British Journal of Pharmacology (1997) 121, 773–781

Differential activation of the epithelial and smooth muscle NK_1 receptors by synthetic tachykinin agonists in guinea-pig trachea

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1 The presence of tachykinin NK_1 receptors have been shown in the epithelium and smooth muscle of guinea-pig airways. Previous data showed that substance P (SP), and the NK_1 receptor agonist, [Sar^9, Met $(O_2)^{11}$]-SP, relax guinea-pig tracheal tube preparations by stimulation of epithelial NK_1 receptors and via nitric oxide (NO) release. However, the selective tachykinin NK_1 receptor agonist, septide, was unable to produce this effect. The aim of the present study was to investigate the ability of a series of SP analogues to stimulate NK_1 receptors of guinea-pig airway epithelium.

2 Isometric tension was recorded in isolated tracheal tube preparations in which compounds were administered intraluminally in the presence of phosphoramidon, indomethacin (both 1 μM) and the tachykinin NK_2 receptor antagonist, SR 48,968 ((**S**)-N-methyl N-(4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl)benzamide) (0.1 μM). Cumulative concentration-response curves were obtained in preparations under resting tone or in preparations precontracted with acetylcholine (ACh, 10 μM).

3 Contractile responses to low concentrations (0.1–10 nM) of substance P (SP) and the selective agonist of NK_1 receptors, [Pro^9]-SP, in non precontracted tracheae were higher in preparations pretreated with the NO-synthase inhibitor, N^G-monomethyl L-arginine (L-NMMA, 100 μM) than in preparations pretreated with its inactive enantiomer D-NMMA (100 μM). Tracheal tube preparations precontracted with ACh and pretreated with D-NMMA were relaxed by low concentrations of SP and [Pro^9]-SP (0.1–10 nM). In contrast, after pretreatment with L-NMMA, SP and [Pro^9]-SP contracted tracheae at all the concentrations tested.

4 Concentration-response curves to the NK_1 receptor agonists, SP methyl ester, [Apa^{9-10}]-SP and [$pGlu^6$] SP (6–11) obtained in non-precontracted tracheae were similar in the presence of either D-NMMA or L-NMMA. SP methyl ester, [Apa^{9-10}]-SP and [$pGlu^6$] SP (6–11) did not produce any relaxation, but instead, cause contractions in tracheal tube preparations precontracted with ACh and pretreated with D-NMMA. Concentration-response curves produced by all these agonists were similar in preparations precontracted with ACh and pretreated with L-NMMA or D-NMMA.

5 In guinea-pig tracheal tube preparations two groups of NK_1 receptor agonists can be distinguished: one group, including [Pro^9]-SP, stimulator epithelial NK_1 receptors, the other group, including SP methyl ester, [Apa^{9-10}]-SP and [$pGlu^6$] SP (6–11), does not. One possible explanation for these findings and for the existence of compounds with a peculiar 'septide-like' pharmacological profile in the guinea-pig trachea could be the recently proposed phenomenon referred to as 'agonist-directed receptor trafficking'.

Keywords: Substance P; septide; NK_1 receptors; tachykinins; airway; epithelium

Introduction

It has been shown that three receptors, namely the NK_1, NK_2 and NK_3, mediate the biological actions of the tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Maggi *et al*., 1993b; Regoli *et al*., 1994). The existence of tachykinin receptor subtypes has also been proposed, although each subtype has been described in a different mammalian species (Maggi *et al*., 1993b; Regoli *et al*., 1994). Pharmacological diversity of NK_1 receptors in different mammals has emerged also with the use of the recently developed non-peptide NK_1 receptor antagonists (Maggi *et al*., 1993b; Regoli *et al*., 1994). In guinea-pig the peculiar pharmacological profile of septide, a selective agonist of the tachykinin NK_1 receptor, has led to the proposal of the existence of an atypical NK_1, 'septide-selective' receptor, supersensitive to the stimulant action of septide (Chassaing *et al*., 1992; Petitet *et al*., 1992; Glowinski, 1995). The observation that different NK_1 receptor antagonists exhibited differential affinities toward effects elicited by septide or other selective NK_1 receptor agonists in guinea-pig (Longmore *et al*., 1994), rat (Meini *et al*., 1994) and rabbit (Hall *et al*., 1994) preparations, further supported the hypothesis of a 'septide-selective' receptor, distinct from the 'typical' NK_1 receptor.

In contrast to these findings, preparations containing NK_1 receptors in which septide was found to be less potent than SP or other NK_1 receptor agonists have been also studied. Thus, SP, [Pro^9]-SP and [Sar^9, Met $(O_2)^{11}$]-SP were found to be more potent than septide in inhibiting *myo*-[^{3}H]-inositol uptake in rat parotid glands (Petitet *et al*., 1992), in stimulating the formation of inositol phosphates (IP) in mouse cultured astrocytes (Beaujouan *et al*., 1992) and in releasing adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in chinese hamster ovarian (CHO) cells expressing the human NK_1 receptor (Sagan *et al*., 1996). Of interest for the present study is the observation that low potency was not unique to septide: different selective agonists for the NK_1 receptor or SP analogues, including SP methyl ester, [$pGlu^6$] SP (6–11) and [Apa^{9-10}]-SP were also less potent than SP, [Pro^9]-SP or [Sar^9, Met $(O_2)^{11}$]-SP in the assays described above (Petitet *et al*., 1992; Sagan *et al*., 1996).

Recently, it has been shown that SP and the selective NK_1 receptor agonist, [Sar^9, Met $(O_2)^{11}$]-SP exert a dual function in tracheal tube preparations: indeed, by stimulating epithelial

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NK_1 receptors and via nitric oxide (NO) release, they cause relaxation and by stimulating NK_1 smooth muscle receptors they cause contraction (Figini *et al*., 1996). The observation that septide was a powerful spasmogen, but was unable to relax this preparation, indicated that NK_1 receptors of the guinea-pig tracheal epithelium were insensitive to septide (Figini *et al*., 1996).

The aim of the present paper was to verify whether in tracheal tube preparations the pharmacological profile of compounds previously defined as 'septide-like' peptides (Sagan *et al*., 1996), and including SP methyl ester, [pGlu^6] SP (6–11) and [Apa$^{9-10}$]-SP, were similar to that of septide and different from that of [Pro^9]-SP (a 'typical' NK_1 receptor agonist). The effect of the chemically unrelated NK_1 receptor antagonists, GR 82334 (Hagan *et al*., 1991) and SR 140333 (Emonds-Alt *et al*., 1993) and of the recently described non peptide NK_3 receptor antagonist, SR 142801 (Emonds-Alt *et al*., 1995) on SP-induced contraction or relaxation was also studied.

Methods

Male guinea pigs (350–400 g) were killed with sodium pentobarbitone (80 mg kg^{-1}, i.p.) and the trachea was isolated and perfused with a Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3. The solution was maintained at 37°C and aerated continuously with a mixture of 95% 0_2-5% CO_2, which keeps the pH of the solution to 7.4. The experiments were performed by use of apparatus and techniques similar to those described previously (Nijkamp *et al*., 1993). Briefly, proximal ends of the trachea were used for the experiments. An organ bath was constructed that allows the independent circulation of fluid within the lumen or around the exterior of the tracheal segment. Two hooks were passed through the trachea wall around two adjacent cartilaginous rings as close as possible to the muscle. The lower hook was fixed; the upper hook was connected to an isometric transducer (Basile, Italy). The tracheal tension was set at an optimal counterweight of 2 g. The inside of the trachea was perfused with Krebs solution at a constant flow rate of 2 ml min^{-1} with a peristaltic pump. All drugs were administered intraluminally. Every 15 min, during the 90 min of the equilibration period, the Krebs buffer was changed on both sides. Thereafter, the intraluminal side of the trachea was perfused for 30 min with N^G-monomethyl-L-arginine (L-NMMA) or N^G-monomethyl-D-arginine (D-NMMA). Cumulative agonist concentration-response curves were constructed by adding increasing concentrations of the agonists as soon as a plateau to the previous concentration had been reached. In experiments in which antagonists were used, these were added intraluminally 15 min before the administration of ACh.

In some experiments, tracheal tube preparations were precontracted with an intraluminal infusion of acetylcholine (ACh, 10 μM). The contraction in response to ACh remained stable for at least 25 min. As soon as such a stable contraction to ACh was obtained, tachykinin NK_1 receptor agonists (0.1 nM–1 μM) were added intraluminally. Each trachea was challenged only once with ACh. In a separate set of experiments, the epithelial layer was removed by a cotton swab (Nijkamp *et al*., 1993). To verify that the tissues were denuded of epithelium, histological examinations were performed. The tissues were fixed by immersion in formaldehyde (4%) and embedded in paraffin blocks. Sections measuring 5 μm were cut and stained with haematoxylin and eosin for histological evaluation. Histological examination showed that the epithelial layer was completely removed, without damaging the lamina propria (data not shown).

Drugs

D-NMMA, L-NMMA, isoprenaline, acetylcholine, indomethacin and phosphoramidon were obtained from Sigma Chemical (U.S.A.). SP, SP methyl ester, [pGlu^6]-SP (6–11), [Pro^9]-SP and GR-82334 (pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Pro(spiro-γ-lactam)Leu-Trp-NH_2) were purchased from Bachem (Switzerland). SR 48968 ((**S**)-N-methyl N-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl)benzamide), SR 140333 ((s)-1-(2[3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo{2.2.2}octane, chloride) and SR 142801 ((**S**)-(N)-1-3(1-benzoyl-3(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide) were gifts of Dr X. Emonds-Alt (Sanofi Recherche, France). [Apa$^{9-10}$]-SP was a gift of Dr S. Lavielle (URA CNRS, Laboratoire de Chimie Organique Biologique, Université P. & M. Curie, Paris, France). All agonists and antagonists were dissolved in dimethylsulphoxide. All the other drugs were dissolved in 0.9% saline. Stock solution of peptides were stored at −20°C. L-NMMA, D-NMMA and ACh were freshly prepared for each experiment.

Statistical analysis

Values in the text and figures are the mean ± s.e.mean. Statistical comparisons were performed by use of Student's t tests for unpaired values. In all cases, a P value of less than 0.05 was considered significant.

Results

Non precontracted tracheal tube preparations

The tachykinin NK_2 receptor antagonist, SR 48,968 (Emonds-Alt *et al*., 1992), was added to eliminate the contribution of NK_2 receptors to the tachykinin-mediated contraction in the guinea-pig airways (Ballati *et al*., 1992; Bertrand *et al*., 1993). The neutral endopeptidase (NEP, E.C. 3.4.24.11) inhibitor, phosphoramidon, was added to block tachykinin metabolism, that in the guinea-pig airways is mainly due to NEP (Nadel, 1991). Addition of SR 48,968 (0.1 μM), and phosphoramidon (1 μM) or D-NMMA (100 μM for 30 min) through the lumen did not affect the tone of guinea-pig isolated tracheal tube preparations (data not shown). However, after the addition of L-NMMA (100 μM for 30 min) tracheal tube preparations developed a sustained increase in tone that reached a maximum at 20 min (167 ± 18 mg, n = 5). In tracheal tube preparations under resting tone, SP, [Pro^9]-SP, SP methyl ester, [Apa$^{9-10}$]-SP and [pGlu^6] SP (6–11) caused a concentration-dependent contraction (Figures 1 and 2). SP and [Pro^9]-SP (0.1–10 μM) caused significantly greater contractions in the presence of L-NMMA than in the presence of D-NMMA (Figure 1). In contrast, contractions in response to SP methyl ester [Apa$^{9-10}$]-SP and [pGlu^6] SP (6–11) were not significantly different in preparations pretreated with D-NMMA or L-NMMA (Figure 2). In epithelium-denuded preparations pretreatment with D-NMMA or L-NMMA did not affect the concentration-response curves in response to [Pro^9]-SP (Figure 1).

Tracheal tube preparations precontracted with acetylcholine

In order to show more clearly the relaxant component of the motor response due to tachykinin NK_1 receptor stimulation some experiments were performed in tracheal tube preparations precontracted with ACh. In these experimental conditions, it was shown previously (Figini *et al*., 1996) that SP and [Sar^9, Met $(O_2)^{11}$]-SP (0.1–10 nM) relaxed guinea-pig tracheae in a NO-dependent manner. As shown previously (Figini *et al*., 1996), contractions induced by ACh (10 μM) were similar in preparations pretreated with D-NMMA or L-NMMA (498 ± 55 mg, n = 6 and 571 ± 65 mg, n = 6, respectively), and after precontraction with Ach and treatment with D-NMMA, addition of increasing concentrations of SP (0.1–10 nM)

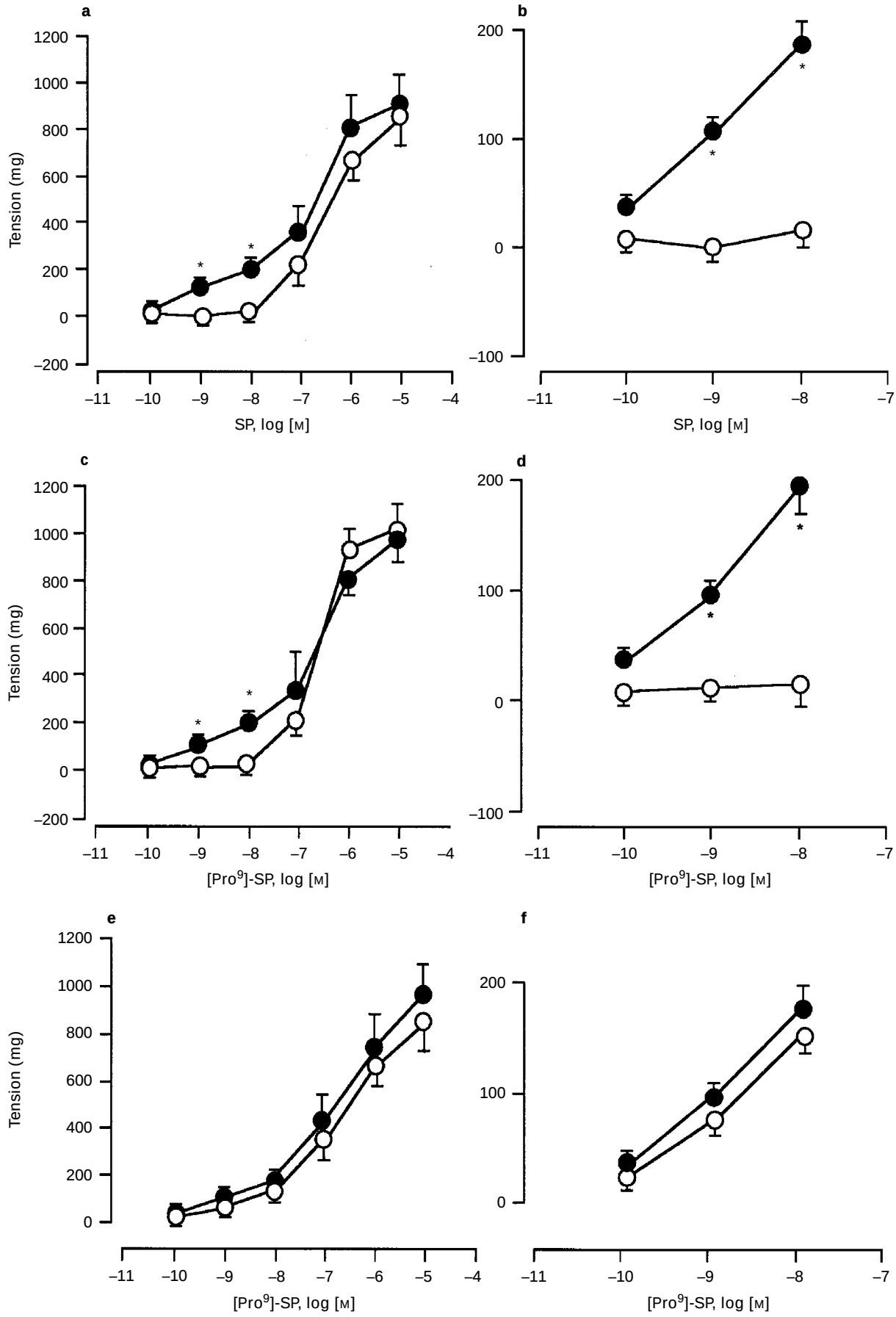

Figure 1 Motor effects of (a,b) substance P (SP) and (c,d,e and f) [Pro^9]-SP in guinea-pig isolated tracheal tube preparations treated with D-NMMA (100 μM, ○) or L-NMMA (100 μM, ●). (e and f) Experiments performed in epithelium-denuded tracheae. Preparations were also treated with phosphoramidon (1 μM), indomethacin (1 μM) and the tachykinin NK_2 receptor antagonist, SR 48968 (0.1 μM). Each point is the mean and vertical lines s.e.mean of 6 experiments. $^*P<0.05$ vs D-NMMA. (b,d and f) Data shown in (a,c and e) enlarged.

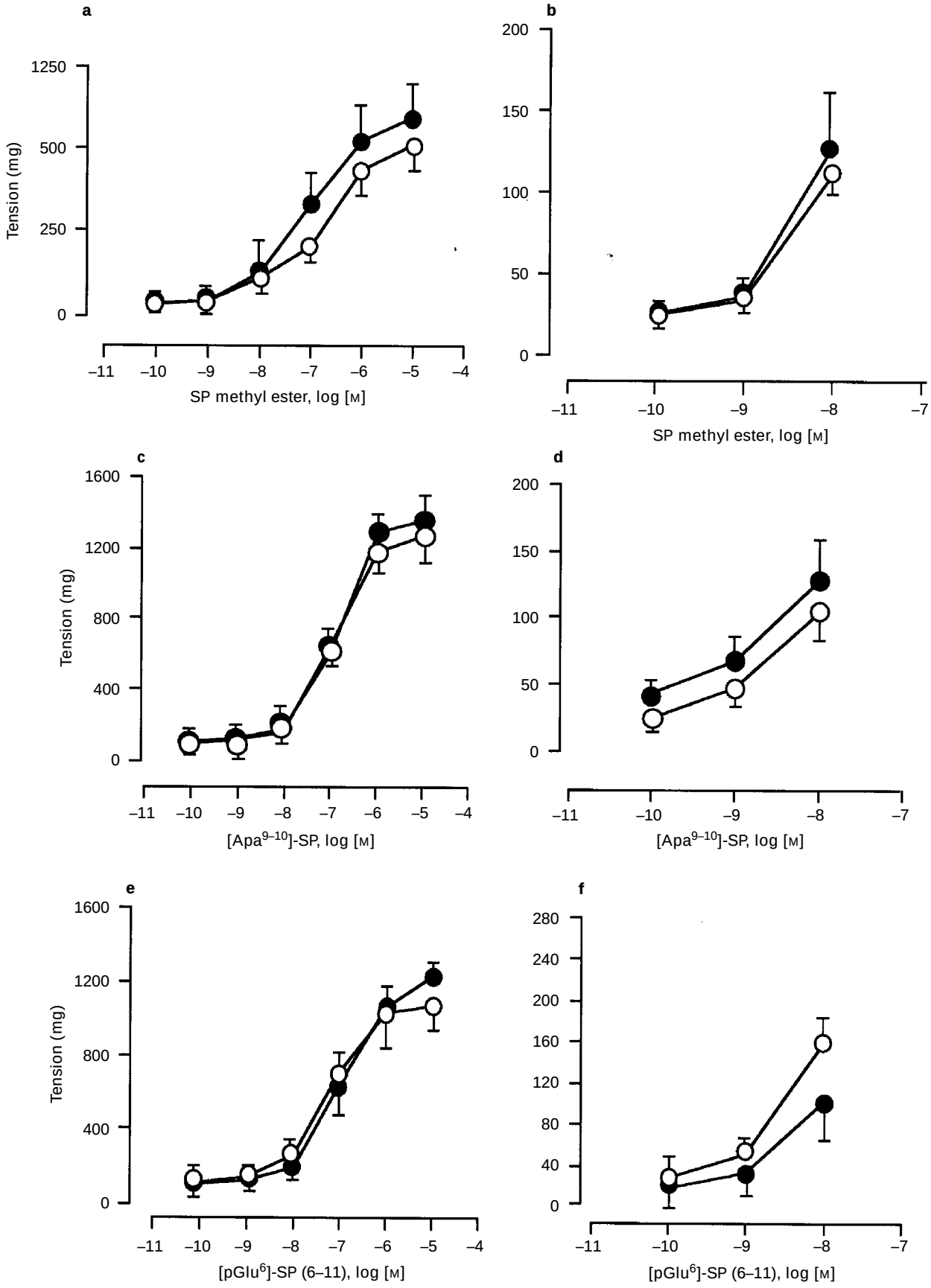

Figure 2 Motor effects of (a,b) substance P (SP) methyl ester, (c,d) [Apa$^{9-10}$]-SP and (e,f) [pGlu^{6}] SP (6–11) in guinea-pig isolated tracheal tube preparations treated with D-NMMA (100 μM, ○) or L-NMMA (100 μM, ●). Preparations were also treated with phosphoramidon (1 μM), indomethacin (1 μM) and the tachykinin NK_2 receptor antagonist, SR 48968 (0.1 μM). Each point is the mean and vertical lines s.e.mean of 5 experiments. *$P<0.05$ vs D-NMMA. (b,d and f) Data shown in (a,c and e) enlarged.

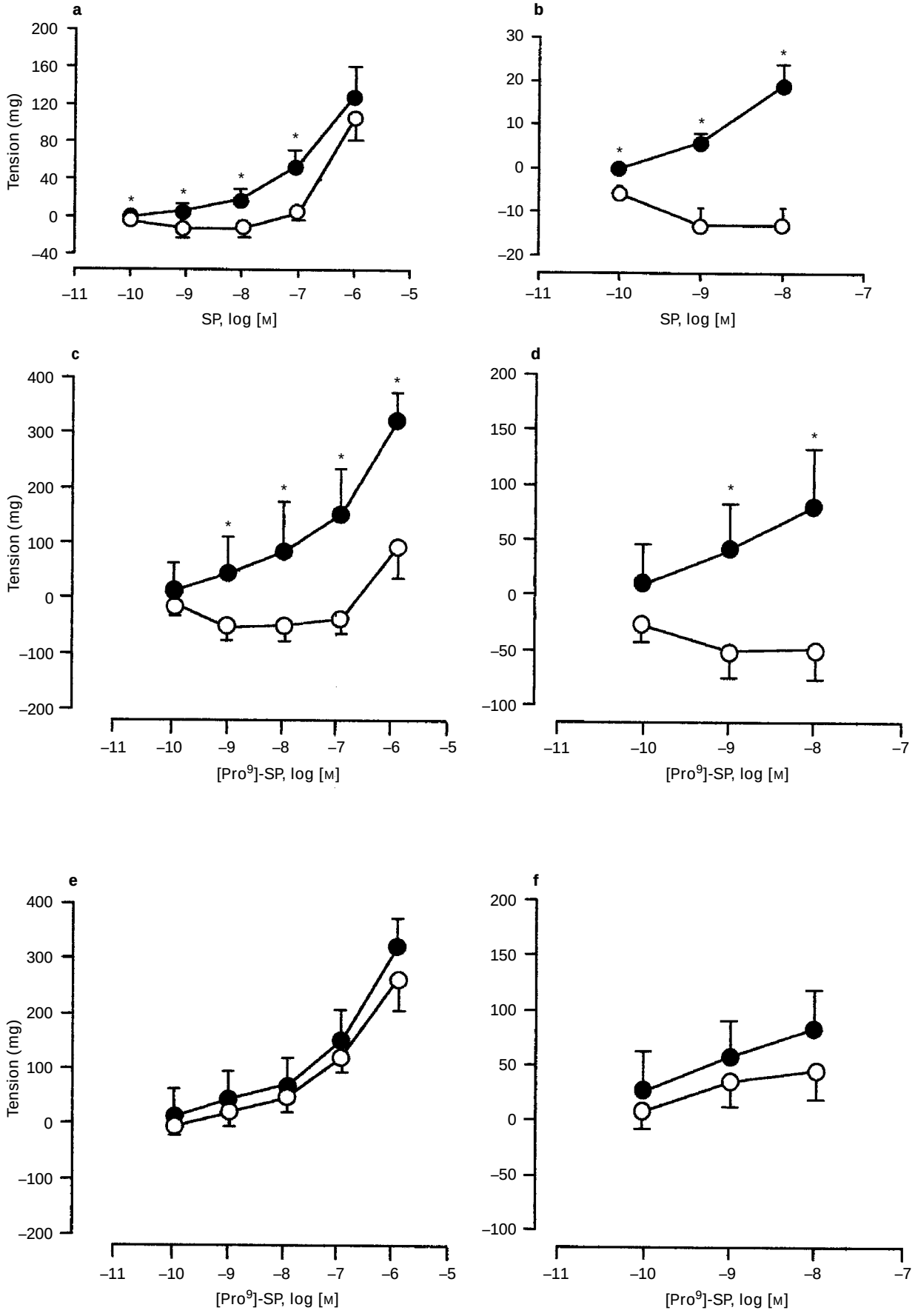

Figure 3 Motor effects of (a,b) substance P (SP) and (c,d,e and f) [Pro^9]-SP in guinea-pig isolated tracheal tube preparations treated with D-NMMA (100 μM, ○) or L-NMMA (100 μM, ●), and precontracted with acetylcholine (10 μM). (e and f) Experiments performed in epithelium-denuded tracheae. Preparations were also treated with phosphoramidon (1 μM), indomethacin (1 μM) and the tachykinin NK_2 receptor antagonist, SR 48968 (0.1 μM). Each point is the mean and vertical lines s.e.mean of 6 experiments. *$P<0.05$ vs D-NMMA. (b,d and f) Data shown in (a,c and e) enlarged.

caused moderate relaxations. Isoprenaline (10 μM) caused similar relaxations in tissues precontracted with ACh (10 μM) and pretreated with either D-NMMA ($n=5$) or L-NMMA ($n=5$) (325 ± 45 mg and 287 ± 32 mg, respectively). SP (100 nM) produced a moderate contraction, whereas, at the highest concentration tested, SP induced a marked contraction (Figure 3). Similarly to SP, [Pro^9]-SP (0.1 – 100 nM) caused a moderate, concentration-related relaxation (Figure 3), and a higher concentration of [Pro^9]-SP (1 μM) caused a contraction (Figure 3). In epithelium-denuded tracheae, and after treatment with L-NMMA, SP or [Pro^9]-SP induced contractions at all the concentrations tested, from 0.1 to 100 nM (Figure 3). In these preparations concentration-dependent contractions in response to [Pro^9]-SP were not different in preparations pretreated with D-NMMA or L-NMMA (Figure 3).

SP methyl ester, [Apa$^{9-10}$]-SP and [pGlu^6] SP (6 – 11) caused concentration-dependent contractions of tracheal tube preparations precontracted with ACh. Concentration-response curves produced by SP methyl ester, [Apa$^{9-10}$]-SP and [pGlu^6] SP (6 – 11) were not different in tracheal tube preparations pretreated with D-NMMA or L-NMMA (Figure 4).

Effect of NK_1 and NK_3 receptor antagonists

Administration of GR-82334 (1 μM) or SR 140333 (1 μM) affected neither the basal tone of tracheal tube preparations, nor the contractile response to ACh (data not shown). GR-82334 (1 μM) reduced significantly the contraction induced by SP (1 μM) in non precontracted tissues and abolished the relaxation produced by SP (10 nM) in tissues precontracted with ACh (Table 1). Similarly, SR 140333 (1 μM) reduced markedly the contraction induced by 1 μM SP ($P<0.01$) in non precontracted tissues and abolished the relaxation produced by SP (10 nM, $P<0.01$) in tissues precontracted with ACh (Table 1). Administration of the tachykinin NK_3 receptor antagonist, SR 142801 (1 μM), did not affect either the basal tone of tracheal tube preparations, or the response to ACh (data not shown). Pretreatment with SR 142801 did not affect either the relaxation produced by 10 nM SP or the contractile response induced by 1 μM SP (Table 1).

Discussion

The present results confirm previous observations that SP causes a relaxation of guinea-pig tracheal tube preparations precontracted with ACh and that this relaxation is changed into a contraction by pretreatment with the NO synthase inhibitor, L-NMMA, or by removal of the epithelium (Figini *et al*., 1996). The selective agonist of NK_1 receptors, [Pro^9]-SP, was also able to relax guinea-pig tracheal tube preparations in a NO- and epithelium-dependent manner. The ability of NO to modulate the motor response to SP and [Pro^9]-SP was further supported by the observation that in non precontracted tracheae, contractions observed in response to low concentrations of SP and [Pro^9]-SP were greater in preparations pretreated with L-NMMA than in preparations pretreated with the inactive enantiomer, D-NMMA.

Previously, it was shown that the contractile response to tachykinins in the guinea-pig trachea could be potentiated by removing the epithelium (Frossard *et al*., 1989). Based on the rank order of potency of agonists, it was suggested that the epithelium-dependent relaxation was mediated by the stimulation of an epithelial NK_1 receptor (Frossard *et al*., 1989). More recently, evidence for epithelial NK_1 receptors that relax the guinea-pig tracheal tube preparations, via NO release, was provided (Figini *et al*., 1996). Thus, in the guinea-pig airways, naturally occurring tachykinins stimulate both relaxant epithelial NK_1 receptors and contractile NK_1 receptors, probably located on smooth muscle cells. [Sar^9, Met $(O_2)^{11}$]-SP, a selective agonist of NK_1 receptors, was also found to exert such a dual effect (Figini *et al*., 1996). The present results show that [Pro^9]-SP, another selective agonist for the NK_1 receptor, is able to relax (in a NO-dependent manner) and to contract tracheal tube preparations, thus indicating that this agonist stimulates both epithelial and smooth muscle NK_1 receptors.

In the same experimental conditions, septide was totally ineffective in relaxing tracheal tube preparations (Figini *et al*., 1996). The present results show that the ability of only contracting (and not relaxing) guinea-pig tracheal tube preparations is not unique to septide: different NK_1 receptor agonists, such as SP methyl ester, [Apa^{9,10}]-SP and [pGlu^6]-SP(6 – 11) were also unable to stimulate epithelial NK_1 receptors, because they did not relax the guinea-pig trachea. Septide has been found to be a more potent spasmogen than other NK_1 receptor agonists in various smooth muscle preparations, including the guinea-pig airways (Maggi *et al*., 1993a; Hall *et al*., 1994; Meini *et al*., 1994). One possible explanation for higher potency of septide and other NK_1 receptor agonists in contracting the guinea-pig airway epithelium is that these compounds do not activate relaxant NK_1 receptors of the guinea-pig airway epithelium. Absence of the relaxant component of the effect of septide may also be involved in the higher potency of NK_1 receptor antagonists at inhibiting contractions of guinea-pig isolated bronchi induced by septide, than those induced by other NK_1 receptor agonists (Longmore *et al*., 1994; Zeng & Burcher, 1994).

The 'septide-selective' NK_1 receptor has been described as a receptor much more sensitive to the spasmogen activity of septide than to that of other tachykinins (Glowinski, 1995). However, there are examples of preparations containing NK_1 receptors in which septide has a potency lower than those of other NK_1 receptor agonists: septide was 50 times less potent than SP or [Sar^9, Met $(O_2)^{11}$]-SP and [Pro^9]-SP in inhibiting the uptake of *myo*-[^{3}H]-inositol in rat parotid glands (Petitet *et al*., 1992), and in the formation of IP in mouse cultured astrocytes (Beaujouan *et al*., 1992). More recently, it was observed that septide was much less potent than SP in promoting the release of cyclic AMP from CHO cells transfected with the human NK_1 receptor (Sagan *et al*., 1996). Of interest for the present discussion is the observation that SP methyl ester, [Apa^{9,10}]-SP and [pGlu^6]-SP(6 – 11) all compounds that, like septide, do not relax guinea-pig tracheal tube preparations, showed a 'septide-like' pharmacological profile in previous studies (Beaujouan *et al*., 1992; Petitet *et al*., 1992; Sagan *et al*., 1996). In contrast, SP and [Sar^9, Met $(O_2)^{11}$]-SP (Figini *et al*., 1996) and [Pro^9]-SP, all compounds that relax tracheal tube preparations, showed high potency in the assays described above (Beaujouan *et al*., 1992; Petitet *et al*., 1992; Sagan, 1996). Thus, the pharmacological profile of 'typical' NK_1 agonists or 'septide-like' peptides appears to be maintained consistently in various preparations of different species.

The reason for the diversity of NK_1 receptors in the epithelium and in the smooth muscle of the guinea-pig trachea is not known. The hypothesis of the existence of NK_1 receptor subtypes in this preparation or in other preparations has not been supported so far by the identification of genetic variants of the NK_1 receptor. The present data showing that different NK_1 receptor antagonists of diverse chemical nature were all equally able to block relaxation and inhibit contraction induced by SP does not favour the hypothesis of the existence of NK_1 receptor subtypes in the guinea-pig trachea. Participation of the NK_3 receptor in the tachykinin-induced contraction or relaxation of tracheal tube preparation also seems unlikely because SR 142801 was without effect on these responses.

In COS cells transfected with the human NK_1 receptor (Pradier *et al*., 1994) septide does not affect the binding of [^{3}H]-RP67580, and the response to septide (but not that to [Pro^9]-SP) is non competitively blocked by NK_1 receptor antagonists. These and other findings led the authors to propose that septide acts on a site distinct from SP on NK_1 receptors (Pradier *et al*., 1994). More recent results showed that septide and [pGlu^6]-SP (6 – 11), although much less potent in releasing cyclic AMP, were as potent as SP, [Sar^9, Met$(O_2)^{11}$]-SP or [Pro^9]-SP in increasing IP levels in CHO cells transfected with the human NK_1 receptor. Sagan *et al*. (1996) could not exclude the pos-

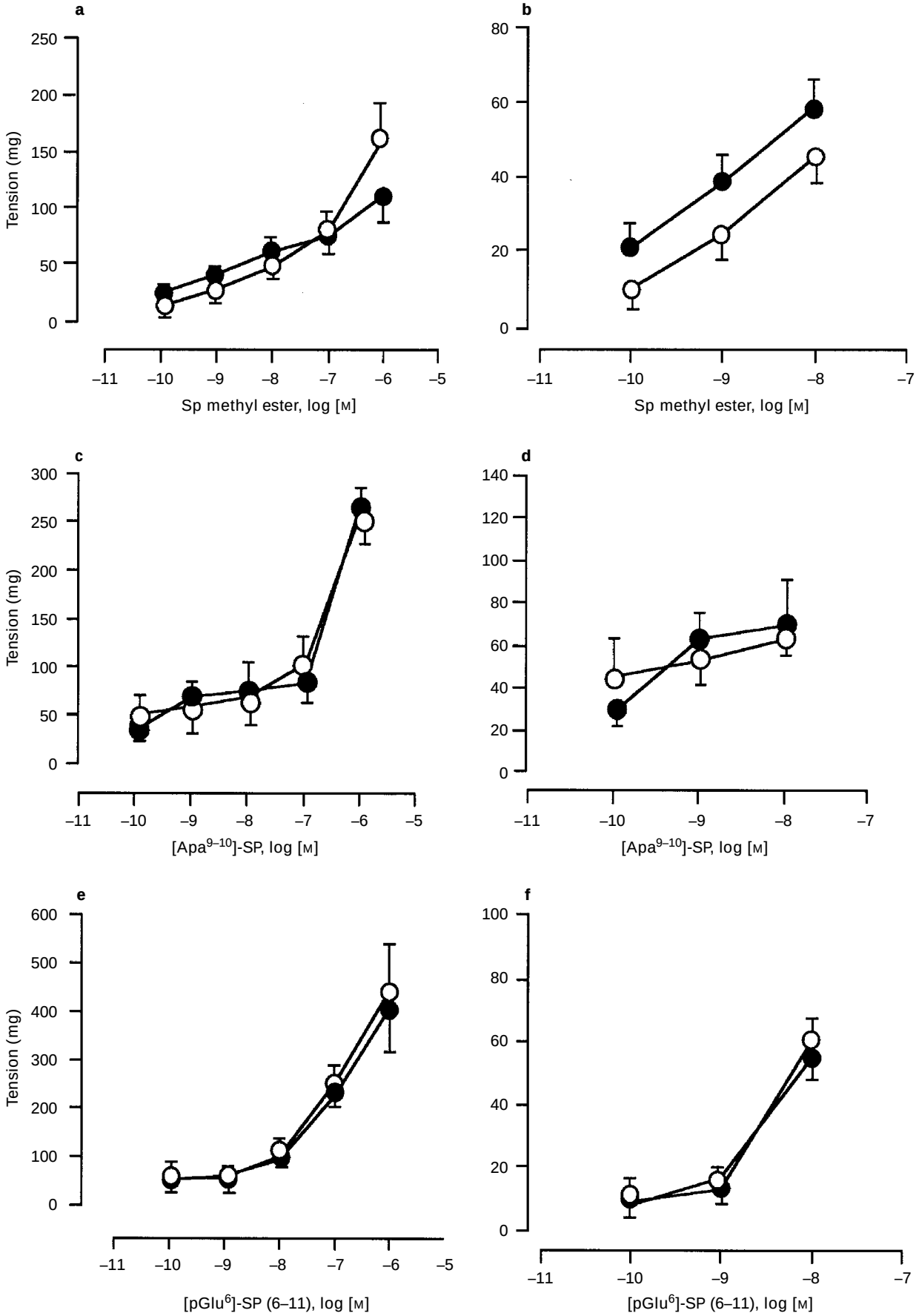

Figure 4 Motor effects of (a,b) substance P (SP) methyl ester, (c,d) [Apa^{9-10}]-SP and (e,f) [$pGlu^6$] SP (6–11) in guinea-pig isolated tracheal tube preparations treated with D-NMMA (100 μM, ○) or L-NMMA (100 μM, ●), and precontracted with acetylcholine (10 μM). Preparations were also treated with phosphoramidon (1 μM), indomethacin (1 μM) and the tachykinin NK_2 receptor antagonist, SR 48968 (0.1 μM). Each point is the mean and vertical lines s.e.mean of 5 experiments. $^*P<0.05$ vs D-NMMA. (b,d and f) Data shown in (a,c and e) enlarged.

Table 1 Effect of tachykinin NK_1 and NK_3 receptor antagonists on substance P-induced relaxation and contraction of guinea-pig tracheal tube preparations

	SP (10 nM)-induced relaxation (mg)	*SP (1 μM)-induced contraction* (mg)
Vehicle	−15±3	144±21
SR 14033 (1 μM)	−2±2*	46±8*
Gr-82334 (1 μM)	−3±2*	54±9*
SR 142801 (1 μM)	−17±4	127±12

Non precontracted and precontracted (acetylcholine, 10 μM) preparations were used to measure contraction and relaxation, respectively. Values are mean±s.e. of at least 5 experiments. *$P<0.01$ vs vehicle.

sibility that expression of different isoforms of the NK_1 receptor in the transfected cells may be the cause for such a diverse response. However, the hypothesis that septide and 'septide-like' peptides, including [pGlu^6]-SP (6–11) may activate NK_1 receptors, thus leading to the activation of one individual intracellular signalling pathway (that causes IP release), but not the pathway that increases cyclic AMP, has been advanced. In contrast, SP and other 'typical' NK_1 receptor agonists, including [Sar^9, Met(O$_2$)^{11}]-SP or [Pro^9]-SP may activate more than one intracellular signalling pathway, thus releasing not only IP, but also cyclic AMP (Sagan *et al*., 1996).

It has been widely recognised that different agonists acting on the same receptor may activate numerous biochemical response pathways. Although this phenomenon may depend on the differential strength of the signal, the possibility that separate agonist-specific receptor states selectively promote G protein coupling in response to activation by different agonists may also be involved (Kenakin, 1995). Various examples in support of this latter hypothesis have been found in the heterologous expression systems, with pituitary adenylate cyclase activating polypeptide receptors (Spengler *et al*., 1993) or NK_1 receptors (Glowinski, 1995; Sagan *et al*., 1996). One possible explanation of the present data would be that, although genetically identical, NK_1 receptors in the epithelium and smooth muscle differ regarding the ability of different agonists to activate differential intracellular signalling pathways: septide, SP methyl ester, [Apa^{9,10}]-SP and [pGlu^6]-SP(6–11) may not be able to activate the signalling pathway associated with epithelial NK_1 receptors that releases NO which eventually causes tracheal relaxation. In contrast SP, [SP^9,Met(O$_2$)^{11}]-SP and [Pro^9]-SP may activate NK_1 receptors and their intracellular signalling pathways both in the epithelium and in the smooth muscle of the guinea-pig airways. If this hypothesis holds true, NK_1 receptors in the epithelium and smooth muscle of the guinea-pig trachea would represent an example in a preparation containing non recombinant receptors of the phenomenon referred to as 'agonist-directed receptor trafficking' (Kenakin, 1995).

This work was supported by a grant from MURST (60%, Florence, Italy).

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(Received February 7, 1997
Accepted March 17, 1997)