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Tachykinin-induced contraction of the guinea-pig isolated oesophageal mucosa is mediated by NK_2 receptors

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1 The tachykinin receptor present in the guinea-pig oesophageal mucosa that mediates contractile responses of the muscularis mucosae has been characterized, using functional *in vitro* experiments.

2 The NK_1 receptor-selective agonist, [$Sar^9(O_2)Met^{11}$]SP and the NK_3 receptor-selective agonists, [$MePhe^7$]-NKB and senktide, produced no response at submicromolar concentrations. The NK_2 receptor-selective agonists, [Nle^{10}]-NKA(4–10), and GR 64,349 produced concentration-dependent contractile effects with pD_2 values of 8.20 ± 0.16 and 8.30 ± 0.15, respectively.

3 The concentration-response curve to the non-selective agonist, NKA ($pD_2 = 8.13 \pm 0.04$) was shifted significantly rightwards only by the NK_2 receptor-selective antagonist, GR 159,897 and was unaffected by the NK_1 receptor-selective antagonist, SR 140,333 and the NK_3 receptor-selective antagonist, SB 222,200.

4 The NK_2 receptor-selective antagonist, GR 159,897, exhibited an apparent competitive antagonism against the NK_2 receptor-selective agonist, GR 64,349 (apparent pK_B value $= 9.29 \pm 0.16$) and against the non-selective agonist, NKA (apparent pK_B value $= 8.71 \pm 0.19$).

5 The NK_2 receptor-selective antagonist, SR 48,968 exhibited a non-competitive antagonism against the NK_2 receptor-selective agonist, [Nle^{10}]-NKA(4–10). The pK_B value was 10.84 ± 0.19.

6 It is concluded that the guinea-pig isolated oesophageal mucosa is a useful preparation for studying the effects of NK_2 receptor-selective agonists and antagonists as the contractile responses to various tachykinins are mediated solely by NK_2 receptors.

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Abbreviations: DMSO, dimethyl sulphoxide; NK, neurokinin; NKA, neurokinin A; NKB, neurokinin B; NPγ, neuropeptide γ; NPK, neuropeptide K; SP, substance P

Introduction

There are five mammalian tachykinins, *viz*., substance P (SP), neurokinin B (NKB), neurokinin A (NKA), neuropeptide K (NPK) and neuropeptide γ (NPγ). SP and NKB respectively activate tachykinin $(NK)_1$ and NK_3 receptors preferentially but not exclusively. NKA, NPK and NPγ are relatively more selective for NK_2 receptors. Tachykinin receptors in the body of the guinea-pig oesophagus have been characterized as predominantly NK_2 and NK_3 receptors (Kerr *et al*., 1997). Atropine was shown to abolish the contractile responses to the NK_3 receptor selective agonist, [$MePhe^7$]-NKB, suggesting that the NK_3 receptors are present on cholinergic neurones, possibly in the myenteric plexus. Immunohistochemical studies carried out by Portbury *et al*. (1996) have shown that NK_2 receptors are located on the surfaces of longitudinally oriented smooth muscle cells of the muscularis mucosae in the guinea-pig oesophagus. There was no NK_2 receptor immunoreactivity of nerve fibre varicosities in oesophageal myenteric ganglia. Thus, it appears that in the guinea-pig oesophagus, the effects mediated by NK_2 receptors are myogenic, whereas those mediated by NK_3 receptors are neurogenic. A number of studies have shown that tachykinins induce contractile effects on the oesophageal muscularis mucosae in various species. While both NK_2 and NK_3 receptor activation results in contractions in the rat (Delany *et al*., 1999) and are myogenic (Stables *et al*., 1991), contractions in the opossum appear to be mediated predominantly by NK_1 receptors (Daniel *et al*., 1989) and are also myogenic in nature (Christensen & Percy, 1984).

It appears very likely that if the guinea-pig oesophagus were stripped of the muscularis externa, which is more easily done in the oesophagus than in the rest of the alimentary tract, then the remaining mucosal preparation would constitute an NK_2 monoreceptorial system for investigating contractile responses of the muscularis mucosae. Such a system would provide a useful bioassay for determining the potencies and the affinities of new NK_2 receptor-selective agonists and antagonists, respectively. Unless appropriate receptor-selective antagonists are used in mixed receptor systems, monoreceptor systems should be used when determining the pA_2 values of antagonists, particularly if the agonist and antagonist are insufficiently selective.

This study shows that tissue from another species can be added to the existing *in vitro* NK_2 receptor systems, such as the rabbit denuded pulmonary artery (Regoli *et al*., 1988), the rat vas deferens, and the hamster urinary bladder (Dion *et al*., 1987), and trachea (Maggi *et al*., 1989). Various human *in vitro* NK_2 monoreceptor systems include the circular smooth muscle of the colon (Croci *et al*., 1998), the bronchus (Sheldrick *et al*., 1995) and the urinary bladder (Giuliani *et al*., 1993). It is important that new tachykinin receptor antagonists are screened in a variety of species as species-dependent tachykinin receptor homologues exist. Further, a pharmacological homology exists between all three types of human and

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guinea-pig tachykinin receptors (Maggi *et al*., 1993; Emonds-Alt *et al*., 1995) which is not the case with the rat. This makes guinea-pig tissue more suitable for studying certain tachykinin receptor antagonists with therapeutic potential for the treatment of pathophysiological conditions in humans.

In this study, the potencies of the endogenous NK_2 receptor preferring agonist, NKA as well as the selective NK_1 ([Sar^9,$Met(O_2)^{11}$]-SP), NK_2 ([Nle^{10}]-NKA(4–10) and GR 64,349) and NK_3 (senktide and [$MePhe^7$]-NKB) receptor agonists, were determined and used for receptor characterization (Drapeau *et al*., 1987; Hagen *et al*., 1991). Since major potency differences can arise from differential rates of metabolism of the various agonists (Sekizawa *et al*., 1987; Stephens-Smith *et al*., 1988; Shore & Drazen, 1989), experiments were performed in the presence of an appropriate cocktail of peptidase inhibitors. The effects of the non-peptide NK_1 (SR 140,333; Emonds-Alt *et al*., 1993), NK_2 (SR 48,968; Emonds-Alt *et al*., 1992 and GR 159,897; Beresford *et al*., 1995) and NK_3 (SR 142,801; Emonds-Alt *et al*., 1995; and SB 222,200; Giardina *et al*., 1996–compound 7b) receptor selective antagonists were also examined.

Methods

Dunkin Hartley guinea-pigs were killed by CO_2 asphyxiation and bleeding. A 2-cm length of oesophagus was removed starting 1 cm from the gastro-oesophageal junction and was opened up by cutting along its dorsal length. With the lumenal side facing up, the oesophagus was laid on a wax slab, and submerged in modified Krebs-Henseleit solution of the following composition (mM): NaCl 116, KCl 5.4, $MgSO_47H_2O$ 0.6, $NaH_2PO_42H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1 and $CaCl_2$ 2.5 gassed with 95% O_2, 5% CO_2. Two distinctive layers of the oesophageal wall were identified. The muscularis externa (dark pink) was pinned down on the wax slab, while the upper mucosal layer (white) was peeled off carefully using forceps and a scalpel blade. The mucosa was then pinned on the wax slab and slit in half longitudinally using a scalpel blade. Some experiments were performed on strips of the whole oesophagus. In these cases the tissue was dissected in the same manner. The strip was pinned out muscularis mucosae side upwards and directly slit in half longitudinally without separating the muscularis mucosae from the muscularis externa. Strips of whole oesophagus or mucosa only were set up in the same manner. Each half of tissue, measuring approximately 2 cm in length, was set up in a 3 ml siliconized (Sigmacote; Sigma, St Louis, MO, U.S.A.) organ bath containing 2.5 ml of the modified Krebs-Henseleit solution, maintained at 30°C to reduce spontaneous activity. Different experiments were performed on each preparation, so that the *n* values refer to the number of animals used.

A tension of 0.5 g weight was applied to the tissue, which was then equilibrated for 1 h, washing every 15 min. When experiments were performed on the whole oesophagus, a tension of 1.5 g weight was applied. Isometric responses were recorded from a Grass FT.03 transducer connected to a MacLab/2e unit and a Macintosh SE computer. Contractile responses were induced to a single maximal concentration of carbachol (10 μM) and repeated until reproducible (usually only two doses). The agonists were studied after the protocols for peptidase inhibitor additions and tachyphylaxis checks were performed (see following sections). Cumulative log concentration-response curves to the agonists were constructed and used to estimate the pD_2 and E_{max} values. In order to determine the apparent pK_B values of the selective antagonists, the cumulative concentration response curves of the selective agonists were repeated after 1 h equilibration with one of various selective antagonists, SR 140,333 (10 nM), SR 48,968 (0.1–30 nM), SB 222,200 (50 nM) and GR 159,897 (100 nM) or after 1 h equilibration with antagonist vehicle, which served as antagonist vehicle-time controls.

Peptidase inhibitors

As preliminary experiments showed that both captopril (10 μM) and thiorphan (10 μM) caused contractions *per se* (ca. 50% of carbachol, peaking around 7–8 min), they were added together to the tissue bath for 8 min, then washed out. When tension had returned to baseline, they were added in the same manner until no response resulted (usually after three additions). After washout, captopril, thiorphan and amastatin (20 μM) were then added together, 30 min prior to each concentration-response curve, since the maximum inhibition of aminopeptidases by amastatin has been reported to require a 30 min equilibration period (Rich *et al*., 1984).

The effect of NKA was tested in the absence of peptidase inhibitors, because it has been shown that these enzyme inhibitors do not significantly affect the response to this peptide in the guinea-pig whole oesophagus preparation (Kerr *et al*., 1997). The effects of senktide, which is protected from peptidases by the succinyl moiety, were also tested in the absence of peptidase inhibitors.

Tachyphylaxis check

After 30 min of equilibration with the peptidase inhibitors and before constructing a cumulative response curve, a single near-maximal concentration of the agonist was added to the bath for 3 min. The response was compared to the response to the same amount in the cumulative method of addition, also in the presence of peptidase inhibitors, as a check for tachyphylaxis. Peptidase inhibitors were absent in the case of NKA and senktide.

Statistics and data evaluation

The values of the responses were normalized as percentages of the response to carbachol (10 μM). Log concentration-response curves to the agonists were plotted and pD_2 and E_{max} values calculated using the program PRISM, version 2.0 (GraphPad Software, San Diego CA, U.S.A.). Points on the graphs represent mean ± s.e.mean. The concentration-response curves were generated by fitting the data to a non-linear regression using the following four-parameter logistic equation:

$$Y = a + \frac{b - a}{1 + 10^{(LogEC_{50} - Log[X])n}}$$

where Y is the response, X is the agonist concentration, a is the minimum asymptote of the curve, b is the maximum asymptote, n is the mid-point of slope and EC_{50} is the concentration of agonist producing 50% of its maximal response (E_{max}).

Significant differences between agonist concentration-response curves in the absence versus presence of antagonists or antagonist-vehicle were calculated using two-way ANOVA. Tachyphylaxis checks were determined using Student's *t*-test for paired data.

Apparent pK_B estimates for antagonists that did not cause a significant reduction in the slope of the agonist concentration-response curve, were determined from individual con-

centration-ratios using the Schild equation (Arunlakshana & Schild, 1959) for competitive inhibition at equilibrium, $pK_B = \log_{10}(CR-1) - \log_{10}[A]$, where CR is the concentration-ratio and [A] the antagonist concentration.

In the case of the non-competitive inhibition produced by SR 48,968 (i.e. significant reduction in agonist E_{max} and slope), the pK_B value was calculated using the equation:

$$K_B = \frac{[B]}{\text{slope} - 1}$$

where [B] is the antagonist concentration (Kenakin, 1997). The slope was determined by plotting the reciprocals of the agonist concentration for four points on the control concentration-response curve against against the reciprocal agonist concentrations for the four corresponding points on the concentration-response curve in the presence of the antagonist. The slope was calculated for each tissue at 0.1 nM ($n=4$) and for each tissue at 0.3 nM ($n=4$) of SR 48,968. Thus, the pK_B that was subsequently calculated represents a mean of eight values.

Drugs and solutions

[Sar^9,Met(O$_2$)^{11}]-SP, NKA, [Nle^{10}]-NKA(4–10), [MePhe^7]-NKB, senktide, amastatin and DL-thiorphan were purchased from Auspep (Melbourne, Australia). Captopril was obtained from Research Biochemicals Incorporated (Natick, MA, U.S.A.). Carbachol was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). SR 140,333, ((*S*)-1-{2-[3-(3,4,- dichlorophenyl)-1-(3-is opropoxyphenylacetyl)piperidin-3yl]ethyl}-4-phenly-1-azoniabicyclo[2.2.2]octane chloride) and SR 48,968, ((*S*)-*N*-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide) were gifts from Sanofi Recherche (Montpellier, France). GR 64,349 ([Lys^3, Gly^8-*R*-γ-lactam-Leu^9]-NKA(3–10)) and GR 159,897 ((*R*)-1-[2-(5-fluoro-1 H-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulphinyl)methyl]piperidine) were gifts from GlaxoWellcome (Hertfordshire, U.K.). SB 222,200, (*S*)-(−)-*N*-(1-Phenylpropyl)-3-methyl-2-phenylquinoline-4-carboxamide) was a gift from SmithKline Beecham (S.p.A. Milan, Italy).

[Sar^9,Met(O$_2$)^{11}]-SP and NKA were dissolved in distilled water, senktide was dissolved in pH = 7.2 buffer (6.045 g KH_2PO_4 and 15 g Na_2HPO_4 in 1.5 l distilled water), [Nle^{10}]-NKA(4–10) was dissolved in dimethyl sulphoxide (DMSO), [MePhe^7]-NKB and GR 64,349 were dissolved in acetic acid 0.02 M and 0.01 M, respectively. They were all made into 2.5 mM stock solutions, diluted further in normal saline and stored frozen in small aliquots. GR 159,897 was dissolved in distilled water. All other non-peptide antagonists were dissolved in absolute alcohol and stored refrigerated as 2.5 mM stock solutions for up to 14 days. Stock solutions of captopril (2.5 mM) made in normal saline were stored refrigerated. Stock solutions of DL-thiorphan (2.5 mM) and amastatin (1.67 mM) in 5% ethanol, and 0.01 M HCl, respectively, were made into aliquots and stored frozen. Carbachol was dissolved in $NaH_2PO_42H_2O$ buffer (pH = 4) as a stock solution of 25 mM. All subsequent dilutions were made in normal saline.

Results

Effects of selective agonists

No contractile responses occurred to cumulative additions of [Sar^9,Met(O$_2$)^{11}]-SP, [MePhe^7]-NKB and senktide at concentrations up to 3 μM (Figure 1a,c). Although, a small contraction occurred in response to 10 μM [MePhe^7]-NKB, the isolated mucosal preparation was insensitive to the other NK_3 receptor-selective agonist, senktide, even at high concentrations (Figure 1c). However, senktide contracted the whole oesophagus with a pD_2 value of 8.89 ± 0.09, and an E_{max} value of 29.78 ± 1.17% ($n=7$) relative to the internal standard, carbachol.

Large responses were elicited by the selective NK_2 receptor agonists, [Nle^{10}]-NKA(4–10) and GR 64,349, which produced maximum responses equivalent to 65.8 ± 3% ($n=14$)

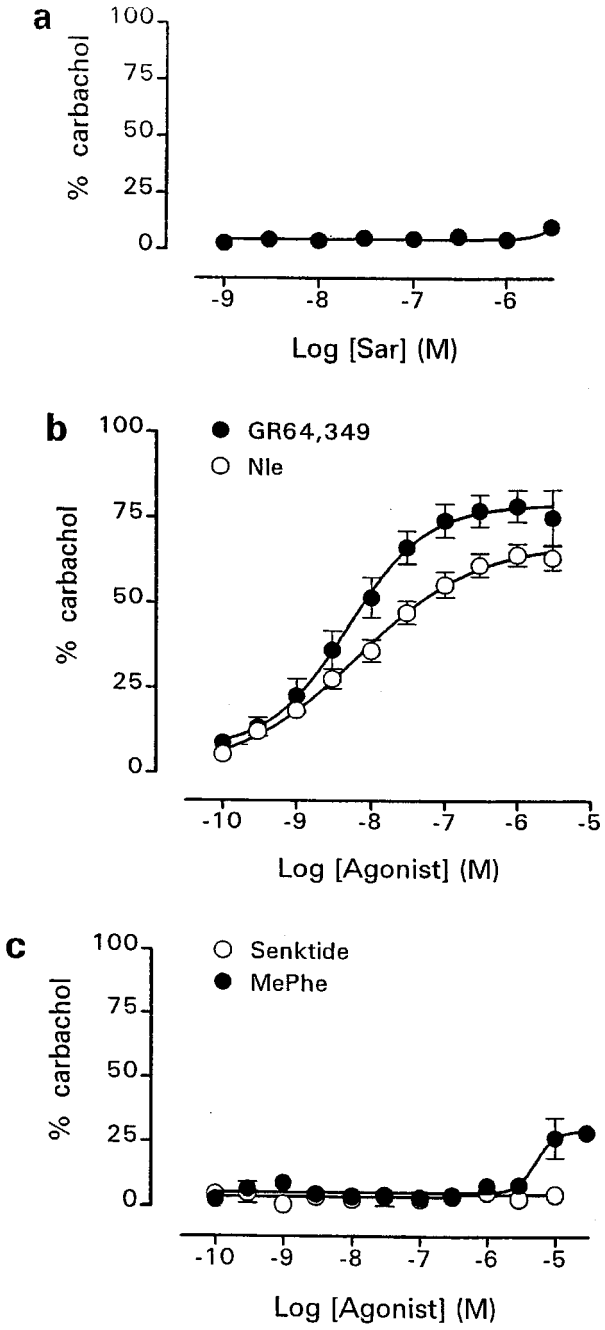

Figure 1 Concentration-response curves for (a) the NK_1 receptor-selective agonist, [Sar^9,Met(O$_2$)]-SP (Sar; $n=3$), (b) the NK_2 receptor-selective agonists, ([Nle^{10}]-NKA(4–10) (Nle; $n=14$), and GR 64,349 ($n=5$) and (c) the NK_3 receptor-selective agonists, [MePhe^7]-NKB (MePhe; $n=4$) and senktide ($n=3$). Vertical lines show s.e.mean. Error bars that are not shown lie within the dimensions of the symbol in this and all other figures.

and 79.6±3.5% ($n=5$) of the internal standard, carbachol, respectively (Figure 1b). The pD_2 values for [Nle^{10}]-NKA(4–10) and GR 64,349 were 8.20±0.16 ($n=14$) and 8.30±0.15 ($n=5$), respectively. DMSO and acetic acid (0.02 M), vehicles for [Nle^{10}]-NKA(4–10) and [MePhe^7]-NKB, respectively, did not contract the muscularis mucosae, nor did they alter the baseline tension of the tissue, at concentrations used to dissolve the agonists ($n=3$).

There were no significant differences ($P>0.05$) between the E_{max} values obtained after the addition of a single near-maximal concentration of each agonist (GR 64,349, 30 nM, $n=4$; NKA 30 nM, $n=11$; [Nle^{10}]-NKA(4–10), 300 nM, $n=8$ in the muscularis mucosae and senktide, 3 nM, $n=5$ in the whole oesophagus) and the responses induced by the same concentrations achieved by cumulative addition of the agonists, indicating an absence of tachyphylaxis. In the isolated muscularis mucosae, a single maximum concentration of [Sar^9,Met(O$_2$)^{11}]-SP (10 nM, $n=3$) and a single maximum concentration of [MePhe^7]-NKB (10 nM, $n=4$) gave no response. The concentration of 10 nM was chosen for the maximal concentration as the work of Kerr *et al.* (1997) showed that the maximal response to both of these agonists at selective concentrations in the whole oesophagus occurred at 10 nM. A single near-maximal concentration of senktide (3 nM, $n=3$), based on the concentration-response curve obtained in the whole oesophagus gave no response in the isolated muscularis mucosae. In addition, preliminary experiments in the isolated mucosa preparation, showed that maximal responses to the agonists remained stable until washout (approximately 10 min).

Effects of NK_1, NK_2 and NK_3 receptor-selective antagonists against NKA

NKA produced a concentration-dependent contractile response with an E_{max} equivalent to 71.4%±1.56% of the internal standard, carbachol and a pD_2 value of 8.13±0.04 ($n=12$).

The NK_1 receptor selective-antagonist, SR 140,333 (10 nM, $n=4$) and the NK_3 receptor-selective antagonist, SB 222,200 (50 nM, $n=4$), did not significantly affect the position or slope of the concentration effect curve to NKA on the guinea-pig oesophageal muscularis mucosae ($P>0.05$, Figure 2a,c). On the other hand, the selective NK_2 receptor antagonist, GR 159,897 (100 nM,) shifted the curve significantly to the right ($P<0.0001$), without suppressing the E_{max} of NKA. (Figure 2b). The apparent pK_B value of GR 159,897 was 8.71±0.19 ($n=4$).

The vehicle for these three antagonists (absolute alcohol), did not alter the concentration-response curve to NKA ($P>0.05$) when incubated for 60 min at a concentration equivalent to that present in the final bath concentration when SB 222,200 was used ($n=3$). Neither the antagonists nor the vehicle modified the baseline tension of the muscosal preparation.

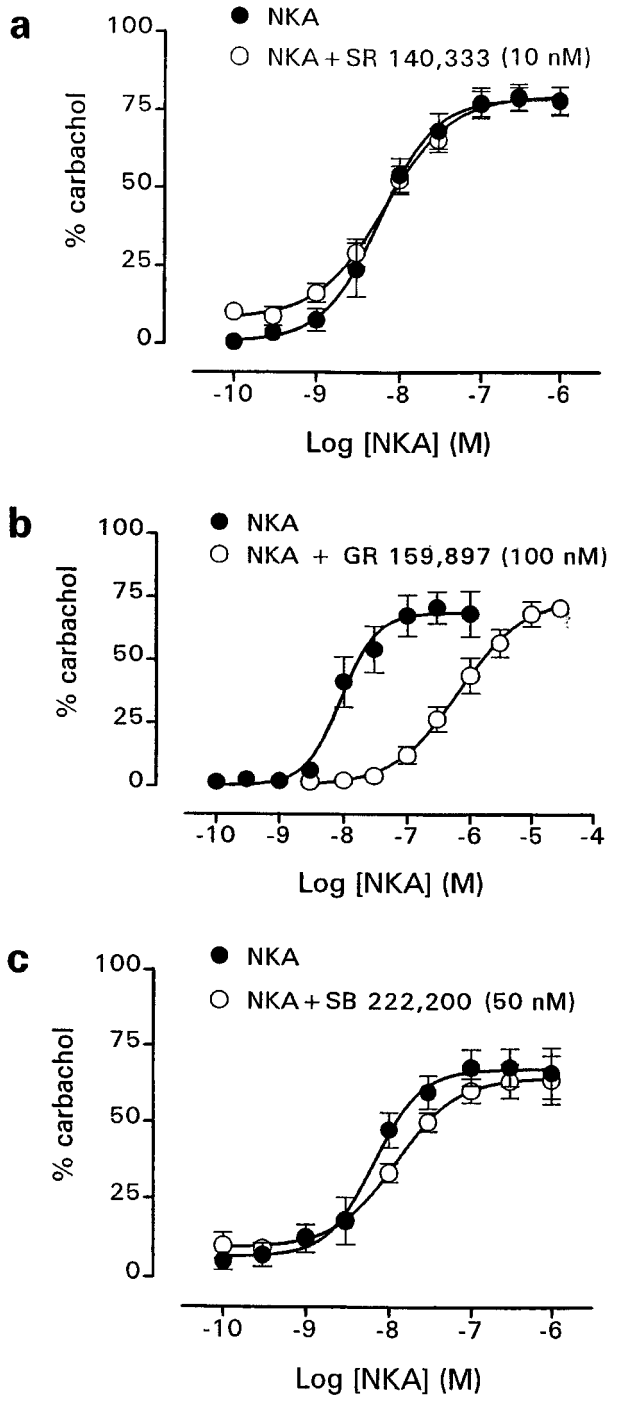

Figure 2 Concentration-response curves for NKA alone ($n=4$) and in the presence of (a) the NK_1 receptor-selective antagonist, SR 140,333 ($n=4$), (b) the NK_2 receptor-selective antagonist, GR 159,897 ($n=4$) and (c) the NK_3 receptor-selective antagonist, SB 222,200 ($n=4$).

Effects of NK_2 receptor-selective antagonists against NK_2 receptor-selective agonists

The NK_2 receptor-selective antagonist, GR 159,897 (100 nM), caused a significant rightwards displacement of the concentration-response curve of the NK_2 receptor-selective agonist, GR 64,349, ($P<0.0001$) without reducing the E_{max} indicating an apparent competitive antagonism (Figure 3a). The apparent pK_B value of GR 159,897 was 9.29±0.16 ($n=5$). There was no significant shift of the concentration-response curve when repeated after 1 h ($n=3$), in the presence of vehicle (distilled water).

The NK_2 receptor-selective antagonist, SR 48,968, significantly shifted the concentration-response curve of the NK_2 receptor-selective agonist, [Nle^{10}]-NKA(4–10) to the right in a concentration-dependent manner. The E_{max} of [Nle^{10}]-NKA(4–10) was reduced by 12.3% at 0.1 nM ($n=4$), 45.6% at 0.3 nM ($n=4$) and by 65.7% at 30 nM ($n=3$), indicating a non-competitive antagonism (Figure 3b). The apparent pK_B value was calculated to be 10.84±0.19 ($n=8$).

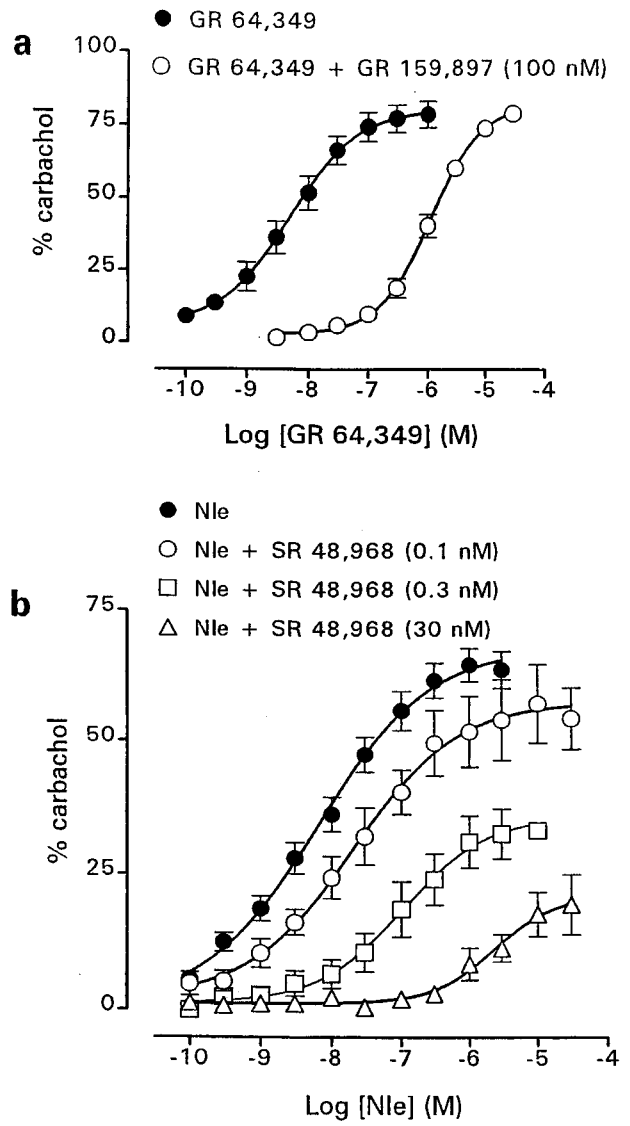

Figure 3 Concentration-response curves for the NK_2 receptor-selective agonists (a) GR 64,349 alone ($n=5$) and in the presence of the NK_2 receptor-selective antagonist, GR 159,897 ($n=5$), and (b) [Nle^{10}]-NKA(4–10) (Nle) alone ($n=14$) and in the presence of the NK_2 receptor-selective antagonist, SR 48,968 ($n=4$ for all concentrations, except for 30 nM, where $n=3$).

The antagonist vehicle-time control ($n=3$) showed no significant alteration of the agonist cumulative concentration-response curve ($P>0.05$). Neither SR 48,968 nor its vehicle (absolute alcohol) altered the baseline tension of the tissue.

Effects of peptidase inhibitors

Amastatin had no effect ($n=21$) on the baseline tension of the oesophageal strips of isolated muscularis mucosae. However, captopril and thiorphan, added together, exhibited gradual, sustained contractile responses that took 7–8 min to reach a plateau. The contraction was 51.7±4.4% ($n=21$) of the internal standard. When the tissue tension had returned to baseline after washing and resting, the re-addition of captopril and thiorphan produced a much lower contractile response (28.1±3.8%, $n=21$). Usually by the third addition of the two peptidase inhibitors, the tissue no longer produced a contractile response.

Discussion

The results of this study indicate that the guinea-pig isolated mucosal preparation is an NK_2 monoreceptor system. The NK_2 receptor-selective agonists, [Nle^{10}]-NKA(4–10) and GR 64,349 were potent in eliciting contractile effects in this preparation, whereas the NK_1 and NK_3 receptor-selective agonists were without effect. Further, the concentration-response curve to the non-selective agonist, NKA, was only shifted significantly rightwards by an NK_2 receptor-selective antagonist, while the NK_1 and NK_3 receptor-selective antagonists had no significant effect.

Agonist effects

No appreciable contractile responses occurred to the NK_1 receptor-selective agonist, [Sar^9, $Met(O_2)^{11}$]-SP nor to the NK_3 receptor-selective agonists, [$MePhe^7$]-NKB and senktide. Although contractile responses did occur in response to [$MePhe^7$]-NKB at concentrations greater than 3 μM, it has been found that [$MePhe^7$]-NKB stimulates NK_2 receptors in micromolar concentrations (Maggi, 1995; Kerr *et al.*, 1997). While the NK_3 receptor-selective agonist, senktide, elicited contractile responses over the nanomolar concentration range in the whole oesophageal preparation, these responses were absent in the isolated mucosal preparation.

Strong contractile responses occurred to the NK_2 receptor-selective agonists, [Nle^{10}]-NKA(4–10) and GR 64,349 as well as to NKA. Kerr *et al.* (1997) found a pD_2 value of 7.2 for [Nle^{10}]-NKA(4–10) in the guinea-pig whole oesophagus. It is suggested that this lower value, compared to that of 8.2 obtained in the current study may have resulted from the 10 fold lower concentrations of the peptidase inhibitors, thiorphan and captopril, used in that study.

Concentration-response curves to GR 64,349 were performed in the presence of peptidase inhibitors since Hagan *et al.* (1991) showed, in experiments on the guinea-pig trachea, that the potency of GR 64,349 was increased 4.8 fold in the presence of the endopeptidase 24.11 inhibitor, phosphoramidon and the aminopeptidase inhibitor, bestatin. The pD_2 value of the NK_2 receptor-selective agonist, GR 64,349 was 8.3 in the current experiments. This is similar to the value of 8.4 obtained by Ireland *et al.* (1991) in the rat colon muscularis mucosae.

In the current experiments, the pD_2 value of 8.1 for the natural tachykinin, NKA, was similar to those obtained, in other NK_2 monoreceptor systems in the absence of peptidase inhibitors, for example 8.2 in the rabbit endothelium-denuded pulmonary artery (Drapeau *et al.*, 1987), and 8.3 in the human colon (Croci *et al.*, 1998).

Antagonist effects

NKA acts preferentially, but not selectively on NK_2 receptors (Regoli *et al.*, 1994). While the NK_2 receptor-selective antagonist, GR 159,897, shifted the concentration-response curve to NKA significantly rightwards, the NK_1 receptor-selective antagonist, SR 140,333 and the NK_3 receptor-selective antagonist SB 222,200 failed to do so. These results provide further evidence for the lack of NK_1 and NK_3 receptors.

The apparent pK_B value for the NK_2 receptor-selective antagonist, GR 159,897, was 8.7 against NKA and 9.3 against GR 64,349. Sheldrick *et al.* (1995) obtained a similar pA_2 value of 8.6 for GR 159,897 in the human bronchus, where contractile effects are mediated only by NK_2 receptors.

The apparent pK_B value for the NK_2 receptor-selective antagonist, SR 48,968 was 10.8. This value is close to that of 10.5 for SR 48,968 in the guinea-pig trachea (Advenier *et al*., 1992). Kerr *et al*. (1997) determined an apparent pK_B value of 8.7 for SR 48,968 in the guinea-pig whole oesophagus. However, they had assumed a competitive antagonism as they were unable to obtain a maximum response to the agonist, [Nle^{10}]-NKA(4–10), in the presence of the antagonist as the agonist vehicle (0.1 M ammonia) at agonist concentrations greater than 3 μM produced a response. In the current experiments, DMSO, which had no effect on the tissue response, was used to dissolve the agonist. These results clearly show that SR 48,968 acts non-competitively. Another reason for the higher pK_B value for SR 48,968 may be that in the isolated mucosa, the receptors are more accessible to the antagonist, than they are in the whole oesophagus when a longer binding time may be required. Advenier *et al*. (1992), showed that the pA_2 value of SR 48,968 can be affected by the incubation time ($pA_2 = 7.5$ at 10 min and 8.1 at 120 min).

The non-competitive nature of the antagonist, SR 48,968 has been demonstrated in other tissues of the guinea-pig, namely the isolated gallbladder and colon (Patacchini *et al*., 1994). These authors showed that the non-parallel rightward shifts of the curves and the depression of the maximal response were dependent on both the antagonist concentration and the incubation time. On the other hand, Maggi *et al*. (1994) had shown that SR 48,968 behaved as a competitive antagonist in the guinea-pig colon. However, Patacchini *et al*. (1994) proposed that the NK_2 receptor-selective agonist, [βAla^8]-NKA(4–10), which was used in the experiments of Maggi *et al*. (1994) was able to stimulate NK_1 receptors at high concentrations. Thus, the nature of the antagonism of SR 48,968 appeared competitive. This phenomenon reinforces the advantage of using monoreceptorial tissues when investigating the effects of new compounds.

Effects of peptidase inhibitors

The peptidase inhibitors, captopril and thiorphan, produced contractile responses and the experimental protocol required the addition and subsequent washing out until these contractions no longer occurred. It is possible that contractile responses to the endogenous peptide(s) no longer occurred because depletion of the peptide(s). It might be argued also that if the endogenous peptide(s) acted on NK_1 or NK_3 receptors to cause desensitization, this would account for the absence of any response to the NK_1 and NK_3 receptor-selective agonists in these experiments. However, this is not the case since the concentration response curves to NKA were performed in the absence of peptidase inhibitors. Thus, if NK_1 or NK_3 receptors were present they would not have been desensitized by the effects of the captopril and thiorphan. The concentration-response curve to NKA would have been expected to be shifted to the right in the presence of either the NK_1 receptor-selective antagonist or the NK_3 receptor-selective antagonist, which, however was not the case. Also, the effects of the NK_3 receptor-selective agonist, senktide, were performed in the absence of peptidase inhibitors and the lack of response in the isolated mucosa cannot, therefore be attributed to NK_3 receptor desensitization by the indirect action of captopril and thiorphan.

Skidgel *et al*. (1984) have shown that other neuropeptides besides the tachykinins, such as neurotensin, angiotensin I and bradykinin are susceptible to degradation by angiotensin converting enzyme (inhibited by captopril) and endopeptidase-24.11 (inhibited by thiorphan). Contractile responses to captopril and thiorphan indicate the presence of one or more endogenous peptides that are slowly released and may be responsible for the maintenance of oesophageal tone. Further work is required to identify the peptides.

In conclusion, the guinea-pig isolated oesophageal mucosa is an NK_2 monoreceptor system. Responses to various NK_2 receptor agonists are reproducible and maintained at a stable plateau showing that desensitization does not occur. Since a pharmacological homology exists between guinea-pig and human tachykinin receptors, this preparation will be useful for screening new NK_2 receptor-selective ligands, both agonists and antagonists, that may be of therapeutic benefit.

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