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Comparison of the effects of epithelium removal and of an enkephalinase inhibitor on the neurokinin-induced contractions of guinea-pig isolated trachea

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1 The influence of epithelium removal and/or thiorphan on the effects of neurokinins (substance P (SP), neurokinin A (NKA), neurokinin B (NKB)) and related peptides on airway contractility was investigated on the guinea-pig isolated trachea.

2 Removing the tracheal epithelium significantly enhanced the sensitivity but not the maximum contractile responses to the peptides.

3 After removal of the epithelial layer, the shifts to the left of the log concentration response curves were greater for SP and SP-OMe (1.62 and 1.94 log units, respectively) than for two SP analogues substituted in position 9 namely [Pro^9]SP sulfone and [β-Ala^4, Sar^9]SP(4-11)sulfone (0.66 and 0.68 log units, respectively). The leftward shifts for compounds related to NKA or NKB lay between 0.58 and 0.73 log units.

4 The leftward shifts of the log concentration-response curves for SP, SP-OMe, [Pro^9]SP sulfone, [β-Ala^4, Sar^9]SP(4-11) sulfone and NKA were of similar magnitude after removal of the epithelium or after pretreatment with thiorphan (10^{-5} M), an enkephalinase inhibitor, in the presence of epithelium. No significant additional shift of the curves to the left was observed with thiorphan plus epithelium removal.

5 The results obtained with the selective agonists for each of the three classes of neurokinin receptor (i.e NK_1, NK_2, NK_3) suggest that the guinea-pig trachea contains receptors for SP and NKA but few if any for NKB.

6 It was concluded that neurokinins and related peptides (especially SP and analogues not substituted in position 9) are degraded by enkephalinase mainly located in the tracheal epithelium and that the addition of thiorphan or epithelium removal results in an inhibition or loss of enkephalinase activity, thereby increasing similarly the potencies of these peptides. It was, therefore, suggested that the supersensitivity to neurokinins produced by epithelium removal was due neither to the elimination of a permeability barrier nor to reduced production of a relaxant factor, but mainly to reduced peptide degradation.

Introduction

The mechanical removal of the epithelial layer increases the sensitivity of the isolated trachea of several species to a variety of bronchoconstrictor agents, such as histamine, acetylcholine, 5-hydroxytryptamine (5-HT) and substance P and to antigenic challenge (Barnes *et al.*, 1985; Flavahan *et al.*, 1985; Goldie *et al.*, 1986; Frossard & Muller, 1986; Hay *et al.*, 1986a, b; Holroyde, 1986; Raeburn *et al.*, 1986; Tschirhart & Landry, 1986; Tschirhart *et al.*, 1987; Butler *et al.*, 1987), and it has been suggested that epithelial cells may act as a diffusion barrier (Holroyde, 1986) or may secrete an inhibitory

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factor which modulates airway smooth muscle tone (Barnes et al., 1985; Flavahan et al., 1985; Frossard & Muller, 1986; Raeburn et al., 1986, Tschirhart & Landry, 1986; Ilhan & Sahin, 1986; Tschirhart et al., 1987; Hay et al., 1987; Butler et al., 1987). In support of the latter suggestion, Frossard & Muller (1986) and Tschirhart & Landry (1986) have shown that a relaxant factor (EpDRF) might be released from airway epithelium during the action of substance P on rat or guinea-pig trachea.

More recently, Advenier et al. (1988) have shown that the increased responsiveness of airway smooth muscle to adenosine following epithelium removal was due to a metabolic role of epithelium. Thus, a third possible mechanism whereby epithelium removal may produce supersensitivity of guinea-pig trachea to various agents was described.

Several enzymes have been found to degrade substance P and these include kininase II (angiotensin converting enzyme, Cascieri et al., 1984), serine proteinase (Hanson & Lovenberg, 1980), acetylcholinesterase (Chubb et al., 1980), and enkephalinase (Skidgel et al., 1984). Immunohistochemical and biochemical studies in human lung tissue have demonstrated that neutral metalloendopeptidase or 'enkephalinase' is localized within the alveolar septa and appears to be concentrated within the epithelial cells (Johnson et al., 1985). Enkephalinase inhibitors potentiate substance P-induced secretion of macromolecules from ferret trachea *in vitro* (Borson et al., 1987) and substance P-induced bronchoconstriction in the guinea-pig *in vivo* (Shore et al., 1987).

Therefore, the principal objective of this study was to compare the effect of epithelium removal and/or of the addition of thiorphan, a potent enkephalinase inhibitor (Roques et al., 1980), on the contractile effect of substance P and related peptides.

Furthermore, as more than one receptor type for neurokinins has been identified in tracheal and bronchial tissues by comparing rank orders of potencies and efficacies of neurokinins (Regoli et al., 1987b; Advenier et al., 1987; Barnes, 1987), and since the epithelium might have differential effects with respect to the agonist concerned, we have investigated the effect of its removal and/or that of thiorphan on the contractile responses to the three mammalian neurokinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). Because of the possible interaction of the naturally occurring peptides with the three classes of receptors (Henry, 1987; Regoli et al., 1987a, b), we also used more selective compounds for each receptor type (i.e. NK_1 = NK-P, NK_2 = NK-A, NK_3 = NK-B) (Table 1). Thus, to characterize further NK_1-receptors, we tested the effects of SP-OMe (Watson et al., 1983), [Pro^9]SP sulfone and [β-Ala^4, Sar^9]SP(4-11)sulfone; for NK_2-receptors, we used NKA (4–10) and [Nle^{10}]-NKA(4–10), and for NK_3-receptors, [$MePhe^7$]-NKB(4–10) (Drapeau et al., 1987a, b).

The present results showed that inhibition of neutral metalloendopeptidase and/or epithelium removal potentiated to the same extent and without any additive effect the responses of the guinea-pig trachea to substance P and related peptides.

Methods

Tissue preparation

Male guinea-pigs (250–350 g) were killed by a blow on the head and exsanguinated. The trachea was removed and placed in Krebs-Henseleit solution of the following composition (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.7. Following removal of adhering fat and connective tissue, the trachea was slit open along its longitudinal axis, diametrically opposite the smooth muscle, and two strips consisting of 3 adjacent cartilage rings were prepared according to the zig-zag method of Emmerson & Mackay (1979).

From one of the strips the epithelium was removed by gently rubbing the luminal surface (over both the smooth muscle and cartilage areas) with a cotton-tipped applicator (Tschirhart & Landry, 1986; Hay et al., 1986a, b; Raeburn et al., 1986); the other strip served as a paired control.

The strips were suspended in 25 ml organ chambers containing Krebs-Henseleit solution at 37°C, gassed with 95% O_2 and 5% CO_2, and were equilibrated under an initial tension of 1.50 g. After incubation for 1.25 h, the resting tension ranged between 0.6 and 1.4 g. Under these conditions, responses to agonists were reproducible. Tension changes were measured isometrically with Gould strain gauges (UC3) and were displayed on a Bryans BS 2H pen recorder.

The efficiency of the method of epithelium removal was verified both histologically and pharmacologically. In histological studies ($n = 10$) paraffin-embedded hematoxylin-eosin and saffran-stained transverse sections of intact and rubbed trachea were prepared. Pharmacological verification of epithelial removal was performed by testing the relaxant effect of arachidonic acid (Tschirhart et al., 1987). Relaxant responses to arachidonic acid (10^{-5} M) were $49.6 \pm 6.1\%$ ($n = 17$) and $3.6 \pm 1.4\%$ ($n = 17$) of maximal relaxation induced by theophylline in unrubbed and rubbed preparations, respectively.

Protocols

In all experiments, the tissues were first contracted to maximal tension with carbachol 10^{-4} M and

Table 1 Primary structures of the neurokinins and related peptides used in the present study

SP	H-Arg-Pro-Lys-Pro-Gln-Phe-Phe-Gly-Leu-Met-NH_2
SP-OMe	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-*CH3*
[Pro^9]SP sulfone	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-*Pro*-Leu-*Met(02)*-NH_2
[β-Ala^4, Sar^9]SP(4–11) sulfone	H-*βAla*-Gln-Gln-Phe-Phe-*Sar*-Leu-*Met(02)*-NH_2
NKA	H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
NKA(4–10)	H-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
[Nle^{10}]NKA(4–10)	H-Asp-Ser-Phe-Val-Gly-Leu-*Nle*-NH_2
NKB	H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
[MePhe^7]NKB(4–10)	H-Asp-Phe-Phe-*MePhe*-Gly-Leu-Met-NH_2

SP = substance P and NKA, NKB = neurokinins A and B, respectively.

maximal relaxation was induced by adding 3×10^{-3} M theophylline. During the following 60 min, the tissues were washed every 15 min and thereafter cumulative concentration-response curves to neurokinins and related peptides were constructed by applying increasing concentrations of these peptides at 5–10 min intervals in logarithmic increments. A period of 60 min was allowed before retesting the maximal response of the tissue using carbachol 10^{-4} M.

To study the effect of neutral metalloendopeptidases, we incubated tracheal segments (with or without epithelium) with thiorphan 10^{-5} M for 30 min before recording the concentration-response curves to neurokinins and related peptides.

The data are expressed in terms of pD_2 and in mg of tension. PD_2 values were derived from the log concentration-effect curves and defined as the negative log of the drug concentration that caused 50% of maximal effect. The maximal effect (E_{max}) was calculated as the maximal increase in tone for each neurokinin or related peptide. These values were evaluated graphically from each experiment. Only one concentration-response curve for a neurokinin was constructed per tracheal strip, the same compound being tested on paired (with and without epithelium) tissues.

Statistical analysis of results

Statistical analysis of the results was performed by use of Student's t test for paired or unpaired data. All values in the text and table are expressed as mean ± s.e. mean. P values less than 0.05 were considered to be significant.

Drugs

The drugs used were: substance P, neurokinin A, B, or neurokinin-related peptides synthesized in the Département de Physiologie et de Pharmacologie, Centre Hospitalier et Universitaire, Sherbrooke, Canada, namely: SP-OMe, [Pro^9]SP(1–11) sulfone, NKA(4–10), [Nle^{10}]NKA(4–10), [MePhe^7]NKB(4–10) and [β-Ala^4, Sar^9]SP(4–11) sulfone (for chemical structures see Table 1). Substance P and neurokinin A and B were also purchased from Novabiochem (Laüfelfingen, Switzerland). [D-Ala^2, Met]enkephalin was from U.C.B. (Brain-L'Alleud, Belgium); thiorphan was the generous gift of Professor B.P. Roques (I.N.S.E.R.M. U 266, Paris, France); theophylline sodium anisate was used as a proprietary injectable solution (Theophylline Bruneau, Paris). Neuropeptides were dissolved in distilled water (except for NKB) at concentrations of 2.5×10^{-4} M to 5×10^{-3} M and kept in small aliquots at −20°C until used. A fresh aliquot was used for each experiment. Peptides were diluted in Krebs solution. Solutions of NKB were prepared in 20% sulpholane and then diluted in Krebs solution. Stock solutions of thiorphan (10^{-1} M) were prepared in dimethyl sulphoxide (DMSO) and then diluted in Krebs solution. Maximal final concentrations of either sulpholane or DMSO achieved in organ baths were found to have no effect on the tracheal tone.

Results

Log concentration-response curves to neurokinins and related peptides in the guinea-pig trachea with and without epithelium

The log concentration-response curves to SP, NKA, NKB and related peptides are presented in Figures 1, 2, 3 and 4. The pD_2 values and maximal effects are shown in Table 2.

On guinea-pig trachea with epithelium, a well-sustained contraction was observed in response to the mammalian neurokinins. The order of potency of these peptides was NKA > NKB > SP with significant differences in pD_2 between NKA, NKB and SP

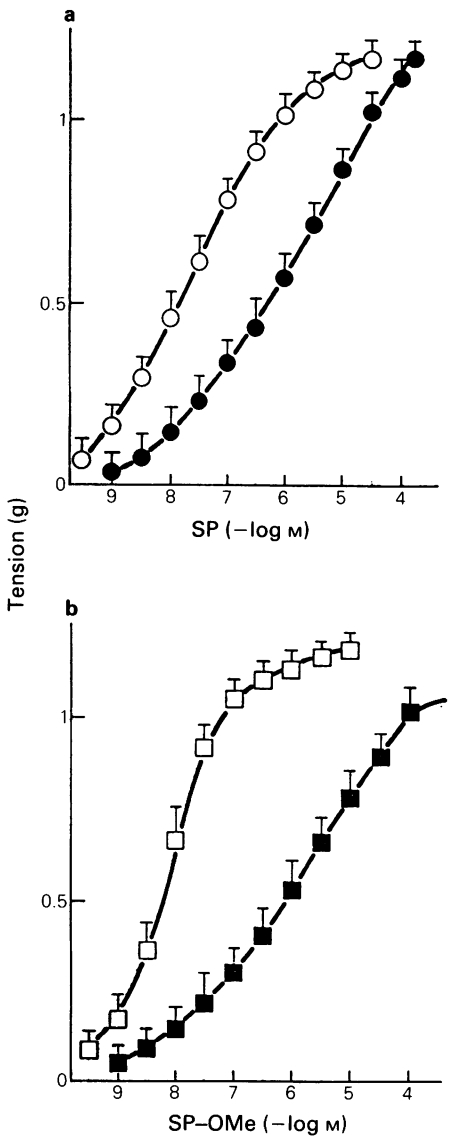

Figure 1 Effects of epithelium removal on concentration-response curves to (a) substance P (SP) and (b) SP-OMe in guinea-pig tracheal strips in the presence (●, ■) and absence (○, □) of epithelium. Each point is the mean with s.e. mean indicated by vertical lines. SP (n = 13); SP-OMe (n = 8).

($P < 0.001$, Student's t test for unpaired data). There was 2.15 log units difference between the pD_2 of SP and NKA. The trachea without epithelium exhibited hypersensitivity to neurokinins compared to the intact preparations since the concentration-response curves for SP, NKA and NKB were all shifted to the

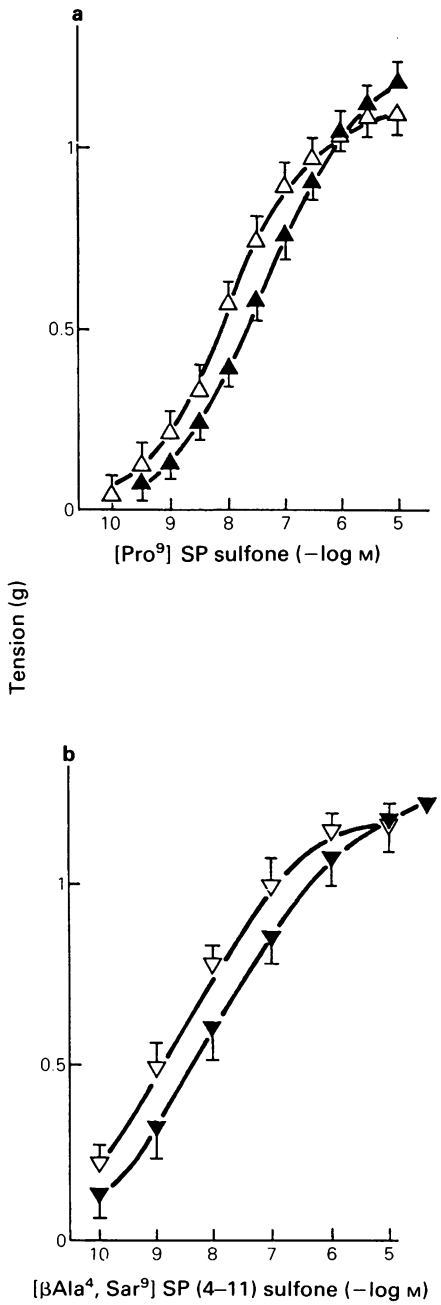

Figure 2 Effects of epithelium removal on concentration response curves to (a) [Pro^9]substance P sulfone ([Pro^9]SP sulfone) and (b) [β-Ala^4, Sar^9]SP(4–11) sulfone in guinea-pig tracheal strips in the presence (▲, ▼) and absence (△, ▽) of epithelium. Each point is the mean with s.e. mean indicated by vertical lines. [Pro^9]SP sulfone (n = 10); [β-Ala^4, Sar^9]SP(4–11) sulfone (n = 5).

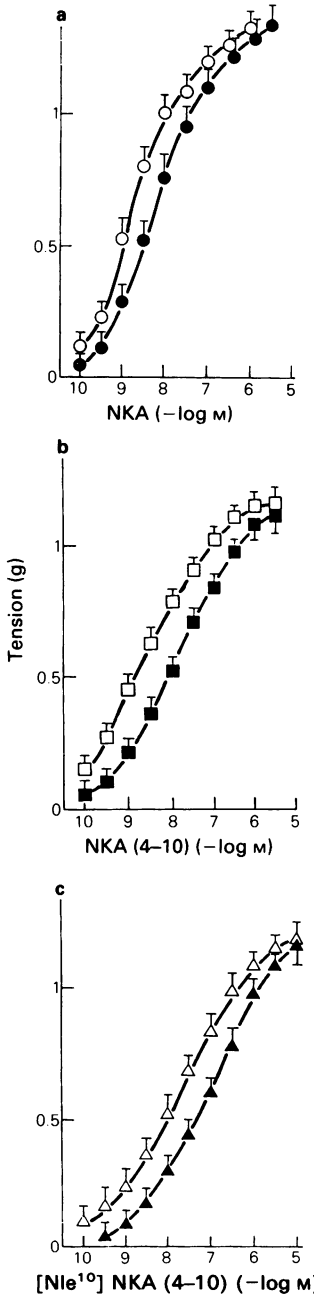

Figure 3 Effects of epithelium removal on concentration-response curves to (a) neurokinin A (NKA), (b) NKA(4–10) and (c) [Nle^{10}]NKA(4–10) in guinea-pig tracheal strips in the presence (●, ■, ▲) and absence (○, □, △) of epithelium. Each point is the mean with s.e. mean indicated by vertical lines. NKA(n = 7); NKA(4–10) (n = 8) and [Nle^{10}]NKA(4–10) (n = 5).

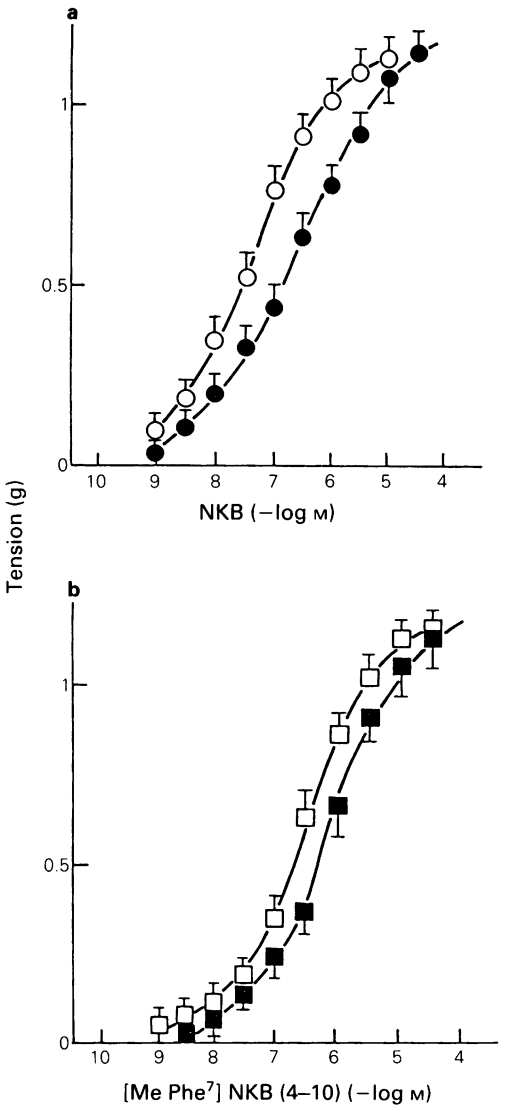

Figure 4 Effects of epithelium removal on concentration-response curves to (a) neurokinin B (NKB) and (b) [Me-Phe^7]NKB(4–10) in guinea-pig tracheal strips in the presence (●, ■) and absence (○, □) of epithelium. Each point is the mean with s.e. mean indicated by vertical lines. NKB (n = 7) and [Me-Phe^7]NKB(4–10) (n = 4).

left. (Figures 1, 3 and 4). The leftward shift was greater for SP (1.62 log units) than for NKA and NKB (0.63 and 0.71 log units respectively). The rank order of potency was NKA > NKB = SP with a less marked, but still significant, difference between SP and NKA (1.16 log units, $P < 0.001$, Student's t test

Table 2 Effect of epithelium removal on the sensitivity (pD_2) and maximal effects (E_{max}) to neurokinins and related peptides in guinea-pig isolated trachea

Peptides	n	pD_2		Shift		E_{max} (mg tension)		
		+E	−E	+E/−E	P	+E	−E	P
SP	13	6.03 ± 0.10	7.65 ± 0.08	1.62	0.001	1239 ± 116	1227 ± 76	NS
SP-OMe	8	5.98 ± 0.09	7.92 ± 0.12	1.94	0.001	1125 ± 104	1209 ± 98	NS
[Pro^9]SP sulfone	10	7.44 ± 0.16	8.10 ± 0.11	0.66	0.001	1204 ± 95	1098 ± 62	NS
[β-Ala^4, Sar^9]SP(4–11) sulfone	5	7.84 ± 0.12	8.52 ± 0.08	0.68	0.008	1236 ± 58	1195 ± 48	NS
NKA	7	8.18 ± 0.09	8.81 ± 0.08	0.63	0.006	1337 ± 119	1296 ± 52	NS
NKA(4–10)	8	7.90 ± 0.12	8.63 ± 0.14	0.73	0.001	1142 ± 79	1167 ± 66	NS
[Nle^{10}]NKA(4–10)	5	7.25 ± 0.26	7.83 ± 0.29	0.58	0.006	1187 ± 91	1209 ± 54	NS
NKB	7	6.83 ± 0.06	7.54 ± 0.05	0.71	0.001	1168 ± 97	1121 ± 72	NS
[MePhe^7]NKB(4–10)	4	5.87 ± 0.21	6.58 ± 0.11	0.71	0.045	1119 ± 96	1151 ± 47	NS

The shifts of the concentration-response curves are expressed in $\log_{10}$ units. Values are mean ± s.e. mean. +E: tissues with epithelium; −E: tissues without epithelium; *P*: statistical significance between pD_2 or E_{max} values (Student's *t* test for paired data). NS: not significant.

for unpaired data). The maximal contractile responses of the tissues to the three spasmogens were not significantly different in intact and epithelium-denuded preparations.

Among the peptides related to substance P, the selective compound [β-Ala^4, Sar^9]SP(4–11) sulfone was the most potent, being 65 fold (1.81 log units) more potent than substance P. The order of potency of the four compounds tested was: [β-Ala^4, Sar^9]SP(4–11) sulfone = [Pro^9]SP sulfone ≫ SP-OMe = SP. There was no significant difference in pD_2 values between [β-Ala^4, Sar^9]SP(4–11) sulfone and [Pro^9]SP sulfone and between SP-OMe and SP (Student's *t* test for unpaired data). After removal of the epithelium [β-Ala^4, Sar^9]SP(4–11) sulfone was significantly more potent than [Pro^9]SP sulfone ($P < 0.025$, Student's *t* test for unpaired data), but no significant difference was observed between the pD_2 values for [Pro^9]SP sulfone, SP-OMe and SP. No significant change in the maximal responses occurred after epithelial removal. However, epithelial removal caused striking variations in the leftward shifts of the concentration-response curves for these SP-related peptides. For SP and SP-OMe, the leftward shifts (1.62 and 1.94 log units, respectively) were greater than those observed with [β-Ala^4, Sar^9]SP(4–11) sulfone and [Pro^9]SP sulfone (0.68 and 0.66 log units, respectively).

For the NKA related peptides, NKA and NKA(4–10) were significantly more potent than [Nle^{10}]NKA(4–10) ($P < 0.004$ and $P < 0.03$, respectively; Student's *t* test for unpaired data). There was no significant difference between the pD_2 values for NKA and NKA(4–10). Epithelial removal did not affect either the order of potency or the average maximal responses. NKB was markedly more potent that the selective agonist for the NK_3-receptor, [MePhe^7]NKB(4–10) ($P < 0.001$, Student's *t* test for unpaired data). As for NKA related peptides, epithelial removal did not affect either the order of potency or the average maximal responses for the NKB related peptides. Finally, it is worth noting that the leftward shifts for NKA and NKB related peptides resulting from epithelium removal were of the same magnitude (between 0.58 and 0.73 log units). These values were close to those found with [β-Ala^4, Sar^9]SP(4–11) sulfone and [Pro^9]SP sulfone, two SP-related peptides with an amino acid substituted in position 9, but much less pronounced than with SP and SP-OMe.

Influence of thiorphan on log concentration-response curves for neurokinins and related peptides

In trachea with intact epithelium, pretreatment with thiorphan (10^{-5} M) resulted in a shift to the left of the log concentration-response curves for SP and SP-OMe and to some extent also of those to [β-Ala^4, Sar^9]SP(4–11) sulfone, [Pro^9]SP sulfone and NKA (compare Figures 1, 2, 3 with Figure 5). In these conditions, the pD_2 values (Table 3) for the four peptides were not significantly different from the corresponding pD_2 values observed on strips without epithelium and not exposed to thiorphan (compare with values in Table 2, Student's *t* test for unpaired data). Maximal effects were similar in preparations with and without epithelium and in the absence and presence of thiorphan.

In the presence of thiorphan, there was no additive effect of epithelium removal since the concentration-response curves on epithelium denuded strips and the corresponding pD_2 values were unchanged by treatment with thiorphan (Table 3, Figure 5).

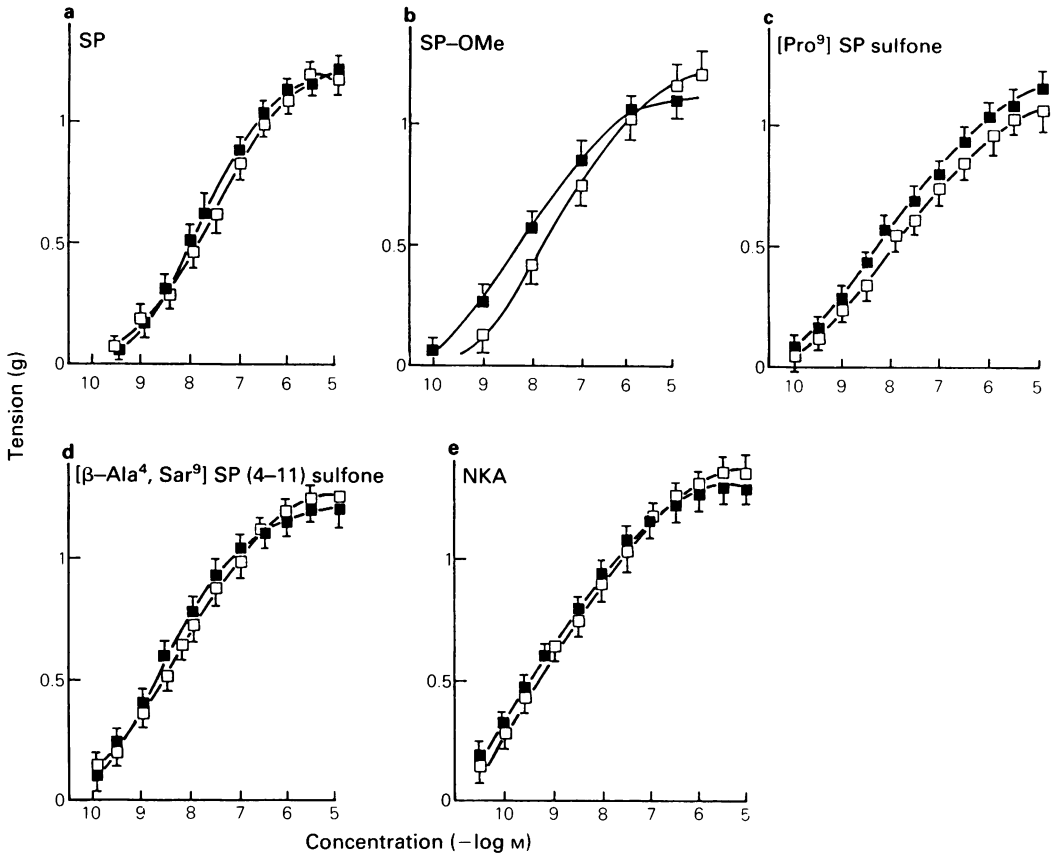

Figure 5 Influence of thiorphan (a) substance P (SP), (b) SP-OMe, (c) [Pro^9]SP sulfone, (d) [β-Ala^4, Sar^9]SP(4–11) sulfone and (e) neurokinin A (NKA) in guinea-pig tracheal strips in the presence (□) and absence (■) of epithelium. Each point is the mean with s.e. mean indicated by vertical lines from the number of experiments indicated in Table 3.

These results suggest that inhibition of a metalloendopeptidase (enkephalinase-like) by thiorphan might potentiate the contractile effect of SP and related peptides. Since such an effect could be mediated indirectly via the release of endogenous enkephalins, we tested the action of [D-Ala^2, Met] enkephalin as a stimulant. [D-Ala^2, Met]enkephalin did not induce any contractile effect when applied in concentrations up to 5×10^{-5} M on trachea with or without epithelium.

Discussion

Our results indicate that epithelium removal potentiates the effects of neurokinins and related peptides with an increase of potency illustrated by the shift to the left of the log concentration-response curves without changes in the maximal responses. However, epithelium removal had differing effects on the contractions induced by the various neurokinins and related peptides. Among the SP-related peptides, the greatest shifts to the left were observed for SP and SP-OMe. In contrast, the shifts for [Pro^9]SP sulfone and [β-Ala^4, Sar^9]SP(4–11) sulfone were of similar size to those for NKA or NKB related peptides. This suggests that SP and SP-OMe might be better substrates than the other peptides for a hypothetical peptidase located in the epithelium. This interpretation is supported by the findings with thiorphan, since the enkephalinase inhibitor potentiated the effects of neurokinins and some related peptides to a similar extent as did epithelium removal; moreover there was no additional effect when thiorphan and epithelium removal were applied together.

Table 3 Effect of thiorphan 10^{-5} M (+T) on the sensitivity (pD_2) and maximal effects (E_{max}) to substance P (SP), neurokinin A (NKA) and related peptides in guinea-pig tracheal strips

Peptides	n	pD_2		Shifts		E_{max} (mg tension)		
		$+E+T$	$-E+T$	$+E/-E$	P	$+E+T$	$-E+T$	P
SP	6	7.57 ± 0.15	7.77 ± 0.12	0.20	NS	1213 ± 54	1223 ± 66	NS
SP-OMe	6	7.87 ± 0.22	8.15 ± 0.20	0.28	NS	1224 ± 105	1094 ± 87	NS
[Pro^9]SP sulfone	4	7.94 ± 0.15	8.14 ± 0.12	0.20	NS	1072 ± 111	1170 ± 78	NS
[β-Ala^4,Sar^9]SP(4–11) sulfone	4	8.34 ± 0.08	8.68 ± 0.13	0.34	NS	1254 ± 74	1181 ± 59	NS
NKA	4	8.76 ± 0.14	9.01 ± 0.04	0.25	NS	1335 ± 87	1298 ± 72	NS

The shifts of the concentration-response curves are expressed in $\log_{10}$ units. Statistical significance between pD_2 or E_{max} values was assessed by t test for unpaired data. Abbreviations: as in Table 2.

The present findings are in accord with those of Tschirhart & Landry (1986) and Grandordy et al. (1988); they showed that epithelium removal potentiates the effects of neurokinins. Furthermore, Grandordy et al. (1988) have also demonstrated that the increase in potency for SP was greater than for NKA and NKB, and that SP, but not NKA or NKB, stimulated phosphoinositide breakdown in epithelium suggesting that receptors for SP may be present in the epithelium. Tschirhart & Landry (1986) have attributed the effect of epithelium removal to the elimination of a relaxant factor (EpDRF) which they have demonstrated by the use of a sandwich protocol similar to that described by Furchgott & Zawadzky (1980). On the other hand, the results obtained with [Pro^9]SP sulfone and [β-Ala^4, Sar^9]SP(4–11) sulfone (two selective agonists for NK_1-receptors; Drapeau et al., 1987a, b) in the present study appear to rule out the hypothesis of the EpDRF. It appears therefore that the epithelium essentially plays a metabolic role in promoting the degradation of neurokinins, since removal of the epithelium or thiorphan treatment increased similarly the sensitivity to neurokinins with no additive effects. A metabolic role of tracheal epithelium has been proposed by Advenier et al. (1988) who have found an increased responsiveness of the guinea-pig trachea to adenosine after epithelium removal but no additive effects of epithelium removal and dipyridamole treatment.

Log concentration-response curves for [Pro^9]SP sulfone and [β-Ala^4, Sar^9]SP(4–11) sulfone were shifted to the left much less than those for SP or SP-OMe by the removal of epithelium: this is possibly due to the fact that enkephalinase cleaves SP between the 9 and 10 positions (Borson et al., 1987), generating the SP(1–9) fragment which has been shown to be inactive in contracting the guinea-pig trachea (Mizrahi et al., 1985). Substitutions of Gly^9 by Pro or Sar might lead to compounds which are not degraded by enkephalinase. The location of enkephalinase in the guinea-pig trachea is not precisely known, but because both epithelium removal and the enkephalinase inhibitor were similarly effective, the enzyme is likely to be located mainly in the epithelium layer, as already shown with immunohistochemical and biochemical studies in human lung by Johnson et al. (1985). Furthermore, the findings that epithelium removal or thiorphan treatment potentiated to a similar degree the analogues of SP substituted in position 9 and the peptides of the NKA and NKB groups suggest that the NKA or NKB related compounds might be partly resistant to enkephalinase as previously shown for SP6–11 (Borson et al., 1987; Blumberg & Teichberg, 1979). As enkephalinase appears to be membrane bound and essentially located on epithelial cells, another possible explanation is that rapid degradation may occur in the vicinity of the SP receptor site on epithelial cells, thereby reducing the active concentration of SP or SP-OMe without altering much the peptide activities on the smooth muscle receptors for NKA.

Although we cannot exclude completely the release of an EpDRF from the tracheal epithelium, our results indicate that epithelium can play a metabolic role in reducing effects of neurokinins: such an interpretation is in contrast with previous findings with several agents including SP which have been found to promote the release of EpDRF (Barnes et al., 1985; Flavahan et al., 1985; Frossard & Muller, 1986; Raeburn et al., 1986, Tschirhart & Landry, 1986; Ilhan & Sahin, 1986; Tschirhart et al., 1987; Hay et al., 1987; Butler et al., 1987). Furthermore, the present findings suggest that, at least in guinea-pig trachea, pharmacological assays of neurokinins and possibly other peptides (bradykinin) should be carried out in the presence of thiorphan, or in tissues without epithelium, in order to avoid the peptide degradation that may influence differently the effects of various peptides.

On preparations with or without epithelium, the

order of potency of the three mammalian neurokinins is in agreement with that observed in other preparations containing receptors for NKA, as previously shown by Regoli *et al.* (1987b) and Advenier *et al.* (1987). However, previously published results on unrubbed guinea-pig trachea (Advenier *et al.*, 1987) are similar to the present results obtained on rubbed preparations. This discrepancy might be explained by differences in the method of preparation of the tracheal strips. In the study of Advenier *et al.* (1987), guinea-pig trachea was spirally cut, while in the present work it was prepared according to the zig-zag method (Emmerson & Mackay, 1979), which is perhaps a more appropriate method to preserve intact epithelium. In addition to the results on the effects of epithelial removal, the contractile responses obtained with specific agonists for each of the three classes of neurokinin receptor suggest the presence in the trachea of two and possibly three classes of receptors, as already identified in other preparations (Regoli, 1987a). Thus, the effects measured with the natural agonists cannot be attributed only to differences in potency for the NK_2-receptor but also to a more complex interaction with other receptor classes which seem to be present in guinea-pig tracheal preparations. For instance, SP-OMe which is essentially inactive on the rabbit pulmonary artery and the rat portal vein, is as active as SP on the guinea-pig trachea. This indicates that the trachea contains receptors for SP (NK_1). On the other hand, [MePhe^7]NKB(4–10) is much less active than NKB, suggesting that the trachea may have few if any NK_3-receptors.

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