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Biological evaluation of substance P antagonists

K. Folkers*, R. Håkanson, J. Hörig**, Xu Jie-Cheng* & S. Leander

Department of Pharmacology, University of Lund, Lund, Sweden, Ferring Arzneimittel Gmbh**, Kiel, FRG and Institute of Biomedical Research*, University of Texas, Austin, Texas, U.S.A.

1 Five undeca- and six C-terminal heptapeptide substance P (SP) analogues were tested for their capacity to block the contractile effect of SP on the guinea-pig isolated taenia coli. They had one feature in common, namely substitutions in positions 7 and 9 in the SP molecule. In the majority of analogues D-tryptophan was used for these substitutions.

2 All analogues tested were found to be competitive antagonists to exogenous SP and to be capable of blocking the electrically induced non-cholinergic, non-adrenergic neuronal contraction of the taenia.

3 Of the undecapeptides, (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}) SP and (D-Arg^1, D-Trp^{7,9}, Leu^{11}) SP (Spantide) had the highest pA_2 value, 7.1–7.2, and the lowest IC_{50} value, 10^{-6} M. The pA_2 values of the heptapeptides were generally lower.

4 Three of the most potent antagonists were tested for specificity and found to block the smooth muscle contraction induced by SP, physalaemin, eledoisin and bombesin but not that induced by bradykinin, carbachol, 5-hydroxytryptamine, histamine, prostaglandins and vasopressin.

5 The SP antagonists were also tested for spasmogenic effect on the taenia and for their capacity to release histamine from rat isolated peritoneal mast cells. The spasmogenic activity displayed by most of the SP antagonists tested is likely to be related to their ability to release histamine since the contractile response was reduced by mepyramine, a histamine H_1-receptor antagonist.

6 (D-Arg^1, D-Trp^{7,9}, Leu^{11}) SP was notable for combining a high antagonistic potency with a weak spasmogenic effect (and poor histamine releasing effect).

Introduction

Antagonists to substance P (SP) have recently been made available (Rackur *et al.*, 1979; Yamaguchi *et al.*, 1979; Folkers *et al.*, 1981; 1982; Rosell & Folkers, 1982; Rosell *et al.*, 1983). They have been found to be specific and competitive antagonists (Leander *et al.*, 1981; Håkanson *et al.*, 1982). However, the first antagonists described had a low potency which limited their usefulness as pharmacological tools. Further, they possessed histamine-releasing properties (Fewtrell *et al.*, 1982; Håkanson *et al.*, 1982; 1983; Sydbom, 1982) which complicated the analysis of their mode of action. Subsequently other SP antagonists have been synthesized in order to eliminate these shortcomings. This paper describes two series of new SP analogues with SP antagonistic properties, undecapeptides and C-terminal heptapeptides, together with the screening procedures that we have found appropriate in the testing of such antagonists. A brief report of this work has appeared elsewhere (Leander *et al.*, 1983).

Methods

Studies on motor effects

Guinea-pig taenia coli preparations, consisting of longitudinal smooth muscle with the attached myenteric plexus (Burnstock *et al.*, 1966), were placed in Krebs solution, kept at 4°C for about 1 h and then mounted vertically on a Perspex holder in a 7 ml organ bath maintained at 37°C. One end was attached to a rigid support and the other to a lever connected via a spring to a Grass FT03 force displacement transducer or to a photoelectric transducer for isotonic registration of mechanical activity, The load on the muscle was set at 0.2 g. The mechanical activity of the preparation was continuously recorded on a Grass model 7 or model 5 polygraph. The bathing fluid had the following composition (mM): NaCl 133, $NaHCO_3$ 16.3. KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8, The solution was bubbled with a gas mixture of 7% CO_2 in O_2 giving a pH of 7.2–7.3.

Concentration-response curves were constructed by adding step-wise increasing amounts of SP (200 μl volumes) to the bath. Below 10^{-7} M three concentrations of SP were tested on each muscle; above 10^{-7} M only one concentration was tested per muscle. Between each application the bath was rinsed, and the next SP concentration was not tested until the effect of the preceding one had been washed away. The contractile response was expressed in relation to that evoked by a standard concentration of carbachol (10^{-5} M). All EC_{50} values were calculated by linear regression analysis of the steepest part of each concentration-response curve. Each value in the concentration-response curves was the mean of at least six separate determinations. The potency of each SP antagonist was assessed from the analysis of a large number of SP concentration-response curves with at least three of the the following concentrations of SP antagonists in the bath (added 5–10 min before SP): 10^{-6} M, 3×10^{-6} M, 10^{-5} M and 3×10^{-5} M. The SP antagonists were added in a volume of 40 μl. The pA_2 values were calculated as described by Arunlakshana & Schild (1959) and by Tallarida *et al.* (1979). The standard error of the slopes of the regression lines of Schild plots (obtained by regression analysis) and the 95% confidence range of the pA_2 values (intercepts on the abscissa scale) were calculated according to Armitage (1971), Rerup (1979) and Tallarida *et al.* (1979).

In another series of experiments, platinum ring electrodes were placed around the muscle with a constant electrode distance of 5 mm and the electrodes were connected to a Grass S4C stimulator for field stimulation with square wave pulses (15 V over the electrodes, 0.5–1 ms duration). The preparations were stimulated with trains of pulses lasting 3 s and with a frequency of 3 Hz, the resting period between stimulations being at least 2 min. Experiments on the capacity of the SP antagonists to block the electrically induced SP-ergic contractions (Leader *et al.*, 1981) were carried out with atropine 10^{-6} M and guanethidine 5×10^{-6} M in the bath. Concentration-response curves were constructed by adding stepwise increasing amounts of the SP antagonist (40 μl volumes) to the bath. Below 10^{-5} M three concentrations of the antagonist were tested on each muscle; above 10^{-5} M only one concentration was tested per muscle. The results were expressed in relation to the electrically induced contraction before addition of the antagonist. The IC_{50} values were calculated as described for the EC_{50} values.

The spasmogenic activity of the various SP antagonists was assessed from the contractions evoked upon the first application of the drug. The contractile response was expressed in relation to that evoked by carbachol 10^{-5} M. Concentration-response curves were constructed and the EC_{50} values were calculated by linear regression analysis of the steepest part of each concentration-response curve. Each value was the mean of at least six separate determinations.

Studies on histamine release

Male Sprague-Dawley rats (freely fed, body weight 300–350 g) were killed by decapitation under light diethyl ether anaesthesia. Peritoneal cells, consisting of 3–6% mast cells (Padawer, 1963; Kurose & Saeki, 1981), were obtained by peritoneal lavage with 10 ml buffered salt solutions (BSS), containing 145 mM NaCl, 2.7 mM KCl and 10% (v/v) Sörensen phosphate buffer ($Na_2HPO_4 + KH_2PO_4$, 67 mM, pH 7.0). After gentle abdominal massage for 3–4 min, the intra-abdominal mast cell-rich fluid was removed with a pipette. Abdominal fluid from 2 rats was pooled and centrifuged at approximately 300 *g* for 10 min at room temperature. The precipitated cells were resuspended in 10 ml BSS to which was added bovine serum albumin 1 mg ml^{-1} (Armour, Kanakee, IL, USA) (BSSA). The cell suspension was washed twice in the BSSA. Finally the cells were suspended in 30 ml BSSA at room temperature and aliquots of 0.9 ml were transferred to plastic test tubes and preincubated for 5 min at 35°C before adding the peptide in a volume of 0.1 ml BSS. The mixture was incubated for 5 min at 35°C and the reaction was interrupted by placing the samples in ice. They were then centrifuged at 600 *g* for 5 min at 0°C. Aliquots (0.1 ml) of the supernatant were taken for direct fluorometric assay of histamine (Rönnberg & Håkanson, 1983). The sediment was taken up in 1 ml redistilled H_2O, centrifuged for 5 min at 0°C and 0.1 ml of the supernatant was assayed for histamine. All determinations were made in duplicate. The amount of histamine released was expressed as a percentage of the total amount of histamine present in the peritoneal cells at the start of the experiment. The results were corrected for 4–5% spontaneous release. Maximal histamine release was close to 80%. Each dose-response curve was constructed from 3–8 separate series of determinations.

Drugs

Full-length SP analogues were synthesized at the Institute of Biomedical Research, University of Texas, Austin, Texas and the heptapeptide SP analogues were prepared at Ferring, Kiel, FRG. All SP analogues were synthesized by techniques analogous to those described elsewhere (Folkers *et al.*, 1981) Their purity was tested by high performance liquid chromatography and found to be better than 96%. Atropine sulphate was from ACO, Stockholm, Sweden and mepyramine maleate from SKF, Welwyn Garden City, England. Physalaemin, eledoisin,

bombesin and bradykinin were provided by Peninsula, Calif., U.S.A., histamine dihydrochloride, carbamoylcholine chloride (carbachol), 5-hydroxytryptamine (5-HT) creatinine sulphate and prostaglandin $F_{2\alpha}$ by Sigma, St. Louis, Mo, U.S.A. Lys^8-vasopressin was a gift from Ferring AB, Malmö, Sweden.

Results

The specificity and potency of the SP antagonists, their ability to inhibit SP-ergic neuronal responses and their contractile activity were tested on the guinea-pig isolated taenia coli. Their ability to release histamine was studied in rat peritoneal mast cells.

All the analogues tested blocked the contractile effects of exogenous SP in a competitive manner (Figure 1), the slopes of the Schild plots being close to

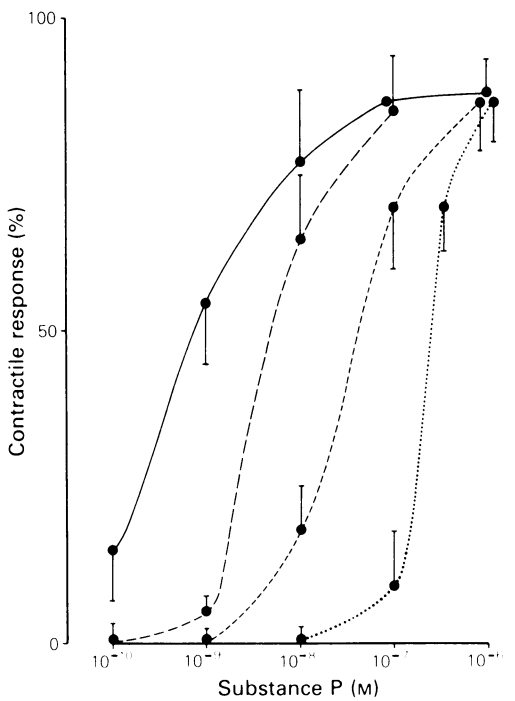

Figure 1 Concentration-response curves showing the contractile effect of substance P (SP) alone (——) or in the presence of increasing concentrations of (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})SP_{1-11} on the guinea-pig taenia coli, (--) 10^{-6} M, (---) 10^{-5} M, (....) 3×10^{-5} M. The response is expressed as percentage of the contraction induced by carbachol 10^{-5} M. In the presence of (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})SP_{1-11} the concentration-response curve was shifted to the right suggesting competitive inhibition. Each curve is constructed from 3–10 experiments. Vertical bars give s.e.mean.

Table 1 Substance P (SP) antagonism and blockade of non-cholinergic, non-adrenergic nerve stimulation by SP analogues

Peptide	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2	pA_2	*95% conf. lim.*	*Slope* (± s.d.).	IC_{50}*(± s.e.). (M)
SP	–	–	–	–	–	–	D-Phe	–	D-Trp	–	–	–	5.8	5.7–5.9	−1.12(±0.02)	$1.0\times10^{-5}\pm0.9\times10^{-6}$
	–	–	–	–	–	–	D-Trp	–	D-Trp	–	–	–	5.6	NA	−0.84(±0.13)	$2.0\times10^{-5}\pm4.3\times10^{-6}$
	–	D-Pro	–	–	–	–	D-Trp	–	D-Trp	–	–	–	6.1	5.9–6.4	−1.00(±0.07)	$1.1\times10^{-5}\pm4.3\times10^{-6}$
	D-Arg	D-Pro	–	–	–	–	D-Trp	–	D-Trp	–	Leu	–	7.2	NA	−0.82(±0.07)	$1.0\times10^{-6}\pm1.7\times10^{-7}$
Spantide	D-Arg	–	–	–	–	–	D-Trp	–	D-Trp	–	Leu	–	7.1	6.7–7.7	−0.98(±0.02)	$1.8\times10^{-6}\pm4.0\times10^{-7}$
					Arg	–	D-Trp	–	D-Trp	–	–	–	6.2	6.1–6.4	−0.96(±0.01)	$2.0\times10^{-6}\pm4.5\times10^{-7}$
					Arg	–	D-Trp-p-Cl-Phe-D-Trp			–	–	–	6.2	6.1–6.3	−0.99(±0.01)	$1.0\times10^{-6}\pm20\times10^{-7}$
					Arg	–	D-p-Vl-Phe		D-Trp	–	–	–	5.8	NA	−0.94(±0.16)	$3.0\times10^{-6}\pm2.7\times10^{-7}$
					Arg	–	D-Trp	–	D-Trp	–	Leu	–	6.1	NA	−0.94(±0.05)	$3.0\times10^{-6}\pm9.0\times10^{-7}$
					Arg	–	D-Trp	–	D-Trp	–	Nle	–	7.1	6.9–7.6	−0.96(±0.02)	$2.0\times10^{-6}\pm1.3\times10^{-7}$
					Arg	–	D-Trp	–	D-Trp	–	Val	–	6.2	NA	−0.89(±0.01)	$2.5\times10^{-6}\pm0.3\times10^{-7}$

– = same amino acid as in SP, NA = not assessed

* Inhibition of the electrically induced, non-cholinergic, non-adrenergic contraction of the isolated taenia coli. The slopes were not significantly different from −1.

−1 (Table 1 and Figure 2). Differences in pA_2 values were observed (Table 1 and Figure 2); (D-Arg^1, D-Trp^{7,9}, Leu^{11})SP_{1-11} ('Spantide') and (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}) SP_{1-11} being the most potent undecapeptide antagonists.

The SP antagonists blocked also the contractile response to electrical stimulation of non-cholinergic, non-adrenergic nerves in the taenia (Figure 3). The concentrations required to give 50% inhibition (IC_{50}) are given in Table 1. (D-Arg^1, D-Trp^{7,9}, Leu^{11})SP_{1-11} and (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}) -SP_{1-11} were among the most potent, having an IC_{50} value of 10^{-6} M. The IC_{50} for the various antagonists varied from 10^{-6} to 3×10^{-5} M. As expected, those that had a high pA_2 value usually had a low IC_{50} value and *vice versa*. The correlation betweeen pA_2 and the negative logarithm of IC_{50} was found to be significant ($P < 0.05$).

The specificity of the antagonism was examined in detail for (D-Pro^2, D-Trp^{7,9})-SP_{1-11}, (D-Arg^1, D-Trp^{7,9}, Leu^{11})SP_{1-11} and (Arg^5, D-Trp^{7,9})SP_{5-11}. These antagonists blocked the contractile effects of SP, physalaemin, eledoisin and bombesin (Table 2 and Figure 4) but not the contractions induced by

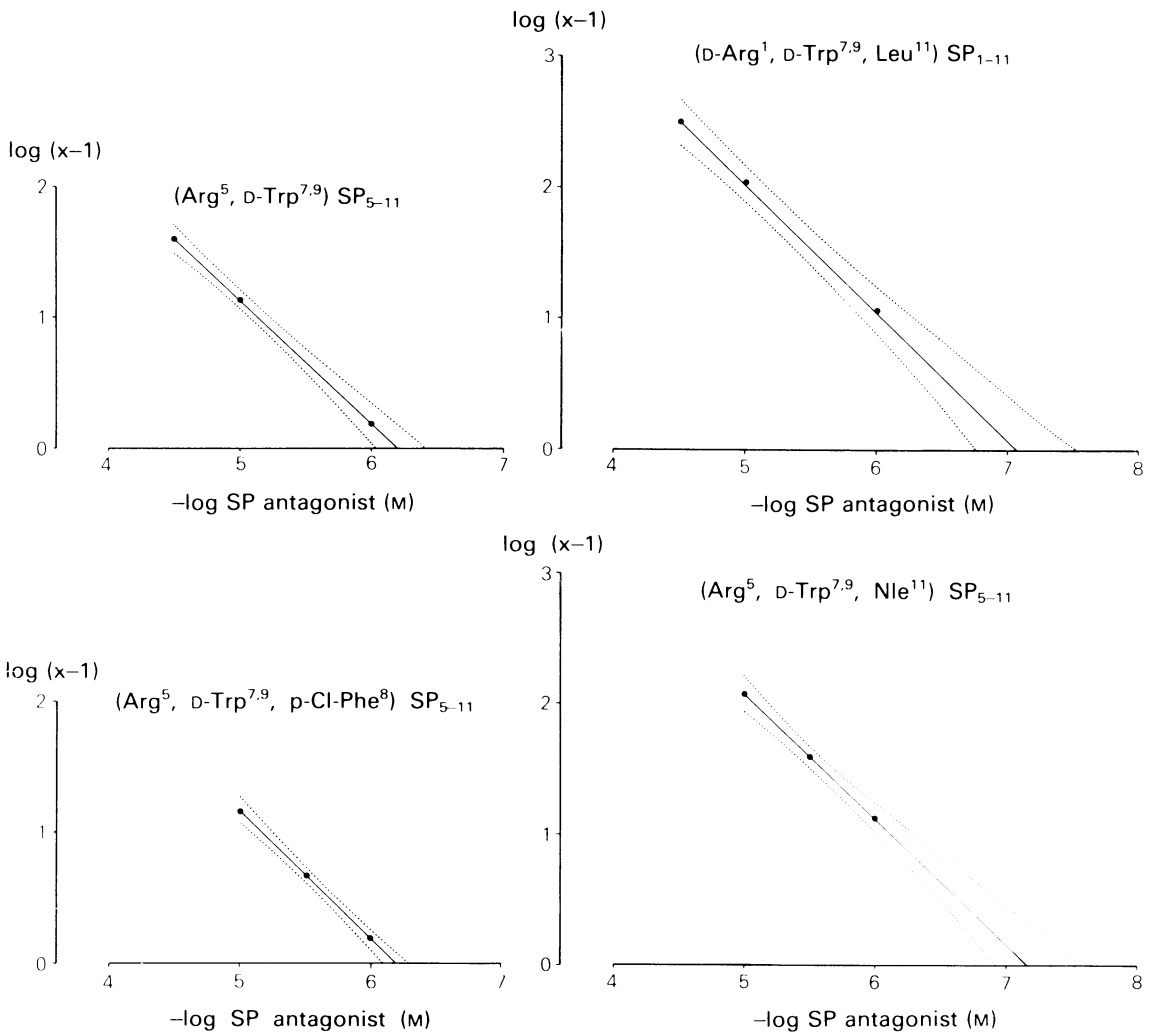

Figure 2 Schild plots calculated from the results of experiments as in Figure 1 with three concentrations of each of the substance P (SP) antagonists indicated. X is the dose-ratio (Tallarida *et al.*, 1979). The intercept on the abscissa scale is the pA_2 value (Table 1). The 95% confidence limits are indicated. The slope of each line was close to −1 (see Table 1).

vasopressin (10^{-7} M), bradykinin (10^{-9} M), histamine (10^{-7} M), prostaglandin E_2 or F_2 (10^{-10} M), 5-HT, or carbachol (10^{-8} M) (not shown).

The capacity of the SP antagonists *per se* to contract the isolated taenia coli (spasmogenic activity) is shown in Table 3. (D-Arg[1], D-Trp[7,9], Leu[11]) -SP_{1-11} and (Arg^5, D-$Trp^{7,9}$) SP_{5-11} displayed a notably low spasmogenic activity. The contractions produced by the SP antagonists could be prevented by adding the histamine H_1-receptor antagonist mepyramine to the bath. All SP antagonists were found to release histamine from rat peritoneal mast cells. The EC_{50} values are given in Table 3.

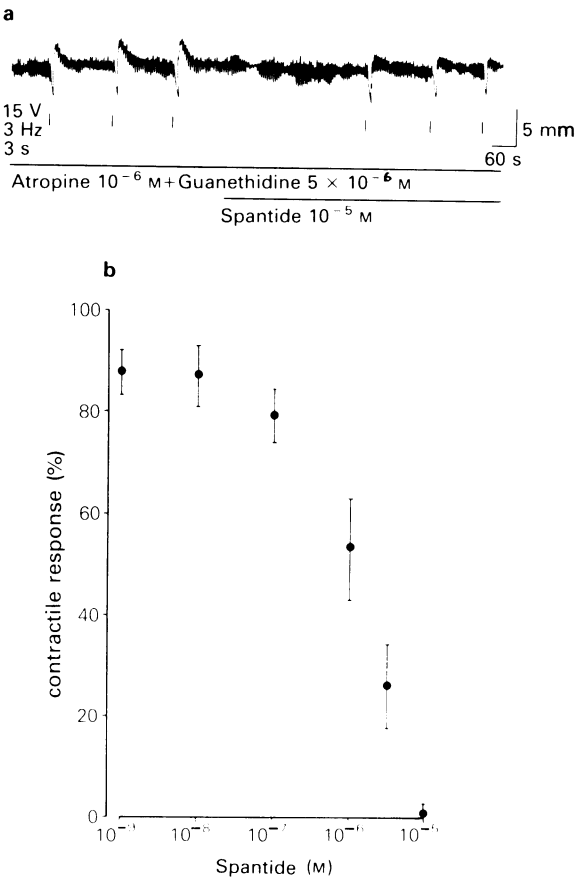

Figure 3 (a) Effect of (D-Arg[1], D-Trp[7,9], Leu[11]) SP_{1-11} (Spantide) on the contractile response of the isolated taenia to electrical stimulation (vertical lines). The muscles were stimulated in the presence of atropine and guanethidine. Note that Spantide blocks the contractile but not the relaxant response to stimulation. Tetrodotoxin (10^{-6} M) blocked both the contractile and the relaxant response (not shown). The tracings shown are typical examples of several experiments. (b) Effect of increasing concentrations of Spantide on the contractile response of the isolated taenia to electrical stimulation. Atropine and guanethidine were present in the bath. The results are expressed as a percentage of the contractile response before adding the antagonist. Each value is the mean of 4–10 experiments. Vertical bars give s.e.mean.

Discussion

The SP analogues tested antagonized the contractile effects of SP, physalaemin and eledoisin on the isolated taenia. SP, physalaemin and eledoisin share the

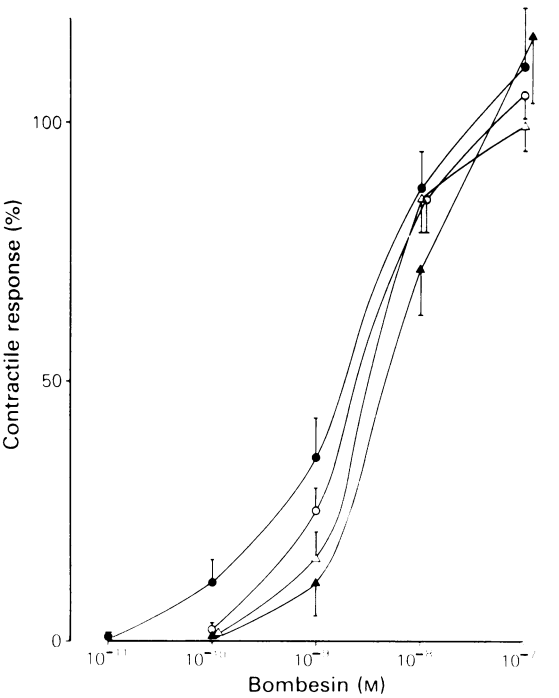

Figure 4 Concentration-response curves showing the contractile effect of bombesin alone (●) or in the presence of 10^{-5} M (D-Pro^2, D-$Trp^{7,9}$)SP_{1-11} (△), (Arg^5, D-$Trp^{7,9}$)SP_{5-11} (○), (D-Arg[1], D-$Trp^{7,9}$, Leu^{11})SP_{1-11} (▲) on the guinea-pig taenia. The response is expressed in relation to the contraction produced by carbachol 10^{-5} M. In the presence of SP antagonists the concentration-response curve was shifted to the right, suggesting competitive inhibition. Bombesin had a strong spasmogenic effect upon first application. This could not be reproduced upon subsequent applications, which gave much lower and quite stable contractile responses. The curves were constructed on the basis of second application of bombesin. Each value is the mean of 3–10 experiments. Vertical bars give s.e.mean

Table 2 Antagonistic specificity of three substance P (SP) analogues expressed as remaining contractile activity as a percentage of control

SP- antagonist	Contractile agent:	*SP*	*Physalaemin*	*Eledoisin*	*Bombesin*
(10^{-5} M)		10^{-9} M	10^{-10} M	10^{-10} M	10^{-9} M
(D-Pro^2, D-Trp^{7,9})-SP$_{1-11}$		(8) 29.1 ± 5.6	(8) 4.8 ± 2.7	(8) 0 ± 0	(8) 46.6 ± 7.0
Spantide (D-Arg^1, D-Trp^{7,9}, Leu^{11})-SP$_{1-11}$		(8) 0 ± 0	(4) 0 ± 0	(11) 4.6 ± 2.5	(10) 32.9 ± 5.2
(Arg^5, D-Trp^{7,9})-SP$_{5-11}$		(13) 38.7 ± 12.9	(8) 56.9 ± 9.9	(8) 34.2 ± 8.8	(7) 72.2 ± 4.9

Values are mean ± s.e.mean; *n* in parentheses

C-terminal bioactive portion and have been referred to collectively as tachykinins (Erspamer, 1981). Recent reports have indicated the existence of two different tachykinin receptor types, one which appears to be preferentially activated by eledoisin ('E' type receptors) and another which is preferentially activated by physalaemin ('P' type receptors) (see Lee *et al.*, 1982). Growcott & Tarpey (1983) observed that (D-Pro^2, D-Trp^{7,9})-SP was more active against eledoisin than against SP and our findings seem to support this view. Interestingly, the response to bombesin was also inhibited by the SP antagonists. This inhibition was apparent only with low concentrations of the agonist. Bombesin shares the two C-terminal amino acids with SP (Erspamer & Melchiorri, 1983) and it is not inconceivable that bombesin may interact with SP receptors. The action of bombesin was not as effectively antagonized as that of SP. The rightward shift of the bombesin dose-response curve to 10^{-5} M antagonists was only one order of magnitude, and it cannot be excluded that the antagonism to bombesin is non-competitive. The contractile responses to vasopressin, bradykinin, histamine, prostaglandins, 5-HT or carbachol were unaffected by the SP antagonists. The antagonism to SP was competitive in nature as indicated by the slopes of the Schild plot (Tallarida *et al.*, 1979) which were close to −1 for all the analogues analyzed. The pA_2 values varied from 5.6 to 7.2. The undecapeptide antagonists with the highest pA_2 values were (D-Arg^1, D-Trp^{7,9}, Leu^{11})SP$_{1-11}$ and (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11})SP$_{1-11}$; they were also the most potent with respect to inhibition of the non-cholinergic, non-adrenergic contraction of the taenia. Of the two, (D-Arg^1, D-Trp^{7,9}, Leu^{11})SP$_{1-11}$ was notable for its virtual lack of spasmogenic properties and poor histamine-releasing effect.

(D-Arg^1, D-Trp^{7,9}, Leu^{11})SP$_{1-11}$ was designed and synthesized by Folkers *et al.*, (chemistry unpublished) and was recently reported to be an effective antagonist (Rosell *et al.*, 1983). The name Spantide (*S*ubstance *P-An*tagonist-Pep*tide*) was proposed.

Most of the SP antagonists contracted the taenia. It has been suggested previously that this may reflect local histamine release rather than an effect on the SP receptors of the smooth muscle, since the response was seen only upon the first application, and since pretreatment with the histamine H_1-receptor antagonists mepyramine abolished the contractile response to the SP antagonists (Håkanson *et al.*, 1982). Furthermore, the SP antagonists were found to cause release of histamine from rat peritoneal mast cells (cf. Håkanson *et al.*, 1983), the EC_{50} for the various antagonists varying from 10^{-6} to 10^{-5} M. On the whole, SP antagonists with strong spasmogenic activity were potent histamine releasers. This was not invariably so, however, possibly suggesting the involvement of mechanisms other than histamine release in the contraction of smooth muscle by the SP antagonists. Conceivably, however, the rate with which SP antagonists release mast cell histamine may be of importance for their contractile effect, a rapid release causing a high local concentration resulting in muscle contraction, whereas a slow release may not generate a sufficiently high local concentration to cause contraction.

For the design of the next generation of SP antagonists, information on the structure-activity relationship is important. All tachykinins of the SP family share certain features, particularly apparent at the C-terminal end (Figure 5). It can be assumed that amino acid sequences which appear in all SP-related peptides are essential for interaction with the receptor and that substitutions in these positions could

Table 3 Spasmogenic activity and histamine release by substance P (SP) analogues

Peptide													*Histamine release* $EC_{50} \pm$ s.e. (M)	*Spasmogenic activity* $EC_{50} \pm$ s.e. (M)	*Intrinsic activity**
SP	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2	$1.0 \times 10^{-5} \pm 3.3 \times 10^{-6}$	–	
	–	–	–	–	–	–	D-Phe	–	D-Trp	–	–	–	$3.4 \times 10^{-7} \pm 1.0 \times 10^{-7}$	$1.0 \times 10^{-5} \pm 8.7 \times 10^{-7}$	4.3
	–	–	–	–	–	–	D-Trp	–	D-Trp	–	–	–	$2.7 \times 10^{-6} \pm 6.6 \times 10^{-7}$	$6.00^{-6} \pm 4.0 \times 10^{-8}$	5.5
	–	D-Pro	–	–	–	–	D-Trp	–	D-Trp	–	–	–	$2.0 \times 10^{-6} \pm 1.4 \times 10^{-7}$	$3.0 \times 10^{-6} \pm 1.0 \times 10^{-6}$	2.8
	D-Arg	D-Pro	–	–	–	–	D-Trp	–	D-Trp	–	Leu	–	$1.5 \times 10^{-6} \pm 1.3 \times 10^{-7}$	$2.0 \times 10^{-6} \pm 1.0 \times 10^{-7}$	6.5
Spantide	D-Arg	–	–	–	–	–	D-Trp	–	D-Trp	–	Leu	–	$4.0 \times 10^{-6} \pm 8.2 \times 10^{-7}$	$2.0 \times 10^{-5} \pm 5.0 \times 10^{-7}$	1.0
					Arg	–	D-Trp	–	D-Trp	–	–	–	$2.0 \times 10^{-6} \pm 1.7 \times 10^{-7}$	$2.0 \times 10^{-5} \pm 1.0 \times 10^{-6}$	0.9
					Arg	–	-D-Trp-p-Cl-Phe-		D-Trp	–	–	–	$1.0 \times 10^{-6} \pm 1.3 \times 10^{-7}$	$1.0 \times 10^{-5} \pm 2.9 \times 19^{-6}$	5.4
					Arg	–	-D-p-Cl-Phe-		D-Trp	–	–	–	$3.0 \times 10^{-6} \pm 1.1 \times 10^{-6}$	$6.0 \times 10^{-6} \pm 4.8 \times 10^{-7}$	3.9
					Arg	–	D-Trp	–	D-Trp	–	Leu	–	$1.0 \times 10^{-6} \pm 2.0 \times 10^{-7}$	No contraction	
					Arg	–	D-Trp	–	D-Trp	–	Nle	–	$2.3 \times 10^{-6} \pm 9.4 \times 10^{-7}$	$7.0 \times 10^{-6} \pm 2.5 \times 10^{-6}$	6.9
					Arg	–	D-Trp	–	D-Trp	–	Val	–	$4.9 \times 10^{-6} \pm 1.6 \times 10^{-6}$	No contraction	

– = same amino acid as in SP

* The contractile response to a maximum dose of Spantide is given as 1.

1	2	3	4	5	6	7	8	9	10	11	
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2 Substance P
Pyr	Pro	Asp	Pro	Asn	Ala	Phe	Tyr	Gly	Leu	Met	NH_2 Uperolein
Pyr	Ala	Asp	Pro	Asn	Lys	Phe	Tyr	Gly	Leu	Met	NH_2 Physalaemin
	Pyr	Asn	Pro	Asn	Arg	Phe	Ile	Gly	Leu	Met	NH_2 Phyllomedusin
Pyr	Pro	Ser	Lys	Asp	Ala	Phe	Ile	Gly	Leu	Met	NH_2 Eledoisin
Asp – Val	Pro	Lys	Ser	Asp	Gln	Phe	Val	Gly	Leu	Met	NH_2 Kassinin
	His	Lys	Thr	Asp	Ser	Phe	Val	Gly	Leu	Met	NH_2 Substance K
	Asp	Met	His	Asp	Phe	Phe	Val	Gly	Leu	Met	NH_2 Neuromedin K

Figure 5 Amino acid sequences of substance P and related tachykinins. Amino acids common to them all are boxed.

cause marked changes in biological activity. Antagonists have resulted from substitutions in positions 7 and 9. D-Tryptophan in these positions seems to be favourable for antagonistic activity and leucine in position 11 seems to add potency (Folkers *et al.*, 1983). D-Proline in position 2 is not essential. (D-Arg[1], D-Trp[7,9], Leu[11]) SP_{1-11} (Spantide) is the most promising of the analogues in view of its high pA_2 value, low IC_{50} value, and low spasmogenic activity. Heptapeptides did not offer advantages over undecapeptide analogues, and with the exception of (Arg[5], D-Trp[7,9], Nle[11])SP_{5-11} their pA_2 values were generally lower.

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