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UNDECA- AND OCTA-PEPTIDE ANTAGONISTS FOR SUBSTANCE P, A STUDY ON THE GUINEA PIG TRACHEA

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Undeca- and octa-peptide analogues of substance P (SP), acting as antagonists in the guinea pig ileum, have been tested on the guinea pig trachea. The antagonistic properties of several octapeptides, particularly of [pro^4,trp^{7,9,10},Phe^{11}]SP-(4-11) ** have been confirmed, while the undecapeptides have been found to be potent stimulants of the trachea. The contractions of the guinea pig trachea in response to several undecapeptides, particularly [pro^2,trp^{7,9},Leu^{11}]SP, undergo rapid tachyphylaxis, are significantly reduced in the presence of diphenhydramine and are not influenced by octapeptide antagonists of SP. These contractions appear to be due to the activation of tissue sites mediating the release of intramural histamine and different from SP receptors. On repeated applications, the stimulant effects of undecapeptides are eliminated and the compounds can be tested as antagonists. Undecapeptide antagonists have been found to be more potent against eledoisin and kassinin than against SP or physalaemin, while the octapeptides are equally active against the four homologues. Both undeca- and octa-peptides seem however to exert a competitive type of antagonism against all the SP-related peptides tested in the present study. Differences of antagonistic affinities have been interpreted as indicative of the existence of two different receptor types for SP and related peptides in the guinea pig trachea. The two receptors are blocked by the octapeptide antagonists, which are not discriminatory, while undecapeptides are particularly active on the receptor subtype which shows high sensitivity for eledoisin and kassinin.

Substance P-related peptides Guinea pig trachea SP antagonists Receptors

1. Introduction

In the course of a systematic study on the effects of substance P and related peptides in various smooth muscle preparations, it was found (Regoli et al., 1983a,b) that the guinea pig trachea presents some interesting features. Firstly, the contraction of the isolated trachea to substance P and related peptides consists of a slow increase of tension which persists at the highest level as long as the peptides are kept in contact with the tissues. In other words, the myotropic effects of SP and its congeners do not show any fading, contrary to what is observed in other preparations (the guinea pig ileum, the rabbit mesenteric vein etc.; Couture and Regoli, 1982). Secondly, some undecapeptide analogues of SP that act as antagonists in other smooth muscle preparations (for instance in the guinea pig ileum) are potent stimulants of the guinea pig trachea, while another type of antagonists, the octapeptides, are completely inactive as stimulants. Thirdly, some SP homologues such as eledoisin and kassinin, are more potent than SP and physalaemin, contrary to what is observed in other tissues (for instance the guinea pig ileum).

The present study was undertaken to investigating further the mechanisms responsible for the

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** All lower case amino acid abbreviations indicate the D-isomers: e.g. pro = D-Pro.

effects described above and to answer the following questions: (i) What is the mechanism by which undecapeptide antagonists stimulate the trachea smooth muscle? (ii) Have these compounds to be considered partial agonists acting on SP receptors, or do they act on other sites and by different mechanisms than SP? (iii) Why are the affinities of eledoisin and kassinin such different from those of SP and physalaemin? (iv) Is there an indication of the existence of two different receptor types for SP and related peptides?

If different receptors for SP are present in the trachea, can these receptor subtypes be identified and characterized by using two types of antagonists, recently developed, namely the undecapeptides (Rosell and Folkers, 1982; Björkroth et al., 1982; Rosell et al., 1983) and the octapeptides (Caranikas et al., 1982; Mizrahi et al., 1982c; Regoli et al., 1984)?

2. Materials and methods

Guinea pigs of either sex, weighing 300-450 g were killed by stunning and exsanguination through sectioning of the carotid arteries. The trachea was rapidly removed and strips were prepared according to Constantine (1965). The strips were suspended in 10 ml organ baths, containing oxygenated (95% O_2 and 5% CO_2), warm (37°C) Krebs solution, whose composition has been given before (Mizrahi et al., 1982a) and then stretched with an initial tension of 3.0 g which was adjusted at 20 min intervals during the equilibration (90-120 min) period. Changes of tension were recorded isometrically with Grass (FT.03C) force transducers connected to a Grass polygraph model 7D.

Contractions of the tracheal strips in response to peptides and non-peptide agents were measured in tissue treated with indomethacin (5.6×10^{-6} M) in order to eliminate the interference of inhibitory prostaglandins, as recommended by Mizrahi et al. (1982a,b). The following peptides were used in the various experiments: Substance P (SP), SP-(4-11), SP-(6-11), Physalaemin (P.), Eledoisin (E.), Kassinin (K.), [pro^2,phe^7,trp^9]SP **, [pro^2,trp^{7,9}]SP and [arg^1,pro^2,trp^{7,9},Leu^{11}]SP. These peptides were purchased from Peninsula Laboratories, while all the other peptides were synthesized in our laboratory. These include: [Pro^2,trp^{7,9,10}]SP, [pro^2, trp^{7,9,10}]SP, [pro^2,trp^{7,9},Leu^{11}]SP and the octapeptides [pro^4,trp^{7,9}]-, [pro^4,trp^{7,9,10}]-, [pro^4,trp^{7,9}, Leu^{11}]-, [pro^4,trp^{7,9,10},Leu^{11}]-, [pro^4,trp^{7,9},Nleu^{11}]-, [pro^4,trp^{7,9,10},Nleu^{11}]-, [pro^4,trp^{7,9},Phe^{11}]-, [pro^4, trp^{7,9,10},Phe^{11}]- and [pro^4,trp^{7,9,10},Trp^{11}]SP-(4-11). Details of the synthesis and purification procedures as well as the basic chemical data of these new peptides have been (Caranikas et al., 1983) or will be described. Histamine HCl (Fisher), acetylcholine HCl (Sigma), diphenhydramine HCl (Parke-Davis) and indomethacin bimaleate (Sigma) were also used.

Peptide antagonists of substance P were tested against SP and related peptides as well as against other agents by applying the antagonists during the plateau of the contraction produced by peptide and nonpeptide agonists (curative protocol) or 8-10 min before contracting the tissue with one or the other types of stimulants. Affinity of antagonists was evaluated in terms of pA_2, and Schild plots were calculated according to Tallarida et al. (1979), in order to find out whether the antagonists were competitive or not.

Peptides and nonpeptide agents used in the study were dissolved in distilled water (1, 5, 10 mg/ml) and stored in small aliquots at −20°C. These aliquots were thawed on the day of the experiment, diluted in Krebs solution and discarded at the end of the day. Indomethacin was dissolved in Trizma base 0.02 M (Sigma).

Concentrations of peptides are expressed in mol of salt, while those of nonpeptide agents are expressed in mol of base. Results are given as means ± S.E. and the statistical significance has been evaluated with the Student's t-test for independent samples. P values less than 0.05 were considered to be significant.

3. Results

3.1. Effects of SP-antagonists

In a first series of experiments, the specificity of SP octapeptide antagonists was tested by comparing the effects of these antagonists against SP,

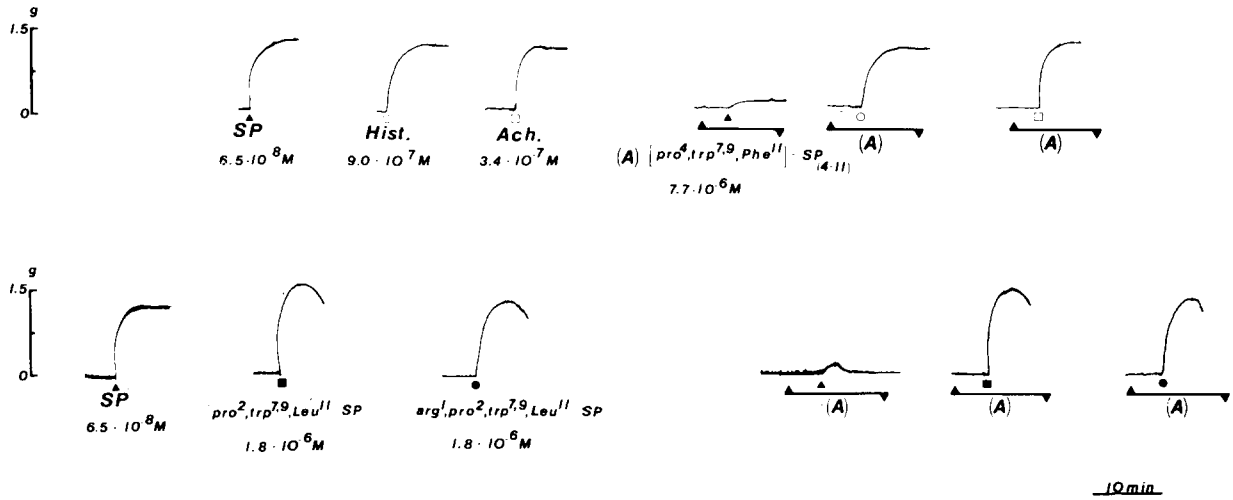

Fig. 1. Myotropic effects of substance P (SP), histamine (Hist.), acetylcholine (ACh). [pro^2,trp^{7,9},Leu^{11}]SP, [arg^1,pro^2,trp^{7,9},Leu^{11}]SP on guinea pig tracheae, non-treated (tracings on the left) or treated with the octapeptide antagonist (A) [pro^4,trp^{7,9},Phe^{11}]SP-(4-11). *Ordinate*: tension in grams (g). *Abscissa*: time in minutes (min).

TABLE 1

Pharmacological effects (contraction expressed in mm) of SP related peptides on the guinea pig isolated trachea.
[a,b,c] Activity of compounds as antagonists are expressed by the pA_2, residual agonistic activity by the fraction of the maximal effects (M.E.) of substance P (SP = 1.0). Reduction of the stimulant effects of some compounds by diphenhydramine (3.3×10^{-6} M) has been measured by comparing the means of 10-15 determinations and the statistical significance has been evaluated with the Student's t-test for independent samples. [d] $P < 0.001$.

	Compound	pA_2 [a]	M.E. [b]	Concentration (M)	Control (mm)	Diphenhydramine [c] (mm)
I	Substance P	-	1.0	3.2×10^{-8}	18.2 ± 2.5	17.3 ± 2.3
II	Physalaemin	-	1.0	4.0×10^{-8}	21.3 ± 2.6	19.6 ± 2.0
III	Eledoisin	-	1.0	1.0×10^{-8}	30.0 ± 2.8	27.1 ± 2.9
IV	[pro^2,phe^7,trp^9]SP	-	1.0	2.2×10^{-6}	20.1 ± 3.3	8.0 ± 1.0 [d]
V	[pro^2,trp^{7,9}]SP	-	1.0	1.5×10^{-6}	29.4 ± 3.1	10.5 ± 1.2 [d]
VI	[pro^2,trp^{7,9,10}]SP	-	0.8	2.1×10^{-6}	13.0 ± 2.0	6.1 ± 0.8 [d]
VII	[Pro^2,trp^{7,9,10}]SP	6.10	0.1	8.5×10^{-6}	-	-
VIII	[pro^2,trp^{7,9},Leu^{11}]SP	-	1.0	1.8×10^{-6}	34.9 ± 3.2	13.4 ± 1.5 [d]
IX	[arg^1,pro^2,trp^{7,9},Leu^{11}]SP	-	0.9	1.8×10^{-6}	25.5 ± 3.5	7.6 ± 0.9 [d]
X	SP-(4-11)	-	1.0	5.0×10^{-8}	17.6 ± 1.3	16.4 ± 1.5
XI	[pro^4,trp^{7,9}]SP-(4-11)	5.65	0.002	2.0×10^{-5}	-	-
XII	[pro^4,trp^{7,9,10}]SP-(4-11)	6.00	0.001	1.4×10^{-5}	-	-
XIII	[pro^4,trp^{7,9},Leu^{11}]SP-(4-11)	6.00	0.1	3.0×10^{-5}	-	-
XIV	[pro^4,trp^{7,9,10},Leu^{11}]SP-(4-11)	5.94	0.001	2.0×10^{-5}	-	-
XV	[pro^4,trp^{7,9},Nleu^{11}]SP-(4-11)	5.92	0.05	1.7×10^{-5}	-	-
XVI	[pro^4,trp^{7,9,10},Nleu^{11}]SP (4-11)	5.70	0	2.0×10^{-5}	-	-
XVII	[pro^4,trp^{7,9},Phe^{11}]SP-(4-11)	6.50	0.001	2.0×10^{-5}	-	-
XVIII	[pro^4,trp^{7,9,10},Phe^{11}]SP-(4-11)	6.71	0.001	2.0×10^{-5}	-	-
XIX	[pro^4,trp^{7,9,10},Trp^{11}]SP-(4-11)	-	1.0	3.6×10^{-7}	38.0 ± 4.7	35.7 ± 4.1

histamine and acetylcholine. As illustrated in fig. 1, [pro^4,trp^{7,9},Phe^{11}]SP-(4-11) reduced significantly the effect of substance P without influencing at all those of the other two agonists. Similar results were obtained with other compounds listed in table 1. It appears therefore that octapeptide antagonists are specific for substance P.

When tested for antagonism, some undecapeptides, such as for instance [pro^2,trp^{7,9},Leu^{11}]SP or [arg^1,pro^2,trp^{7,9},Leu^{11}]SP induced strong contractions which however were not maintained at a stable plateau, as illustrated at the bottom of fig. 1. Surprisingly, these contractile effects are different from that of SP, in that they are not influenced at all in the presence of the octapeptide antagonist [pro^4,trp^{7,9},Phe^{11}]SP-(4-11) (fig. 1). These preliminary observations were extended to several other SP-related peptides and the results obtained are summarised in table 1.

A series of undecapeptide analogues of SP, exerting antagonistic effects on other preparations, according to data reported in the literature (Rosell and Folkers, 1982; Björkroth et al., 1982; Rosell et al., 1983) and obtained in our laboratory (Mizrahi et al., 1983; Regoli et al., 1984) are potent stimulants of the guinea pig isolated trachea and produce contractions of approximately the same intensity as those by substance P. On the contrary octapeptide antagonists are either inactive or show very little activity, as for instance [pro^4,trp^{7,9}, Leu^{11}]SP-(4-11). The only octapeptide active as an agonist is compound XIX of table 1, which is practically a full agonist and has no antagonistic properties against SP on the guinea pig trachea.

3.2. Mechanism of the contractile effects of undecapeptide antagonists

In an attempt to identify the mechanism by which the undecapeptide antagonists stimulate the trachea and produce contractions which are not to be repeated twice in the same tissue (tachyphylaxis?), diphenhydramine was applied, either before or at the top of the contraction induced by substance P, by its homologues physalaemin and eledoisin as well as by several undecapeptides (table 1). As shown in table 1, and in the tracings of fig. 2, diphenhydramine reduced significantly the effects of several undecapeptides without changing significantly (very little in fact) the contractions evoked by SP, physalaemin, eledoisin, by compound no. VII and by the octapeptide [pro^4,

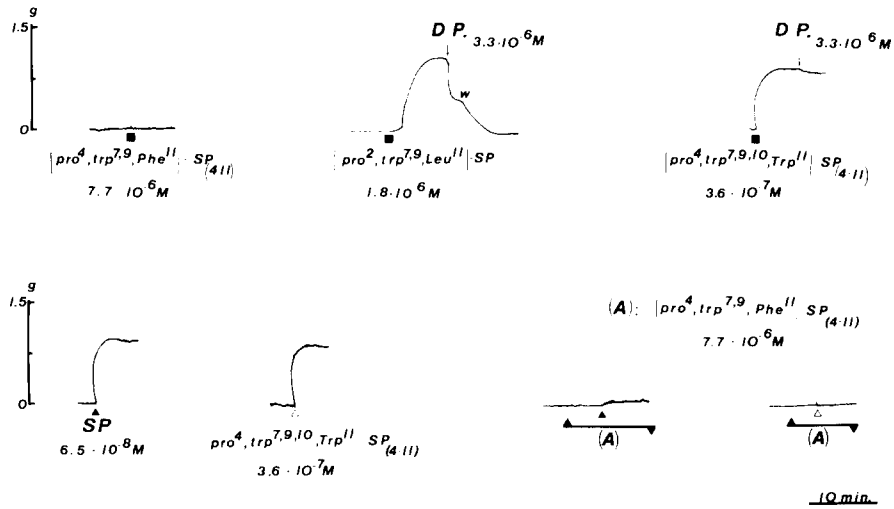

Fig. 2. *Top tracings.* Effects of octa and undecapeptide analogues of SP on isolated guinea pig tracheae as influenced by the curative application of diphenhydramine (3.3×10^{-6} M). *Bottom tracings.* Contractions of the guinea pig trachea in response to SP and to [pro^4,trp^{7,9,10},Trp^{11}]SP-(4-11) in the absence and presence of (A), the antagonist [pro^4,trp^{7,9},Phe^{11}]SP-(4-11). *Ordinate*: tension in grams (g). *Abscissa*: time in minutes (min).

trp^{7,9,10},Trp^{11}]SP-(4-11). This compound appears to act on the same site as substance P since its stimulant effect (a) is maintained at a stable plateau, similar to that of substance P, (b) is not influenced by diphenhydramine, applied curatively (fig. 2) and (c) is inhibited by [pro^4,trp^{7,9},Phe^{11}]SP-(4-11), when used at a concentration sufficient to antagonise substance P (fig. 2, tracings at the bottom).

The results described above suggest that undecapeptide antagonists of SP contract the guinea pig isolated trachea by promoting the release of histamine, because their effects are significantly reduced in the presence of diphenhydramine. In a few experiments [pro^2,trp^{7,9},Leu^{11}]SP and other undecapeptide antagonists were found to produce vasodilation in the rat hind quarter, perfused with saline according to Skofitsch et al. (1983): this effect was practically abolished by diphenhydramine. Octapeptide antagonists were found to be inactive on this preparation.

The replacement of Met^{11} by Trp, in association with the other modifications which are needed to produce antagonism, eliminate completely the antagonistic effect and confer to the octapeptide (compound XIX of table 1) the property of a full agonist, which appears to act on the same site as substance P.

This conclusion is further supported by the results summarised in table 2, in which the octapeptide antagonist [pro^4,trp^{7,9,10},Phe^{11}]SP-(4-11) is shown to be active against substance P, its homologues, some active C-terminal sequences, such as SP-(4-11), SP-(6-11) and [pro^4,trp^{7,9,10}, Trp^{11}]SP-(4-11). The same antagonist is practically inactive against the undecapeptides, suggesting that these compounds may stimulate the guinea pig trachea by a mechanism which is different from that utilised by SP, its fragments and homologues.

3.3. Do substance P-related peptides activate one or more than one receptor type on the guinea pig trachea?

In order to enable the evaluation of the affinities of undecapeptides as antagonists of SP in the guinea pig trachea, the protocol illustrated in fig. 3 was applied to several compounds. The experiment began by recording the contraction of the trachea in response to SP, eledoisin, or other peptides (fig. 3). Thereafter, one of the undecapeptide antagonists was applied and kept in contact with the tissue for several minutes. After washing out the compound, SP or one of the other agonists were tested again. In general their effects were found to be unchanged (fig. 3). Thereafter, the undecapeptide antagonist was applied for the second time and it was found to be inactive (tachyphylaxis?). Substance P eledoisin or other SP-related peptides were then applied in the presence of the undecapeptide antagonists. At rela-

TABLE 2

Antagonist effects of [pro^4,trp^{7,9,10},Phe^{11}]SP-(4-11) on substance P and related peptides.

[a] Millimiters of contraction: values indicate means ± S.E. of at least 6 determinations. [b] $P < 0.001$.

Compound	Concentration (M)	No antagonist	Antagonist (7.75×10^{-6} M)
Substance P	3.2×10^{-8}	18.2 ± 2.5 [a]	1.5 ± 0.1 [b]
Eledoisin	1.0×10^{-8}	30.0 ± 2.8	7.5 ± 1.0 [b]
Physalaemin	4.0×10^{-8}	21.3 ± 2.6	3.2 ± 0.05 [b]
SP-(4-11)	5.0×10^{-8}	17.6 ± 1.3	1.6 ± 0.09 [b]
SP-(6-11)	1.3×10^{-8}	31.8 ± 3.9	0 [b]
[pro^2,phe^7,trp^9]SP	2.2×10^{-6}	20.1 ± 3.3	14.8 ± 2.1
[pro^2,trp^{7,9}]SP	1.5×10^{-6}	29.4 ± 3.1	33.1 ± 4.4
[pro^2,trp^{7,9},Leu^{11}]SP	1.8×10^{-6}	34.9 ± 3.2	34.0 ± 6.4
[arg^1,pro^2,trp^{7,9},Leu^{11}]SP	1.8×10^{-6}	25.5 ± 3.5	30.7 ± 5.8
[pro^4,trp^{7,9,10},Trp^{11}]SP-(4-11)	3.6×10^{-6}	38.0 ± 4.7	3.0 ± 0.5 [b]

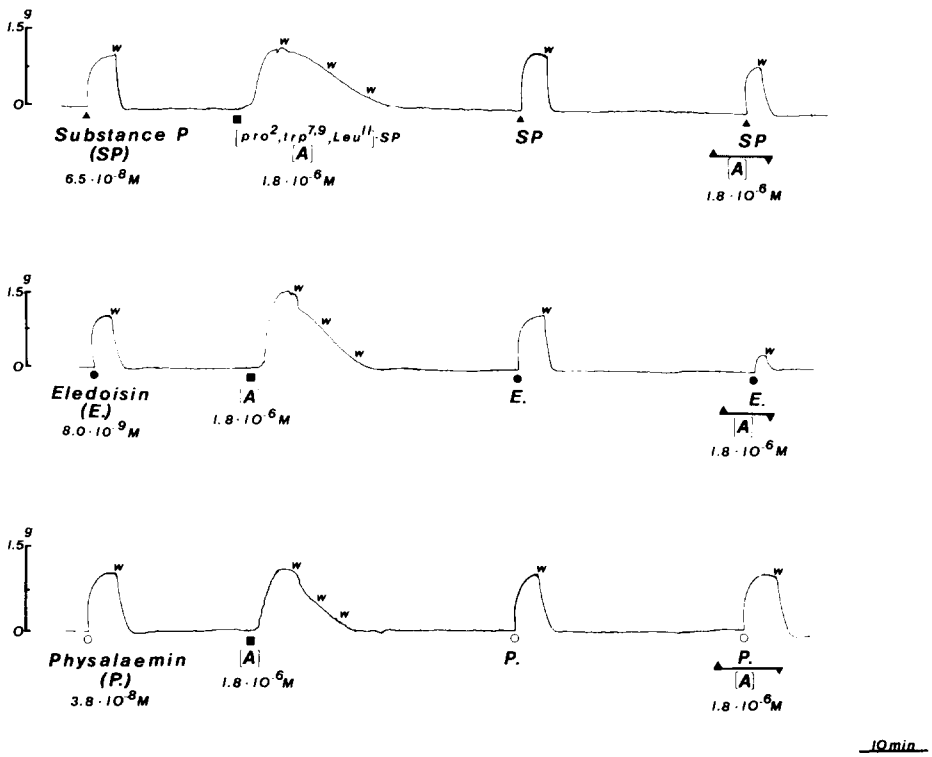

Fig. 3. Contractions of the guinea pig trachea in response to SP, eledoisin and physalaemin before and after treatment with a high concentration of [pro^2,trp^{7,9},Leu^{11}]SP. The same concentration of the undecapeptide analogue was applied a second time and found to be without effect. In his presence, SP, eledoisin, and physalaemin were tested again: only eledoisin was inhibited. *Ordinate*: tension in grams (g). *Abscissa*: time in minutes (min).

tively low concentrations (5×10^{-7} M) these compounds were found to reduce significantly the effects of eledoisin and kassinin while leaving practically unchanged those of SP and physalaemin.

These results were taken as an indication that SP and related peptides may act on two different receptor subtypes, the first activated by eledoisin and kassinin, the second activated by SP and physalaemin. While octapeptide antagonists are active on the two receptor subtypes and reduce the effect of all the SP homologues, low concentrations of undecapeptide antagonists inhibit only the contractions induced by kassinin and eledoisin. Higher concentrations ($> 10^{-6}$ M) of undecapeptides are required to reduce the effects of SP and physalaemin.

In order to further substantiate these observations, pA_2 values of octapeptide and undecapeptide antagonists were measured and the results are summarised in table 3.

The two antagonists show important differences of affinities when challenged with SP, physalae-

TABLE 3

Apparent affinities (pA_2) of SP antagonists on the guinea pig trachea.
A *: [pro^4,trp^{7,9,10},Phe^{11}]SP-(4-11); B **: [pro^2,trp^{7,9},Leu^{11}]SP-(1-11). Slope of Schild plots calculated from at least 3 points. Each point was obtained from at least 6 determinations. In parentheses the pD_2 values of the agonists.

Agonist		A *	B **	Slope	
				A *	B **
Substance P	(6.75)	6.71	5.60	1.08	1.17
Eledoisin	(7.40)	6.30	6.30	1.09	1.13
Physalaemin	(6.85)	6.80	5.30	0.92	0.87
Kassinin	(7.90)	6.38	6.40	1.10	1.07

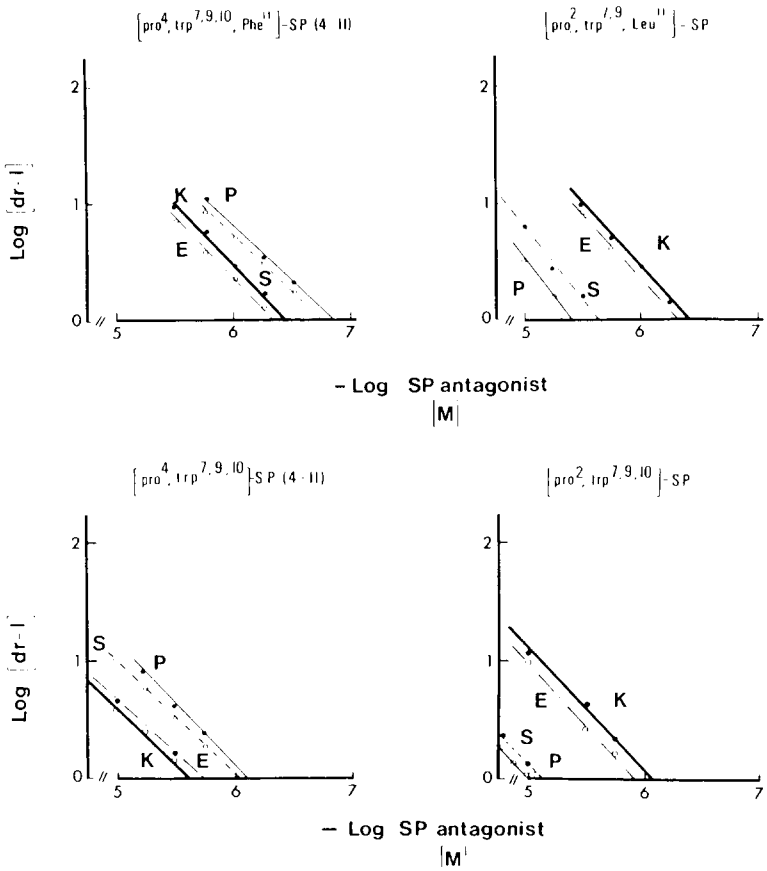

Fig. 4. Schild plots obtained with four SP antagonists on the guinea pig isolated trachea. The plots were calculated from at least 3 points and eachpoint is the mean of 6 determinations. S: substance P; P: physalaemin; E: eledoisin; K: kassinin. *Abscissa*: $-\log$ in molar (M) of antagonist concentration. *Ordinate*: log of dose ratio -1 (dr -1).

min, eledoisin and kassinin. The affinities of the octapeptide are definitely higher than those of the undecapeptide by more than 1 log unit, when measured against SP or physalaemin. On the contrary, when challenged with kassinin and eledoisin, the two antagonists show very similar affinities. Slope of Schild plots, calculated according to Tallarida et al. (1979) are near to unity, suggesting that the two antagonists may exert a competitive type of antagonism against SP, physalaemin, eledoisin and kassinin.

TABLE 4

Affinities as agonists and antagonists of SP and related peptides in the guinea pig trachea.

Agonist	Affinity pD_2	Antagonist A * pA_2	Affinity B **	Difference
Eledoisin	7.40	6.30	6.30	0
Kassinin	7.90	6.38	6.40	0.02
Substance P	6.75	6.70	5.60	1.1
Physalaemin	6.85	6.80	5.30	1.5
Difference	0.65-1.05	0.4	0.8-1.0	

A * and B **: same as in table 3.

Schild plots, obtained with two octapeptide and two undecapeptide antagonists, using three or four different concentrations of each antagonist, are illustrated in fig. 4. Because the two antagonists compared in the upper part of fig. 4 were different not only for the C-terminal end but also by the number of trp residues, used to produce antagonism, affinities were measured with two

other antagonists, namely the octapeptide [pro^4,trp^{7,9,10}]SP-(4-11) and the undecapeptide [pro^2,trp^{7,9,10}]SP which differ only by their N-terminals. The results summarised at the bottom of fig. 4 confirm essentially those obtained with the other two antagonists and show that pA_2 values (given by the intersection of the lines with the abscissa), measured with the octa- and the undeca-peptide antagonists against kassinin and eledoisin are very close (they differ by no more than 0.03 log unit), while the values measured against substance P and physalaemin are definitely lower (by approximately 1 log unit).

When interpreted in view of receptor classification, the data of table 3, obtained with the most potent antagonists for substance P available at present, and those of fig. 4 suggest the existence of two different receptor subtypes for SP and related peptides in the guinea pig trachea (Regoli et al., 1983a,b).

4. Discussion

The present results suggest that SP-related peptides bring about contractions of the guinea pig isolated trachea by various mechanisms. Firstly, the effects of SP and some of its potent C-terminal fragments are not influenced by antagonists of catecholamines, acetylcholine and other endogenous agents, including histamine (Mizrahi et al., 1982a,b). If there is any release of histamine by SP and related peptides from the guinea pig trachea, this effect appears to be irrelevant or of very little importance, in contrast with the observations made by several authors on various other systems in which SP has been shown to be a fairly potent releaser of histamine (Foreman and Jordan, 1981, rat mast cells; Erjavec et al., 1981, rat hindquarters; Foreman and Jordan, 1983, human skin).

However, when modifications are made at the N-terminal part of the SP molecule, the ability to release histamine can be improved, particularly by replacing Pro^2 with pro. As shown by the results of table 1, and in particular by the comparison of the data obtained with compounds VI and VII, the conformational change induced by the dextro-isomer in position 2 appears to be the major factor responsible for the release of histamine. The present results are in accord with the findings of Lundberg et al. (1983) in the guinea pig trachea, whereby the stimulatory effect of [arg^1,pro^{7,9}, Leu^{11}]SP was blocked by anti-H_1 antagonists. Other modifications, required to change the pharmacological spectrum of undecapeptides from that of agonists to that of antagonists, do not seem to play any role for the release of histamine. This effect depends on the N-terminal three-peptide sequence and does not occur with octapeptide agonists and antagonists. Compound XIX of table 1, for instance, is a full agonist but its contractile effect is not significantly modified by diphenhydramine and appears to be due to the stimulation of SP receptors, because it is blocked by a SP antagonist, as shown in table 2. In addition to being partially antagonised by diphenhydramine, peptides acting indirectly through the release of histamine, show marked tachyphylaxis, as if the endogenous mediator were present in limited quantity and were rapidly exhausted. Indeed, on repeated applications, these undecapeptides become inactive and therefore can be tested as antagonists, using the protocol illustrated in fig. 3. Surprisingly, these undecapeptides show higher affinities as antagonists against eledoisin and kassinin, compared to physalaemin and substance P. On the other hand, affinities of octapeptide antagonists do not show such differences (table 4) and eventually these antagonists are more potent against substance P and physalaemin than against eledoisin and kassinin. The data summarised in table 4 and the differences calculated between the affinities of the two antagonists against the four homologues, substance P, physalaemin, eledoisin and kassinin show that the undecapeptide is much more selective than the octapeptide. In fact, the first compounds show pA_2 values much inferior (by more than 1 log unit) when challenged with SP and physalaemin, than with eledoisin and kassinin. These results suggest the existence of two different receptor types, one which appears to be preferentially activated by eledoisin and kassinin and the other which is possibly activated by the four homologues. The first receptor shows high affinity (pD_2 7.4-7.9) for eledoisin and kassinin and it is blocked by both octapeptide and undecapeptide

antagonists, which appears to be equally active (pA_2 values vary between 6.3 and 6.4) but have affinities definitely lower than the agonists (by at least 1 log unit). This indicates that, in order to block the effect of kassinin and eledoisin on the first receptor, the antagonist has to be applied in concentration at least ten times higher than the agonist.

The other receptor, activated presumably by the four homologues, show apparent affinity constant of 6.75-6.85, lower (by 0.55-1.05 log unit) than the first one and it is blocked much more easily by the octapeptide than by the undecapeptide antagonists. The affinities of the first group of antagonists are very close to those of SP and physalaemin, suggesting that the competition agonist-antagonist is occurring at the molecular ratio of one by one. On the contrary, the undecapeptide antagonists are much less active and concentrations at least ten times higher than those of the agonists are required to reduce the effects of SP and physalaemin.

The present findings agree with other recent reports by Rosell et al. (1983) who have tested the antagonistic property of [arg^1,pro^2,trp^{7,9},Leu^{11}]SP in the guinea pig ileum and in the hamster urinary bladder. They found that the antagonist is active on the guinea pig ileum but is inactive on the hamster urinary bladder. The present results are also similar to those reported by Growcott and Tarpey, who tested the partial agonist [pro^2, trp^{7,9}]SP on the guinea pig ileum treated with atropine (SP-P) and on the guinea pig vas deferens (SP-E). They found that [pro^2,trp^{7,9}]SP was ten times more active against eledoisin than against substance P and suggested the existence of a receptor site specific for eledoisin and different from that of SP and physalaemin. More recent studies by the same authors (Growcott et al., 1983) have further substantiated this interpretation, by showing that the receptor activated by eledoisin is blocked, while that activated by SP and physalaemin is not altered by phenoxybenzamine.

Finally, in a recent report, Lin and Musacchio (1983) have shown that phenoxybenzamine significantly reduces the binding of kassinin to receptor sites in the guinea pig ileum. These and other findings support the interpretation that a receptor site, specific for eledoisin and kassinin and possibly different from that for substance P and physalaemin may be present in several tissues. The application of the first classification criterion (the order of potency of agonists), particularly of homologues appears to be useful for the identification of the two receptor sites. These sites have been further identified with undecapeptide antagonists, which appear to be selective and show high affinity for one of them. Data obtained with the undecapeptides have been compared with those of octapeptide antagonists, a group of compounds which do not discriminate between the two receptor sites, but still are as potent antagonists as the undecapeptides.

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