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Receptors mediating tachykinin-induced contractile responses in guinea-pig trachea

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1 The classification of tachykinin receptors in the guinea-pig trachea has been investigated. This was of interest because, from previous studies, it was not clear whether the guinea-pig trachea contains either a mixture of NK_1 and NK_2 receptors or, alternatively, a single type of novel tachykinin receptor.

2 In the present study, the guinea-pig trachea was contracted by tachykinin agonists selective for NK_1 receptors (substance P methylester (SPOMe) and GR73632) or NK_2 receptors (GR64349) but not NK_3 receptors (senktide).

3 Against SPOMe and GR73632, the NK_1 antagonist, GR71251, behaved as a reversible competitive antagonist having apparent affinity (pK_B 7.05 vs SPOMe) consistent with action at NK_1 receptors. GR71251 (3 μM) did not antagonize responses to GR64349.

4 The NK_2 antagonists L-659,877 and Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (R396) antagonized GR64349 although only R396 appeared to behave competitively (pK_B 5.73). Neither L-659,877 (30 μM) nor R396 (30 μM) blocked responses to SPOMe.

5 For L-659,877 and R396, comparison was made between activity in guinea-pig trachea and in preparations known to contain tachykinin receptors predominantly of the NK_2 type. In the rabbit trachea, both L-659,877 and R396 had effects similar to those in guinea-pig trachea. In contrast, in the rat colon muscularis mucosae, both L-659,877 and R396 appeared to behave competitively with pK_B values against GR64349 of 7.83 and 6.90 respectively.

6 It is concluded that in guinea-pig trachea, contractile responses can be induced by activation of both NK_1 and NK_2 receptors. The present data are discussed with reference to the proposed existence of subtypes of the NK_2 receptor.

Keywords: Tachykinin receptors; receptor subtypes; guinea-pig trachea; rabbit trachea; rabbit aorta; rat colon muscularis mucosae

Introduction

Recently, considerable interest has been shown in the possible involvement of tachykinins in the pathogenesis of asthma. Activation of tachykinin receptors in the airways could lead to bronchoconstriction, hyperreactivity and inappropriate secretion of mucus (see for example Saria *et al.*, 1985; Barnes, 1986; 1989; Martling *et al.*, 1987; Crimi & Mistretta, 1989; Joos, 1989; Rogers *et al.*, 1989). Elucidation of the precise role (if any) of tachykinins in asthma is likely to be facilitated by an understanding of the tachykinin receptors present in airways.

In the present study, an investigation has been made of the tachykinin receptors mediating contraction of the guinea-pig isolated trachea. This tissue was of interest since, in previous studies, two distinct hypotheses had been formulated: the guinea-pig trachea was proposed to contain either a mixture of NK_1 and NK_2 receptors (Ireland *et al.*, 1988) or alternatively, a single type of novel tachykinin receptor termed 'NK_4' (McKnight *et al.*, 1987; Maguire *et al.*, 1989). This is of particular significance since it has been suggested that this latter novel receptor may exist also in the central nervous system (see Dourish *et al.*, 1988a,b).

Certain NK_1 or NK_2 antagonists are reported to be inactive in the guinea-pig trachea (see Maguire *et al.*, 1989), consistent with the notion that the tachykinin receptors in this tissue may indeed be novel. However, the question of whether they belong to one or more than one type is still open. The present study was performed in an attempt to answer this latter point, essential before meaningful receptor classification can be undertaken. Use was made of recently described agonists and antagonists selective for either NK_1 or NK_2 receptors.

During the course of the study, it was found that on the guinea-pig trachea, the NK_2 antagonists tested were only weakly active against the NK_2 selective agonist GR64349 (Hagan *et al.*, 1989). It was considered that this could indicate activity of GR64349 at non-NK_2 receptors. Therefore, comparison was made of the activity of the NK_2 antagonists against both GR64349 and the naturally-occurring agonist neurokinin A (NKA) in preparations shown to contain tachykinin receptors predominantly of the NK_2 type: rabbit trachea, endothelium-denuded rabbit thoracic aorta and rat colon muscularis mucosae.

A preliminary account of some of the work described here has been presented to the British Pharmacological Society (Ireland *et al.*, 1990).

Methods

Animals

Male guinea-pigs (300–600 g, Dunkin-Hartley strain, Porcellus), male rabbits (2–3 kg, New Zealand White strain, Froxfield) and male rats (250–500 g, AHA strain, Glaxo) were used. Guinea-pigs and rats were killed by concussion followed by exsanguination, whilst rabbits were killed with a captive-bolt gun; required tissues were excised immediately.

Guinea-pig trachea and rabbit trachea

Guinea-pig trachea was prepared according to the method of Constantine (1965). Briefly, each excised trachea was mounted on a glass rod (approximately 2 mm diameter) and cut to yield a spiral strip which was divided longitudinally to provide up to four individual preparations. Rabbit trachea was also mounted on the rod but was cut into rings 3–5 mm wide.

No attempt was made to assess whether the epithelium was intact in these preparations. However, in a preliminary study,

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examination of guinea-pig trachea preparations that had been stained with hematoxylin and eosin and embedded in paraffin suggested that the epithelium was likely to be present although not necessarily intact.

Rabbit aorta

Each rabbit thoracic aorta was threaded over a Perspex rod (approximate diameter 2 mm) to remove the endothelium (see Furchgott, 1983) and then cut to yield rings approximately 2–3 mm wide.

Rat colon muscularis mucosae

Rat colon muscularis mucosae (RCMM) were prepared as described by Bailey & Jordan (1984).

Use of isolated tissues

Tissue preparations were mounted in glass organ baths of approximately 3 ml capacity (trachea or RCMM) or 5 ml capacity (aorta), filled with physiological medium, gassed with CO_2 (5%) in O_2 and maintained at 37 ± 1°C. To reduce adsorption of peptides, all glassware was pre-treated with dichlorodimethylsilane (Aldrich).

Contraction of guinea-pig trachea, rabbit trachea or rabbit aorta was recorded isometrically via Grass FT.03 transducers, with a basal tension of approximately 0.02N (rabbit aorta) or 0.01N (other tissues). Contraction of RCMM preparations was recorded isotonically via Chemlab CS-IT100 transducers, pre-loaded with approximately 0.005N.

Experimental design

In tracheal preparations, concentration-contraction response curves to agonists were constructed by cumulative addition; in aorta and RCMM, non-cumulative addition of serially-increasing concentrations was used. On each preparation, at least two concentration-response curves were constructed to the chosen agonist. Tissues were used in the study only if two consecutive curves were found to have EC_{50} values consistent to within a factor of 2 and maxima reproducible to within about $\pm 15\%$.

Routinely, antagonists were pre-equilibrated with the tissue for 15 min. Adequacy of this period was verified in separate experiments by comparison of results obtained after 15 min or 60 min pre-equilibration. Each preparation was exposed to one concentration of antagonist only. In control preparations not exposed to antagonist, EC_{50} values for agonist concentration-response curves were reproducible to within 2 fold. No measurements were made of reversibility of effect on wash-out of antagonist.

Data analysis

For agonists, EC_{50} values and maxima of concentration-response curves were estimated by the curve-fitting programme "Allfit" (De Lean *et al.*, 1977). EC_{50} values are quoted as the mean ($\pm$ s.e.mean) of single determinations made on at least four preparations of a given tissue, each obtained from a separate animal.

Antagonist-induced parallel displacement of agonist concentration-response curves was quantified as the ratio of equi-active molar concentrations. These were estimated graphically at the level of the half-maximal response. Estimates of $\log_{10}$(concentration-ratio $-$ 1) were plotted against $\log_{10}$(concentration of antagonist). Provided that such plots were linear and had gradients not significantly different from unity, the apparent affinity (pK_B) of the antagonist was estimated as the mean ($\pm$ s.e.mean) of the individual values:

$$pK_B = \log(\text{concentration-ratio} - 1) - \log(\text{molar concentration of antagonist})$$

For each concentration of antagonist, data were obtained from a minimum of four separate preparations of the chosen tissue.

Solutions

Modified Tyrode solution was used for experiments on guinea-pig trachea, rabbit trachea and RCMM. It had the following composition (in mM): NaCl 137, KCl 2.8, $NaHCO_3$ 11.9, $MgCl_2$ 2.1, NaH_2PO_4 0.32, $CaCl_2$ 1.8 and glucose 5.6. For rabbit aorta preparations, modified Krebs-Henseleit medium was used. The composition was (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.5 and glucose 11. Media were prepared in glass-distilled water and A.R.-grade reagents were used (BDH). The following drugs (each at 1 μM) were added routinely to prevent possible indirect effects of peptides: atropine sulphate (Sigma), indomethacin (Sigma), mepyramine maleate (May & Baker) and methysergide hydrogen maleate (Sandoz). For trachea preparations only, the medium contained the peptidase inhibitors phosphoramidon (1 μM; Peninsula) and bestatin (100 μM; Peninsula) since in guinea-pig airways, peptidase inhibitors have a marked effect on the activities of a number of tachykinin agonists (see Devillier *et al.*, 1988; Stephens-Smith *et al.*, 1988; Djokic *et al.*, 1989; Frossard *et al.*, 1989; Maggi *et al.*, 1990a).

Drugs

Substance P (SP), substance P methylester (SPOMe), neurokinin A (NKA), eledoisin and senktide were purchased from Peninsula or Cambridge Research Biochemicals; L-659,877 (cyclo(Gln-Trp-Phe-Gly-Leu-Met)) from Cambridge Research Biochemicals. Other compounds were synthesized in the Department of Medicinal Chemistry, Glaxo Group Research Ltd., Greenford. These were GR71251 ([D-Pro^9[spiro-γ-lactam]Leu^{10},Trp^{11}]$SP_{(1-11)}$); GR64349 ([Lys^3,Gly^8-**R**-γ-lactam-Leu^9]$NKA_{(3-10)}$); GR73632 (δaminovaleryl[Pro^9,N-MeLeu^{10}]$SP_{(7-11)}$); δaminovaleryl[L-Pro^9]$SP_{(7-11)}$; δaminovaleryl[D-Pro^9]$SP_{(7-11)}$; [pGlu^6,Gly^9-**R**-γ-lactam-Leu^{10}]$SP_{(6-11)}$ (Cascieri *et al.*, 1986) and Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (Maggi *et al.*, 1990b; R396). The identity and concentration of peptides were confirmed as described by Brown *et al.* (1986).

Concentrated solutions (5–10 mM) of each compound were divided into aliquots and stored frozen (-20°C) under nitrogen in plastic tubes. Aliquots were diluted immediately before use. Solutions of L-659,877, R396, eledoisin and senktide were prepared in dimethysulphoxide (DMSO; BDH); other compounds were dissolved in 0.01 M acetic acid (BDH). Stock solutions of all compounds, with the exception of L-659,877, could be diluted with physiological solution without causing visible precipitation. For L-659,877, it was necessary to include DMSO (up to 1.0%) to prevent precipitation. In all cases, the concentration of solvent was limited such that it had no effect on agonist-induced responses.

Results

On all preparations, the effects of antagonists (GR71251, R396 or L-659,877) were not increased by pre-incubation for 60 min rather than the standard 15 min. This was taken to indicate that complete equilibration had been achieved within 15 min. For analysis, data for both pre-incubation periods have been combined.

Guinea-pig trachea

On the guinea-pig trachea, contractile responses were induced by low concentrations of a number of tachykinin agonists (see Table 1). Of particular note were the high activities of the NK_1-selective agonists SPOMe and GR73632 and of the

Table 1 Mean EC_{50} values for tachykinin agonist-induced contractile responses

Compound	*Mean EC_{50} (nM)* Guinea-pig trachea	Guinea-pig ileum (NK_1)	RCMM (NK_2)	Rat portal vein (NK_3)
SPOMe	38.0 ± 10.0	7.7 ± 1.7	>100,000	>30,000
GR73632	4.9 ± 0.5	4.0 ± 0.5	961 ± 175	>15,000
δAva[L-Pro^9]$SP_{(7-11)}$	5.4 ± 0.5	13.4 ± 2.4	13,000	>10,000
GR64349	2.9 ± 0.2	4237 ± 1295	3.7 ± 0.6	117 ± 446
δAva[D-Pro^9]$SP_{(7-11)}$	12.8 ± 1.4	830 ± 410	92.0 ± 24.2	>30,000
SP	98.4 ± 3.0	5.6 ± 0.7	167 ± 20	7500 ± 1200
Neurokinin A	2.4 ± 1.0	13.7 ± 2.1	1.7 ± 0.3	563 ± 99
[pGlu^6,Gly^9-**R**-γ-lactam-Leu^{10}]$SP_{(6-11)}$	3.9 ± 0.3	30.3 ± 4.2	68.5 ± 11.9	435 ± 45
Eledoisin	25.0 ± 9.7	12.4 ± 2.2	13.0 ± 2.4	ND
Senktide	>10,000	>30,000	>30,000	11.8 ± 0.9

Values are mean ± s.e.mean.
On each preparation, concentration-response curves for active agonists were approximately parallel to those obtained with the chosen standard [pGlu^6,Gly^9-**R**-γ-lactam-Leu^{10}]$SP_{(6-11)}$ on guinea-pig trachea, substance P (SP) on guinea-pig ileum, neurokinin A (NKA) on rat colon muscularis mucosae (RCMM) or senktide on rat portal vein); maxima were similar. An EC_{50} value preceded by > indicates that at the concentration indicated, the agonist caused a response smaller than half the maximum to the standard. Each value is the mean (± s.e.mean) of single determinations in at least four separate preparations each obtained from a different animal. Data for guinea-pig ileum, RCMM and rat portal vein are from Ireland *et al.* (1988) or Hagan *et al.* (1990).
Abbreviations: SPOMe: substance P methylester; δAva; δaminovaleryl; ND: not determined.

NK_2-selective agonist GR64349. In addition, there was only a small difference between the activities of the L-proline and D-proline isomers of δaminovaleryl [Pro^9]$SP_{(7-11)}$ (Table 1). Of the compounds tested, only senktide, which showed the greatest selectivity for NK_3 receptors, was inactive (Table 1).

GR71251 (0.1–30 μM) behaved as a reversible competitive antagonist of contractile responses induced by SPOMe (Figure 1). Apparent affinity, expressed as a pK_B value, was 7.05 ± 0.06 ($n = 26$). When tested at a single concentration (3 μM), GR71251 antagonized, by an approximately equal extent, responses induced by the NK_1-selective agonists SPOMe and GR73632 but had negligible effect against a number of other tachykinins including the NK_2-selective agonist GR64349 (Table 2).

The NK_2 antagonist R396 (10–50 μM) caused parallel rightward displacement of the concentration-response curve to GR64349 (Figure 2). The Schild plot constructed from the antagonism data approximated to a straight line with gradient 0.96 (95% confidence limits 0.64–1.27, Figure 3). The mean pK_B was 5.73 ± 0.04 ($n = 19$). R396 (30 μM) had no significant effect against contraction of the guinea-pig trachea induced by SPOMe (mean concentration-ratio (95% confidence limits) 1.2 (1.0–1.4), $n = 6$).

Like R396, L-659,877 (0.3–10 μM) caused parallel rightward displacement of the concentration-response curve to GR64349. However, this effect was not clearly concentration-dependent: the Schild plot had a gradient of 0.28 (0.08–0.49)

Table 2 Effect of GR71251 (3 μM) on tachykinin-induced contraction of the guinea-pig isolated trachea

Tachykinin agonist	*GR71251 (3 μM)* Mean concentration-ratio
Substance P methylester	38.1 (15.7–92.8)
GR73632	42.3 (23.7–75.5)
GR64349	2.4 (1.5–3.8)
δAva[D-Pro^9]$SP_{(1-11)}$	3.3 (1.8–6.1)
Neurokinin A	1.8 (0.9–3.4)
[pGlu^6,Gly^9-R-γ-lactam-Leu^{10}]$SP_{(6-11)}$	3.6 (2.7–5.2)
Eledoisin	6.2 (3.7–10.3)

Each result is the mean (95% confidence limits) of single determinations in at least four individual tissues, each obtained from a separate guinea-pig.
Abbreviations: δAva: δaminovaleryl; SP: substance P.

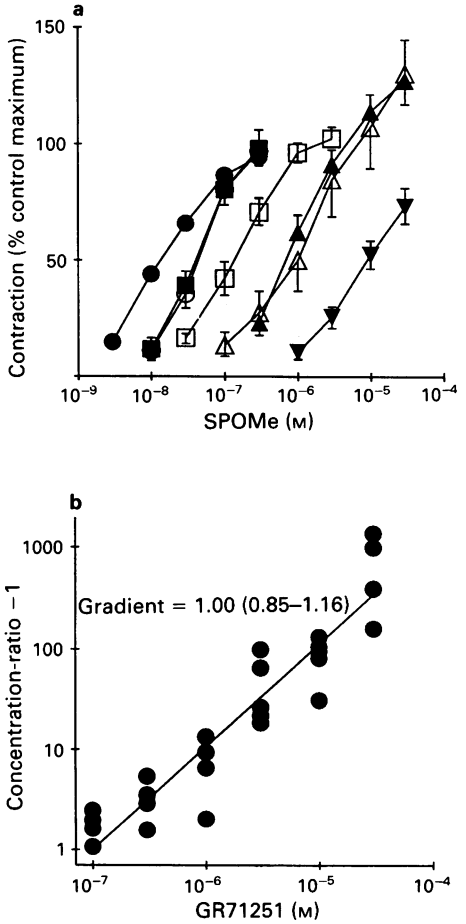

Figure 1 Antagonism by GR71251 of contractile responses induced by substance P methylester (SPOMe) in guinea-pig isolated trachea. In (a) symbols indicate control responses (●) or the presence of GR71251 at 0.1 (○), 0.3 (■), 1.0 (□), 3.0 (▲), 10.0 (△) or 30.0 μM (▼). Each point is the mean with the vertical line indicating the s.e.mean of single determinations in at least four separate tissues. (b) Schild plot of [concentration-ratio − 1] against [concentration of GR71251]. Each point represents the result obtained in a separate tissue. The gradient (95% confidence limits) of the best-fit straight line was determined by linear regression analysis.

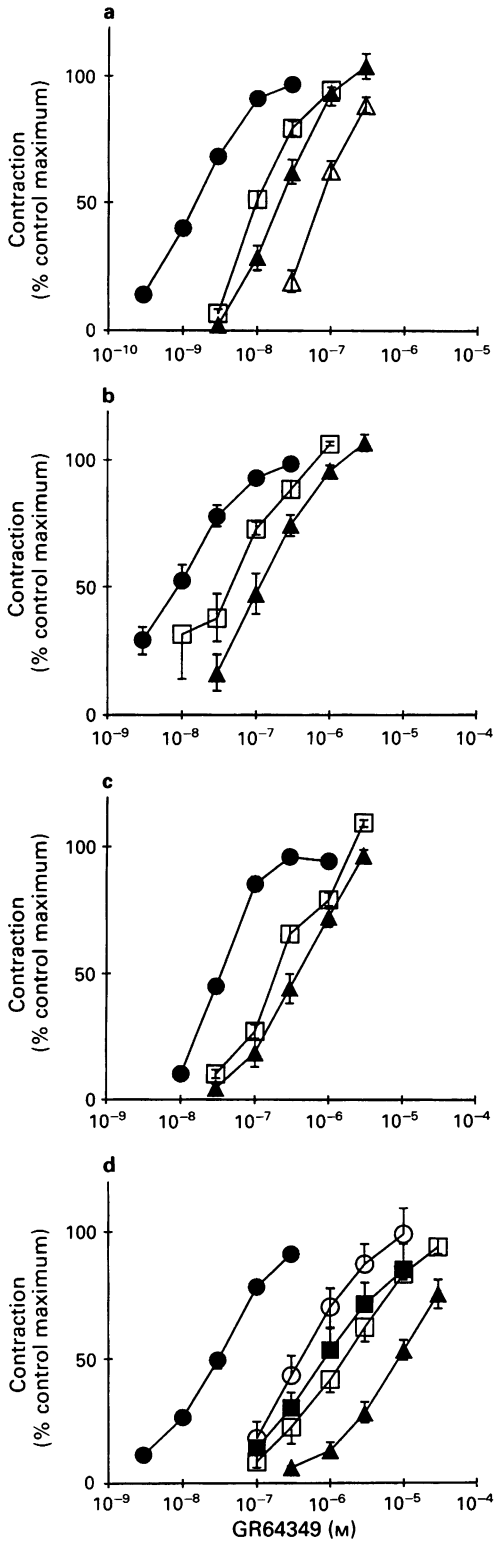

Figure 2 Antagonism by Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (R396) of contractile responses induced by GR64349 in (a) guinea-pig trachea, (b) rabbit trachea, (c) rabbit thoracic aorta or (d) rat colon muscularis mucosae. Symbols indicate control responses (●) or the presence of antagonist at 1.0 (○), 3.0 (■), 10.0 (□), 30.0 (▲) or 50.0 μM (△). Each point is the mean, with vertical lines indicating the s.e.mean, of single determinations in at least four separate preparations.

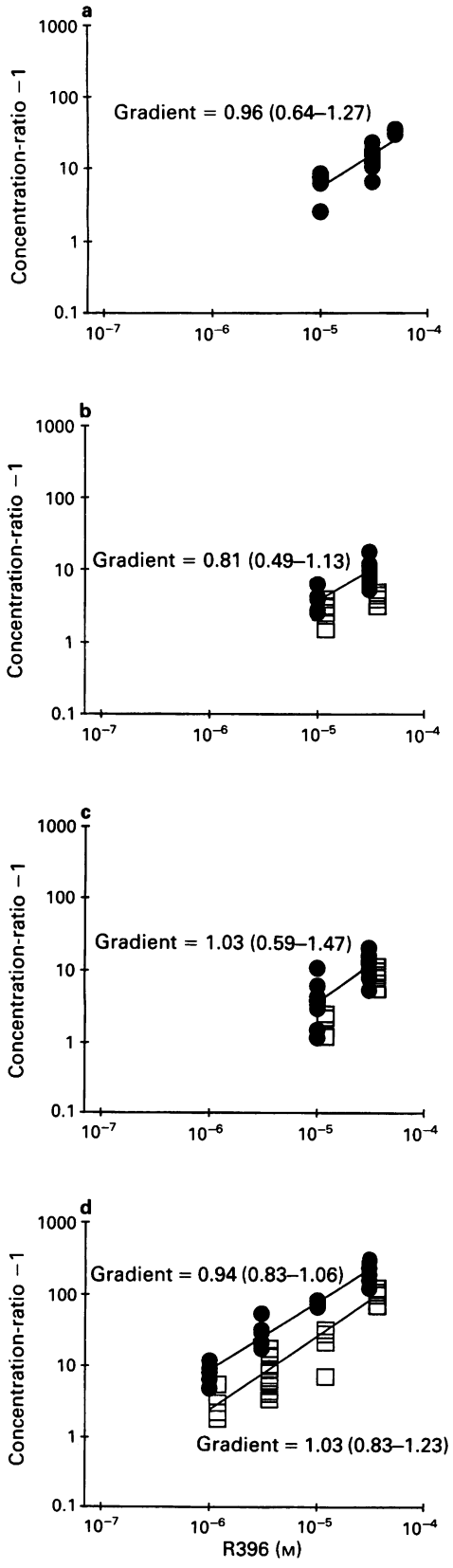

Figure 3 Schild plots of [concentration-ratio − 1] against [concentration of antagonist] for antagonism by Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (R396) of tachykinin agonist induced contraction in (a) guinea-pig trachea, (b) rabbit trachea, (c) rabbit thoracic aorta or (d) rat colon muscularis mucosae. Each point represents data obtained from a separate preparation. Results were obtained with GR64349 (●) or neurokinin A (□) as agonist. Note that to avoid overlap, data obtained with neurokinin A are shown displaced 1.2 fold to the right. For each type of preparation, the gradient of the best-fit straight line (95% confidence limits) was determined by linear regression analysis.

(Figures 4, 5). At a very high concentration, L-659,877 (30 μM) appeared to reduce the amplitude of the maximum response to GR64349 (Figure 4) but had no effect against contractile responses induced by SPOMe (mean concentration-ratio 1.0 (0.4–2.5), $n = 4$).

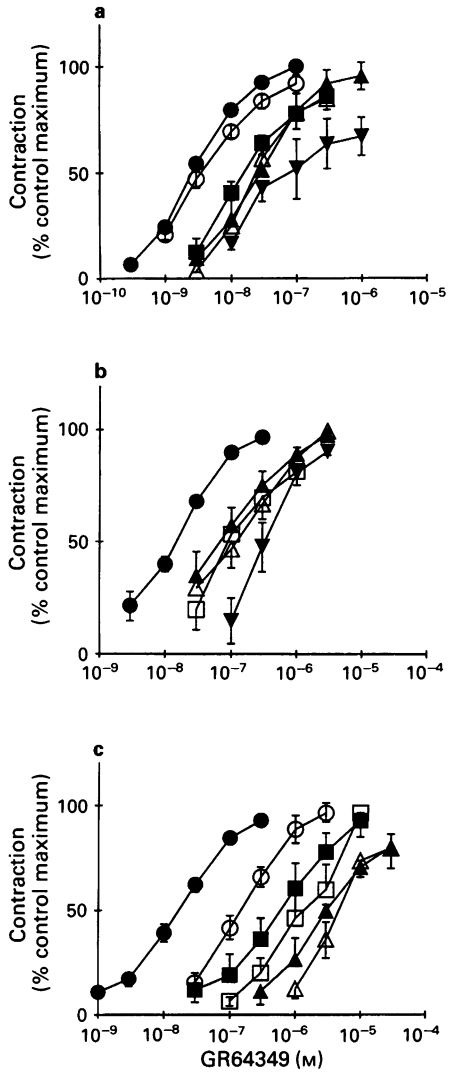

Figure 4 Antagonism by L-659,877 of contractile responses induced by GR64349 in (a) guinea-pig trachea, (b) rabbit trachea or (c) rat colon muscularis mucosae. Symbols indicate control responses (●) or the presence of antagonist at 0.1 (○), 0.3 (■), 1.0 (□), 3.0 (▲), 10.0 (△) or 30.0 μM (▼). Each point is the mean, with vertical lines indicating the s.e.mean, of single determinations in at least four separate preparations.

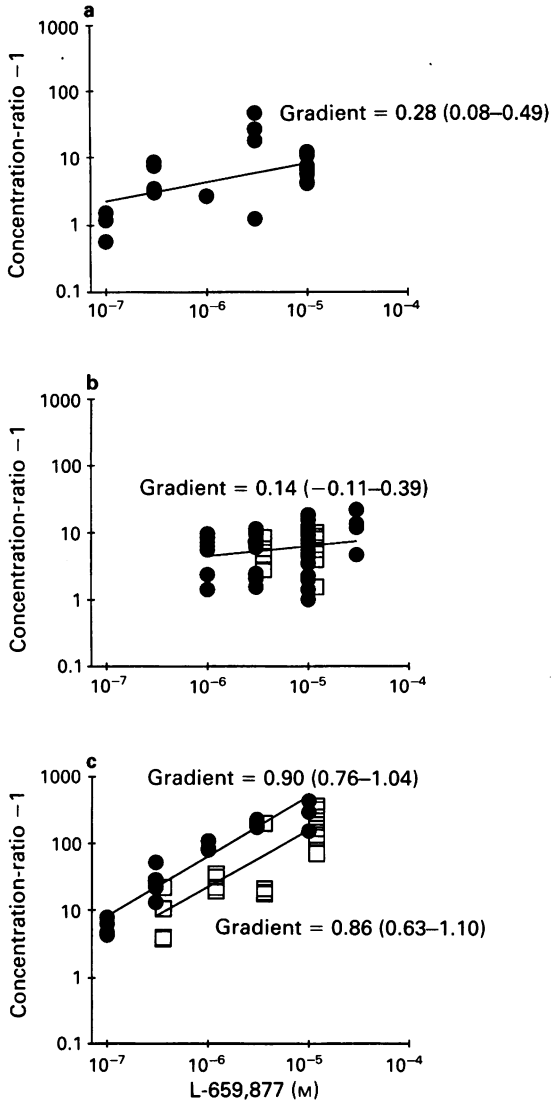

Figure 5 Schild plots of [concentration-ratio − 1] against [concentration of antagonist] for antagonism by L-659,877 of tachykinin agonist-induced contraction in (a) guinea-pig trachea, (b) rabbit trachea or (c) rat colon muscularis mucosae. Each point represents data from a separate preparation. Results were obtained with GR64349 (●) or neurokinin A (□) as agonist. Note that to avoid overlap, data obtained with neurokinin A are shown displaced 1.2 fold to the right. For each type of preparation, the gradient of the best-fit straight line (95% confidence limits) was determined by linear regression analysis.

Rabbit aorta, rabbit trachea and rat colon muscularis mucosae (RCMM)

Rabbit aorta preparations were contracted by the NK_2-selective agonist GR64349 but not the NK_1-selective agonists SPOMe or GR73632 nor by the NK_3-selective agonist senktide. In addition, when pre-contracted with phenylephrine (0.3 μM), no relaxation was observed on addition of SPOMe (1.0 μM) or GR64349 (1.0 μM) (cf. Zawadzki *et al.*, 1981). Therefore, the pharmacology of this preparation resembles that of the endothelium-denuded rabbit pulmonary artery which appears to respond to stimulation of tachykinin receptors of the NK_2 type only (D'Orleans-Juste *et al.*, 1985) as do

Table 3 Estimates of apparent affinity for NK_2 antagonists

		Apparent affinity (mean pK_B)			
Antagonist	*Agonist*	Guinea-pig trachea	Rabbit trachea	Rabbit aorta	RCMM
R396	GR64349	5.73 ± 0.04	5.51 ± 0.03	5.58 ± 0.05	6.90 ± 0.03
R396	NKA	ND	5.28 ± 0.05	5.40 ± 0.04	6.40 ± 0.04
L-659,877	GR64349	NC	NC	ND	7.83 ± 0.05
L-659,877	NKA	ND	NC	ND	7.29 ± 0.07

Data were calculated for the experiments illustrated in Figures 3 and 5. Estimates of apparent affinity (pK_B) are the mean ± s.e.mean of single determinations in at least 11 separate preparations.
Abbreviations: RCMM: rat colon muscularis mucosae; NKA; neurokinin A; ND: could not be determined (see text); NC: could not be calculated (gradient of Schild plot less than unity; $P < 0.05$).

the rabbit trachea (see Cook et al., 1990) and the RCMM (see Bailey et al., 1986; Burcher et al., 1986).

On the rabbit trachea, rabbit aorta and RCMM, R396 (10–30 μM) behaved as a reversible competitive antagonist of contractile responses induced by either GR64349 or NKA (Figures 2, 3). Shifts of the concentration-response curves tended to appear greater when GR64349 rather than NKA was used as agonist, although the magnitude of such agonist-dependence was small (see Figure 3, Table 3). However, irrespective of the agonist used, R396 had markedly greater activity in the RCMM than in the rabbit tissues (Table 3).

On the rabbit trachea, L-659,877 (1–30 μM) caused parallel rightward displacements of concentration-response curves to GR64349 or NKA although the magnitude of displacement was not clearly concentration-dependent (Figures 4, 5). In contrast, in RCMM, L-659,877 (0.3–10 μM) appeared to behave competitively (Figures 4, 5); estimates of apparent affinity are given in Table 3. The rabbit aorta appeared more sensitive than the other tissue preparations to the effects of the solvent (DMSO) necessary to prevent precipitation of L-659,877. For this reason, the highest concentration of L-659,877 that could be tested on rabbit aorta was 3 μM (in 0.3% DMSO (v/v)): this caused a mean rightward displacement of the concentration-response curves to GR64349 of 3.94 (95% confidence limits 3.49–4.46; $n = 5$). As with R396, on the RCMM, there was a tendency for L-659,877 to appear more active against GR64349 than NKA although the differences were small (Figure 5, Table 3).

Discussion

On the guinea-pig isolated trachea, tachykinin agonists were found to induce contractile responses: of the compounds tested, only the NK_3-selective agonist senktide (Wormser et al., 1986) was found to be inactive. This latter result suggests that in the smooth muscle of the trachea, few NK_3 receptors are coupled to this response. It was not possible to draw firm conclusions from the activities of the other agonists: data were not consistent with the presence of a single population of either NK_1 or NK_2 receptors. Thus, low concentrations of either NK_1-selective agonists (SPOME, GR73632 or δaminovaleryl[L-Pro^9]SP$_{(7-11)}$) or NK_2-selective agonists (GR64349 or δaminovaleryl[D-Pro^9]SP$_{(7-11)}$) were active. Although these data are consistent with the presence of a mixture of NK_1 and NK_2 receptors, an alternative explanation, that the guinea-pig trachea contains a single type of novel tachykinin receptor (Maguire et al., 1989) was also considered.

To evaluate these alternative hypotheses, examination was made of the effects of selective NK_1 or NK_2 antagonists against responses induced by SPOMe or GR64349, these being, respectively, the most NK_1- or NK_2-selective of the agonists examined. GR71251 behaved as a reversible competitive antagonist of SPOMe-induced contraction with apparent affinity (pK_B 7.05) comparable with that exhibited at NK_1 receptors in other tissues (7.72, guinea-pig ileum; 7.06, rabbit aorta; Hagan et al., 1990), but quite different from that measured at NK_2 receptors (pK_B 4.8; Ward et al., 1990). The relatively small differences in apparent affinity of GR71251 in the three NK_1 receptor containing preparations remains to be explained, as does the observation that in guinea-pig trachea, another NK_1 antagonist, L-668,169, is inactive against SPOMe (Maguire et al., 1989) despite having a pA_2 of 7.0 against NK_1 receptor-mediated contraction of the guinea-pig ileum (McKnight et al., 1988).

In the present study on the guinea-pig trachea, GR71251 was an effective antagonist of responses induced by the NK_1-selective agonist GR73632, with apparent affinity comparable to that shown against SPOMe. In contrast, GR71251 was markedly less active against other tachykinin agonists, including GR64349, all of which have appreciable activity at NK_2 receptors (see Tables 1, 2). These data are inconsistent with the hypothesis that in the guinea-pig trachea, responses to tachykinin agonists are mediated by a single type of receptor. Rather, they are consistent with the presence of an NK_1 receptor plus another receptor which, classified according to the effects of the agonists used, resembles an NK_2 receptor. Further characterization of this latter receptor was attempted with two NK_2 antagonists: L-659,877 (Williams et al., 1988) and Ac-Leu-Asp-Gln-Trp-Phe-Gly-NH_2 (Maggi et al., 1990b; R396); GR64349 was used as agonist.

L-659,877 was a weak antagonist of GR64349-induced contraction and its effects were not clearly concentration-dependent. In contrast, R396 did behave as a reversible competitive antagonist of GR64349 although its apparent affinity was low (pK_B 5.73). Interestingly this value is close to that obtained for R395 at NK_2 receptors in rabbit pulmonary artery (pK_B 5.42 vs NKA) but much lower than at NK_2 receptors in hamster trachea (pK_B 7.63 vs NKA) (Maggi et al., 1990b). However, before concluding that the present data contribute to evidence for subtypes of the NK_2 receptor, it was considered important to exclude alternative explanations. Of particular concern was the possibility that the low activity of L-659,877 or R396 resulted from GR64349 having activity at some type of receptor which would not be activated by a naturally-occurring agonist such as NKA. This question was addressed by measuring the effects of the two antagonists in tissue preparations which appear to contain tachykinin receptors of the NK_2 type only and in which activity against GR64349 and NKA could be compared without the complication of needing concomitant NK_1 receptor blockade. The preparations used were rabbit trachea (see Cook et al., 1990), endothelium-denuded rabbit thoracic aorta (see Results) and the rat colon muscularis mucosae (RCMM; see Bailey et al., 1986; Burcher et al., 1986).

In each of these preparations, the effects of the antagonists were not increased by using NKA as agonist rather than GR64349. Indeed, the converse tended to occur. Tachykinin receptor antagonists have been found to exhibit agonist-dependence in other tissue preparations which seem to contain only one type of tachykinin receptor (see Bailey, 1985; Bailey et al., 1986; Hall & Morton, 1991) but definitive explanation of such behaviour is awaited. In the rabbit trachea, both L-659,877 and R396 had effects qualitatively and quantitatively similar to those in guinea-pig trachea. In the rabbit aorta, R396 antagonized responses to GR64349 or NKA with apparent affinities comparable to those in rabbit trachea, guinea-pig trachea and also rabbit pulmonary artery (Maggi et al., 1990b). In contrast, in RCMM, R396 had apparent affinity (pK_B 6.90) intermediate between that observed on the guinea-pig or rabbit tissues and that reported at NK_2 receptors in hamster trachea (pK_B 7.63; Maggi et al., 1990b). The RCMM too was the only preparation of those examined in which L-659,877 caused clearly concentration-dependent antagonism of responses to GR64349 or NKA and had activity (pK_B 7.83 vs GR64349) comparable to that at NK_2 receptors in rat vas deferens (pA_2 8.0; McKnight et al., 1988). The behaviour of L-659,877 remains to be explained. In the rabbit aorta, it was not possible to test a range of concentrations of L-659,877 due to its low aqueous solubility and the apparent sensitivity of this preparation to organic solvent (DMSO). Nevertheless, L-659,877 (3 μM) was clearly less active in the rabbit aorta than in the RCMM.

The present results show that the effects of NK_2 antagonists can be tissue-dependent and, as such, confirm the conclusion of Maggi et al. (1990b). Such tissue-dependence was shown not to be a consequence of incomplete equilibration of the antagonist and appeared independent of the agonist used. Further, it is considered unlikely that it resulted from different rates of inactivation of the antagonist since tissue preparations likely to metabolize tachykinins were treated with peptidase inhibitors (see also Maggi et al., 1990b).

In conclusion, the results obtained in the present study suggest that in the guinea-pig trachea, tachykinin-induced

contraction can be mediated via a mixture of NK_1 and NK_2 receptors. They are not consistent with the notion that such contraction is mediated via a single population of a novel type of tachykinin receptor. The present data support the possible existence of subtypes of the NK_2 receptor: in terms of the effects of the NK_2 antagonists examined, the NK_2 receptors in guinea-pig trachea resemble those in rabbit trachea, rabbit thoracic aorta and rabbit pulmonary artery but seem distinct from those in hamster trachea and RCMM.

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