, N.P., COATS, S.R. & DRAZEN, J.M. (1988). Substance P-induced bronchoconstriction in the guinea pig. *Am. Rev. Resp. Dis.*, **137**, 331–336.

THOMPSON, J.E. & SHEPPARD, D. (1988). Phosphoramidon potentiates the increase in lung resistance mediated by tachykinins in guinea pigs. *Am. Rev. Resp. Dis.*, **137**, 337–340.

WATSON, S.P., SANDBERG, B.E.B., HANLEY, M.R. & IVERSEN, L.L. (1983). Tissue selectivity of substance P alkyl esters: suggesting multiple receptors. *Eur. J. Pharmacol.*, **87**, 77–84.

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