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# A tachykinin antagonist inhibits gastric emptying and gastrointestinal transit in the rat

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**1** The effect of a substance P antagonist, [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (SPA), on gastric emptying and gastrointestinal transit in the rat was studied in order to elucidate a possible physiological role of endogenous substance P and other tachykinins in gastrointestinal motility. SPA was given by intraperitoneal injection concurrently with the intragastric administration of a test meal containing charcoal and $^{51}$Cr.

**2** Examination 15 min after the test meal showed that SPA (0.13–1.3 $\mu$mol kg$^{-1}$) inhibited gastric emptying and gastrointestinal transit in a dose-dependent manner.

**3** The inhibitory effect of SPA on gastric emptying and gastrointestinal transit remained unchanged after pretreatment of rats with mepyramine (8.7 $\mu$mol kg$^{-1}$) plus cimetidine (19.8 $\mu$mol kg$^{-1}$) or with guanethidine (67 $\mu$mol kg$^{-1}$).

**4** Since a full examination of SPA as a specific tachykinin antagonist was not possible *in vivo*, SPA was also tested on circular muscle strips from the rat gastric corpus *in vitro*. Submaximal contractions in response to bombesin or bethanechol were not reduced by SPA (50 $\mu$M), whereas those in response to substance P were inhibited.

**5** The results suggest that SPA inhibits gastric emptying and gastrointestinal transit by interfering with the action of tachykinins released from enteric nerves and that endogenous tachykinins are involved in the regulation of gastrointestinal motility.

## Introduction

The observation that small intestinal transit in the rat, unlike that in man and dog, is minimally affected by atropine, has been taken to suggest that the primary stimulant neurotransmitter in the rat small intestine is not acetylcholine (Ruwart *et al.*, 1979). A body of evidence (for a review see Barthó & Holzer, 1985) indicates that substance P or a related peptide (tachykinin) may be a major non-cholinergic excitatory neurotransmitter of the enteric nervous system. Experimental findings obtained with desensitization against substance P and with substance P antagonists indeed suggest that a substance P-like peptide is involved in the physiological regulation of peristalsis in the isolated small intestine of the guinea-pig (Barthó *et al.*, 1982; Yokoyama & North, 1983; Costa *et al.*, 1985). Accordingly, substance P and the related mammalian tachykinin, neurokinin A (substance K), potently affect gastrointestinal propulsion in the rat *in vivo* (Mangel & Koegel, 1984; Holzer, 1985). The nature of their effects, stimulation or inhibition of propulsion, seems to depend on whether their contractile action on the stomach or that on the pylorus prevails.

The present study attempted to provide evidence for a role of endogenous tachykinins in the maintenance of gastrointestinal motility *in vivo*, by examining the effect of a tachykinin antagonist on gastric emptying and gastrointestinal transit in the rat. This approach, however, is somewhat problematic, since relatively high doses of tachykinin antagonists inhibit responses not only to tachykinins but also to bombesin (Jensen *et al.*, 1984; Yachnis *et al.*, 1984) and may exert an unspecific neurosuppressive (local anaesthetic) effect (Karlsson *et al.*, 1984; Post *et al.*, 1985). Hence caution has to be exercised when alterations of physiological

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functions produced by a tachykinin antagonist are to be interpreted. Therefore, we also sought to provide evidence that the antagonist used in this study is a specific tachykinin antagonist in the rat digestive tract.

## Methods

### *Assay of gastrointestinal propulsion*

Adult Sprague-Dawley rats (strain OFA-SD, Forschungsinstitut für Versuchstierzucht, Himberg, Austria), of either sex and 180–230 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. Gastric emptying and gastrointestinal transit were assessed by the transport of a test meal containing the two non-absorbable markers, charcoal and $^{51}Cr$. This method has been previously described in detail (Holzer, 1985). The test meal (1 ml) was given intragastrically by means of a stomach tube while the rats were kept under ether anaesthesia but it was ensured that the animals regained the righting reflex within 1 min after feeding. Fifteen min later the rats were killed. Gastric emptying was expressed by the quotient $(I/(I + S)) \times 100\%$, where I denotes the amount of radioactivity which was found in the small intestine at the time the rats were killed, and S denotes the amount of radioactivity which had remained in the stomach (Ruwart *et al.*, 1979). Gastrointestinal transit was expressed by the quotient $(A/B) \times 100\%$, where A denotes the length of the small intestine which was traversed by the charcoal marker, and B denotes the total length of the small intestine (Green, 1959; Howd *et al.*, 1978; Ruwart *et al.*, 1979).

### *Contractions of circular muscle strips from the gastric corpus*

Muscle strips (length: 10–12 mm, width: about 2 mm) were cut, in a circular direction, from the isolated corpus of the rat, freed of the mucosa, and suspended in an organ bath (capacity: 5 ml). The bath contained Krebs solution (composition in mM: NaCl 118.0, KCl 4.69, $MgSO_4$ 1.18, $CaCl_2$ 2.5, $KH_2PO_4$ 1.18, $NaHCO_3$ 25.0, glucose 11.1) maintained at 37°C and continuously bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. The strips were kept under a resting tension of 8 mN, and contractions were recorded isometrically by means of a force displacement transducer (K 30, Hugo Sachs Elektronik, Freiburg, F.R.G.).

Agonists (bethanechol, bombesin, substance P) were left in contact with the tissue until no further increase in tension developed. The agonists were then washed out and the experiment continued after tension had returned to baseline levels. Its was noted, however, that either the strips often failed to relax completely despite repeated washings or the basal tension slightly increased during the experiment, whereas the tension attained in the presence of a given concentration of an agonist stayed very constant over the time of the experiments (usually about 4 h). The tachykinin antagonist [D-Pro$^2$, D-Trp$^{7,9}$]-substance P was tested against concentrations of the agonists that produced contractions of about 70% of the maximal response to the respective agonist. Since the antagonist, at the concentration tested (50 μM), slightly increased muscle tension by itself and this effect sometimes appeared largest at the first contact with the tissue, the strips were first exposed to the antagonist for 10 min and then washed until tension returned to the baseline. Then the effect of the antagonist on the tension responses to the agonists was tested, the antagonist being added to the bath 5 min before the agonists. The order of application of the various agonists was different in each experiment.

### *Solutions for the* in vivo *experiments*

Bombesin (CRB, Cambridge, U.K.), spantide ([D-Arg$^1$, D-Trp$^{7,9}$, Leu$^{11}$]-substance P; Peninsula, Belmont, CA, U.S.A.), substance P (Serva, Heidelberg, F.R.G.), and [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (Ferring, Malmö, Sweden, and Novabiochem, Läufelfingen, Switzerland) were dissolved in 0.01 M acetic acid (1–1.3 mM). The solutions used for the i.p. administration of the peptides were usually prepared by diluting the peptide stock solutions with Tyrode solution. Only in the case of the two substance P antagonist analogues were the stock solutions used for injection of 1 μmol kg$^{-1}$ spantide and 1.3 μmol kg$^{-1}$ [D-Pro$^2$, D-Trp$^{7,9}$]-substance P. Control rats received equivalent volumes (1 ml kg$^{-1}$) of 0.01 M acetic acid in this instance; otherwise Tyrode solution was administered to vehicle controls. The solutions of cimetidine and mepyramine (SK & F, Welwyn Garden City) and of guanethidine (Ciba-Geigy, Basel, Switzerland) used for injection were made in Tyrode solution. The composition of the Tyrode solution and of the test meal was the same as previously described (Holzer, 1985).

All drugs were administered in a constant volume of 1 ml kg$^{-1}$. Substance P, bombesin, the two substance P antagonists, or the appropriate vehicle were administered at the time of the intragastric administration of the test meal. Mepyramine (8.7 μmol kg$^{-1}$) plus cimetidine (19.8 μmol kg$^{-1}$) were injected i.p. 10 min before administration of the test meal. Guanethidine was given in two doses of 33.5 μmol kg$^{-1}$, each was injected s.c. 18 and 2 h before administration of the test meal.

### *Solutions for the* in vitro *experiments*

Bombesin, substance P, and [D-Pro$^2$, D-Trp$^{7,9}$]-sub-

stance P were dissolved in 0.01 M acetic acid (1–5 mM) and, when necessary, diluted with Krebs solution. Bethanechol (Schuchardt, München, F.R.G.) was dissolved and diluted in Krebs solution. The stock solution of the tachykinin antagonist was added to the bath at a volume of 50 µl; in parallel experiments it was ascertained that addition of 50 µl 0.01 M acetic acid had no effect on the contractile activity of the muscle strips.

*Statistics*

All data are presented as means ± s.e.mean. Statistically significant differences were evaluated by the Mann-Whitney *U* test (two tailed; *in vivo* experiments) or by the paired *t* test (one-tailed; *in vitro* experiments). *P* values $< 0.05$ were regarded as significant.

## Results

*Effect of [D-Pro², D-Trp⁷·⁹]-substance P and spantide on gastric emptying and gastrointestinal transit*

Intraperitoneal injection of [D-Pro², D-Trp⁷·⁹]-substance P inhibited gastric emptying and gastrointestinal transit in a dose-dependent manner (Figure 1) but relatively high doses were needed to produce this effect. Thus, 0.13 µmol kg$^{-1}$ of the antagonist reduced gastric emptying significantly but did not significantly depress gastrointestinal transit whereas both gastric emptying and gastrointestinal transit were profoundly inhibited by 1.3 µmol kg$^{-1}$ of the antagonist (Figure 1). The vehicle for the lower dose of the antagonist was Tyrode solution and that for the higher dose was 0.01 M acetic acid, but gastric emptying and gastrointestinal transit did not differ significantly between the two vehicle control groups (Figure 1). The length of the small intestine was also not affected by these treatments: it was 111 ± 4 cm ($n = 6$) in the control rats treated with Tyrode solution vehicle, 112 ± 1 cm ($n = 9$) in the control rats treated with acetic acid vehicle, 109 ± 3 cm ($n = 6$) in the rats treated with 0.13 µmol kg$^{-1}$ [D-Pro², D-Trp⁷·⁹]-substance P, and 110 ± 2 cm ($n = 9$) in the rats treated with 1.3 µmol kg$^{-1}$ [D-Pro², D-Trp⁷·⁹]-substance P.

Another tachykinin antagonist, spantide ([D-Arg¹, D-Trp⁷·⁹, Leu¹¹]-substance P) at a dose of 1 µmol kg$^{-1}$, had an effect similar to that of [D-Pro², D-Trp⁷·⁹]-substance P: it reduced gastric emptying from 55.8 ± 5.6 % ($n = 9$) to 24.6 ± 8.9 % ($n = 5$; $P < 0.05$) and gastrointestinal transit from 55.3 ± 2.3 % ($n = 9$) to 19.4 ± 1.9 % ($n = 5$; $P < 0.01$).

*Effect of [D-Pro², D-Trp⁷·⁹]-substance P in rats pretreated with mepyramine plus cimetidine or with guanethidine*

Pretreatment of rats with mepyramine (8.7 µmol kg$^{-1}$) plus cimetidine (19.8 µmol kg$^{-1}$) had no effect on gastric emptying and gastrointestinal transit (Figure 2). Pretreatment with guanethidine (total dose: 67 µmol kg$^{-1}$) significantly enhanced gastric emptying (Figure 2) although in this series of experiments this was not accompanied by an altered gastrointestinal transit (see also Holzer, 1985). Neither treatment changed the inhibitory effect of [D-Pro², D-Trp⁷·⁹]-substance P (1.3 µmol kg$^{-1}$; Figure 2).

*Effect of [D-Pro², D-Trp⁷·⁹]-substance P on the motility responses to substance P and bombesin* in vivo

As described previously (Holzer, 1985), i.p. injection of substance P (74 nmol kg$^{-1}$) enhanced both gastric

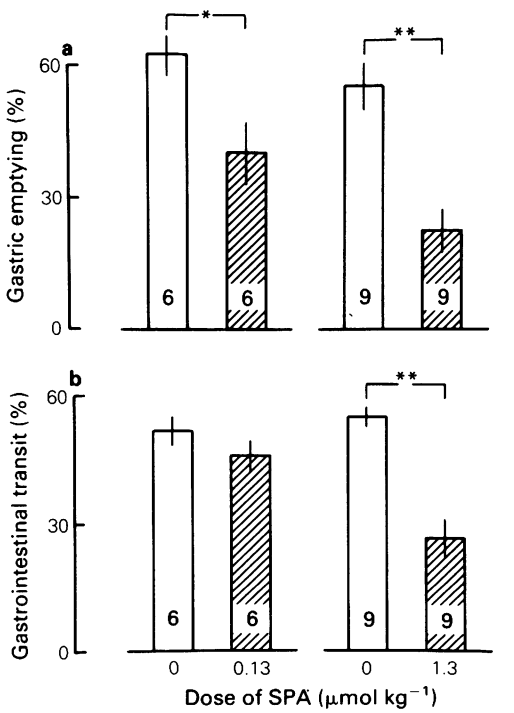


**Figure 1** (a) Gastric emptying and (b) gastrointestinal transit in the rat as measured 15 min after the intraperitoneal injection of vehicle or [D-Pro², D-Trp⁷·⁹]-substance P (SPA). The vehicle for 0.13 µmol kg$^{-1}$ SPA was Tyrode solution that for 1.3 µmol kg$^{-1}$ SPA was 0.01 M acetic acid. Each column shows the mean, with vertical lines representing s.e.mean, *n* indicated by number in each column. $*P < 0.05$, $**P < 0.01$ vs respective vehicle controls as indicated.

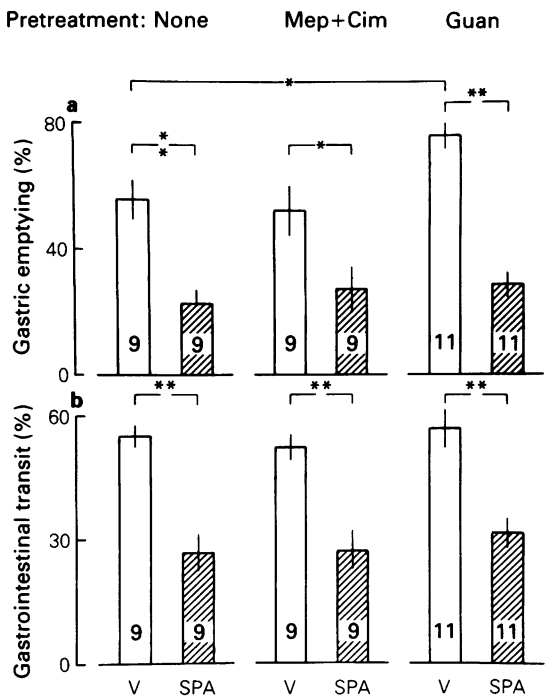


**Figure 2** (a) Gastric emptying and (b) gastrointestinal transit as measured 15 min after the intraperitoneal injection of vehicle (V) or [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (SPA, 1.3 μmol kg$^{-1}$) in untreated rats and in rats pretreated either with mepyramine (Mep, 8.7 μmol kg$^{-1}$) plus cimetidine (Cim, 19.8 μmol kg$^{-1}$) or with guanethidine (Guan, 67 μmol kg$^{-1}$). Each column represents the mean with vertical lines showing s.e.mean, *n* indicated by number in each column. $*P < 0.05$, $**P < 0.01$ vs respective vehicle controls as indicated.

emptying and gastrointestinal transit when measured 15 min after administration of the test meal (Figure 3). This effect of substance P was abolished when substance P (74 nmol kg$^{-1}$) was injected together with [D-Pro$^2$, D-Trp$^{7,9}$]-substance P at a dose (1.3 μmol kg$^{-1}$) which depressed gastric emptying and gastrointestinal transit on its own (Figure 3).

Bombesin, when administered intraperitoneally at a dose of 62 nmol kg$^{-1}$, significantly depressed gastrointestinal transit, whereas gastric emptying was not changed (Figure 4). Visual inspection revealed that bombesin produced a strong contracture of the stomach whereas duodenum and proximal jejunum appeared distended. These observations are consistent with the findings that, in the rat, bombesin contracts the stomach but relaxes the duodenum (Bertaccini, 1982). The motor effect of bombesin on the stomach seemed not to be altered by a concomitant injection of 1.3 μmol kg$^{-1}$ [D-Pro$^2$, D-Trp$^{7,9}$]-substance P, since bombesin still produced a contracture of the stomach and restored gastric emptying to normal as compared with rats treated with the tachykinin antagonist alone. [D-Pro$^2$, D-Trp$^{7,9}$]-substance P, which itself inhibited gastrointestinal transit, did not affect the inhibitory action of bombesin on gastrointestinal transit (Figure 4).

### *Effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P on the isolated gastric corpus*

Substance P, bombesin, and bethanechol all contracted the circular muscle of the rat gastric corpus but differed markedly with respect to potency and maximal increases in tension. The concentrations of these substances which produced a contraction being about 70% of the maximal response to the respective agonist are given in Table 1. When [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (50 μM) was administered to the circular muscle strips, it also caused a slight and sustained

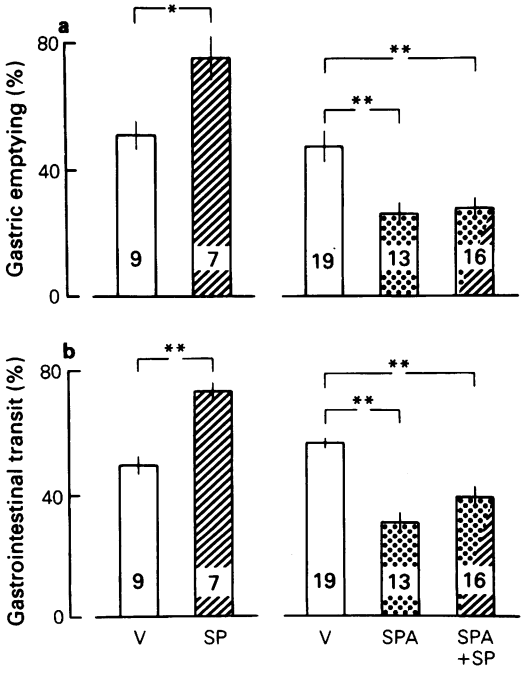


**Figure 3** (a) Gastric emptying and (b) gastrointestinal transit as measured 15 min after the intraperitoneal injection of vehicle (V), substance P (SP, 74 nmol kg$^{-1}$), or [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (SPA, 1.3 μmol kg$^{-1}$), alone or in combination. Each column represents the mean with vertical lines showing s.e.mean, *n* indicated by number in each column. $*P < 0.05$, $**P < 0.01$ vs respective vehicle controls as indicated.

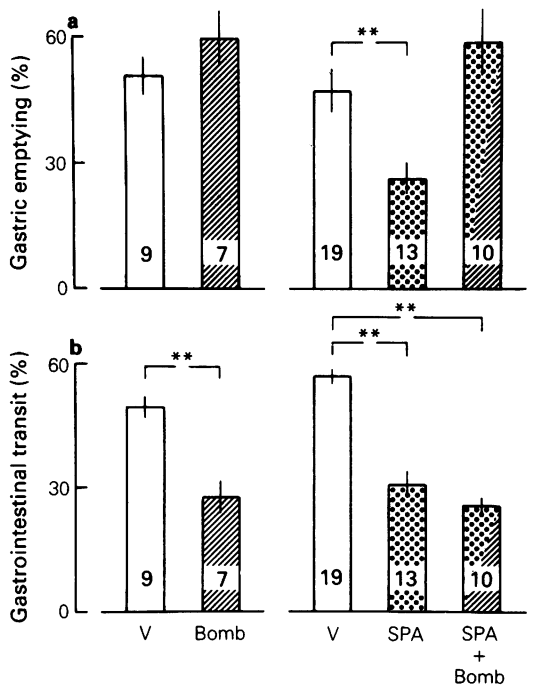


**Figure 4** (a) Gastric emptying and (b) gastrointestinal transit as measured 15 min after the intraperitoneal injection of vehicle (V), bombesin (Bomb 62 nmol $kg^{-1}$), or [D-$Pro^2$, D-$Trp^{7,9}$]-substance P (SPA, 1.3 μmol $kg^{-1}$), alone or in combination. Each column represents the mean with vertical lines showing s.e.mean, *n* indicated by number in each column. $^{**}P < 0.01$ vs respective vehicle controls as indicated.

increase in tension. The tension levels attained in the presence of substance P (1 μM) were, however, significantly reduced by [D-$Pro^2$, D-$Trp^{7,9}$]-substance P whereas those attained in the presence of bombesin or bethanechol were not (Table 1).

The rise in tension produced by [D-$Pro^2$, D-$Trp^{7,9}$]-substance P (50 μM) was not affected by atropine (0.6 μM). In the absence of atropine, [D-$Pro^2$, D-$Trp^{7,9}$]-substance P increased the tension from 10.8 ± 2.3 mN to 22.5 ± 5.6 mN ($n = 6$), whereas in the presence of atropine (added to the bath 4 min before the addition of the tachykinin antagonist), [D-$Pro^2$, D-$Trp^{7,9}$]-substance P increased the tension from 14.5 ± 4.0 mN to 24.1 ± 6.4 mN ($n = 6$). Atropine (0.6 μM) abolished the contraction caused by bethanechol (30 μM, $n = 6$) but did not alter the response to bombesin (30 nM, $n = 6$). Histamine antagonists were not tested against the contraction produced by the tachykinin antagonist, since histamine (100 μM) failed to evoke a contractile response in the circular muscle of the rat gastric corpus ($n = 4$).

## Discussion

The present results show that tachykinin antagonists inhibit gastric emptying and gastrointestinal transit in the rat. This depressant effect appears to result primarily from inhibition of gastric emptying; whether inhibition of small intestinal transit is also involved cannot be deduced from the present data. The interpretation of the effect of [D-$Pro^2$, D-$Trp^{7,9}$]-substance P depends critically on the specificity of this compound as a tachykinin antagonist.

Several arguments support the conclusion that [D-$Pro^2$, D-$Trp^{7,9}$]-substance P depresses gastric emptying and gastrointestinal transit by interfering with endogenous tachykinins, but some limitations of this interpretation need also be discussed. (1) A possible activation of the sympathetic nervous system induced

**Table 1** Effect of the tachykinin antagonist [D-$Pro^2$, D-$Trp^{7,9}$]-substance P (50 μM) on isometric contractions of circular muscle strips from the rat gastric corpus

| | *Substance P (1 μM)* | *Bombesin (0.03–0.1 μM)* | *Bethanechol (10–30 μM)* |
|---|---|---|---|
| Basal | 8.9 ± 1.1 | 8.0 ± 0.6 | 10.5 ± 1.0 |
| Agonist | 28.3 ± 3.0 | 26.6 ± 3.9 | 48.5 ± 3.6 |
| Basal | 10.0 ± 0.7 | 10.4 ± 1.2 | 10.6 ± 0.7 |
| Antagonist | 13.7 ± 1.2 | 14.1 ± 1.6 | 15.3 ± 1.6 |
| Antagonist plus agonist | 19.2 ± 2.5* | 26.9 ± 3.7 | 58.7 ± 6.5 |

The values given refer to the tension in mN measured in the absence (basal) or presence of agonist and/or antagonist. The antagonist was added to the bath 5 min before exposure of the strips to the agonists. Means ± s.e.mean are shown, $n = 5$. $^{*}P < 0.01$ versus tension in the presence of the agonist alone.

by tachykinin antagonists has been ruled out by the lack of effect of the sympathetic neurone blocking drug guanethidine. (2) [D-Pro$^2$, D-Trp$^{7,9}$]-substance P and other tachykinin antagonists are able to release histamine from mast cells (Sydbom, 1982; Håkanson *et al.*, 1983; Skofitsch *et al.*, 1983). However since combined treatment with the histamine antagonists, mepyramine and cimetidine, did not alter the inhibitory effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P on gastric emptying and gastrointestinal transit, this mechanism of action can also be excluded. (3) Although the *in vivo* results with substance P and bombesin are not totally conclusive, they show that at least the motor effects of bombesin on the stomach are not inhibited by the tachykinin antagonist at a dose which prevents the stimulant motor effects of substance P and depresses gastric emptying and gastrointestinal transit on its own. Thus, the doses of antagonist used in this study seem to lack a bombesin-antagonistic action which has been found to limit the specificity of tachykinin antagonists (Jensen *et al.*, 1984; Yachnis *et al.*, 1984). (4) Since a further examination of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P *in vivo* was not possible because of the cost of this compound, its specificity as a tachykinin antagonist was also tested on circular muscle strips from the rat gastric corpus. If it is assumed that the volume of the extracellular fluid in the rat is similar to that in man (0.25 l kg$^{-1}$; Bowman & Rand, 1980) and that the tachykinin antagonist will easily distribute into this compartment, a dose of 1.3 μmol kg$^{-1}$ would yield a concentration of about 5 μM in the extracellular fluid. Since the concentration of the antagonist in the peritoneal fluid will be considerably higher than 5 μM initially after the injection, a ten fold higher concentration of the antagonist (50 μM) was used for the *in vitro* test. At this concentration, the antagonist seemed to be specific for substance P although, because of the small contractile activity of the antagonist, it cannot totally be ruled out that bombesin was also slightly antagonized. These findings in the rat are consistent with previous findings in the guinea-pig intestine where concentrations of up to 80 μM [D-Pro$^2$, D-Trp$^{7,9}$]-substance P seem to be specific for substance P (Leander *et al.*, 1981; Barthó *et al.*, 1982). However, the finding that in the guinea-pig intestine [D-Pro$^2$, D-Trp$^{7,9}$]-substance P and some related analogues are effective antagonists only at tachykinin receptors located on the smooth muscle and not on those located on enteric neurones (Nemeth *et al.*, 1983; Costa *et al.*, 1985; Kilbinger *et al.*, 1986) needs to be taken into consideration, although this does not apply to all substance P antagonists (Featherstone *et al.*, 1986).

The small contraction produced by [D-Pro$^2$, D-Trp$^{7,9}$]-substance P in the circular muscle of the rat gastric corpus may indicate a partial agonist activity, a property which has been previously described for some substance P antagonists on other preparations (Hawcock *et al.*, 1982; Bailey & Jordan, 1984; Featherstone *et al.*, 1986). Although the contractile effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P was not further examined, it seems unlikely to be due to release of histamine, since histamine is ineffective in producing a contraction of this preparation. Release of acetylcholine, an action which in the guinea-pig intestine is exerted by some tachykinin antagonists (Hawcock *et* al., 1982; Featherstone *et al.*, 1986), has also been ruled out.

Another limitation to the usefulness of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P and other tachykinin antagonists is related to their unspecific neurosuppressive (local anaesthetic) action in high concentrations. Although not directly excluded in the present study it is improbable that such an action could have contributed to the inhibitory effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P on gastric emptying and gastrointestinal transit in the rat. This conclusion is based on the findings that only very high concentrations ($>100$ μM) of tachykinin antagonists block nerve conduction in the frog (Karlsson *et al.*, 1984) and rat sciatic nerve (Post *et al.*, 1985) and that no evidence for a neurosuppressive effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P (10–80 μM) could be found in a number of smooth muscle preparations (Leander *et al.*, 1981; Barthó *et al.*, 1982; Leander & Håkanson, 1985).

Taken overall, the present data suggest that the inhibitory effect of [D-Pro$^2$, D-Trp$^{7,9}$]-substance P on gastric emptying and gastrointestinal transit is primarily due to interference with endogenous tachykinins released from enteric nerves. If so, this provides further evidence that substance P and/or related mammalian tachykinins (e.g. neurokinin A) play a physiological role in the regulation of gastrointestinal propulsive motility. The observation that gastric emptying particularly is decreased by [D-Pro$^2$, D-Trp$^{7,9}$]-substance P is consistent with the previous finding that substance P and neurokinin A influence gastrointestinal propulsion primarily by their effect on the stomach and pylorus (Holzer, 1985).

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