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GR159897, a potent non-peptide antagonist at tachykinin NK_2 receptors

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Abstract

GR159897 ((*R*)-1-[2-(5-fluoro-1*H*-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]piperidine) is a novel, highly potent and selective non-peptide antagonist at tachykinin NK_2 receptors. GR159897 inhibited binding of the NK_2 receptor antagonist radioligand [^{3}H]cyclohexylcarbonyl-Gly-Ala-(D)Trp-Phe-NMe_2 ([^{3}H]GR100679) to human ileum NK_2 receptors transfected into Chinese hamster ovary cells (pK_i 9.5) and to rat colon membranes (pK_i 10.0). GR159897 was a competitive antagonist of contractions induced by the NK_2 receptor agonist [Lys^3,Gly^8-*R*-γ-lactam-Leu^9]neurokinin A-(3–10) (GR64349) in guinea-pig trachea (pA_2 8.7), and had negligible activity at human NK_1 receptors transfected into Chinese hamster ovary cells (pK_i 5.3), NK_1 receptors in guinea-pig trachea ($pK_B < 5$) or NK_3 receptors in guinea-pig cerebral cortex ($pK_i < 5$). In vivo, in the anaesthetised guinea-pig, GR159897 (0.12 $mg \cdot kg^{-1}$ i.v.) potently antagonised bronchoconstriction induced by GR64349 (dose-ratio = 28), with a long duration of action (3 h). GR159897 should be a useful tool for studying the physiological and pathophysiological role of tachykinin NK_2 receptor activation.

Keywords: Tachykinin receptor; Tachykinin NK_2 receptor; Neurokinin A; GR159897

1. Introduction

Receptors for the mammalian tachykinins, substance P, neurokinin A and neurokinin B, have been classified into three types, NK_1, NK_2 and NK_3. Tachykinin NK_2 receptors, at which neurokinin A has highest affinity, are widely distributed in the periphery, where they mediate a number of actions, most notably smooth muscle contraction in gastrointestinal, respiratory, genito-urinary and vascular systems (for review see Maggi et al., 1993). Although they appear to have a limited distribution within the central nervous system, they do seem to be involved in a number of central functions, including nociception, water homeostasis and locomotor stimulation (Hagan et al., 1993). Recently, an anxiolytic-like action of antagonists at NK_2 receptors has been reported in putative rodent models of anxiety (Stratton et al., 1993).

A number of peptidic antagonists at NK_2 receptors have been described (Table 1). While several structurally diverse non-peptide antagonists at NK_1 receptors have been identified (Table 1), to date only one non-peptide antagonist at NK_2 receptors, SR48968 (Emonds-Alt et al., 1992; Table 1), has been described. We now report the in vitro pharmacology of (*R*)-1-[2-(5-fluoro-1*H*-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]piperidine (GR159897; Fig. 1), which is a novel, low molecular weight, non-peptide antagonist at tachykinin NK_2 receptors. GR159897 was developed using a combination of rational design and directed screening (Cooper et al., 1994). Using a deletion-optimisation approach, we previously identified low molecular weight peptide antagonists with high affinity and selectivity for NK_2 receptors (Smith et al., 1993). Using these compounds as templates, directed screening to yield weak non-peptide antagonists at NK_2 receptors and subsequent optimisation of these leads resulted in the development of GR159897.

Studies using a range of antagonists at NK_2 receptors in a number of species have indicated that NK_2

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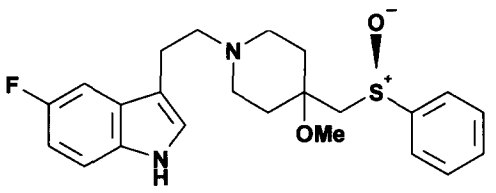

Fig. 1. Structure of GR159897.

antagonist affinities are species-dependent. There is evidence that NK_2 receptors can largely be resolved into two groups, those in human, cow, rabbit and guinea-pig being distinct from those in rat and hamster (Maggi et al., 1993). GR100679 is a potent and selective tetrapeptide antagonist at NK_2 receptors (NK_2 pK_B rat colon 9.1; Smith et al., 1993). We have previously demonstrated that [^{3}H]GR100679 binds with high affinity (K_D 0.6 nM) to recombinant NK_2 receptors from human ileum, expressed in Chinese hamster ovary (CHO) cells (hNK_2-CHO; Hagan et al., 1993). In the present study, we have determined the potency of the non-peptide antagonist, GR159897, at NK_2 receptors in species which are representative of each of the subgroups of NK_2 receptors. Thus, we have determined the affinity of GR159897 for [^{3}H]GR100679 binding sites in hNK_2-CHO cells and also in rat colon membranes, a well characterised NK_2 receptor-containing preparation (Hagan et al., 1991b; Ireland et al., 1991). In addition, we have determined the ability of GR159897 to antagonise contractions induced by the selective NK_2 agonist [Lys^3,Gly^8-*R*-γ-lactam-Leu^9]neurokinin A-(3–10) (GR64349; Hagan et al., 1991b) in guinea-pig isolated trachea. We have evaluated the activity of GR159897 in vivo by measuring its ability to antagonise GR64349-induced bronchoconstriction in the anaesthetised guinea-pig. Since GR159897 has some structural similarities to the antihypertensive agent, indoramin (Holmes and Sorkin, 1986), a compound with well-established blocking activity at 5-HT receptors and α_1-adrenoceptors, we have examined the specificity of GR159897 with respect to these two receptor types. A preliminary account of some of these findings has been presented to the British Pharmacological Society (Ball et al., 1994).

2. Materials and methods

2.1. Materials

[^{3}H]GR100679 (70–90 Ci · mmol^{-1}) was prepared by Amersham. [^{3}H]Succinyl-[Asp^6,N-MePhe^8]-substance P-(6–11) ([^{3}H]senktide; 83 Ci · mmol^{-1}), [^{3}H](3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone ([^{3}H]GR65630; 60–70 Ci · mmol^{-1}) and [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT; 150–200 Ci · mmol^{-1}) were purchased from Du Pont and [^{3}H]substance P (127 Ci · mmol^{-1}) from Cambridge Research Biochemicals. GR159897, GR100679, GR94800, CP99994 and (±)-SR48968 were synthesised by Medicinal Chemistry, Glaxo Research and Development. GR64349 was obtained from Peptide and Protein Research, University of Exeter. Substance P methyl ester and senktide were purchased from Peninsula, prostaglandin $F_{2\alpha}$ from Upjohn, methysergide maleate from Sandoz, mepyramine maleate from Rhône Poulenc, atropine sulphate, indomethacin, phenylephrine and phosphoramidon from Sigma, ketamine hydrochloride (Vetalar) from Parke-Davis, xylazine (Rompun) from Bayer, and sodium pentobarbitone (Sagatal) from Rhône Mérieux.

2.2. Methods

2.2.1. [^{3}H]GR100679 binding assays

To prepare rat (male, LH Glaxo) colon membranes, the entire colon was homogenised in Tris HCl (20 mM; pH 7.4), containing EDTA (10 mM) and centrifuged at 48 000 × *g* for 15 min at 4°C. The pellet was homogenised in Tris HCl (50 mM; pH 7.4), containing NaCl (120 mM), incubated on ice for 30 min and centrifuged at 48 000 × *g* for 15 min at 4°C. The pellet was homogenised (15 mg wet weight · ml^{-1}) in Tris HCl (50 mM; pH 7.4) assay buffer, containing NaCl

Table 1
Some tachykinin NK_1 and NK_2 receptor antagonists

Receptor	Chemical structure	Code	Reference
NK_1	(+)-(2*S*,3*S*)-3-(2-Methoxy-benzylamino)-2-phenylpiperidine	CP99994	McLean et al., 1993
NK_1	(3α*R*,7α*R*)-7,7-Diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one	RP67580	Garret et al., 1991
NK_1	(*S*)-1-[2-[3-(3,4-Dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl]-4--phenyl-1-azoniabicyclo[2.2.2]octane · chloride	SR140333	Emonds-Alt et al., 1993a
NK_2	Cyclo(Gln-Trp-Phe-Gly-Leu-Met)	L659877	Williams et al., 1988
NK_2	Phenylcarbonyl-Ala-Ala-(D)Trp-Phe-(D)Pro-Pro-Nle · NH_2	GR94800	McElroy et al., 1992
NK_2	Cyclohexylcarbonyl-Gly-Ala-(D)Trp-Phe-NMe_2	GR100679	Smith et al., 1993
NK_2	(*S*)-*N*-Methyl-*N*[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]--benzamide	SR48968	Emonds-Alt et al., 1992

(100 mM), $MgCl_2$ (2 mM), bovine serum albumin (0.02%) and phosphoramidon (1 μM) and filtered through nylon mesh (300 μm). Freshly prepared membranes (200 μl) were incubated at 22°C for 90 min with [^{3}H]GR100679 (0.5 nM) and GR159897 (0.01 nM–10 μM). The reaction was terminated by vacuum filtration through Whatman GF/B filters pre-soaked in bovine serum albumin (0.5%). Filters were washed (5 × 1 ml) with ice-cold Tris HCl assay buffer and bound radioactivity determined. Chinese hamster ovary (CHO) cells were stably transfected with the human ileum NK_2 receptor (hNK_2-CHO) and [^{3}H]GR100679 binding to whole CHO cells performed as previously described (Hagan et al., 1993). Confluent cells (2.5×10^5) were incubated in Tris HCl assay buffer (composition as for rat colon assay) at 22°C for 90 min with [^{3}H]GR100679 (1 nM) and GR159897 (0.01 nM–10 μM). Non-specific binding was defined using GR94800 (1 μM). Bound and free ligand were separated by washing cells (3 × 200 μl) in ice-cold Tris buffer and radioactivity in the assay well determined.

2.2.2. [^{3}H]Substance P binding to hNK_1-CHO membranes

The affinity of GR159897 for NK_1 receptors was determined using a [^{3}H]substance P binding assay in CHO cells stably transfected with the human NK_1 receptor (hNK_1-CHO). Membranes were prepared in a Hepes (50 mM; pH 7.4) buffer essentially as described by Cascieri et al. (1992) and incubated (25 μg protein) for 40 min with [^{3}H]substance P (0.7 nM) in the presence of GR159897 (0.1–100 μM). Non-specific binding was defined using CP99994 (1 μM). The reaction was terminated by vacuum filtration and bound radioactivity determined.

2.2.3. [^{3}H]Senktide binding to guinea-pig cortical membranes

The affinity of GR159897 for NK_3 receptors was determined in a [^{3}H]senktide binding assay in guinea-pig cerebral cortical membranes, performed essentially as described by Guard et al. (1990). Membranes (8 mg per tube) were incubated at 22°C for 60 min with [^{3}H]senktide (1 nM) in the presence of GR159897 (0.1–100 μM). Non-specific binding was defined using senktide (1 μM). The reaction was terminated by vacuum filtration and bound radioactivity determined.

2.2.4. Guinea-pig isolated trachea

Tracheal strips (2–3 cartilage rings thick) were obtained from guinea-pigs (male, Dunkin Hartley, Porcellus, 300–800 g). The lumen of the trachea was rubbed with a moist cotton bud to remove the epithelium, an effect that was confirmed histologically. Tracheal strips were mounted for isometric tension recording in silanised organ baths containing modified Krebs-Henseleit (Krebs) solution (mM: NaCl (118.4), KCl (4.7), $NaHCO_3$ (25), $MgSO_4 \cdot 7H_2O$ (0.6), KH_2PO_4 (1.2), D-glucose (11.1), $CaCl_2 \cdot 6H_2O$ (1.3)), maintained at 37°C and gassed with 95% O_2/5% CO_2. The Krebs solution also contained indomethacin (2.8 μM), atropine (1 μM), mepyramine (1 μM) and methysergide (1 μM) in an attempt to prevent any indirect actions of tachykinins. A resting tension of 1 g was applied to the tissues, which was maintained throughout the course of the experiment. The neutral endopeptidase inhibitor, phosphoramidon (1 μM), was added to the Krebs solution approximately 15 min prior to the addition of test drugs, in order to prevent any tissue metabolism of tachykinins. Cumulative concentration-effect curves to the NK_1 receptor selective agonist, substance P methyl ester (1–300 nM), the NK_2 receptor selective agonist, GR64349 (1–300 nM), or prostaglandin $F_{2\alpha}$ (0.1–10 μM) were repeated until constant sensitivity was obtained. A final concentration-effect curve was then constructed in the presence (45 min pre-equilibration) of GR159897 (0.01–10 μM).

2.2.5. 5-HT and α_1-adrenoceptor selectivity studies

The affinity of GR159897 for 5-HT_{1A} receptors was determined in a [^{3}H]8-OH-DPAT binding assay in rat hippocampal membranes, performed essentially as described by Mir et al. (1988). 5-HT_3 receptor affinity was determined in a [^{3}H]GR65630 binding assay in membranes prepared from rat entorhinal cortex (Kilpatrick et al., 1987). The potency of GR159897 at 5-HT_2 receptors and α_1-adrenoceptors was assessed by its ability to antagonise contractions in rabbit thoracic aorta induced by 5-HT (Apperley et al., 1976) and phenylephrine (Stubbs et al., 1991), respectively.

2.2.6. Anaesthetised guinea-pig

Guinea-pigs (source as above) were anaesthetised with ketamine (40 mg · kg^{-1} i.m.), xylazine (8 mg · kg^{-1} i.m.), and sodium pentobarbitone (30 mg · kg^{-1} i.p.). Animals were artificially respired (8 ml · kg^{-1} · breath^{-1}, and 50 breaths · min^{-1}), and tracheal inflation pressure was measured as an index of bronchoconstriction. The left carotid artery was cannulated to measure blood pressure, from which heart rate was derived, and the right jugular vein was cannulated to allow i.v. drug administration. Body temperature was maintained at 38°C. Sequential dose-response curves to i.v. GR64349 (27–276 ng · kg^{-1}) were repeated until constant increases in tracheal inflation pressure were obtained (2 curves). GR159897 (0.12 mg · kg^{-1} i.v.) was then administered, after which single doses of GR64349 (92–4600 ng · kg^{-1}) were administered at 2–44 min intervals, from which dose-ratios were calculated. Values of dose-ratio were obtained by dividing the dose of GR64349 administered after the antagonist, GR159897, by that required to produce the same response before

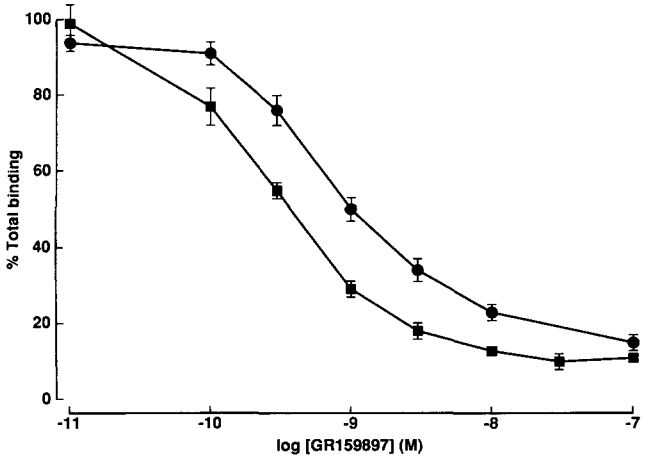

Fig. 2. Inhibition by GR159897 of [^{3}H]GR100679 binding to hNK$_2$-CHO cells (●) or rat colon membranes (■). Results are expressed as a percentage inhibition of total binding. Data shown are mean ± S.E.M. obtained in 10 (hNK$_2$-CHO) and 5 (rat colon) separate experiments, with each point determined in triplicate.

GR159897 (control equi-effective dose). The control equi-effective dose was determined from the final dose-response curve to GR64349 prior to administration of GR159897. Duration of action was defined as the time over which GR159897 caused a dose-ratio of ≥ 5.

2.2.7. Data analysis

Functional data in guinea-pig trachea and the in vivo potency of GR159897 in the anaesthetised guinea-pig are expressed as mean values with 95% confidence limits. All other results are expressed as mean ± S.E.M. Binding data were analysed using the curve-fitting program ALLFIT and IC_{50} and pK_i values determined. The affinity of GR159897 in guinea-pig trachea was expressed as a pA_2 value determined as described by Arunlakshana and Schild (1959). The apparent affinity (pK_B) of GR159897 in other functional assays was estimated as described previously (Ireland et al., 1991).

3. Results

3.1. Radioligand binding studies

GR159897 competed for binding of [^{3}H]GR100679 to hNK$_2$-CHO cells with a pK_i of 9.51 ± 0.07 ($n = 10$) and a slope (1.06 ± 0.04) not significantly different from unity (Fig. 2). GR159897 inhibited binding of [^{3}H]GR100679 to rat colon membranes with a pK_i of 10.00 ± 0.10 ($n = 5$) and a slope (1.04 ± 0.10) not significantly different from unity (Fig. 2).

Comparative data for the ability of tachykinin NK$_1$ and NK$_2$ receptor agonists and antagonists to compete for [^{3}H]GR100679 binding sites in hNK$_2$-CHO cells and rat colon membranes are given in Tables 2 and 3. In rat colon membranes, those agonists for which competition curves could be constructed (neurokinin A, GR64349 and neurokinin B) competed for [^{3}H]GR100679 binding with slopes which were significantly less than unity ($P < 0.05$). Since calculation of inhibition constants (K_i) was therefore inappropriate, IC_{50} values are given in Table 2. In no case was the slope of the antagonist competition curve significantly different to unity ($P > 0.05$).

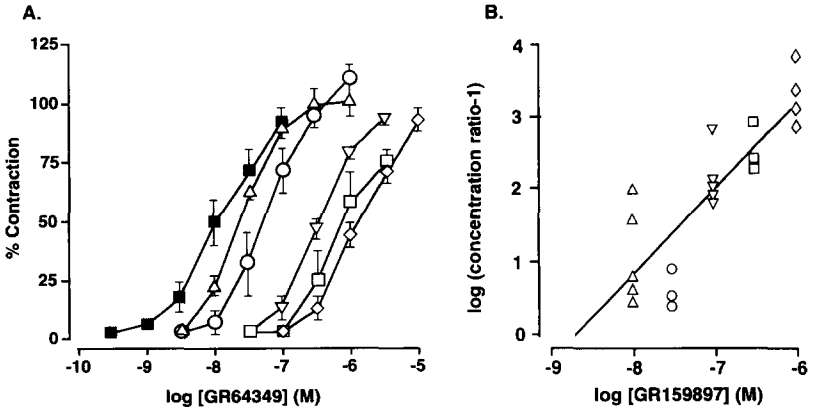

Fig 3. Antagonism by GR159897 of contractions induced by GR64349 in guinea-pig isolated trachea. A: Cumulative concentration-effect curves were constructed to GR64349 in the absence (■), and in the presence of GR159897 10 nM (△), 30 nM (○), 100 nM (▽), 300 nM (□) and 1 μM (◇). Data shown are mean ± S.E.M. The control curve represents the means of time-matched curves constructed on a non-GR159897 treated preparation included in each experiment. Responses of all curves are calculated as a percentage of the response obtained to GR64349 (300 nM) in the preceding control curve for each preparation. B: Schild analysis for GR159897 antagonism of GR64349-induced responses in guinea-pig trachea. Data were derived from the experiments illustrated in the upper panel. Each point represents data obtained from a separate preparation.

Table 2
Potencies of tachykinin NK_1 and NK_2 receptor agonists to compete for binding of [^{3}H]GR100679 to hNK_2-CHO cells and rat colon (RC) membranes

Agonist	hNK_2-CHO K_i (nM)[a]	RC pK_i IC_{50} (nM)
Neurokinin A	1.2	288 ± 96 (0.53 ± 0.03*)
GR64349	8.6	2856 ± 819 (0.55 ± 0.06*)
Neurokinin B	22	4430 ± 1027 (0.75 ± 0.04*)
Substance P	4792	> 30 μM
Substance P methyl ester	4648	> 30 μM
Senktide	> 30 μM	> 30 μM

[a] K_i values from Hagan et al., 1993. Slope values were not significantly different to unity. Results in rat colon membranes are IC_{50} values ± S.E.M. from 3–6 separate experiments performed in triplicate. Slope values are indicated in parentheses. *$P < 0.05$.

3.2. Functional assay

In guinea-pig trachea, GR64349, substance P methyl ester and prostaglandin $F_{2\alpha}$ caused concentration-related contractions. GR64349-induced concentration-contraction curves were displaced to the right by GR159897 (0.01–10 μM) in a concentration-dependent and parallel manner, with no change in the maximum response (Fig. 3). Schild analysis yielded a pA_2 of 8.72 (95% confidence limits 8.31–9.40), with a slope not significantly different from unity (1.16, 0.84–1.47; $n = 6$; Fig. 3). GR159897 (10 μM) did not antagonise NK_1 receptor-mediated contractions induced by substance P methyl ester ($pK_B < 5.00$; $n = 4$), or those induced by prostaglandin $F_{2\alpha}$ ($pK_B < 5.00$; $n = 4$).

3.3. Specificity studies

GR159897 had negligible affinity for tachykinin NK_1 (hNK_1-CHO) or NK_3 (guinea-pig cerebral cortex) receptor binding sites, with pK_i values of 5.34 ± 0.04 ($n = 3$) and < 5.00 ($n = 2$), respectively. GR159897 had little affinity for 5-HT_{1A} ([^{3}H]8-OH-DPAT) binding sites in rat hippocampus ($pK_i = 6.06 \pm 0.11$; $n = 3$) and was inactive at 5-HT_3 binding sites ([^{3}H]GR65630) in rat entorhinal cortex ($pK_i < 5.00$, $n = 2$). Furthermore, in rabbit thoracic aorta, GR159897 did not antagonise 5-HT_2 receptor mediated contractions ($pK_B < 6.00$, $n = 2$), and was only weakly active against α_1-adrenoceptor mediated contractions (pK_B 6.78 ± 0.11; $n = 4$; Fig. 4).

Table 3
Affinities of tachykinin NK_1 and NK_2 receptor antagonists to compete for binding of [^{3}H]GR100679 to hNK_2-CHO cells and rat colon (RC) membranes

Antagonist	hNK_2-CHO pK_i	RC pK_i
GR159897	9.51 ± 0.07	10.00 ± 0.10
(±)-SR48968	9.9 [a]	8.82 ± 0.10
GR94800	9.9 [a]	9.35 ± 0.10
GR100679	8.9 [a]	9.47 ± 0.06
MEN10376	8.6 [a]	6.52 ± 0.04
L-659,877	7.3 [a]	7.96 ± 0.02
(±)-CP96345	5.2 [a]	< 5
GR82334	< 5	< 5

Results are pK_i values ± S.E.M. from 3–10 separate experiments performed in triplicate. Slope values were not significantly different from unity. [a] Data from Hagan et al., 1993. MEN10376 = [Tyr^5,D-Trp^{6,8,9},Lys^{10}]neurokinin A-(4–10), GR82334 = [D-Pro^9[spiro-γ-lactam]-Leu^{10},Trp^{11}]physalaemin-(6–11), Hagan et al., 1991a.

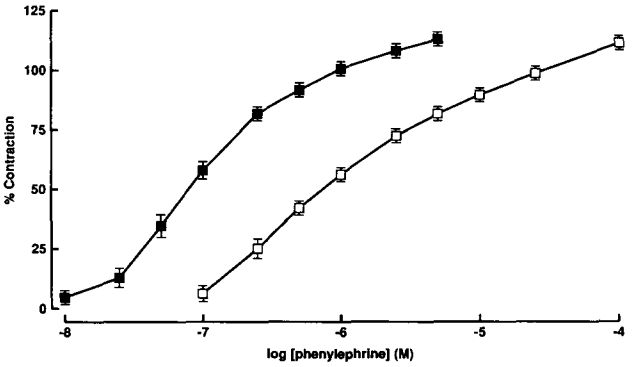

Fig. 4. Antagonism by GR159897 of contractions induced by phenylephrine in rabbit isolated thoracic aorta. Cumulative concentration-effect curves were constructed to phenylephrine in the absence (■) and in the presence of GR159897 1 μM (□). Data shown are mean ± S.E.M. The control curve represents the means of time-matched curves constructed on a non-GR159897 treated preparation included in each experiment. Responses of all curves are calculated as a percentage of the maximum response obtained to phenylephrine in the preceding control curve for each preparation.

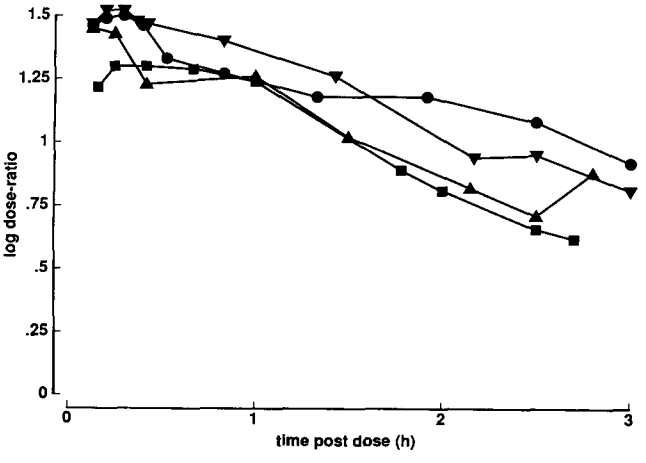

Fig. 5. Duration of antagonism by GR159897 of bronchoconstriction induced by GR64349 in four individual anaesthetised guinea-pigs. GR64349-induced increases in tracheal inflation pressure were repeated at intervals, first before, and then at intervals after the administration of GR159897 (0.12 mg·kg^{-1} i.v.), so that antagonist dose-ratios could be calculated at intervals after dosing. The degree of antagonism is expressed as a log of the dose-ratio obtained and is plotted against time. Each curve represents the duration of this antagonism in a single animal.

3.4. In vivo study

In the anaesthetised guinea-pig, GR64349 (27–276 $ng \cdot kg^{-1}$) caused dose-related increases in tracheal inflation pressure (dose causing 100% increase in tracheal inflation pressure = 127 ± 32 $ng \cdot kg^{-1}$ i.v.; $n = 4$). GR64349-induced bronchoconstriction was antagonised by GR159897 (0.12 $mg \cdot kg^{-1}$ i.v.) with a maximum dose-ratio of 28 (19–40, $n = 5$), which was obtained at 13 ± 2 min after the administration of GR159897, and had a duration of action of 3.0 ± 0.3 h (Fig. 5).

4. Discussion

GR159897 is a novel, non-peptide antagonist at tachykinin NK_2 receptors. It has high affinity for NK_2 receptors derived from human ileum and transfected into CHO cells (pK_i 9.5), and in rat colon (pK_i 10.0). In a functional preparation from a third species, the guinea-pig isolated trachea, GR159897 potently antagonised contractions evoked by the NK_2 receptor agonist, GR64349, in a concentration-dependent and competitive manner (pA_2 8.7).

The affinity of GR159897 for NK_2 receptors in human ileum and rat colon was determined using the NK_2 receptor antagonist radioligand [^{3}H]GR100679 (Hagan et al., 1993). Both naturallly occurring and synthetic, receptor-selective tachykinin agonists inhibited [^{3}H]GR100679 binding to rat colon membranes with the expected rank order of potency for binding to NK_2 receptors, neurokinin A > GR64349 > neurokinin B > substance P = substance P methyl ester = senktide. This is identical to their rank order of potency to antagonise neurokinin A-induced contractions of rat colon (Ireland et al., 1991) and to compete for [^{3}H]GR100679 binding sites in hNK_2-CHO cells (Hagan et al., 1993). However, the absolute estimates of agonist potency were substantially lower in the rat colon binding assay and slopes of inhibition curves were significantly less than one. Lower agonist potencies when competing against an antagonist rather than an agonist radioligand are consistent with previous observations, for example binding of the NK_1 antagonist radioligand [^{3}H]CP96345 to guinea-pig brain membranes (McLean et al., 1991). This is most likely because NK_2 receptors are G-protein coupled and can exist in two affinity states. While antagonists normally bind to these states with equal affinity, agonists preferentially bind to the G-protein coupled, high affinity state of the receptor and have lower affinity for the receptor in its uncoupled state. Therefore, agonists differentially displace [^{3}H]GR100679 binding, resulting in biphasic competition curves and lower apparent IC_{50} values. Agonists competed for [^{3}H]GR100679 binding sites in whole hNK_2-CHO cells with slopes of unity and affinities very similar to those observed in functional assays (Hagan et al., 1993), suggesting that under these experimental conditions, the majority of NK_2 receptors are in the G-protein coupled high affinity state. Indeed, we have observed that the profile of agonist binding to [^{3}H]GR100679 binding sites in membranes prepared from hNK_2-CHO cells is very similar to that observed in rat colon membranes (unpublished observations), sugggesting that the degree of G-protein coupling is related to the method of cell or tissue preparation and the integrity of the cell. Interestingly, Emonds-Alt and co-workers (1993b), using a manganese-containing, sodium-free assay buffer, reported that agonists competed with high affinity for [^{3}H]SR48968 binding sites in membranes prepared from rat duodenum and hamster urinary bladder, but were somewhat weaker in guinea-pig ileum membranes, implying that the proportion of G-protein coupled receptors in membrane preparations is dependent on the ionic environment and may vary between different tissues and/or species.

Studies with peptidic tachykinin NK_2 receptor antagonists have indicated that NK_2 receptors can be subdivided into two distinct species variants, those in human, bovine, rabbit and guinea-pig tissues, where MEN10376 is more potent than L-659,877, being different to those in rat and hamster, where the converse order of potency is observed (see Maggi et al., 1993). Results obtained using the NK_2 receptor antagonist radioligand [^{3}H]GR100679 support these conclusions. Thus, we previously demonstrated that the human ileum NK_2 receptor was similar to that found in rabbit and guinea-pig, with a rank order of antagonist potencies (±)-SR48968 ≥ GR94800 > GR100679 > MEN10376 > L-659,877 ≫ (±)-CP,96,345 ≥ GR82334 (Hagan et al., 1993). In the present study, we report a rank order of potencies in rat colon membranes of GR159897 > GR100679 ≥ GR94800 > (±)-SR48968 > L-659,877 > MEN10376 ≫ (±)-CP,96,345 ≥ GR82334. The NK_1 receptor antagonists (±)-CP,96,345 and GR82334 were without effect in either preparation. The rank order of antagonist affinities in rat colon membranes is in agreement with their potencies to antagonise NK_2 receptor-mediated responses in hamster trachea (Maggi et al., 1993) and to compete for [^{3}H]SR48968 binding sites in rat duodenum (Emonds-Alt et al., 1993b). In agreement with previous observations in human gut (Maggi et al., 1993), MEN10376 had higher affinity than L-659,877 for [^{3}H]GR100679 binding sites in hNK_2-CHO cells (Hagan et al., 1993), while the converse order of potency was observed in rat colon membranes. Interestingly, the heptapeptide NK_2 receptor antagonist, GR94800 (McElroy et al., 1992), was more potent in hNK_2-CHO cells than in rat colon membranes, while GR100679 (Smith et al., 1993)

exhibited the converse order of potency. To date, the only other non-peptide NK_2 receptor antagonist that has appeared in the literature is SR48968 (Emonds-Alt et al., 1992), which was developed by optimisation of a random screen lead. Although GR159897 and SR48968 are both highly potent antagonists at NK_2 receptors, they show some differences in their relative potencies at NK_2 receptors in different species. Thus, while GR159897 is slightly more potent at NK_2 receptors in rat compared to those in human or guinea-pig, SR48968 has the converse selectivity (Advenier et al., 1992, see also present results). There is overall 86% sequence identity between NK_2 receptors in human (gastric tissue or trachea) and rat (stomach) tissues (Gerard et al., 1990; Sasai and Nakanishi, 1989). Greatest structural differences are observed at the N- and C-termini. The human protein has a 14 amino-acid extension at the C-terminus and two N-linked glycosylation sites in the extracellular N-terminal region compared to one glycosylation site in the rat. However, the biological significances of such differences are unknown, and a site-directed mutagenesis approach is required to establish the molecular basis of inter-species differences in affinities of antagonists for NK_2 receptors.

Like SR48968 (Emonds-Alt et al., 1992), GR159897 has little or no activity at NK_1 receptors in human and guinea-pig. In addition, GR159897 has little or no affinity ($pK_i < 5$) for NK_3 receptors in guinea-pig cortex. In contrast, SR48968 has some activity at NK_3 receptors in human and guinea-pig but not in rat (pIC_{50} 6.5; Chung et al., 1994; Petitet et al., 1993), indicating the existence of species differences in NK_3 receptors. GR159897 has little or no activity at prostanoid EP_1, 5-HT_{1A}, 5-HT_2 or 5-HT_3 receptors, and is only weakly active at α_1-adrenoceptors.

In vivo, GR159897 administered i.v. is a potent and long acting antagonist at NK_2 receptors in the anaesthetised guinea-pig, with a similar order of potency to SR48968 (Emonds-Alt et al., 1992). However, a precise quantitative comparison of the potencies and duration of action of the two compounds is not possible as different methodologies have been used; Emonds-Alt and colleagues determined antagonist activity as percentage inhibition of the bronchoconstrictor response to a single dose of agonist, whereas in the present study, it has been determined in terms of rightward displacement of an agonist-induced bronchoconstriction dose-response curve. In addition to antagonism of NK_2 agonist-induced bronchoconstriction in guinea-pigs, we have recently demonstrated that GR159897 has anxiolytic-like activity in a range of putative models of anxiety in both rats and marmosets following peripheral and central administration (Stratton et al., 1994).

Thus, GR159897 is a highly potent and selective antagonist at NK_2 receptors, and has good in vivo activity, and penetrates the CNS. GR159897 should be a useful tool for studying the physiological and pathophysiological role of NK_2 receptor activation, for example in respiratory tract, bladder and CNS.

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References

Advenier, C., N. Rouissi, Q.T. Nguyen, X. Emonds-Alt, J.C. Breliere, G. Neliat, E. Naline and D. Regoli, 1992, Neurokinin A (NK-2) receptor revisited with SR48,968 a potent nonpeptide antagonist, Biochem. Biophys. Res. Commun. 184, 1418.

Apperley, E., P.P.A. Humphrey and G.P. Levy, 1976, Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta, Br. J. Pharmacol. 58, 211.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Ball, D.I., I.J.M. Beresford, G.P.A. Wren, Y.D. Pendry, R.L.G. Sheldrick, D.M. Walsh, M.P. Turpin, R.M. Hagan and R.A. Coleman, 1994, In vitro and in vivo pharmacology of the non-peptide antagonist at tachykinin NK_2-receptors, GR159897, Br. J. Pharmacol. 112 (Suppl.), 48P.

Cascieri, M.A., E. Ber, T.M. Fong, S. Sadowski, A. Bansal, C., Swain, E. Seward, B. Frances, D. Burns and C.D. Strader, 1992, Characterization of the binding of a potent, selective, radioiodinated antagonist to the human neurokinin-1 receptor, Mol. Pharmacol. 42, 458.

Chung, F.-Z., L.-H. Wu, M.A. Vartanian, K.J. Watling, S. Guard, G.N. Woodruff and D.L. Oxender, 1994, The non-peptide tachykinin NK_2 receptor antagonist SR48968 interacts with human, but not rat, cloned tachykinin NK_3 receptors, Biochem. Biophys. Res. Commun. 198, 967.

Cooper, A.W.J., H.S. Adams, R. Bell, P.M. Gore, A.B. McElroy, J.M. Pritchard, P.W. Smith and P. Ward, 1994, GR159897 and related analogues as highly potent, orally active non-peptide neurokinin NK_2 receptor antagonists, Bioorg. Med. Chem. Lett. 4, 1951.

Emonds-Alt, X., P. Vilain, P. Goulaouic, V. Proietto, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Brelière, 1992, A potent and selective nonpeptide antagonist of the neurokinin A (NK-2) receptor, Life Sci. 50, PL101.

Emonds-Alt, X., J.D. Doutremepuich, M. Heaulme, G. Neliat, V. Santucci, R. Steinberg, P. Vilain, D. Bichon, J.P. Ducoux, V. Proietto, D. Van Broeck, P. Soubrie, G. Le Fur and J.C. Breliere, 1993a, In vitro and in vivo biological activites of SR140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist, Eur. J. Pharmacol. 250, 403.

Emonds-Alt, X., F. Golliot, P. Pointeau, G. Le Fur and J.C. Breliere, 1993b, Characterization of the binding sites of [^{3}H]SR48968, a potent nonpeptide radioligand antagonist of the neurokinin-2 receptor, Biochem. Biophys. Res. Commun. 191, 1172.

Garret, C., A. Carruette, V. Fardin, S. Moussaoui, J.F. Peyronel, J.C.

Blanchard and P.M. Laduron, 1991, Pharmacology properties of a potent and selective nonpeptide substance P antagonist, Proc. Natl. Acad. Sci. USA 88, 10208.

Gerard, N.P., R.L. Eddy, T.B. Shows and C. Gerard, 1990, The human neurokinin A (substance K) receptor, J. Biol. Chem. 265, 20455.

Guard, S., S.P. Watson, J.E. Maggio, H. Phon Too and K.J. Watling, 1990, Pharmacological analysis of [^{3}H]-senktide binding to NK_3 tachykinin receptors in guinea-pig ileum longitudinal muscle-myenteric plexus and cerebral cortex membranes, Br. J. Pharmacol. 99, 767.

Hagan, R.M., S.J. Ireland, F. Bailey, C. McBride, C.C. Jordan and P. Ward, 1991a, A spirolactam conformationally-constrained analogue of physalaemin which is a peptidase-resistant, selective neurokinin NK-1 receptor antagonist, Br. J. Pharmacol. 102, 168P.

Hagan, R.M., S.J. Ireland, C.C. Jordan, I.J.M. Beresford, M.J. Deal and P. Ward, 1991b, Receptor-selective, peptidase-resistant agonists at neurokinin NK-1 and NK-2 receptors: new tools for investigating neurokinin function, Neuropeptides 19, 127.

Hagan, R.M., I.J.M. Beresford, J. Stables, J. Dupere, C.M. Stubbs, P.J. Elliott, R.L.G. Sheldrick, A. Chollet, E. Kawashima, A.B. McElroy and P. Ward, 1993, Characterisation, CNS distribution and function of NK_2 receptors studied using potent NK_2 receptor antagonists, Regul. Pept. 46, 9.

Holmes, B. and E.M. Sorkin, 1986, Indoramin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension and related vascular, cardiovascular and airway diseases, Drugs 31, 467.

Ireland, S.J., F. Bailey, A. Cook, R.M. Hagan, C.C. Jordan and M.L. Stephens-Smith, 1991, Receptors mediating tachykinin-induced contractile responses in guinea-pig trachea, Br. J. Pharmacol. 103, 1463.

Kilpatrick, G.J., B.J. Jones and M.B. Tyers, 1987, Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding, Nature 330, 746.

Maggi, C.A., R. Patacchini, P. Rovero and A. Giachetti, 1993, Tachykinin receptors and tachykinin receptor antagonists, J. Auton. Pharmacol. 13, 23.

McElroy, A.B., S.P. Clegg, M.J. Deal, G.B. Ewan, R.M. Hagan, S.J. Ireland, C.C. Jordan, B. Porter, B.C. Ross, P. Ward and A.R. Whittington, 1992, Highly potent and selective heptapeptide antagonists of the neurokinin NK-2 receptor, J. Med. Chem. 35, 2582.

McLean, S., A.H. Ganong, T.F. Seeger, D.J. Bryce, K.G. Pratt, L.S. Reynolds, C.J. Siok, J.A. Lowe, III and J. Heym, 1991, Activity and distribution of binding sites in brain of a nonpeptide substance P (NK_1) receptor antagonist, Science 251, 437.

McLean, S., R.M. Snider, M.C. Desai, T. Rosen, D.K. Bryce, K.P. Longo, A.W. Schmidt and J. Heym, 1993, CP-99,994, a nonpeptide antagonist of the tachykinin NK_1 receptor, Regul. Pept. 46, 329.

Mir, A.K., M. Hibert, M.D. Tricklebank, D.N. Middlemiss, E.J. Kidd and J.R. Fozard, 1988, MDL 72832: a potent and stereoselective ligand at central and peripheral 5-HT_{1A} receptors, Eur. J. Pharmacol. 149, 107.

Petitet, F., J.-C. Beaujouan, M. Saffroy, Y. Torrens and J. Glowinski, 1993, The nonpeptide NK_2 antagonist SR48968 is also a NK_3 antagonist in the guinea-pig but not in the rat, Biochem. Biophys. Res. Commun. 191, 180.

Sasai, Y. and S. Nakanishi, 1989, Molecular characterization of rat substance K receptor and its mRNA, Biochem. Biophys. Res. Commun. 165, 695.

Smith, P.W., A.B. McElroy, J.M. Pritchard, M.J. Deal, G.B. Ewan, R.M. Hagan, S.J. Ireland, D. Ball, I. Beresford, R. Sheldrick, C.C. Jordan and P. Ward, 1993, Low molecular weight neurokinin NK_2 antagonists, Bioorg. Med. Chem. Lett. 3, 931.

Stratton, S.C., I.J.M. Beresford, F.J. Harvey, M.P. Turpin, R.M. Hagan and M.B. Tyers, 1993, Anxiolytic activity of tachykinin NK_2 receptor antagonists in the mouse light-dark box, Eur. J. Pharmacol. 250, R11.

Stratton, S.C., I.J.M. Beresford and R.M. Hagan, 1994, GR159897, a potent non-peptide tachykinin NK_2 receptor antagonist, releases suppressed behaviours in a novel aversive environment, Br. J. Pharmacol. 112 (Suppl.), 49P.

Stubbs, C.M., D. Trezise, H.E. Connor and W. Feniuk, 1991, Vasodilator effect of 8-OH-DPAT in the isolated perfused mesenteric bed of the rat: no evidence for involvement of 5-HT_{1A} receptors, J. Auton. Pharmacol. 11, 237.

Williams, B.J., N.R. Curtis, A.T. McKnight, J. Maguire, A. Foster and R. Tridgett, 1988, Development of NK-2 selective antagonists, Regul. Pept. 22, 189.