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Characterization of tachykinin receptors in isolated basilar arteries of guinea-pig

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1 In circular lengths cut from the basilar artery of guinea-pig (0.2–0.3 mm o.d.) relaxations induced by substance P and neurokinin A were highly susceptible to mechanical damage of the endothelium by rubbing. The precontraction induced by prostaglandin $F_{2\alpha}$ but not that of 124 mM potassium was reduced considerably by the rubbing procedure.

2 Concentration-dependent relaxations were evoked by tachykinin agonists in the following order of potency: substance P = physalaemin > neurokinin A > eledoisin. Physalaemin was, however, a partial agonist, giving only half the maximum relaxation as compared to the other tachykinins.

3 The two putative tachykinin receptor antagonists, spantide ([D-Arg^1, D-Trp^{7,9}, D-Leu^{11}] substance P) and [D-Pro^2, D-Trp^{7,9}] substance P, shifted the concentration-dependent relaxations of substance P to the right in a parallel manner. Calculation of pA_2 values and Schild plot analysis revealed pA_2 values of 7.4–7.6 for spantide and 6.9–7.0 for [D-Pro^2, D-Trp^{7,9}] substance P, irrespective of whether substance P or neurokinin A was used as agonist. The pA_2 values and the Schild plot analysis suggest a specific interaction between tachykinin agonists and antagonists that follow a simple bimolecular process.

4 The results suggest the presence of tachykinin receptors of the 'SP-P' type in guinea-pig basilar arteries which, for induction of relaxation, involves the release of an endothelium-derived relaxing factor.

Introduction

Substance P (SP) is regarded as a possible neurotransmitter in a population of primary sensory afferents (Lembeck & Gamse, 1982). SP has been localized to thin, probably unmyelinated nerve fibres in many tissues, e.g. skin, dental pulp and anterior segment of the eye, and these may represent peripheral branches of sensory neurones (cf. Hökfelt *et al.*, 1975; Tervo *et al.*, 1981). Cerebral arteries and veins, choroid plexus and dura mater are supplied with perivascular SP fibres (Edvinsson *et al.*, 1981; Uddman *et al.*, 1981) which, at least partly, originate in the trigeminal ganglion (Steiger *et al.*, 1982; Liu-Chen *et al.*, 1983; Uddman *et al.*, 1985). The cerebrovascular SP fibres may have a dual function, involving both a sensory and a vasomotor role since capsaicin, known to deplete certain sensory nerve endings (Jancso *et al.*, 1967) depletes SP from cerebrovascular nerves both *in vitro* (Moscowitz *et al.*, 1983) and *in situ* (Duckles & Buck, 1982). There is evidence that SP may be involved in the vascular effects caused by antidromic activation of sensory afferents (Lembeck & Holzer, 1979); these effects include vasodilatation (Gazelius & Olgart, 1980) and an inflammatory response, e.g. in the eye with increased intraocular pressure and breakdown of the blood-aqueous barrier (Bill *et al.*, 1979).

In the cerebral circulation SP has been found to cause relaxation of major cerebral arteries, pial arterioles and veins both *in vitro* and *in situ* (Edvinsson *et al.*, 1981; 1982). There is however no systematic characterization of the cerebrovascular SP receptors, which to a large extent is due to limited experimental tools. In the present study we have attempted to characterize the tachykinin receptor responsible for relaxation of the guinea-pig basilar artery using a sensitive *in vitro* technique (Högestätt *et al.*, 1983) and utilizing two recently developed substance P analogues with antagonistic properties on tachykinin receptors (Rosell & Folkers, 1983).

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Methods

Young guinea-pigs (body weight 200–250 g) of either sex were decapitated under ether anaesthesia. The brain was removed and the basilar artery dissected free under an operation microscope. The basilar artery was cut into four, cylindrical segments (2–3 mm long; 0.2–0.3 mm o.d.) which were examined immediately or, occasionally, stored in a cold, aerated buffer solution for up to 24 h. For examination each segment was mounted on two, L-shaped metal prongs (0.1 mm in diameter), one of which was connected to a force displacement transducer attached to a Grass polygraph for continuous recording of the isometric tension, and the other to a displacement device (Högestätt *et al.*, 1983). The position of the other holder could be changed by means of a movable unit allowing fine adjustments of the vascular tension by varying the distance between the metal prongs.

The mounted specimens were immersed in temperature-controlled (37°C) tissue baths containing a Krebs buffer solution of the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$, 1.5 and glucose 11. The solution was continuously gassed with 5% CO_2 in O_2 giving a pH of 7.4.

A tension of 2 mN was applied to the arterial segments and they were allowed to stabilize at this level for 1.5 h. The contractile capacity of the vessel segments was examined by exposure to a potassium-rich (60 mM) buffer solution which had the same composition as the standard buffer solution except that some of the NaCl was exchanged for an equimolar concentration of KCl. Only after two reproducible contractions (8.7 ± 0.5 mN, $n = 73$) had been achieved were the vessels used for further studies (variation less than 10%).

Agonists

When agonists were tested neither SP nor neurokinin A (NKA) were able to induce relaxation of vessels at the resting level of tension. Therefore, the vessels were precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (3×10^{-6} M) which induced strong and stable contraction of the basilar artery segments by 6.3 ± 0.5 mN ($n = 73$). During this contraction tachykinin receptor agonists were added in increasing concentrations to the tissue bath (10^{-11}–10^{-6} M), which invariably resulted in relaxation.

Blockade experiments

The SP antagonists [D-Arg^1, D-Trp^{7,9}, D-Leu^{11}]SP (spantide) and [D-Pro^2, D-Trp^{7,9}]SP were used in blockade experiments and added to the tissue bath in different concentrations within the range 1×10^{-7}–3×10^{-6} M. Vessel segments were exposed only once to SP or NKA. Four experiments were run in parallel. One concentration of antagonist was given to three of the four test segments; the fourth segment was used as control (i.e. relaxing response without blockade). Thus, after an incubation period of 15–20 min with the SP blocker, the vessels were precontracted with $PGF_{2\alpha}$ and the relaxant response to SP or NKA was examined. Results are given below as a percentage of the $PGF_{2\alpha}$-induced contraction; maximum amount of relaxation (I_{max}) and concentration resulting in half maximum relaxation (IC_{50}). The concentration ratio (CR) was obtained as the ratio of the IC_{50} value in the presence and in the absence of a given concentration of antagonist (B). The dissociation constant (or pA_2) was calculated as described by Arunlakshana & Schild (1959) and modified by Tallarida *et al.* (1979): $pA_2 = \log_{10} (CR - 1)/B$ and shown graphically by a Schild plot. The data below are expressed as mean values ± s.e.mean. Comparison between mean values was performed using Student's *t* test.

Endothelium removal experiments

The endothelium was removed in some tests by rubbing the intimal surface with a stainless steel tube, inserted via one cut end of the basilar artery. The vessels were, except for this procedure, treated in the same way as the unrubbed. Potassium induced a contraction that was somewhat weaker in rubbed arteries (6.0 ± 1.1 mN, $n = 12$) as compared to the unrubbed arteries. $PGF_{2\alpha}$, however, induced a significantly weaker contraction of the rubbed basilar arteries 1.3 ± 0.3 mN ($n = 19$) compared to the unrubbed arteries (6.3 ± 0.5 mN, $n = 78$) ($P < 0.001$). Verification of the absence of endothelium was always checked by the lack of a dilatatory response to acetylcholine (Furchgott & Zawadzki, 1980).

Drugs

The following were used: acetylcholine, eledoisin, substance P, physalaemin (Sigma, U.S.A.), spantide ([D-Arg^1, D-Trp^{7,9}, D-Leu^{11}]SP), and [D-Pro^2, D-Trp^{7,9}]SP (both kind gifts from Ferring AB, Malmö, Sweden), neurokinin A (CRB, England), prostaglandin $F_{2\alpha}$ (Amoglandin, Astra, Sweden).

The peptides were dissolved in distilled water, further diluted in 0.9% saline and used in the experiments within 30 min to avoid possible breakdown or other unknown phenomena. $PGF_{2\alpha}$ and acetylcholine were dissolved and diluted in 0.9% saline. All concentrations described below are the final molar concentration in the tissue bath during the experiments.

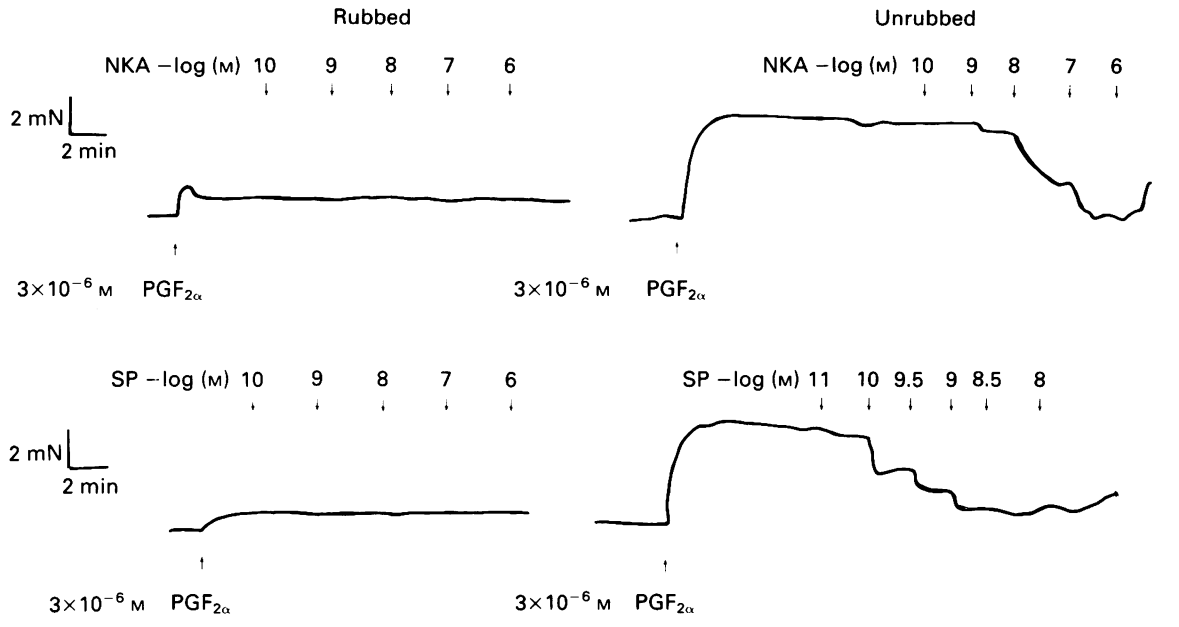

Figure 1 Relaxant responses to substance P (SP) and neurokinin A (NKA) of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-precontracted rubbed and unrubbed cerebral vessels. Peptide concentration in tissue bath is given as −log (M).

Results

Agonists

SP and NKA, given in increasing concentrations, induced concentration-dependent relaxations of the $PGF_{2\alpha}$-precontracted basilar arteries (Figure 1). The contraction induced by $PGF_{2\alpha}$ was significantly less in arteries in which the endothelium had been removed prior to the experiment (see Methods). In these arteries SP and NKA failed to induce a significant relaxation (Figure 1).

In unrubbed arteries SP and NKA induced the same maximum relaxation; however, on a molar basis SP was about 30 times more potent than NKA (Figure 2). Other members of the tachykinin family tested, eledoisin (E) and physalaemin (Phys), also induced relaxant responses (Table 1). The order of potency for the compounds were: SP = Phys > NKA > E. Although physalaemin was as potent as SP it was not a full agonist as the I_{max} was only 48% of the maximum effect of SP.

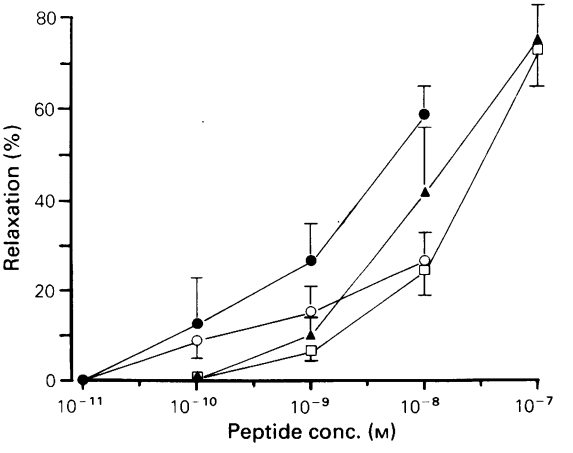

Figure 2 Relaxation in % of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-contraction on guinea-pig basilar arteries induced by increasing concentrations of substance P (●), neurokinin A (▲), physalaemin (○) and eledoisin (□). Mean values are given with s.e.mean shown by vertical lines. Each curve is based on experiments on 7–8 arterial segments.

Blockade experiments

Since the two antagonistic agents used are modified forms of SP, their own capacities for relaxing the

Table 1 Relaxant response of prostaglandin $F_{2\alpha}$-precontracted guinea-pig basilar arteries to various tachykinin receptor agonists

	pD_2	I_{max}	r.p.
Physalaemin	9.6 ± 0.4	27.2 ± 6.7*	1.67 (0.6)
Substance P	9.4 ± 0.2	65.7 ± 6.8	1
Neurokinin A	7.9 ± 0.3	84.8 ± 5.2	0.04 (27.5)
Eledoisin	7.8 ± 0.1	73.9 ± 9.1	0.03 (40.0)

Values given represent the mean values ± s.e.mean of 8–10 experiments in each group.
I_{max} for physalaemin is significantly lower than the I_{max} of the other tachykinins tested: $P < 0.001$.

basilar arteries were first examined by cumulative addition to $PGF_{2\alpha}$-precontracted vessels. Neither spantide nor [D-Pro^2, D-Trp^{7,9}]SP was able to induce relaxation of the vessel segments in the concentrations used in the experiments below (up to 3×10^{-6} M).

Spantide, given in the concentration range 10^{-7} to 10^{-6} M, caused a parallel shift of the relaxant response elicited by SP and NKA (Figure 3a,b) without any alteration of the I_{max} of the agonists. Schild plots revealed regression lines with slopes that did not differ significantly from unity, thus resulting in the following pA_2 values: 7.6 ± 0.3 for SP and 7.4 ± 1 for NKA (Figure 5a).

[D-Pro^2, D-Trp^{7,9}]SP was tested in concentrations between 1×10^{-7} and 3×10^{-6} M. This resulted in a parallel shift to the right of the concentration-response of SP and NKA (Figure 4a,b). The antagonist did not significantly change the I_{max} for the relaxation caused by SP or NKA. This resulted in a slope of the Schild plots that did not differ from unity (Figure 5b). The pA_2 values did not differ significantly for the two tachykinins: 6.9 ± 0.1 for SP and 7.0 ± 0.2 for NKA.

Discussion

In the present study, evidence is provided that not only SP but also NKA require an intact endothelium for induction of relaxation of $PGF_{2\alpha}$-precontracted cerebral arteries. The reason why the tachykinin receptor appears to be located in the endothelial cells and/or to induce release of an endothelium-derived relaxing factor to produce dilatation is still an enigma (Furchgott, 1983). It has been observed that neither adenylate cyclase (Edvinsson *et al.*, 1985) nor cyclo-oxygenase (Bolton & Clapp, 1986) are involved in the SP-induced vascular response. However, there are reports showing increased levels of cyclic GMP with endothelium-dependent relaxations (Rapaport & Murad, 1983) and recently it was shown that haemoglobin (a blocker of guanylate cyclase) may abolish SP-induced relaxation in guinea-pig mesenteric arteries (Bolton & Clapp, 1986).

Current pharmacological studies have provided

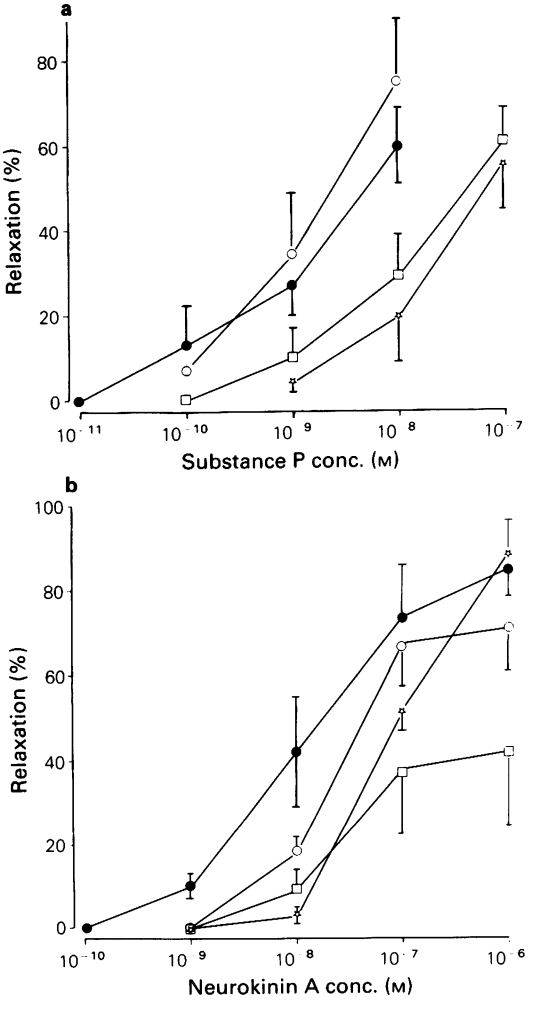

Figure 3 Relaxant response to substance P (a) and neurokinin A (b) and effects of various concentrations of spantide: (○) 10^{-7} M, (□) 3×10^{-7} M, and (☆) 10^{-6} M, on guinea-pig cerebral vessels; (●) control. The antagonist was given 15–20 min before the relaxing agents. Mean values are shown with s.e.mean shown by vertical lines ($n = 6$).

evidence for the existence of two types of tachykinin receptors (Lee *et al.*, 1982; Sandberg & Iversen, 1982). These initial studies were based on the potency pattern of the contractile activity of tachykinins. The 'SP-P' subtype is present in tissues where all tachykinins are approximately equipotent (Phys > SP > E). The 'SP-E' subtype is present in tissues where eledoisin is more potent than either SP and Phys. The pattern of agonistic relaxant activity in the guinea-pig basilar artery, shown in this study, conforms to the 'SP-P' subtype of tachykinin receptors according to the above classification. In the dog carotid artery the relative potency for relaxation is: SP = Phys > E (Regoli *et al.*, 1984a,b) which compares favourably with our data, while the rabbit mesenteric vein shows a different order of potency; E > Phys > SP. It should, however, be noted that Phys is not a full agonist in the guinea-pig basilar artery since it produces only half the relaxation which is seen with SP (Table 1).

The recent availability of radiolabelled tachykinins has provided new ways of examining the multiplicity of the tachykinin receptors, i.e. by ligand binding and by autoradiographic studies in brain and peripheral tissues. This has resulted in the suggestion that three

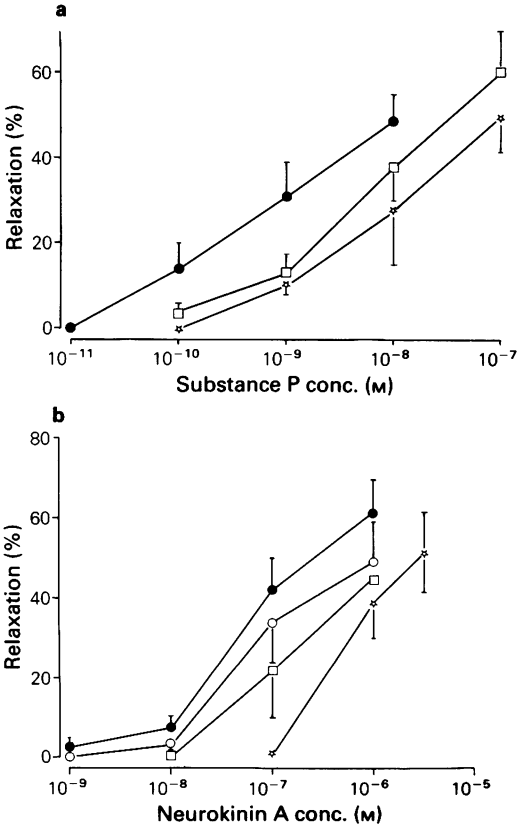

Figure 4 Relaxant response to substance P (a) and neurokinin A (b) and effects of various concentrations of [D-Pro^2, D-Trp^{7,9}]SP: (○) 10^{-7} M, (□) 10^{-6} M and (☆) 3×10^{-6} M, on guinea-pig cerebral arteries; (●) control. The antagonist was given 15–20 min before the relaxing agents. Mean values are given with s.e.mean shown by vertical lines ($n = 6$).

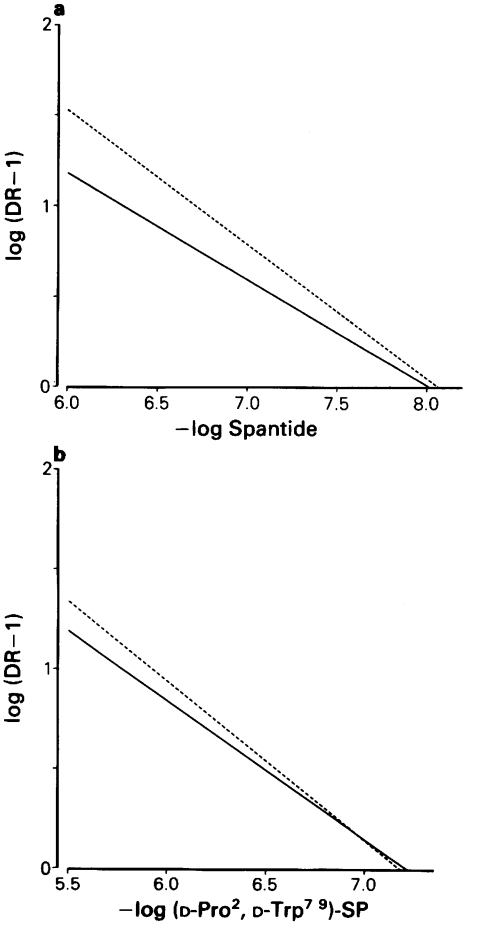

Figure 5 Schild plots from experiments on guinea-pig basilar arteries using (a) spantide, (b) [D-Pro^2-D-Trp^{7,9}]SP as antagonists of the relaxant responses of substance P (broken line) or neurokinin A (unbroken line). The graphically obtained pA_2 value (− log antagonist concentration of the intercept of the straight line with the abscissa scale) was 8.1 for substance P and 8.0 for neurokinin A in (a), the equation for the best fit line being $y = -0.75x + 6.1$ for the former and $y = -0.57x + 4.6$ for the latter; in (b) pA_2 for substance P was 7.2, $y = -0.61x + 5.7$ and pA_2 for neurokinin A was 7.2, $y = -0.67x + 5.1$.

types of tachykinin receptors exist: a 'SP-P' receptor in most tissues examined (Lee *et al.*, 1983; Buck *et al.*, 1984b; Park *et al.*, 1984). A binding site corresponding to a 'SP-E' profile has been discovered in rat cerebral cortex (Beajouan *et al.*, 1984; Cascieri *et al.*, 1985), while the third type is seen in gastrointestinal and urinary bladder smooth muscle membranes (Buck *et al.*, 1984a,b). The latter type of tachykinin receptor has a preference for NKA. In the present study NKA has a much lower affinity for the receptor site than SP but shows the same I_{max}. Further support for their interaction with the same receptor site is obtained from the blockade experiments where the pA_2 values for spantide and [D-Pro^2, D-Trp^{7,9}]SP are identical whether SP or NKA is used as agonist.

Although a number of SP analogues containing D-amino acid residues have been synthesized in recent years and used to block biological effects of tachykinins they have so far not proved useful for characterization of tachykinin receptor subtypes. The SP analogues used in the present study have agonistic effects albeit in concentrations above 10^{-6} M. Since only lower concentrations were used in the blockade experiments the rightward shift of the relaxant responses to SP and NKA is not obscured by a reduction in I_{max}. Schild plots have confirmed that there is an interaction between the tachykinins and the SP analogues which appears to follow a simple bimolecular process according to receptor theory (Arunlakshana & Schild, 1959). Meticulous studies by Regoli and colleagues (Regoli *et al.*, 1984a,b) on a series of *in vitro* preparations, have revealed that some SP analogues with similar structure to those used in the present study exhibit high pA_2 values against SP. However, for [D-Pro^2, D-Trp^{7,9}]SP the reported pA_2 values are around 5.5 and for spantide about 6.0. However, the pA_2 values observed here in guinea-pig basilar arteries are about 1.5 units higher. This difference may be due to a variation in regional receptor sensitivity, a well known phenomenon for other receptor-antagonist interactions, e.g. α-adrenoceptors (see Skärby *et al.*, 1983).

The present studies have revealed that the guinea-pig basilar artery may provide a sensitive model tissue for analysis of tachykinin receptor antagonists. The data suggest that the guinea-pig basilar artery is supplied with tachykinin receptors that are of the 'SP-P' subtype.

Note added in proof

At an International Congress on substance P and neurokinins in Montreal (July 1986) a new nomenclature for tachykinin receptors was suggested. According to this, the 'SP-P' type of tachykinin receptors should be labelled neurokinin$_1$ receptors.

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