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In vitro and in vivo predictors of the anti-emetic activity of tachykinin NK_1 receptor antagonists

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Abstract

The ability of tachykinin NK_1 receptor antagonists to inhibit GR73632 (D-Ala-[L-Pro^9,Me-Leu^8]substance P-(7–11))-induced foot tapping in gerbils was employed as an indirect measure of brain penetration and this was compared with their ability to prevent acute emesis induced by cisplatin in ferrets. (±)-GR203040 ((2*S*,3*S* and 2*R*,3*R*)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine), CP-99,994 ((2*S*,3*S*)-*cis*-3-(2-methoxybenzylamino)-2-phenyl piperidine) dihydrochloride), and L-742,694 (2-(*S*)-(3,5-bis(trifluoromethyl)benzyloxy)-3-(*S*)-phenyl-4-(5-(3-oxo-1,2,4-triazolo)methylmorpholine) potently inhibited GR73632-induced foot tapping ($ID_{50} \leq 0.85$ mg/kg), and acute retching induced by cisplatin ($ID_{50} \leq 0.18$ mg/kg). RPR100893 ((3*aS*,4*S*,7*aS*)-7,7-diphenyl-4-(2-methoxyphenyl)-2-[(*S*)-2-(2-methoxyphenyl)proprionyl] perhydroisoindol-4-ol) was not a potent antagonist of retching (ID_{50} 4.1 mg/kg) or foot tapping ($ID_{50} > 10$ mg/kg). High doses (3–10 mg/kg) of CGP49823 ((2*R*,4*S*)-2-benzyl-1-(3,5-dimethylbenzoyl)-*N*-[(4-quinolinyl)methyl]-4-piperineamine) dihydrochloride), FK888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-propyl]-*N*-methyl-*N*-phenylmethyl-L-3-(2-naphthyl)-alaninamide), and LY303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidinyl)piperidin-1-yl)acetyl)amino]propane) were required to inhibit foot tapping; these agents were not anti-emetic in this dose range. SR140333 ((*S*)-1-{2-[3-(3,4-dichlorphenyl)-1 (3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl}-4-phenyl-1 azaniabicyclo [2.2.2]octane; 3–10 mg/kg) failed to inhibit foot tapping or emesis. Affinities for the human and ferret tachykinin NK_1 receptor were highly correlated ($r = 0.93$, $P = 0.0008$). Inhibition of foot tapping in gerbils, but not NK_1 receptor binding affinity, predicted anti-emetic activity in ferrets ($r = 0.75$, $P < 0.01$). These findings confirm that the anti-emetic activity of tachykinin NK_1 receptor antagonists is dependent on brain penetration.

Keywords: Anti-emetic; Brain penetration; Tachykinin NK_1 receptor

1. Introduction

Severe nausea and vomiting is a distressing side-effect of cytotoxic drug and radiation therapy which contributes to poor patient compliance and limits the use of aggressive cancer treatment regimens. Cytotoxic drugs elicit an immediate emetic response on the day of therapy (acute emesis) and also protracted vomiting lasting up to 5 days thereafter (delayed emesis). Some relief from acute emesis can be achieved using cocktails of anti-emetic drugs, especially dexamethasone combined with high doses of metoclopramide, but these may themselves elicit unacceptable iatrogenic side-effects (Andrews et al., 1988). The introduction of 5-HT_3 receptor antagonists into clinical practice in the early 1990s represented an important advance in the development of well-tolerated drugs specifically designed to prevent the severe emesis caused by antineoplastic therapy (Bunce et al., 1991; Aapro, 1991). However, whilst 5-HT_3 receptor antagonists such as ondansetron and granisetron control the acute phase of nausea and vomiting induced by emetogenic cytotoxins in at least 60% of patients, emesis is not fully blocked in the residual population (Aapro, 1991; Yarker and McTavish, 1994), and

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5-HT_3 receptor antagonists are less effective at preventing the delayed emesis associated with chemotherapy (De Mulder et al., 1990; Butcher, 1993). The clinical utility of 5-HT_3 receptor antagonists is further limited by their lack of efficacy against centrally acting emetogens such as morphine and apomorphine (Andrews and Bhandari, 1993).

Recently, tachykinin NK_1 receptor antagonists have emerged as an important new class of broad-spectrum anti-emetic agents in preclinical assays of acute emesis. One such compound, the phenylpiperidine CP-99,994, completely abolished cisplatin-induced retching and vomiting in ferrets (Bountra et al., 1993; Watson et al., 1995a). Unlike the limited profile of anti-emetic activity exhibited by 5-HT_3 receptor antagonists, CP-99,994 also inhibited retching and emesis in ferrets challenged with a wide range of both peripheral and central emetic stimuli (Bountra et al., 1993; Tattersall et al., 1994; Watson et al., 1995a). The enantioselectivity of the effects of CP-99,994 (Tattersall et al., 1993, 1994; Watson et al., 1995a), and the low affinity of this compound for other neurotransmitter receptors in vitro (McLean et al., 1993), confirm the tachykinin NK_1 receptor specificity of this anti-emetic activity. The ability of CP-99,994 to block both peripherally and centrally acting emetogens, and the demonstration that direct injection of CP-99,994 into the region of the nucleus tractus solitarius inhibited cisplatin-induced emesis in ferrets (Gardner et al., 1994), suggest that the anti-emetic activity of tachykinin NK_1 receptor antagonists is centrally mediated. This proposal has been confirmed recently by the use of a quaternised tryptophan ketone L-743,310, a poorly brain-penetrant tachykinin NK_1 receptor antagonist, which was able to prevent cisplatin-induced retching in ferrets when infused directly into the central nervous system (CNS), but not when it was injected intravenously (Tattersall et al., 1996). Thus, the ability of tachykinin NK_1 receptor antagonists to block emesis when they are administered systemically appears to be critically dependent on their ability to cross the blood–brain barrier. The central locus of action of tachykinin NK_1 receptor antagonists indicates a fundamental difference in the mechanisms underlying their anti-emetic activity from that of 5-HT_3 receptor antagonists, which are believed to act mainly in the periphery on vagal afferent fibres (Sanger, 1992), although blockade of central 5-HT_3 receptors may also be involved (Higgins et al., 1989).

In addition to the predicted clinical utility of tachykinin NK_1 receptor antagonists to control emesis, other possible therapeutic uses suggested from preclinical investigations include rheumatoid arthritis, asthma, migraine, pain and psychiatric conditions (reviewed by Longmore et al., 1995). A large number of structurally diverse antagonists which possess high (nM) affinity for the human tachykinin NK_1 receptor have been evaluated in preclinical assays and in clinical trials for these various conditions. These include the recently described morpholine-based antagonist L-742,694 (Hale et al., 1996); the phenylpiperidine GR203040 (Beattie et al., 1995); the piperidineamine CGP 49823 (Vassout et al., 1994); the aryl amino acid derivative LY303870 (Gitter et al., 1995); the *N*-acylated 3-(3,4-dichlorophenyl)piperidine SR140333 (Emonds-Alt et al., 1993); the triarylperhydroisoindol RPR100893 (Tabart and Peyronel, 1994), and the dipeptide FK888 (Hagiwara et al., 1993). Access of tachykinin NK_1 receptor antagonists into the brain is clearly a prerequisite for clinical efficacy in CNS disorders (emesis, pain, psychiatry), but not in conditions associated with inflammation of peripheral tissues. However, only limited information is presently available describing the in vivo pharmacology of these compounds in assays that require brain penetration.

Recently, we described the use of tachykinin NK_1 agonist-induced foot tapping in gerbils as a simple in vivo functional assay for CNS penetration of tachykinin NK_1 receptor antagonists (Rupniak and Williams, 1994). Like the ferret, the pharmacology of the gerbil tachykinin NK_1 receptor appears to resemble that of the human receptor (Beresford et al., 1991). Central infusion of tachykinin NK_1 receptor agonists in gerbils elicits a vigorous and readily quantifiable rhythmic stamping or tapping of the hindfeet which can be inhibited by systemic administration of brain-penetrant tachykinin NK_1 receptor antagonists (Graham et al., 1993; Vassout et al., 1994; Bristow and Young, 1994; Rupniak and Williams, 1994). In contrast, the poorly CNS-penetrant compound, L-743,310, failed to inhibit foot tapping at a dose which completely blocked chromodacryorrhoea evoked by stimulation of peripheral tachykinin NK_1 receptors (Rupniak and Williams, 1994). In the present studies, we have directly compared the ability of the currently available antagonists to block central tachykinin NK_1 receptors in vivo, and examine whether activity in the gerbil foot tapping assay is predictive of anti-emetic efficacy in ferrets.

2. Materials and methods

2.1. Displacement of ^{125}I-[Tyr^8]substance P binding to cloned human tachykinin NK_1 receptors and to ferret brain membranes in vitro

Tachykinin NK_1 receptor binding assays were performed in intact Chinese hamster ovary (CHO) cells expressing the human tachykinin NK_1 receptor using a modification of the assay conditions described by Cascieri et al. (1992). The receptor was expressed at a level of 3×10^5 receptors per cell. Cells were grown in monolayer culture, detached from the plate with enzyme-free cell dissociation solution (Speciality Media), and washed prior to use in the assay. ^{125}I-[Tyr^8]substance P (0.1 nM, 2000 Ci/mmol; New England Nuclear) was incubated in the presence or absence of test compounds (dissolved in 5 μl dimethyl sulphoxide, DMSO) with 5×10^4 CHO cells. Ligand binding was performed in 0.25 ml of 50 mM Tris-HCl, pH 7.5,

containing 5 mM $MnCl_2$, 150 mM NaCl, 0.02% bovine serum albumin (Sigma), 40 μg/ml bacitracin, 0.01 mM phosphoramidon and 4 μg/ml leupeptin. The incubation proceeded at room temperature until equilibrium was achieved (> 40 min) and the receptor-ligand complex was harvested by filtration over GF/C filters presoaked in 0.1% polyethylenimine using a Tomtek 96-well harvester. Nonspecific binding was determined using excess substance P (1 μM) and represented < 10% of total binding. Affinity for the ferret tachykinin NK_1 receptor was similarly determined using synaptosomal membranes (50 μg) purified from ferret brain cortex by discontinuous sucrose gradient centrifugation as previously described (Cascieri et al., 1985). Receptor binding data were analysed using the Prism software package (Graph Pad).

2.2. Inhibition of GR73632-induced foot tapping in gerbils

2.2.1. Intravenous administration

The ability of tachykinin NK_1 receptor antagonists to inhibit foot tapping was determined as described previously (Rupniak and Williams, 1994). Male or female Mongolian gerbils (35–70 g; Leeds University) were anaesthetised by inhalation of an isoflurane/oxygen mixture to permit exposure of the jugular vein through a skin incision in the neck, using blunt dissection to clear surrounding salivary gland and connective tissues. Test compounds or vehicle were administered using an injection volume of 5 ml/kg i.v. The wound was closed and a second skin incision was made in the midline of the scalp to expose the skull. The highly selective, peptidase-resistant tachykinin NK_1 receptor agonist GR73632 (D-Ala-[L-Pro^9,$Me\text{-}Leu^8$]substance P-(7–11); Hagan et al., 1991) was infused directly into the cerebral ventricles (3 pmol in 5 μl i.c.v.) by vertical insertion of a cuffed 27-gauge needle to a depth of 4.5 mm below bregma. The scalp incision was closed and the animal allowed to recover from anaesthesia in a clear Perspex observation box (25 × 20 × 20 cm). The duration of hind foot tapping was then recorded continuously for 5 min using a stopclock. The time lapse from induction to recovery of anaesthesia, with intervening i.v. and i.c.v. injections, was 3–4 min per animal. Between 3 and 9 animals received one dose of each test compound or vehicle.

The ability of CP-99,994 to inhibit GR73632-induced foot tapping following i.v. administration has been published previously (Rupniak and Williams, 1994); these data are included here for the purpose of comparison with other tachykinin NK_1 receptor antagonists, and for comparison with the activity of CP-99,994 in this assay following oral administration.

2.2.2. Oral activity

The ability of test compounds to inhibit foot tapping following oral administration (up to 30 mg/kg) in conscious gerbils 1 h before i.c.v. infusion of GR73632 (3 pmol) was evaluated as an indirect measure of their oral bioavailability in this species. FK888 was not evaluated orally because insufficient compound was available.

2.3. Inhibition of acute retching induced by cisplatin in ferrets

Acute emesis studies were performed using the method described by Tattersall et al. (1993). Male fitch or albino ferrets (1.1–1.8 kg; Froxfield Farms) were prefed with approximately 100 g of commercially available cat food at least 20 min prior to initiation of experiments. Under halothane/oxygen anaesthesia, the left jugular vein was cannulated and test compounds (or vehicle) were administered at doses up to 10 mg/kg i.v., usually in a volume of 1 ml/kg. After 3 min, cisplatin (10 mg/kg) was administered via the same route. The cannula was then removed, the vein ligated and the skin incision closed. Animals were returned to individual cages and after recovery from anaesthesia (10–20 min), the number of retches was recorded continuously for 4 h after the administration of cisplatin. Between 3 and 6 animals received each dose of test compound or vehicle.

The anti-emetic activity of CP-99,994 in this assay has been described in full previously (Tattersall et al., 1993); retching data are presented here for comparison with other compounds.

2.4. Preparation of test compounds

L-742,694 (2-(*S*)-(3,5-Bis(trifluoromethyl)benzyloxy)-3-(*S*)-phenyl-4-(5-(3-oxo-1,2,4-triazolo)methylmorpholine) was synthesized by the Department of Basic and Medicinal Chemistry, Merck Research Laboratories, Rahway, USA, as described by Hale et al. (1996). (±)-GR203040 ((2*S*,3*S* and 2*R*,3*R*)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine), CP-99,994 ((2*S*,3*S*)-*cis*-3-(2-methoxybenzylamino)-2-phenyl piperidine) dihydrochloride, CGP 49823 ((2*R*,4*S*)-2-benzyl-1-(3,5-dimethylbenzoyl)-*N*-[(4-quinolinyl)methyl]-4-piperineamine) dihydrochloride), FK888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-propyl]-*N*-methyl-*N*-phenylmethyl-L-3-(2-naphthyl)-alaninamide), LY303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1*H*-indol-3-yl)-2-[*N*-(2-(4-(piperidinyl)piperidin-1-yl)acetyl)amino]propane), SR140333 ((*S*)-1-{2-[3-(3,4-dichlorphenyl)-1 (3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl}-4-phenyl-1 azaniabicyclo [2.2.2]octane) and RPR100893 ((3*aS*,4*S*,7*aS*)-7,7-diphenyl-4-(2-methoxyphenyl)-2-[(*S*)-2-(2-methoxyphenyl)proprionyl] perhydroisoindol-4-ol) were synthesized by the Departments of Basic and Medicinal Chemistry of MSD in Harlow, UK and Rahway, USA.

For in vivo administration, L-742,694, (±)-GR203040, CP-99,994 and CGP 49823 were dissolved in aqueous vehicles (0.1 M hydrochloric acid or water). FK888 and LY3030870 were dissolved in 5% PEG 300/5% Tween

80/90% water. SR140333 and RPR100893 were either formulated in a mixture of 50% PEG 400/50% 0.1 M hydrochloric acid (gerbil experiments) or administered in solution with PEG 300 (ferrets).

2.5. Statistical analysis

Data from in vivo experiments were subjected to one-way analysis of variance (ANOVA), followed by Dunnett's or Newman-Keuls multiple comparison *t*-tests using BMDP statistical software (BBN Software Products Corporation). Results are expressed as a percentage of values obtained in vehicle-treated control animals. ID_{50} values were calculated by non-linear least-squares regression analysis of mean data using GraFit (Erithacus Software). Correlation coefficients comparing the activity of test compounds in in vitro and in vivo assays were calculated by simple linear regression analysis using BMDP statistical software.

3. Results

3.1. Inhibition of ^{125}I-[Tyr^8]substance P binding to cloned human tachykinin NK_1 receptors and to ferret brain membranes by tachykinin NK_1 receptor antagonists in vitro

Binding of ^{125}I-[Tyr^8]substance P to the human tachykinin NK_1 receptor expressed in CHO cells was inhibited by all of the tachykinin NK_1 receptor antagonists examined in a concentration-dependent and competitive manner. These compounds all exhibited high affinity binding to the cloned human tachykinin NK_1 receptor in vitro, with IC_{50} values in the concentration range from high pM (SR140333, (±)-GR203040, L-742,694, LY303870, FK888 and CP-99,994) to low nM (CGP 49823 and RPR100893; Table 1).

Specific ^{125}I-[Tyr^8]substance P binding to tachykinin NK_1 receptors in ferret neocortical membranes was also displaced with all compounds examined in the pM to nM concentration range, although the affinities were generally lower for membrane preparations from ferret cerebral cortex than for CHO cells expressing the human tachykinin NK_1 receptor. The rank order of affinities of the 8 antagonists was similar in the human and ferret assays, indicating analogous human and ferret tachykinin NK_1 receptor pharmacology, and confirming the very high affinity of these compounds for both the human and the ferret tachykinin NK_1 receptor in vitro (Table 1). Linear regression analysis confirmed that there was an excellent correlation between the affinity of the antagonists for the human and the ferret tachykinin NK_1 receptor in vitro ($r = 0.93$, $P = 0.0008$).

Table 1
Inhibition of ^{125}I-[Tyr^8]substance P (0.1 nM) binding to cloned human tachykinin NK_1 receptors in CHO cells and to ferret brain membranes by tachykinin NK_1 receptor antagonists in vitro

Compound	Cloned human tachykinin NK_1 receptors IC_{50} (nM)	Ferret cerebral cortex membranes IC_{50} (nM)
SR140333	0.04	0.5
(±)-GR203040	0.08	0.25
L-742,694	0.09	0.25
LY303870	0.40	3.0
FK888	0.51	2.8
CP-99,994	0.53	3.0
CGP 49823	1.0	9.5
RPR100893	1.5	28.5

3.2. Inhibition of GR73632-induced foot tapping in gerbils

3.2.1. Intravenous administration

Central infusion of 3 pmol i.c.v. of GR73632 caused virtually continuous foot tapping throughout the 5 min period of observation in vehicle-treated control animals. Foot tapping was dose-dependently and completely inhibited by intravenous injection of (±)-GR203040 (0.01–0.1 mg/kg; $F(3,8) = 163.0$, $P < 0.001$) or CP-99,994 (0.03–0.3 mg/kg; $F(3,20) = 16.8$, $P < 0.001$) immediately before i.c.v. infusion of GR73632. These compounds were the most potent inhibitors of foot tapping examined, with ID_{50} values of 0.04 and 0.06 mg/kg i.v., respectively. Similarly, GR73632-induced foot tapping was inhibited dose-dependently by i.v. injection of L-742,694 (0.3–3 mg/kg; $F(3,26) = 16.6$, $P < 0.001$), CGP 49823 (1–10 mg/kg; $F(3,8) = 51.6$, $P < 0.001$) and the peptide NK_1 receptor antagonist FK888 (1–10 mg/kg; $F(3,8) = 30.5$, $P < 0.001$), with ID_{50} values of 0.85, 2.28 and 3.7 mg/kg i.v., respectively. In contrast, LY303870 (1–10 mg/kg i.v.) partially attenuated but did not fully block foot tapping ($F(3,9) = 4.1$, $P = 0.04$); the ID_{50} of this compound was estimated to be 11 mg/kg i.v. (Fig. 1).

Unlike these compounds, foot tapping was not blocked following i.v. administration of SR140333 (10 mg/kg; $F(1,5) = 1.3$, $P = 0.30$), and was only weakly inhibited (< 14%) by RPRP100893 (10 mg/kg; $F(1,4) = 12.6$, $P = 0.02$; Fig. 1).

Linear regression analysis revealed no correlation between the binding affinity of the antagonists for the human tachykinin NK_1 receptor in vitro and their rank order potency to inhibit foot tapping in vivo ($r = 0.29$, $P = 0.49$).

3.2.2. Oral administration

L-742,694 was the most potent inhibitor of foot tapping of the compounds tested when administered orally 1 h before GR73632 (3 pmol i.c.v.). L-742,694 caused a dose-dependent and complete inhibition of foot tapping in the dose range 0.03–3 mg/kg ($F(2,25) = 18.1$, $P < 0.001$), with an ID_{50} of 0.41 mg/kg p.o. (±)-GR203040 also inhibited foot tapping following oral administration ($F(4,13) = 19.4$, $P < 0.001$), but the ID_{50} of 8.10 mg/kg p.o. was considerably higher than that observed after i.v. administration (> 200-fold increase; Fig. 2). Unlike these

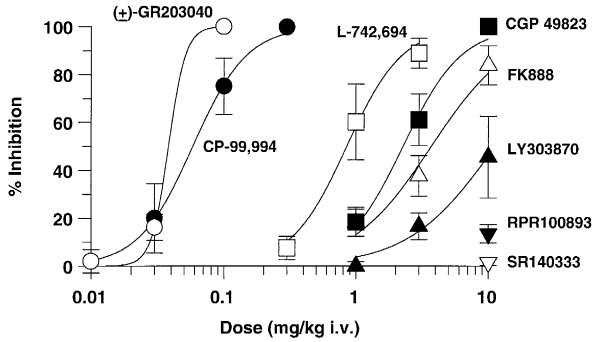

Fig. 1. Inhibition of foot tapping in gerbils by intravenous administration of tachykinin NK_1 receptor antagonists (0.01–10 mg/kg) immediately prior to i.c.v. infusion of the tachykinin NK_1 receptor agonist GR73632 (3 pmol). The duration of foot tapping was recorded for 5 min after recovery from anaesthesia and is expressed as a percentage of values observed in vehicle-treated control animals. Between 3–6 animals received one dose of each test compound or vehicle. The ID_{50} was calculated by non-linear least-squares regression analysis of mean data.

compounds, foot tapping was not inhibited following oral administration of 10 mg/kg of either CP-99,994 ($F(1,4) = 0.01$, $P = 0.95$) or CGP 49823 ($F(1,4) = 1.22$, $P = 0.33$; Fig. 3). LY303870, SR140333 and RPR100893 were not evaluated orally because of their weak activity in this assay after intravenous administration.

3.3. Inhibition of acute retching induced by cisplatin in ferrets

There was a marked difference in the ability of the tachykinin NK_1 receptor antagonists to prevent the acute emetic response to cisplatin in ferrets. At the doses tested, only 4 of the 8 compounds examined caused a dose-dependent and complete inhibition of retching following intravenous administration. In order of potency, these were: (±)-GR203040 (0.06–1 mg/kg; $F(4,13) = 11.21$, $P < 0.001$), CP-99,994 (0.3–3 mg/kg; $F(3,22) = 29.87$, $P < 0.001$), L-742,694 (0.3–3 mg/kg; $F(3,12) = 54.22$, $P < 0.001$), and RPR100893 (1–10 mg/kg; $F(3,12) = 6.01$, $P = 0.001$; Fig. 3). The most potent anti-emetic compounds examined were (±)-GR203040 (ID_{50} 0.05 mg/kg i.v.), L-742,694 (ID_{50} 0.18 mg/kg i.v.) and CP-99,994 (ID_{50} 0.18 mg/kg i.v.). Considerably higher doses of RPR100893 were required to prevent retching (ID_{50} 4.1 mg/kg i.v.).

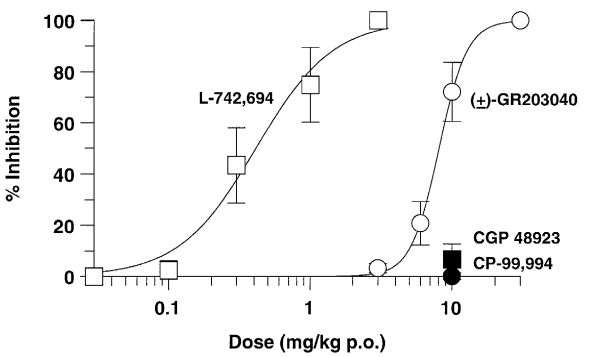

Fig. 2. Ability of selected tachykinin NK_1 receptor antagonists to inhibit GR73632 (3 pmol i.c.v.)-induced foot tapping in gerbils following oral administration 1 h previously. The duration of foot tapping was recorded for 5 min after recovery from anaesthesia and is expressed as a percentage of values observed in vehicle-treated control animals. ID_{50} values were calculated using non-linear least-squares regression analysis of mean data.

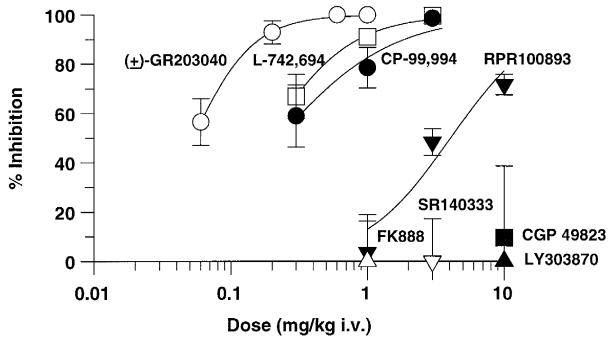

Fig. 3. Prevention of acute cisplatin-induced retching in ferrets by i.v. administration of tachykinin NK_1 receptor antagonists. The number of retches was recorded continuously for 4 h after the administration of cisplatin (10 mg/kg i.v.). Between 3 and 6 animals received each dose of test compound or vehicle. ID_{50} values were calculated by non-linear least-squares regression analysis of mean data.

Unlike these compounds, there was no inhibition of cisplatin-induced retching in ferrets treated with 1 mg/kg i.v. of FK888 ($F(1,4) = 0.89$, $P = 0.40$); higher doses could not be examined because of limitations of compound availability. Similarly, there was no inhibition of retching following administration of 3 mg/kg i.v. of SR140333 ($F(1,6) = 0$, $P = 0.99$); higher doses were not examined because of toxicity. CGP 49823 and LY303870 at 10 mg/kg i.v. failed to inhibit retching ($F(1,4) = 0.10$, $P = 0.77$ for CGP 49823; $F(1,4) = 0.18$, $P = 0.70$ for LY303870; Fig. 3).

Since an ID_{50} for inhibition of emesis was not obtained for all compounds tested, the rank order of potency of the

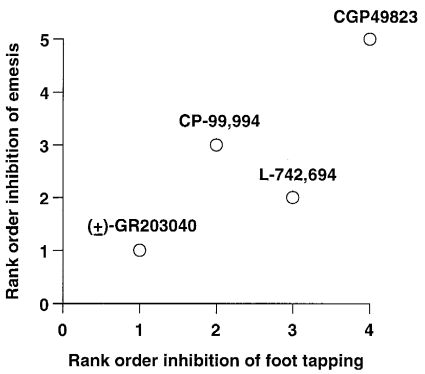

Fig. 4. Correlation between the ability of tachykinin NK_1 receptor antagonists to inhibit foot tapping in gerbils and cisplatin-induced emesis in ferrets. Compounds were ranked based on their order of potency to inhibit these behaviours. Ranked scores were then subjected to Spearman's rank correlation analysis.

tachykinin NK_1 receptor antagonists was instead determined: (±)-GR203040 > L-742,694 > CP-99,994 > RPR100893 ≫ SR140333 = CGP 49823 = LY303870. FK888 was not tested at a sufficiently high dose to permit ranking. Regression analysis revealed that the binding affinity of these compounds for neither the human nor the ferret tachykinin NK_1 receptor in vitro was predictive of their rank potency to inhibit cisplatin-induced retching in vivo ($r = 0.38$, $P = 0.40$, and $r = 0.28$, $P = 0.54$, respectively). In contrast, there was a good correlation between the rank order of potency to inhibit foot tapping in gerbils and rank order of anti-emetic activity in ferrets ($r = 0.75$, $P < 0.01$, Spearman's rank correlation analysis; see Fig. 4).

4. Discussion

The present findings confirm evidence from previous studies (Gardner et al., 1994; Tattersall et al., 1996) that the anti-emetic activity of tachykinin NK_1 receptor antagonists is critically dependent upon their ability to cross the blood–brain barrier. The ability of a range of structurally diverse compounds to inhibit tachykinin NK_1 agonist-induced foot tapping in gerbils, a centrally mediated behaviour (Rupniak and Williams, 1994), was highly predictive of their ability to prevent cisplatin-induced retching in ferrets, and provides a rapid and simple in vivo assay for CNS penetration. Whilst the use of stereoisomers, such as CP-99,994 and CP-100,263, confirms that high (≤ nM) affinity binding to the NK_1 receptor is the mechanism responsible for blocking emesis (Tattersall et al., 1993; Watson et al., 1995a,b), the affinity of compounds for the tachykinin NK_1 receptor in vitro alone is poorly predictive of anti-emetic activity in the absence of brain penetration data (Tattersall et al., 1996; present study). Inhibition of foot tapping in gerbils can also provide an indirect measure of the oral bioavailability of tachykinin NK_1 receptor antagonists. Finally, the predictive validity of anti-emetic effects obtained with tachykinin NK_1 receptor antagonists in ferrets for their clinical utility in man is greatly strengthened by the excellent correlation between the affinity of a wide range of antagonists for the human and the ferret tachykinin NK_1 receptor in vitro (Beresford et al., 1991; Tattersall et al., 1996; present study). Although slight differences were observed in the absolute affinity values for the test compounds at human and ferret receptors, these are probably due to differences in the amounts of membrane protein required in the two assays. The apparent affinity of selected compounds for the human tachykinin NK_1 receptor expressed in CHO cells is reduced when excess protein is added to the assay (unpublished observations).

CP-99,994, (±)-GR203040 and L-742,694 were extremely effective inhibitors of the acute retching response to cisplatin in ferrets. The impressive anti-emetic activity of the phenylpiperidine CP-99,994 appears attributable to the ease and speed of entry of this compound into the brain, as demonstrated using an in situ brain perfusion assay in rats (Shepheard et al., 1995), and by the potent inhibition of foot tapping in gerbils when CP-99,994 was administered intravenously immediately before central infusion of GR73632 (Rupniak and Williams, 1994). However, the rapid loss of inhibition of foot tapping during the first 2 h after i.v. administration of CP-99,994 appears to mirror the rate of decay of the plasma drug concentration (Rupniak et al., 1996), indicating that rapid efflux of this compound occurs from the CNS as plasma drug levels fall. The short duration of action of CP-99,994 in the CNS, and the critical dependence of brain drug levels on the plasma concentration, may explain the need to use continuous intravenous infusions of CP-99,994 in order to investigate its analgesic effects in man (Suarez et al., 1994; Dionne et al., 1996) and anti-emetic activity in dogs (Watson et al., 1995a,b). CP-99,994 has been superseded in clinical development by CP-122,721, a potent non-competitive, orally active tachykinin NK_1 receptor antagonist (McLean et al., 1996) with broad-spectrum anti-emetic activity in ferrets (Gonsalves et al., 1996).

Another major limitation for the clinical development of CP-99,994 is its lack of oral bioavailability, believed to be a consequence of extensive first-pass metabolism (Ward et al., 1995). Metabolic stabilisation of this molecule to prevent hydroxylation of the benzylamine gave rise to the structurally related tetrazole, GR203040. Like CP-99,994, GR203040 exhibits potent, broad-spectrum anti-emetic activity in ferrets (Gardner et al., 1995; present study), but is also orally bioavailable in dogs (Ward et al., 1995). Unlike CP-99,994, (±)-GR203040 also inhibited foot tapping when administered orally; however, this required doses approximately 200 times higher than were effective by the i.v. route, indicating that significant first-pass metabolism may remain a liability for this compound in some species. Our findings suggest that scope remains for further optimisation of the in vivo pharmacology of tachykinin NK_1 receptor antagonists in order to fully exploit their clinical potential as anti-emetics. This is illustrated by the morpholine L-742,694, a compound with comparable anti-emetic potency to CP-99,994 in ferrets, but with excellent oral bioavailability.

The ability to achieve sustained blockade of central tachykinin NK_1 receptors in the absence of high plasma drug concentrations in vivo was also recently described using the piperidine-ether based tachykinin NK_1 receptor antagonist L-733,060 (Rupniak et al., 1996). Such compounds have the advantage over short-acting agents that unwanted nonspecific pharmacological effects in peripheral tissues (e.g., ion channel blockade) incurred at high plasma concentrations can be minimised. It might be anticipated that a long duration of central tachykinin NK_1 receptor blockade might be advantageous to prevent breakthrough emesis during the delayed phase following cyto-

toxic drug therapy. Since the majority (> 70%) of retching and vomiting in ferrets occurs within the first 4 h of challenging with cisplatin (Rudd and Naylor, 1994), animal assays for anti-emetics routinely evaluate efficacy only during the acute cytotoxic insult. Recently, using observation periods extended for up to 72 h, Rudd et al. (1994) and Milano et al. (1995) have demonstrated the presence of both an acute and a delayed emetic response to cisplatin in ferrets and pigs, respectively. Interestingly, whilst the 5-HT_3 receptor antagonists ondansetron and granisetron were highly effective inhibitors of the acute emetic response to cisplatin, delayed retching and vomiting in both species was attenuated, but not fully blocked (Rudd and Naylor, 1994; Milano et al., 1995), a finding consistent with clinical experience. In contrast, repeated administration of CP-99,994 was able to markedly attenuate both acute and delayed emesis induced by cisplatin in ferrets (Watson et al., 1995b; Rudd et al., 1996). Preliminary clinical data also indicate that CP-122,721 may protect against both acute and delayed emesis in man (Kris et al., 1996), and that tachykinin NK_1 receptor antagonists may therefore provide more complete relief from emesis than is possible through other currently available mechanistic approaches.

The acute anti-emetic activity of the other compounds examined in the present study was considerably less impressive than that of the 3 antagonists described above. Whilst not a potent anti-emetic, RPR100893 was unexpectedly active in the cisplatin assay considering its weak inhibition of foot tapping in gerbils. A likely explanation for this discrepancy is that RPR100893 may cross the blood–brain barrier relatively slowly, and its ability to inhibit foot tapping may therefore have been underestimated using the extremely short pretreatment time employed here. This interpretation appears to be compatible with evidence for CNS penetration obtained using an ex vivo binding assay in guinea-pigs 1 h after oral administration of high doses (10–30 mg/kg) of RPR100893 (Flamand and Fardin, 1995). However, for FK888, CGP 49823 and LY303870, the lack of anti-emetic activity was predictable given their ability to block foot tapping only at high intravenous doses. The lack of anti-emetic activity of FK888 in ferrets probably reflects the metabolic instability and poor CNS penetration of peptide molecules in vivo. Similarly, the peptide tachykinin NK_1 receptor antagonist GR82334 was unable to inhibit cisplatin-induced retching in ferrets following systemic administration, whilst direct central injection markedly attenuated retching (Gardner et al., 1994). The weak activity of CGP 49823 observed in the present study is consistent with the findings of Vassout et al. (1994) that doses of approximately 50 mg/kg p.o. were required to inhibit NK_1 agonist-induced foot tapping in gerbils. Our findings using LY303870 are also consistent with the 2–30 mg/kg dose range required to inhibit scratching elicited by intrathecal injection of a tachykinin NK_1 receptor agonist in mice based on a preliminary report by Iyengar et al. (1995), and contrast with the μg/kg potency of this compound to inhibit tachykinin NK_1 agonist-mediated effects in peripheral tissues (Gitter et al., 1995).

The failure of SR140333 to inhibit either foot tapping or emesis resembles the profile of the poorly brain-penetrant tachykinin NK_1 antagonist L-743,310 in these assays (Rupniak and Williams, 1994; Tattersall et al., 1996). The failure of this and other tachykinin NK_1 antagonists examined to inhibit emesis in ferrets was not attributable to a lower affinity of these compounds for the tachykinin NK_1 receptor expressed in this species; indeed, SR140333 had the highest affinity of all the compounds examined for both the human and ferret receptor in vitro. Our findings are not consistent with evidence that SR140333 is centrally active based on its ability to inhibit scratching induced by i.c.v. infusion of tachykinin NK_1 agonists in mice with an ID_{50} of less than 1 mg/kg i.p. (Jung et al., 1994). Other evidence also suggests centrally mediated effects of SR140333 in tests of nociception; in anaesthetised rats, SR140333 potently inhibited thermal hyperalgesia (60 μg/kg i.p.; Jung et al., 1994), and the responses of thalamic neurones to noxious pinch (ID_{50} = 0.2 μg/kg i.v.; Emonds-Alt et al., 1993). However, the antinociceptive effects observed by Emonds-Alt et al. showed only marginal enantioselectivity, and therefore cannot be attributed to selective blockade of central tachykinin NK_1 receptors. Nonspecific antinociceptive activity attributable to ion channel blockade is exhibited by many other tachykinin NK_1 receptor antagonists, including RP67580 (Rupniak et al., 1995), CP-96,345 (Nagahisa et al., 1992) and CP-99,994 (Smith et al., 1994; Rupniak et al., 1995).

In conclusion, our findings provide confirmation of the anti-emetic activity of brain-penetrant tachykinin NK_1 receptor antagonists, and demonstrate that inhibition of tachykinin NK_1 agonist-induced foot tapping in gerbils provides a simple in vivo assay for CNS penetration which is highly predictive of anti-emetic activity in ferrets. Good CNS penetration would also be predicted to be advantageous for migraine therapy by virtue of the central antinociceptive activity of tachykinin NK_1 antagonists in the trigeminal nucleus caudalis (Shepheard et al., 1995) which would be anticipated to give more rapid pain relief than might be accomplished through the inhibition of dural plasma extravasation in the periphery alone.

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