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Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat

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1 Substance P and substance K (neurokinin A) (dose range: 0.08–80 nmol kg^{-1}) were tested for their effects on gastrointestinal propulsion in the rat. The peptides were given by intraperitoneal injection concurrently with the intragastric administration of a test meal containing charcoal and ^{51}Cr.

2 Examination 3 min after the test meal showed that high doses of substance P (> 0.74 nmol kg^{-1}) and substance K (> 8.8 nmol kg^{-1}) inhibited gastric emptying and gastrointestinal transit. This inhibitory effect was changed to a stimulant effect by pretreatment of the rats with atropine (3.5 μmol kg^{-1}). Guanethidine pretreatment (67 μmol kg^{-1}) revealed a facilitatory effect of low doses of the two tachykinins (about 1 nmol kg^{-1}) on gastrointestinal propulsion.

3 Examination 15 min after the test meal demonstrated that substance P (> 0.74 nmol kg^{-1}) dose-dependently enhanced gastrointestinal propulsion, an effect that was also seen after atropine pretreatment. Low doses of substance K (about 1 nmol kg^{-1}) also stimulated gastrointestinal propulsion but this effect was abolished by atropine. In addition, atropine pretreatment revealed a stimulant effect of high doses of substance K (88 nmol kg^{-1}) on gastric emptying.

4 These results show that the effects of substance P and substance K on gastrointestinal propulsion vary with dose and time and involve, at least partly, activation of the autonomic nervous system.

Introduction

In a number of species substance P has been found to stimulate gastrointestinal contractions both *in vivo* and *in vitro* (see Bertaccini, 1982), and there is experimental evidence that an endogenous substance P-like peptide is involved in the physiological regulation of peristalsis, at least in the guinea-pig isolated small intestine (Barthó *et al.*, 1982; Yokoyama & North, 1983; Costa *et al.*, 1985). In the anaesthetized rat, substance P was reported to cause contractions of the stomach and pylorus (Bertaccini & Coruzzi, 1977). However, it is not yet clear in which way this effect might influence gastrointestinal propulsion, since intraperitoneal injections of substance P were found either to accelerate gastric emptying (Mangel & Keogel, 1984) or to be ineffective (Bertaccini *et al.*, 1981). The present study was therefore carried out to investigate systematically the time- and dose-dependence of the effect of substance P on gastrointestinal propulsion. Substance P was also tested in rats pretreated with either atropine or guanethidine so as to reveal a possible involvement of the autonomic nervous system.

The effects of substance P were analysed in parallel with those of a closely related peptide, substance K (neurokinin A). Substantial concentrations of this tachykinin have been detected in the rat digestive tract (Maggio & Hunter, 1984; Theodorsson-Norheim *et al.*, 1984). Since one of the two preprotachykinin messenger RNAs thus far isolated from mammalian tissue encodes both substance P and substance K (Nawa *et al.*, 1983) it is likely that these two tachykinins may co-exist in the same neurones. It is, however, an open question as to what extent their effects and functions overlap or differ.

Methods

Adult Sprague-Dawley rats (strain OFA-SD, Forschungsinstitut für Versuchstierzucht, Himberg, Austria), of either sex and 200–250 g body weight, were used in all experiments. The animals were deprived of food for 20 h before experimentation but allowed free access to tap water.

Assay

Gastric emptying and gastrointestinal transit were assessed by the gastrointestinal propulsion of a test meal containing the two non-absorbable markers charcoal and ^{51}Cr. The test meal (1 ml) was given intragastrically by means of a stomach tube. During the administration of the test meal the rats were kept under ether anaesthesia but it was ensured that they regained the righting reflex within 1 min after feeding. At the time intervals specified in the results section, the rats were killed by cervical dislocation, and the stomach and small intestine were exposed rapidly by laparotomy. The stomach was occluded at the pylorus and cardia, and the front of the charcoal suspension in the small intestine, which was detected visually, was also marked by a ligation. *Gastrointestinal transit* is expressed as the percentage of the length traversed by the charcoal marker divided by the total length of the small intestine. The ^{51}Cr radioactivity contents of the stomach and small intestine were then determined by γ-counting. *Gastric emptying* is defined as the percentage of the radioactivity contained in the small intestine at the time the rats were killed divided by the total radioactivity recovered from the stomach and small intestine.

Arterial blood pressure

Rats were anaesthetized with urethane (13.5 mmol kg^{-1}, i.p.), and one carotid artery was cannulated and connected to a Statham pressure transducer for blood pressure recording.

Drug administration

All drugs were administered in a constant volume of 1 ml kg^{-1}. Substance P and substance K were injected intraperitoneally at the time of the intragastric administration of the test meal. Control animals received Tyrode solution, 1 ml kg^{-1}, intraperitoneally. In a separate series of experiments rats were pretreated with atropine (3.5 μmol kg^{-1}, i.p.) 15 min before administration of the test meal. In a further group of experiments, rats were pretreated with a total of 67 μmol kg^{-1} guanethidine in two equal doses which were injected subcutaneously 18 and 2 h before administration of the test meal.

Substances and solutions

Substance P (Serva, Heidelberg, F.R.G.) and substance K (neurokinin A) (Bachem, Basel, Switzerland) were dissolved in 0.01 M acetic acid (1 mg ml^{-1}). The solutions used for the i.p. administration of the peptides were prepared by diluting the peptide stock solutions with Tyrode solution (composition in mM: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6). The solutions of atropine (Merck, Darmstadt, F.R.G.) and guanethidine (Ciba-Geigy, Basel, Switzerland) used for injection were also made with Tyrode solution.

The charcoal meal was an aqueous suspension of 1% (w/w) hydroxypropyl methylcellulose and 10% (w/w) carbon black (Howd *et al.*, 1978). Carbon black was obtained from Merck (Darmstadt, F.R.G.) and hydroxypropyl methylcellulose from Sigma (München, F.R.G.; viscosity of a 2% solution: 50 centipoises). The meal contained in addition approximately 65 MBq l^{-1} of ^{51}Cr (Amersham, U.K.; corresponding to about 60 nM Na_2CrO_4). The suspension was continuously stirred and prewarmed to body temperature.

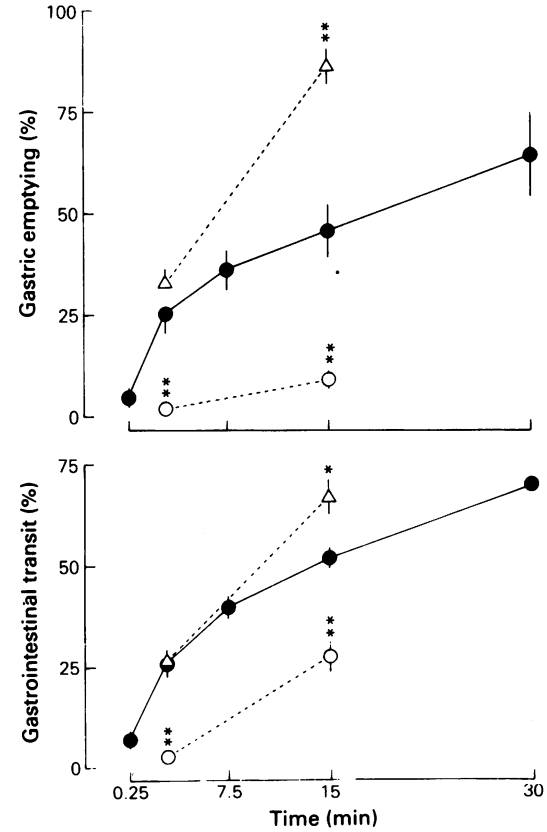

Figure 1 Time course of gastric emptying and gastrointestinal transit following intragastric administration of a test meal in untreated rats and in rats pretreated either with atropine (3.5 μmol kg^{-1}, i.p., ○) or guanethidine (67 μmol kg^{-1}, s.c., △); no drug (●). Means of 6–15 rats are shown with s.e.mean indicated by vertical lines. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. untreated rats.

Statistics

All values are expressed as means ± s.e.mean. Statistically significant differences were evaluated by one way analysis of variance in conjunction with the Duncan multiple range test. P values < 0.05 were regarded as significant.

Results

Effect of ether anaesthesia during feeding on gastrointestinal motility

Neither gastric emptying nor gastrointestinal transit differed when the test meal was given to conscious animals or to rats anaesthetized with ether. Three min after the meal was given to conscious rats, gastric emptying amounted to 27.2 ± 5.9% ($n = 7$) and gastrointestinal transit to 26.4 ± 5.4% ($n = 7$). When the meal was given to anaesthetized rats, gastric emptying was 25.4 ± 5.1% ($n = 14$) and gastrointestinal transit 26.7 ± 2.4% ($n = 14$). Therefore all other experiments described in this paper were performed with rats kept under ether anaesthesia during feeding.

Effect of atropine and guanethidine on gastrointestinal motility

The time course of gastric emptying and gastrointestinal transit from 0.25–30 min after application of the test meal is illustrated in Figure 1. Pretreatment of the rats with atropine (3.5 μmol kg^{-1}) markedly inhibited gastric emptying and gastrointestinal transit. The observation that the slope of the gastrointestinal transit curve was not altered by atropine (Figure 1) suggests that atropine inhibited primarily gastric emptying but did not itself reduce the transit of the marker through the small intestine, as previously shown by Ruwart *et al.* (1979). Guanethidine (67 μmol kg^{-1}) enhanced gastric emptying and gastrointestinal transit preferentially at longer time intervals (15 min) after feeding.

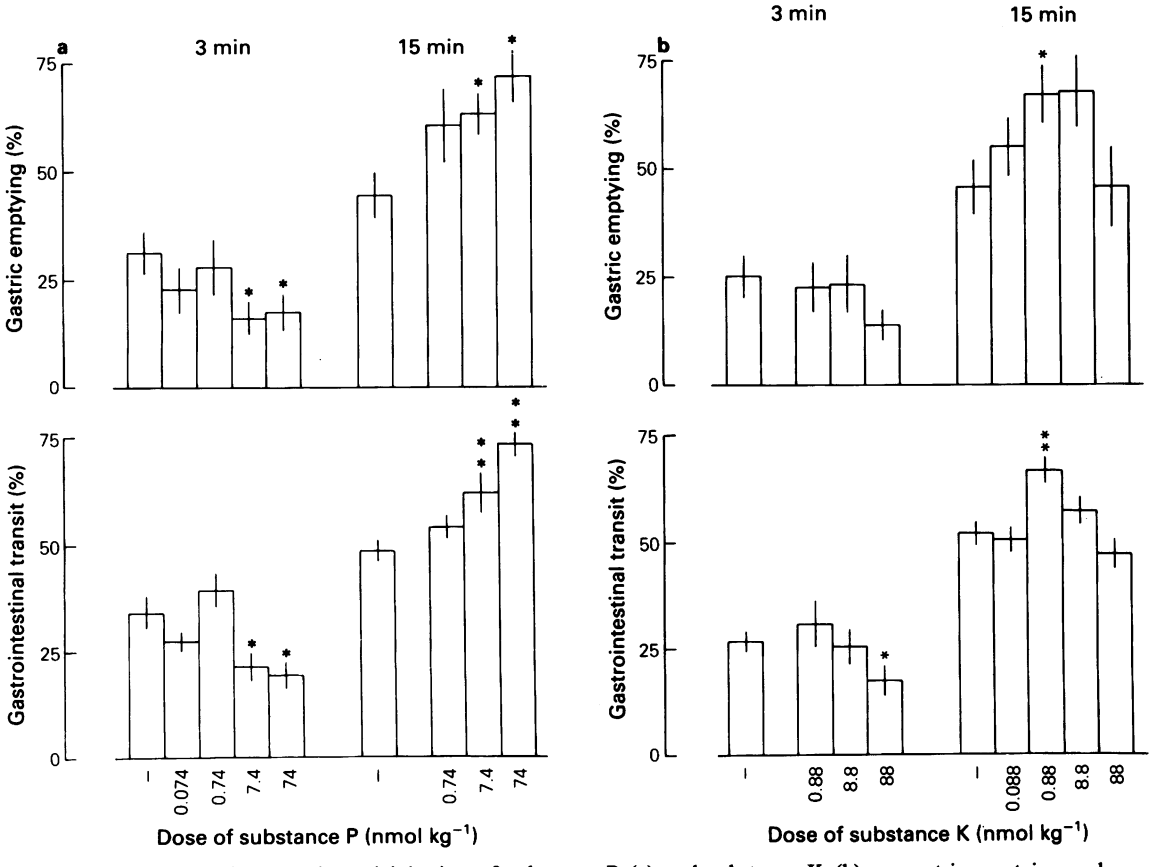

Figure 2 Effect of intraperitoneal injection of substance P (a) and substance K (b) on gastric emptying and gastrointestinal transit in the rat as measured 3 and 15 min after intragastric administration of a test meal. Means of 7–17 rats are shown with s.e.mean indicated by vertical lines. $*P < 0.05$, $**P < 0.01$, vs. vehicle controls.

Effect of substance P and substance K on gastrointestinal motility

The effects of substance P and substance K on gastric emptying and gastrointestinal transit were both dose- and time-dependent (Figure 2a and b). Three min after the concurrent application of the test meal and the peptides a dose-dependent inhibition of gastric emptying and gastrointestinal transit was observed with both tachykinins. Substance P seemed to be more potent in this respect than substance K since a dose of 7.4 nmol kg^{-1} substance P was sufficient to decrease gastric emptying and gastrointestinal transit significantly whereas the effect of 88 nmol kg^{-1} substance K on gastric emptying was still not significant, although a clear tendency towards inhibition was seen with this dose. Gastrointestinal transit was, however, significantly depressed by 88 nmol kg^{-1} substance K (Figure 2b).

Examination of gastrointestinal propulsion 7.5 min after feeding showed that substance P (74 nmol kg^{-1}) no longer inhibited gastrointestinal motility but tended to increase both gastric emptying and gastrointestinal transit, although only the effect on gastrointestinal transit was statistically significant ($n = 9$, data not shown).

Fifteen min after feeding clear increases in gastrointestinal propulsion were observed with both substance P and substance K (Figure 2a and b). However, the dose-dependence of these effects was again different for the two tachykinins. Substance P increased gastric emptying and gastrointestinal transit in a strict dose-dependent manner, the highest dose (74 nmol kg^{-1}) producing the largest increases. In contrast, a significant increase in gastric emptying and gastrointestinal transit was seen only with a rather low dose of substance K (0.88 nmol kg^{-1}), whereas higher doses failed to alter gastrointestinal motility significantly. One explanation for this lack of effect could be derived from the visual observation that higher doses of

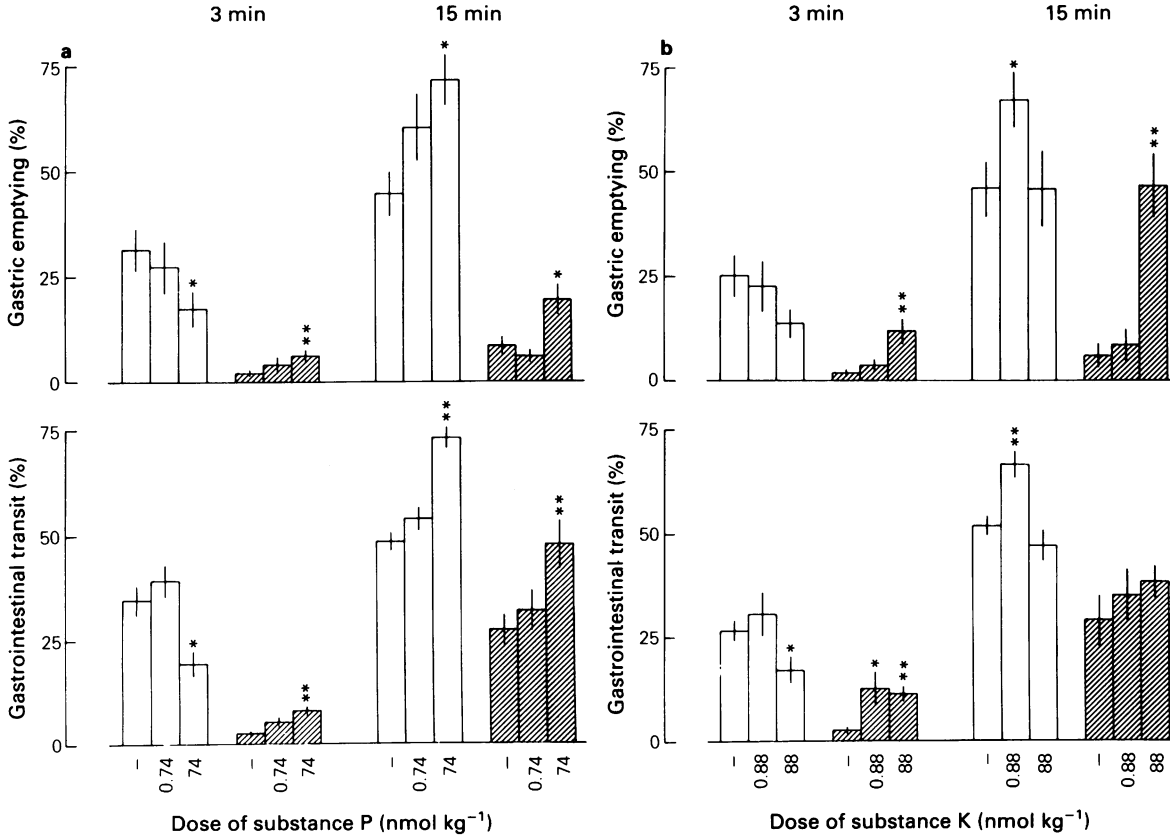

Figure 3 Effect of intraperitoneal injection of substance P (a) and substance K (b) on gastric emptying and gastrointestinal transit as measured 3 and 15 min after intragastric administration of a test meal in untreated rats (open columns) and in rats pretreated with atropine (3.5 μmol kg^{-1}, i.p.) (hatched columns). Means of 8–17 rats are shown with s.e.mean indicated by vertical lines. $*P < 0.05$, $**P < 0.01$, vs. respective vehicle controls.

substance K (88 nmol kg^{-1}) produced a strong spasm of the circular muscle of stomach and small intestine, an effect which was still apparent 15 min after injection of the peptide. Such a spasm of the circular muscle was not seen with substance P.

Effect of substance P and substance K in rats pretreated with atropine

Pretreatment of rats with atropine (3.5 μmol kg^{-1}) had a profound effect on the changes in gastrointestinal motility produced by the two tachykinins (Figure 3a and b). When examined 3 min after the concurrent application of the test meal and the peptides, a dose-dependent increase in gastric emptying and gastrointestinal transit was seen with both tachykinins, compared with a decrease in gastrointestinal propulsion in the absence of atropine. When gastrointestinal propulsion was measured 15 min after feeding it was observed that high doses of substance P (74 nmol kg^{-1}) enhanced gastric emptying and gastrointestinal transit whether or not the rats had been pretreated with atropine. In contrast, the effect of substance K was altered by atropine in two distinct ways. Firstly, the increase in gastric emptying and gastrointestinal transit produced by low doses of substance K (0.88 nmol kg^{-1}) was abolished by atropine. Secondly, high doses of substance K (88 nmol kg^{-1}) which did not alter gastrointestinal propulsion in the absence of atropine, markedly enhanced gastric emptying in rats pretreated with atropine (Figure 3b). However, gastrointestinal transit remained unchanged, probably because even in the presence of atropine, substance K caused a spasm of the gastrointestinal circular muscle.

Effect of substance P and substance K in rats pretreated with guanethidine

Since pretreatment of rats with guanethidine (67 μmol kg^{-1}) markedly increased gastrointestinal propulsion at longer time intervals (15 min) after feeding, the effect of the two tachykinins was examined only 3 min after feeding, at a time when gastrointestinal propulsion was not yet significantly altered by guanethidine (Figure 1). Pretreatment of rats with guanethidine revealed a strong facilitatory effect of low doses of substance P (0.74 nmol kg^{-1}) on both gastric emptying and gastrointestinal transit (Figure 4). The inhibitory effect of 74 nmol kg^{-1} substance P on gastric emptying remained unchanged in rats pretreated with guanethidine whereas the concomitant inhibition of gastrointestinal transit, seen in the absence of guanethidine, was abolished.

Similar but less pronounced effects were observed with substance K. Again, a low dose of substance K (0.88 nmol kg^{-1}) was found to significantly increase

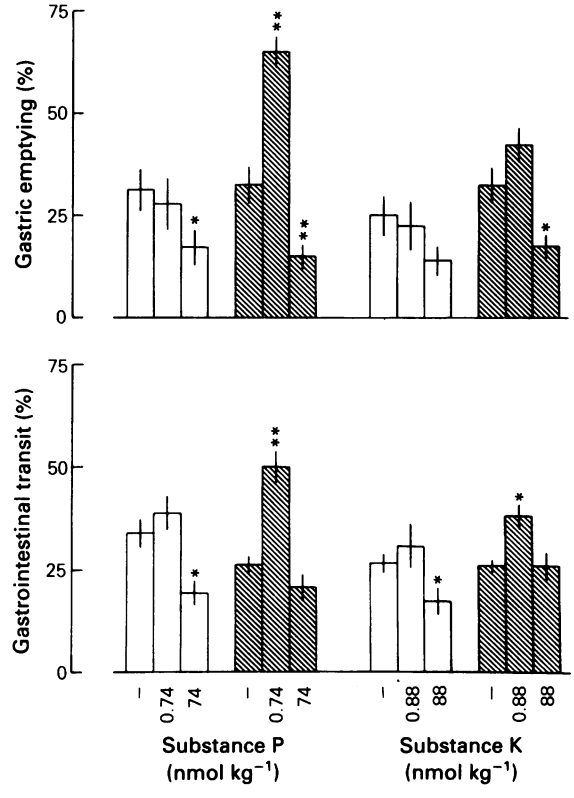

Figure 4 Effect of intraperitoneal injection of substance P and substance K on gastric emptying and gastrointestinal transit as measured 3 min after intragastric administration of a test meal in untreated rats (open columns) and in rats pretreated with guanethidine (67 μmol kg^{-1}, s.c.) (hatched columns). Means of 6–17 rats are shown with s.e.mean indicated by vertical lines. $*P<0.05$, $**P<0.01$, vs. respective vehicle controls.

gastrointestinal transit in rats pretreated with guanethidine, with a similar yet not significant tendency for gastric emptying (Figure 4). The inhibitory effect of 88 nmol kg^{-1} substance K on gastric emptying was enhanced by guanethidine, whereas the inhibition of gastrointestinal transit seen in the absence of guanethidine was abolished.

Effect of substance P and substance K on arterial blood pressure

Within 1–2 min after the intraperitoneal injection of high doses of substance P (74 nmol kg^{-1}) or substance K (88 nmol kg^{-1}) an intense reddening of the paws, ears and the nose tip was usually observed, an effect which in the following 5–10 min gradually disappeared. For this reason the arterial blood pressure in response to intraperitoneal injections of substance P

Table 1 Effect of intraperitoneal injection of substance P or substance K on mean arterial blood pressure

Substance	*Dose* (nmol kg^{-1})	*Decrease in blood pressure* (kPa)	*Time to maximal hypotensive effect* (s)	*Duration of hypotensive effect* (s)
Substance P	0.74	0 (5)	–	–
	7.4	0.51 ± 0.17 (5)	94 ± 22 (4)	216 ± 54 (3)
	74	1.69 ± 0.23 (5)	57 ± 11 (5)	234 ± 46 (5)
Substance K	0.88	0.27 ± 0.27 (5)	126 (1)	243 (1)
	8.8	0.92 ± 0.35 (5)	128 ± 10 (4)	410 ± 92 (3)
	88	2.00 ± 0.37 (5)	85 ± 13 (5)	354 ± 50 (4)

Means ± s.e.mean, number of measurements in parentheses.

and substance K was monitored in rats anaesthetized with urethane. Table 1 shows that both tachykinins lowered the blood pressure in a dose-dependent manner, substance K being appearently slightly more potent than substance P.

Discussion

The present results show that substance P and substance K can both stimulate and inhibit gastrointestinal propulsion, the prevailing effect depending on dose and time interval after injection of the peptides. The results are not directly comparable with those of Bertaccini *et al.* (1981) and Mangel & Koegel (1984) because these authors injected substance P 5 min before administration of the test meal. Since with this protocol a possible early effect of the peptides, which might be different from that seen later, could be missed, the peptides were given simultaneously with the test meal in the present study.

In discussing the present findings it has to be borne in mind that the gastrointestinal effects of the intraperitoneally injected peptides will to some degree be determined by their pharmacokinetic behaviour (noticeably distribution and metabolism) and by other systemic effects, e.g. cardiovascular responses. However, the blood pressure effects of the two tachykinins in rats anaesthetized with urethane but also in conscious rats (Bertaccini *et al.*, 1981) were very modest and hence probably not a primary factor in determining the gastrointestinal responses to the peptides. The observation that the hypotensive effect of substance P following i.p. injection was much smaller than after i.v. administration (Nawa *et al.*, 1984) can be explained by the high capacity of the liver for elimination of substance P from the circulation (Lembeck *et al.*, 1978). The finding that after intravenous injection, substance P was about 20 times more potent in lowering blood pressure than substance K (Nawa *et al.*, 1984) whereas the two compounds were approximately equieffective on intraperitoneal administration in this study points to different pharmacokinetic properties of the two tachykinins. One conceivable interpretation is that substance K is more slowly degraded than substance P as might be inferred from the more prolonged hypotensive effect of substance K (Table 1) and the observation of a spasm of the gastrointestinal circular muscle even 15 min after injection of substance K. Degradation and elimination of the peptides will also determine the duration of their gastrointestinal effects. It thus seems plausible that Bertaccini *et al.* (1981) failed to see a significant effect of substance P (22-220 nmol kg^{-1}) on gastric emptying because they examined the animals 25 min after injection of the peptide, whereas Mangel & Keogel (1984), who examined the animals 20 min after the injection of substance P (7.4 nmol kg^{-1}), still saw a stimulant effect. Synopsis of these and the present results indicates that a stimulant effect of substance P on gastric emptying can be observed between approximately 7.5 and 20 min after its i.p. injection.

The dose-dependent inhibition of gastric emptying seen 3 min after injection of substance P or substance K is likely to be due to a contraction of the pylorus (Bertaccini & Coruzzi, 1977; Bertaccini *et al.*, 1981). Since this inhibitory effect was reversed by atropine to a facilitatory effect it would appear that the action on the pylorus involves activation of cholinergic neurones. The situation in the rat seems thus to be similar to that in the cat, where substance P also depresses transpyloric flow in an atropine-sensitive manner (Edin *et al.*, 1980; Lidberg *et al.*, 1983). However, substance P also causes contraction of the stomach (Bertaccini & Coruzzi, 1977). Since a change in gastric emptying represents the net effect of changes in the motor activity of both the stomach and pylorus it can be concluded that, after atropine pretreatment, the effect of the two tachykinins on the stomach prevailed over that on the pylorus, thus leading to enhanced gastric emptying. The action on the stomach appears independent of the activation of muscarinic cholinoceptors although this does not exclude the participation of cholinergic neurones. Blockade of

adrenergic nerves with guanethidine did not alter the inhibitory effect of the two tachykinins on gastric emptying but abolished that on gastrointestinal transit. This suggests that although normally gastrointestinal transit is the net result of gastric emptying and intestinal transit and therefore gastrointestinal transit changes in parallel with gastric emptying (see Figure 1), gastric emptying and intestinal transit are differentially controlled by sympathetic nerves. A discussion of these control mechanisms is beyond the scope of this paper. Noticeable was the unmasking of a clear stimulant effect of low doses of either tachykinin on gastrointestinal propulsion by guanethidine pretreatment. This could mean that lower doses can elicit contractions of the stomach than are needed to contract the pylorus and that the effects of the two tachykinins on the stomach (and intestine) are inhibited to a larger extent by sympathetic nerves than is the effect on the pylorus.

The dose-dependent stimulation of gastrointestinal propulsion seen 15 min after injection of substance P shows that its action on the pylorus wears off quickly while that on the stomach and possibly on the intestine, is sustained for longer periods. The stimulant effect again did not involve release of acetylcholine acting through muscarinic receptors. Stimulant effects were also encountered with substance K but those of higher doses were apparently cut short by a strong spasmogenic action on gastrointestinal circular muscle, which in some way must have counteracted coordinated propulsion. Furthermore, the mechanism of action of substance K differed from that of substance P in that cholinergic neurones appeared to be involved in the stimulant effect of low doses of substance K. The finding that atropine revealed a clear facilitatory effect of high doses of substance K on gastric emptying whereas gastrointestinal transit remained unaltered suggests that the cholinergic effect of substance K on the pylorus is more sustained than that of substance P and can be observed even 15 min after injection of the peptide.

In summary, this study has shown that the prevalence of the motor effect of substance P and substance K on the stomach or on the pylorus is likely to determine whether stimulation or inhibition of gastrointestinal propulsion is observed. While a participation of the autonomic nervous system in some of the effects of the two tachykinins has been demonstrated it is not yet clear whether extrinsic and/or intrinsic (enteric) autonomic nerves are involved. The study also underlines the need for further experiments aimed at clarifying the role of endogenous tachykinins in gastrointestinal motility.

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References

BARTHÓ, L., HOLZER, P., DONNERER, J. & LEMBECK, F. (1982). Evidence for the involvement of substance P in the atropine-resistant peristalsis of the guinea-pig ileum. *Neurosci. Lett.*, **32,** 69–74.

BERTACCINI, G. (1982). Substance P. In *Handbook of Experimental Pharmacology*, vol. 59/II. ed. Bertaccini, G. pp. 85–105. Berlin: Springer.

BERTACCINI, G. & CORUZZI, G. (1977). Action of some natural peptides on the stomach of the anaesthetized rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **298,** 163–166.

BERTACCINI, G., DE CASTIGLIONE, R. & SCARPIGNATO, C. (1981). Effect of substance P and its natural analogues on gastric emptying of the conscious rat. *Br. J. Pharmac.*, **72,** 221–223.

COSTA, M., FURNESS, J.B., PULLIN, C.O. & BORNSTEIN, J. (1985). Substance P enteric neurons mediate non-cholinergic transmission to the circular muscle of the guinea-pig intestine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **328,** 446–453.

EDIN, R., LUNDBERG, J.M., LIDBERG, P., DAHLSTRÖM, A. & AHLMAN, H. (1980). Atropine sensitive contractile motor effects of substance P on the feline pylorus and stomach in vivo. *Acta physiol. scand.*, **110,** 207–209.

HOWD, R.A., ADAMOVICS, A. & PALEKAR, A. (1978). Naloxone and intestinal motility. *Experientia*, **34,** 1310–1311.

LEMBECK, F., HOLZER, P., SCHWEDITSCH, M. & GAMSE, R. (1978). Elimination of substance P from the circulation of the rat and its inhibition by bacitracin. *Naunyn-Schmiedebergs Arch. Pharmac.*, **305,** 9–16.

LIDBERG, P., DAHLSTRÖM, A., LUNDBERG, J.M. & AHLMAN, H. (1983). Different modes of action of substance P in the motor control of the feline stomach and pylorus. *Regul. Pept.*, **7,** 41–52.

MAGGIO, J.E. & HUNTER, J.C. (1984). Regional distribution of kassinin-like immunoreactivity in rat central and peripheral tissues and the effect of capsaicin. *Brain Res.*, **307,** 370–373.

MANGEL, A.W. & KOEGEL, A. (1984). Effects of peptides on gastric emptying. *Am. J. Physiol.*, **246,** G342–G345.

NAWA, H., DOTEUCHI, M., IGANO, K., INOUYE, K. & NAKANISHI, S. (1984). Substance K: a novel mammalian tachykinin that differs from substance P in its pharmacological profile. *Life Sci.*, **34,** 1153–1160.

NAWA, H., HIROSE, T., TAKASHIMA, H., INAYAMA, S. & NAKANISHI, S. (1983). Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor. *Nature, Lond.*, **306,** 32–36.

RUWART, M.J., KLEPPER, M.S. & RUSH, B.D. (1979). Evidence for noncholinergic mediation of small intestinal transit in the rat. *J. Pharmac. exp. Ther.*, **209,** 462–465.

THEODORSSON-NORHEIM, E., BRODIN, E., NORHEIM, I. & ROSELL, S. (1984). Antisera raised against eledoisin and kassinin detect immunoreactive material in rat tissue extracts: tissue distribution and chromatographic characterization. *Regul. Pept.*, **9,** 229–244.

YOKOYAMA, S. & NORTH, R.A. (1983). Electrical activity of longitudinal and circular muscle during peristalsis. *Am. J. Physiol.*, **244,** G83–G88.

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