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Inhibition of emesis by tachykinin NK_1 receptor antagonists in *Suncus murinus* (house musk shrew)

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Abstract

The anti-emetic potential of CP-122,721 ((+)-2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine), CP-99,994 ((+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine), CP-100,263 ((−)-(2*R*,3*R*)-3-(2-methoxybenzylamino)-2-phenylpiperidine), RP 67580 ((3*R*, 7a*R*)-7, 7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)ethyl] po-hydroisoindol-4-one), FK 888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-in-dole-3-yl) carbonyl-L-propyl]-*N*- methyl-*N*-phenylmethyl-l-3-(2-naphthyl)-alaninamide) and GR 82334 ([D-Pro^9\{spiro-g-lactam\}Leu^{10}]-physalaemin-(1–11)) was investigated to inhibit nicotine (5 mg/kg, s.c.)-, copper sulphate pentahydrate (120 mg/kg, intragastric)- and motion (4 cm horizontal displacement at 1 Hz for 5 min)-induced emesis in *Suncus murinus*. A 30 min intraperitoneal pre-treatment with CP-122,721, CP-99,994, RP 67580 and FK 888 significantly ($P < 0.05$) antagonized nicotine-induced emesis with ID_{50} values of 2.1, 2.3, 13.5 and 19.2 mg/kg, respectively CP-100,263, the less active enantiomer of CP-99,994, was inactive at doses up to 10 mg/kg. Infusion of GR 82334, CP-122,721, CP-99,994 and FK 888 into the dorsal vagal complex of the hindbrain also antagonized nicotine-induced emesis yielding ID_{50} values of 1.1, 3.0, 3.3 and 58.0 μg/dorsal vagal complex, respectively RP 67580 and CP-100,263 were inactive. RP 67580 and FK 888 failed to antagonize copper sulphate-induced emesis but CP-122,721 and CP-99,994 were active yielding ID_{50} values of 2.2 and 3.0 mg/kg, i.p., respectively. CP-99,994 also completely prevented motion-induced emesis at 10 mg/kg, i.p. ($P < 0.05$) and RP 67580 produced a significant reduction of motion-induced emesis at 10 mg/kg, i.p. ($P < 0.05$). These studies provide evidence of a central site of action of tachykinin NK_1 receptor antagonists to inhibit nicotine-induced emesis in *S. murinus* and confirm the broad profile of inhibitory action. The rank order of potency of the antagonists following the intra-dorsal vagal complex administration suggests that the *S. murinus* tachykinin NK_1 receptor has a unique pharmacological profile.

Keywords: Tachykinin NK_1 receptor; Emesis; *(Suncus murinus)*

1. Introduction

Evidence has accumulated to indicate the importance of substance P in the emetic reflex. For example, tachykinin NK_1 receptor antagonists, such as CP-99,994 ((2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine; McLean et al., 1993) and GR 203040 ((2*S*, 3*S*)-2-methoxy-5-tetrazol-l-yl-benzyl)-(2-phenyl-piperidin-3-yl)-amine; Beattie et al., 1995), are highly effective in reducing the emesis induced by a wide variety of challenges including centrally- (e.g., apomorphine, morphine, and nicotine) and peripherally-acting emetics (e.g., intragastric copper sulphate) and provocative motion (Bountra et al., 1993; Gardner et al., 1995a, 1996; Lucot et al., 1997; Tattersall et al., 1993, 1994, 1995). The pre-clinical studies have yielded information relevant to the clinical utilization and mechanism of action of the compounds. Only tachykinin NK_1 receptor antagonists that penetrate the blood brain barrier are active to antagonise emesis and a site of anti-emetic action within the vicinity of the nucleus tractus solitarius has been proposed (Tattersall et al., 1996). The nucleus tractus solitarius is a logical site for a 'broad spectrum' anti-emetic agent to act since it is a site at which vagal afferents from the gastrointestinal tract converge with inputs from the area postrema (the classical 'chemoreceptor trigger zone' for emesis) and other brain areas associated with emesis control (Leslie and Reynolds, 1992; Tattersall et al., 1996).

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Most of the pre-clinical investigations into the role of tachykinin NK_1 receptor antagonists to inhibit drug-induced emesis have been conducted in the ferret, a species that has a human-like tachykinin NK_1 receptor (Tattersall et al., 1996). However, a few studies have used *Suncus murinus* (the house musk shrew), a species of insectivore, since this animal can be easily utilized to assess the anti-emetic potential of drugs to inhibit motion-induced emesis (Ueno et al., 1988; Gardner et al., 1995a,b). There are however, known species differences in the pharmacology of the tachykinin NK_1 receptor, particularly between human and rodents (Gitter et al., 1991; Fardin and Garret, 1991; Beresford et al., 1992). The species differences are seen with RP 67580 ((3*R*, 7a*R*)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)ethyl] po-hydroisoindol-4-one), a compound that displays approximately 20 times higher affinity for rodent-like ($K_i = 3$ nM) than for human-like tachykinin NK_1 receptors ($K_i = 56$ nM; see Tattersall et al., 1996). In *S. murinus*, it is interesting that RP 67580 is only active to reduce emesis when used at relatively high doses and radioligand binding studies indicate a very low affinity ($K_i > 1$ μM) for *S. murinus* tachykinin NK_1 receptors (Tattersall et al., 1995). Taken together, these results strongly indicate a further species difference for the tachykinin NK_1 receptor and suggest that *S. murinus* may not be ideally suitable to screen for active tachykinin NK_1 receptor antagonists to prevent emesis in man.

In the present studies, we decided to investigate further the pharmacology and role of the tachykinin NK_1 receptor in *S. murinus* by using a range of potent and selective tachykinin NK_1 receptor antagonists. The tachykinin NK_1 receptor antagonists used were CP-99,994 (McLean et al., 1993), CP-100,263 ((2*R*,3*R*)-3-(2-methoxybenzylamino)-2-phenylpiperidine; the less active enantiomer of CP-99,994), CP-122,721 ((2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine; McLean et al., 1996), FK 888 ((N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indole-3-yl) carbonyl-L-propyl] -*N*-methyl-*N*- phenylmethyl-L -3-(2-naphthyl)-alaninamide; Fujii et al., 1992), RP 67580 (Garret et al., 1991) and GR 82334 ([D-Pro^9\{spiro-g-lactam\}Leu^{10}]-physalaemin-(1–11); Hagan et al., 1991) and their rank order of anti-emetic potency was determined against nicotine-, copper sulphate- and provocative motion-induced emesis. Some of the investigations utilized an intracerebral administration of the antagonists into the dorsal vagal complex of the brainstem to inhibit emesis. The dorsal vagal complex essentially comprises the area postrema, nucleus tractus solitarius and dorsal motor nucleus of the vagus nerve (Leslie and Reynolds, 1992). The studies may be considered important to characterize the tachykinin NK_1 receptors in the emesis models since the direct delivery of compounds into the brainstem avoids the logistical problems associated with the use of drugs that have poor central nervous system penetration. The studies should help to clarify if the *S. murinus* tachykinin NK_1 receptor is 'human-like', 'rat-like' or completely novel.

2. Materials and methods

2.1. Animals

The experiments were performed on adult male or female *S. murinus* (30–85 g), bred at the Chinese University of Hong Kong. The breeding animals were originally obtained from the Central Institute for Experimental Animals (Kanagawa, Japan) in 1993. Prior to the experiments, they were housed in a temperature controlled room at 24 ± 1°C under artificial lighting, with lights on between 0700 h and 1730 h. They were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, USA). All experiments were conducted in accordance with the Animal Research Ethics Committee, The Chinese University of Hong Kong.

2.2. Measurement of emesis

On the day of the experiment, the animals were transferred to clear perspex observation chambers ($21 \times 14 \times 13$ cm^3) for the assessment of emetic behaviour. Animal behaviour was recorded by a trained observer. Episodes of emesis were characterized by rhythmic abdominal contractions which are either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e., vomiting) or not associated with the passage of material (i.e., retching movements). Episodes of retching and/or vomiting (bouts) were considered separate when an animal changed its location in the observation chamber, or when the interval between retches and/or vomits exceeded 2 s.

2.3. Stereotaxic surgery

The stereotaxic surgery performed in *S. murinus* was similar to that previously carried out in the ferret (see Tattersall et al., 1996). Animals were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.) and placed into a stereotaxic frame equipped with custom made ear-bars and mouth pieces (David Kopf Instruments, Tujunga, USA). An incision was made in the skin from just behind the nose to the back of the head and the temporalis muscles on either side of the sagittal crest were displaced. The skull areas in the immediate vicinity of the crest were then cleared of connective tissue. A burr hole was made midline 0.3 mm from the posterior edge of the surface of the skull and a guide cannula (23 gauge) was lowered into the brain to a 2 mm depth below the surface of the dura (i.e., 2 mm above the dorsal vagal complex) and fixed with dental cement to a brass anchor screw that was secured to the skull. Once the cement had dried, the operative area was closed with a number of interrupted stitches around the guide cannula. Animals were allowed 72 h to recover from the operative procedure prior to the commencement of the emesis studies.

2.4. Drug-induced emesis studies

The effect of nicotine (0–10 mg/kg, s.c.) or intragastric copper sulphate pentahydrate solution (0–200 mg/kg) was investigated over a 30 min observation period to ascertain the optimum doses of the emetogens to use in the anti-emetic studies.

To assess the anti-emetic potential of an intra-dorsal vagal complex administration of tachykinin NK_1 receptor antagonists, the animals were first injected subcutaneously with nicotine and then an injection needle (30 gauge) was immediately inserted into the dorsal vagal complex via the guide cannula. After 3 min, the animals received a 4 min intra-dorsal vagal complex infusion of drug or vehicle (1 μl/min) and were monitored for the development of retching and/or vomiting episodes. At the end of the 30 min observation period, the animals were anaesthetized with pentobarbitone (60 mg/kg, i.p.) and 2 μl of Evans Blue dye was infused into the site of injection. We injected 2 μl of dye since this volume enabled a more accurate assessment of the centre of the site of drug infusion than the 4 μl volume used for the actual drug administration studies. The brains were then fixed in situ by cardiac perfusion with phosphate buffered paraformaldehyde and dissected for histological examination using a binocular light microscope (×10 magnification) to confirm the site of injection.

Selected tachykinin NK_1 receptor antagonists were also administered intraperitoneally as a 30 min pre-treatment to assess the effect of a peripheral administration of the drugs on the emesis induced by either nicotine or copper sulphate.

2.4.1. Motion-induced emesis studies

In these experiments, the animals were initially screened for their susceptibility to motion-induced emesis 2 weeks prior to receiving the antagonists on the cross-over. On the first occasion, the animals received an intraperitoneal injection of the respective tachykinin NK_1 receptor antagonist vehicle followed by a 5 min motion-induced emesis test. To conduct the test, the animals were placed on a linear reciprocating desktop shaker (Heidolph Promax 2020, Labplant, England) that was set to produce a 4 cm horizontal displacement, delivered at 1 Hz, as previously described (Gardner et al., 1995a,b; Ueno et al., 1988). *S. murinus* that failed to retch or vomit during the 5 min test were excluded from the studies. On the cross-over, the 'motion-sensitive' animals were randomized to receive either an intraperitoneal administration of tachykinin NK_1 receptor antagonist or respective vehicle 30 min prior to the motion test.

2.5. Statistical analysis

The total number of episodes was recorded in each animal following the administration of the respective emetogens or the start of the motion-induced emesis test. For the drug-induced emesis experiments, the significance of difference between treatments was assessed by One-way analysis of variance (ANOVA) followed by a Fisher's Protected Least Significant Difference (PLSD) test (Statsview®, Abacus Concepts, USA). To assess an effect on motion-induced emesis the data was subject to analysis by a repeated measures two-factor ANOVA with comparisons of specified means by Planned Contrasts (Super-ANOVA®, Abacus Concepts, USA). The latter procedure is very efficient for comparing a limited subset of possible contrasts. This is useful for testing hypotheses about data that are more specific than the hypothesis automatically tested for by each term in the ANOVA model (Gagnon et al., 1989). Differences were considered significant when $P < 0.05$. ID_{50} values were determined on the mean data by non-linear regressional analysis (Kailidagraph™, Synergy Software, USA).

2.6. Drug formulation

Both (−)-nicotine di-D-tartrate (Research Biochemicals International) and copper sulphate pentahydrate (British Drug Houses) were dissolved in distilled water and administered in a volume of 2 ml/kg. CP-99,994 (Pfizer), CP-100,263 (Pfizer), CP-122-721 (Pfizer) and GR 82334 (Research Biochemicals International) were dissolved in saline (0.9% w/v). RP 67580 (Rhône-Poulenc Rorer) was first dissolved with a few drops of 0.1 M hydrochloric acid and was made up in NaCl (0.9%w/v; the final pH was adjusted to 6 with 0.1 M NaOH). FK 888 (Fujisawa Pharmaceutical) was formulated in polyethylene glycol 200 for the peripheral studies. For the central administration studies, FK 888 was formulated in a solution of 2:1

Table 1
The emetic action of nicotine (1.25–10 mg/kg s.c.) and copper sulphate pentahydrate solution (40–200 mg/kg intragastric) in *S. murinus* during a 30 min observation period

	Latency (min)	Episodes	RV/*T*
Nicotine (mg/kg)			
0.0	–	0.0 ± 0.0	0/3
1.25	–	0.0 ± 0.0	0/3
2.5	11.3 ± 3	10.7 ± 5.0	3/3
5.0	4.4 ± 2.0	20.0 ± 1.2[a]	3/3
10.0	5.1 ± 0.7	13.3 ± 3.2	3/3
$CuSO_4 \cdot 5H_2O$ (mg/kg)			
0.0	–	0.0 ± 0.0	0/4
40.0	2.1 ± 0.4	8.5 ± 0.7	4/4
80.0	2.7 ± 0.6	11.0 ± 4.5	4/4
120.0	1.5 ± 0.2	20.0 ± 2.4[a]	4/4
200.0	2.1 ± 0.3	17.0 ± 4.3[a]	4/4

Data represent the mean ± S.E.M. of 3–4 determinations.
RV/*T* indicates the number of animals either retching or vomiting (RV) out of the number of animals tested (*T*).
Significant differences between the respective vehicle treatments (0.0 mg/kg) and nicotine or copper sulphate pentahydrate treated animals are indicated as [a]$P < 0.05$ (One-way ANOVA followed by Fisher's PLSD test).

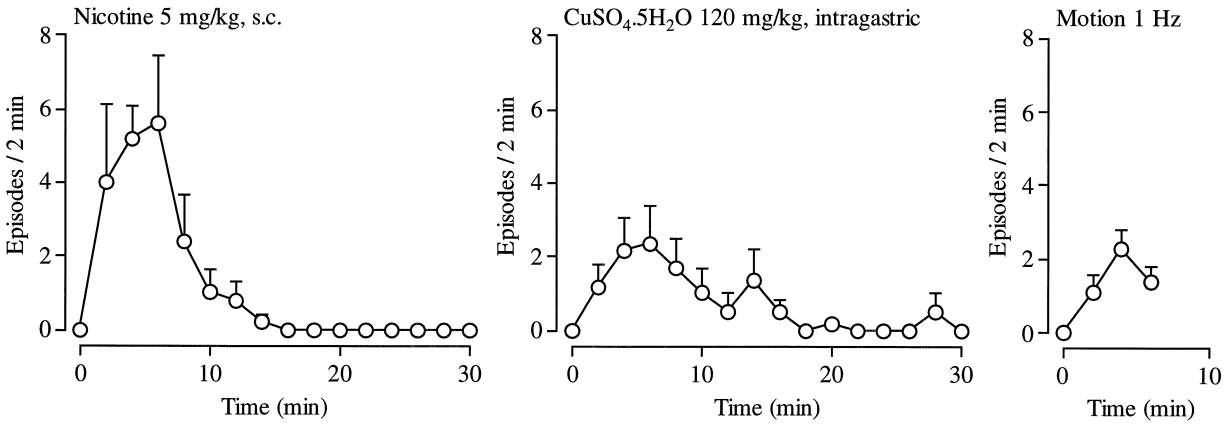

Fig. 1. The emetic profile of nicotine (5 mg/kg, s.c.), copper sulphate pentahydrate (120 mg/kg, intragastric) and motion (4 cm horizontal displacement, 1 Hz for 5 min) in *S. murinus*. Values are the means ± S.E.M. of 5–14 determinations.

dimethyl sulfoxide:saline (0.9% w/v). For the peripheral administration studies, CP-99,994, CP-100,263, CP-122-721 and FK 888 were administered in a volume of 2 ml/kg; RP 67580 was administered in a volume of 4 ml/kg. Doses are expressed as the free base weight unless otherwise indicated.

3. Results

3.1. Drug-induced emesis

The subcutaneous administration of nicotine produced emesis at doses as low as 2.5 mg/kg and was maximally effective at 5 mg/kg; higher doses were associated with a reduction of emesis (Table 1). A dose of nicotine 5 mg/kg, s.c., was selected for the anti-emetic studies. This dose reliably induced emesis within 4.4 ± 2.0 min of injection and comprised 20.0 ± 1.2 episodes; the duration of emesis (from the first to the last episodes) was 6.0 ± 1.8 min (Fig. 1).

The intragastric administration of copper sulphate pentahydrate produced emesis at doses as low as 40 mg/kg within approximately 2 min of administration. Higher doses (up to 200 mg/kg) were also associated with emesis (Table 1). A dose of copper sulphate pentahydrate 120 mg/kg was selected for the anti-emetic studies and produced emesis within 1.5 ± 0.2 min of administration and

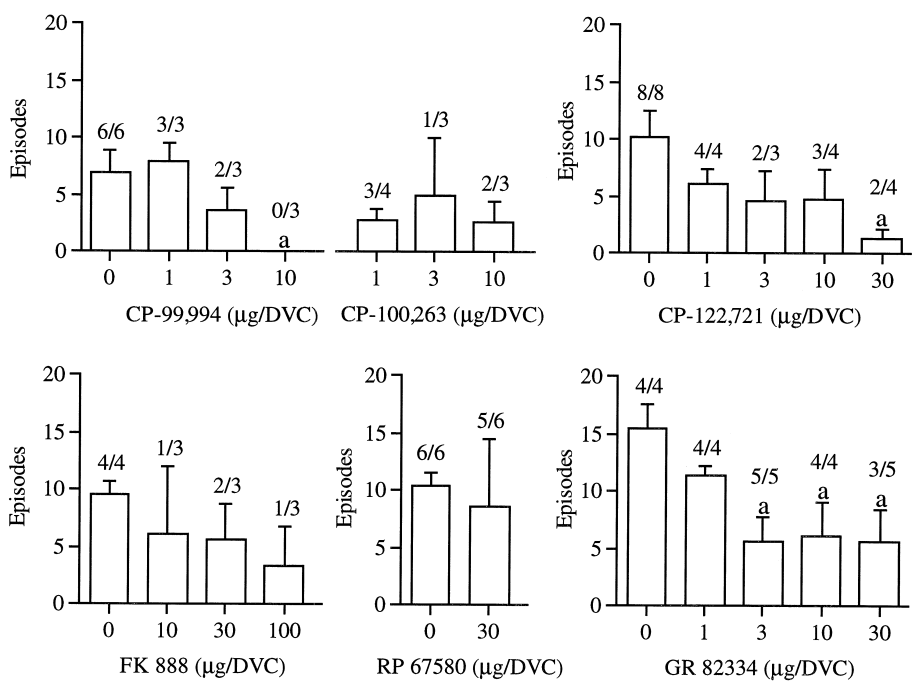

Fig. 2. The effect of an intra-dorsal vagal complex (DVC) administration of tachykinin NK_1 receptor antagonists on nicotine (5 mg/kg, s.c.)-induced emesis in *S. murinus*. Values are the means ± S.E.M. of 3–8 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences from vehicle treatment during the 3–30 min observation period are indicated as $^{a}P < 0.05$ (One-way ANOVA followed by a post-hoc Fisher's PLSD test).

comprised 20.0 ± 2.4 episodes; the duration of action was 12.2 ± 2.6 min (Fig. 1).

3.2. Antagonism of nicotine-induced emesis

3.2.1. Central administration of antagonists

The site of injection of the antagonist or vehicle was confirmed by the infusion of Evans Blue dye at the end of the experiment. Only data from animals showing bilateral staining of the primary target site restricted to the dorsal vagal complex were analyzed (see Won et al., 1998, for a description of brainstem anatomy in *S. murinus*); data from brains showing extensive staining of the central canal were rejected. In a preliminary set of experiments, the intra-dorsal vagal complex infusion of saline (1 μl/min) or 2:1 dimethyl sulfoxide:saline (1 μl/min), for 4 min, was associated with a reduction of exploratory behaviour. It is possible that the intra-dorsal vagal complex infusion technique and/or surgery may have interfered with the emetic potential of nicotine since only approximately 7–15 episodes of retching and or vomiting could be observed (Fig. 2) compared to the 17–24 episodes observed in normal non-operated animals (Fig. 3). While it is possible that the physical infusion of vehicle into the brain may have affected the emetic reflex, it is also possible that the handling of the animals to insert and remove the infusion needle could have also interfered with the retching and vomiting response. Nevertheless, infusion of CP-99,994 inhibited nicotine-induced emesis at doses as low as 10 μg/dorsal vagal complex ($P < 0.05$) but its less active enantiomer, CP-100,263, was without significant effect ($P > 0.05$) over a similar dose range and CP-122,721 produced a significant 88% reduction of emesis at 30 μg/dorsal vagal complex (Fig. 2; $P < 0.05$). There was a non-significant trend for FK 888 to reduce dose-dependently nicotine-induced emesis (the reduction at a dose of 100 μg/dorsal vagal complex was 65%, $P > 0.05$) but RP 67580 was essentially inactive at 30 μg/dorsal vagal complex (Fig. 2; $P > 0.05$). GR 82334 was active to antagonise emesis at doses as low as 3 μg/dorsal vagal complex (Fig. 2; $P < 0.05$) but could only produce a 64% maximum reduction of emesis when tested at higher doses (up to 30 μg/dorsal vagal complex, $P < 0.05$).

Based on ID_{50} values, the rank order of potency of the antagonists to reduce emesis was: GR 82334 (ID_{50} = 1.12 μg/dorsal vagal complex) > CP-122,721 (ID_{50} = 3.0 μg/dorsal vagal complex) > CP-99,994 (ID_{50} = 3.3 μg/dorsal vagal complex) > FK 888 (ID_{50} = 58 μg/dorsal vagal complex). The ID_{50} values for CP-100,263 and RP 67580 could not be calculated but RP 67580 appeared less potent than FK 888 to inhibit emesis.

3.2.2. Peripheral administration of antagonists

CP-99,994, CP-122,721 and RP 67580 dose dependently reduced nicotine-induced emesis (Fig. 3; $P < 0.05$). The anti-emetic effects of CP122,721 and CP-99,994 were evident at 1 to 3 mg/kg, i.p. but with RP 67580 only significantly inhibiting emesis at 30 mg/kg, i.p. (Fig. 3; $P < 0.05$). Similarly, FK 888 was only active to antagonise nicotine-induced emesis when used at 30 mg/kg, i.p. and

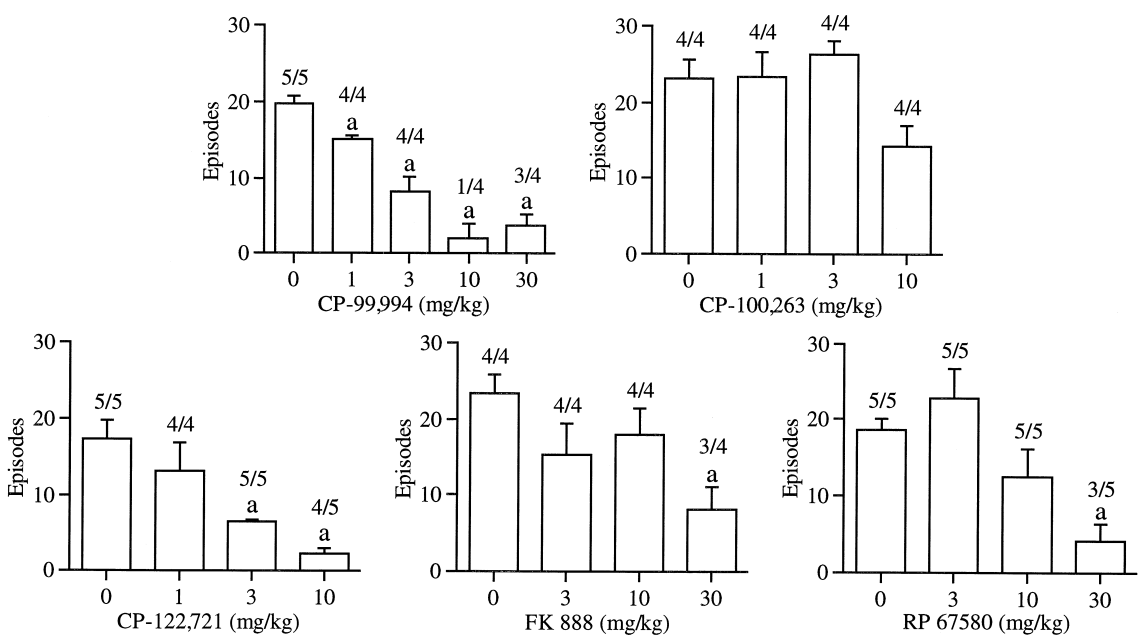

Fig. 3. The effect of an intraperitoneal administration of tachykinin NK_1 receptor antagonists on nicotine (5 mg/kg, s.c.)-induced emesis in *S. murinus*. Values are the means ± S.E.M. of 4–5 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences from vehicle treatment are indicated as $^{a}P < 0.05$ (One-way ANOVA followed by a post-hoc Fisher's PLSD test).

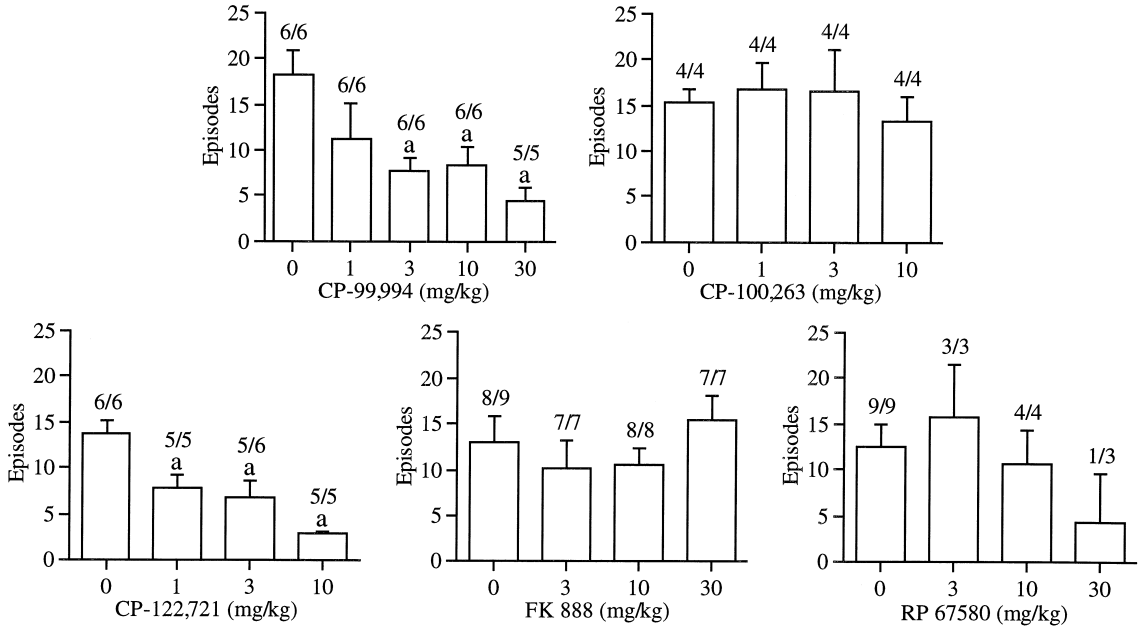

Fig. 4. The effect of an intraperitoneal administration of tachykinin NK_1 receptor antagonists on copper sulphate pentahydrate (120 mg/kg, intragastric)-induced emesis in *S. murinus*. Values are the means ± S.E.M. of 3–9 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences from vehicle treatment are indicated as $^{a}P < 0.05$ (One-way ANOVA followed by a post-hoc Fisher's PLSD test).

produced a 65% reduction in the number of emetic episodes (Fig. 3; $P < 0.05$). The anti-emetic effects of CP-99,994 and CP-122,721 were comparable at 10 mg/kg, i.p. with 89 ($P < 0.05$) and 87% ($P < 0.05$) reductions, respectively of the number of emetic episodes but CP-100,263 only produced a 39% reduction that was not statistically significant (Fig. 3; $P > 0.05$). Based on ID_{50} values, the rank order of potency of the antagonists to reduce emesis was: CP-122,721 ($ID_{50} = 2.1$ mg/kg) ≥ CP-99,994 ($ID_{50} = 2.3$ mg/kg) > RP 67580 ($ID_{50} = 13.5$ mg/kg) > FK 888 ($ID_{50} = 19.2$ mg/kg). The ID_{50} value of CP-100,263 could not be determined.

3.3. Antagonism of copper sulphate-induced emesis

Only CP-99,994 and CP-122,721 were capable of significantly reducing copper sulphate-induced emesis ($P < 0.05$) and CP-100,263 and FK 888 were inactive ($P > 0.05$)

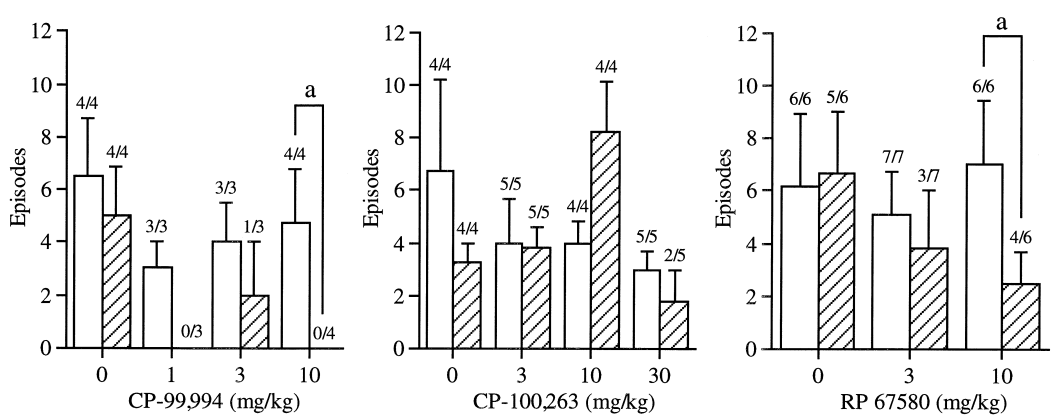

Fig. 5. The effect of tachykinin NK_1 receptor antagonists on motion (4 cm horizontal displacement at 1 Hz for 5 min)-induced emesis in *S. murinus*. Values are the means ± S.E.M. of 3–7 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences between data generated in the pre-test (open histograms) and test phase of the experiment (hatched histograms) are indicated as $^{a}P < 0.05$ (repeated measures two-factor ANOVA with comparisons of means by Planned Contrasts).

at the doses tested (Fig. 4). RP 67580 displayed a trend to antagonise dose dependently copper sulphate-induced emesis but this was not statistically significant (Fig. 4; $P > 0.05$). At the doses tested, none of the antagonists were capable of completely preventing the emesis induced by copper sulphate. The maximum reduction observed was 79.5% by CP-122,721 at 10 mg/kg, i.p.

Based on ID_{50} values, the rank order of potency of the antagonists to reduce emesis was: CP-122,721 ($ID_{50} = 2.2$ mg/kg) ≥ CP-99,994 ($ID_{50} = 3.0$ mg/kg) > RP 67580 ($ID_{50} = 28.6$ mg/kg). The ID_{50} values for CP-100,263 and FK 888 could not be calculated but both antagonists appeared less potent than RP 67580 to inhibit emesis.

3.4. Antagonism of motion-induced emesis

Analysis of the control data generated from the first arm of the motion experiments revealed a latency to onset of emesis of 2.2 ± 0.3 min comprising 6.4 ± 1.5 episodes during the 5 min test interval (see Fig. 1 for a profile of the emetic response). After randomization of the animals to receive vehicle or drug administration, the control animals exhibited emesis following a latency of 2.0 ± 0.3 min and comprised 5.2 ± 1.2 episodes on the second motion test (Fig. 5).

We used a repeated measures two-factor ANOVA with comparisons of specified means by Planned Contrasts to avoid misinterpreting changes caused by simple desensitization of the animals to the motion stimulus as a drug effect. Using this approach, analysis of the data showed that CP-99,994 completely prevented emesis in four out of four animals at the doses of 1 (this was not statistically significant; $P > 0.05$) and 10 mg/kg, i.p. ($P < 0.05$). However, CP-100,263 only inhibited emesis in two out of five animals at the high dose of 30 mg/kg, i.p. (this was not statistically significant, $P > 0.05$; Fig. 5). RP 67580 produced a dose-related antagonism of motion-induced emesis that was statistically significant at 10 mg/kg, i.p. causing a 64% reduction in the number of emetic episodes (four out of six animals were protected from retching and/or vomiting, $P < 0.05$; Fig. 5).

4. Discussion

The present studies have used a number of selective and potent tachykinin NK_1 receptor antagonists to characterize the tachykinin NK_1 receptors in the emetic reflex of *S. murinus*. Using an intra-dorsal vagal complex administration, we demonstrated a central site of anti-emetic action of tachykinin NK_1 receptor antagonists that is consistent with the mechanism previously described for inhibition of vomiting in the ferret (Gardner et al., 1994; Tattersall et al., 1996). In our studies, GR 82334, CP-122,721 and CP-99,994 were potent inhibitors of nicotine-induced emesis following injection into the dorsal vagal complex and FK 888 was less active. The ability of CP-99,994 to antagonize the emesis induced by three emetic challenges with purported different mechanisms of action confirm the broad anti-emetic potential of the tachykinin NK_1 receptor antagonists. Importantly, these studies revealed that RP 67580 was inactive following central administration, confirming the suspicion that the *S. murinus* tachykinin NK_1 receptor has a unique pharmacological profile (see Tattersall et al., 1995).

However, while RP 67580 was inactive to inhibit nicotine-induced emesis following an intra-dorsal vagal complex injection, it was capable of antagonizing emesis following peripheral administration. A significant 78% reduction of nicotine-induced emesis was observed at 30 mg/kg, i.p. which is similar to the level of inhibition reported by Tattersall et al. (1995). We have, therefore, established that RP 67580 is not likely to be acting at the dorsal vagal complex to suppress emesis and given the poor penetration of this compound into the central nervous system (V. Fardin, Personal communication), a peripheral mechanism of action may be involved. While the precise mechanism of action of RP 67580 to suppress nicotine-induced emesis in *S. murinus* is unknown, it has been hypothesized to involve a block of Ca^{2+} channels (Rupniak et al., 1993; Tattersall et al., 1995). Such a mechanism is possible and further studies using selective calcium channel blockers could be designed to help resolve the mechanism. Certainly, the studies may be interesting given the trend by RP 67580 to also reduce copper sulphate-induced emesis and to antagonise motion-induced emesis. Of further importance to the classification of tachykinin NK_1 receptors in *S. murinus* was the similar inhibition of emesis by CP-122,721 and CP-99,994. There was an equipotent reduction of nicotine-induced emesis either by intra-dorsal vagal complex (ID_{50} values ranged from 3.0–3.3 μg/dorsal vagal complex) or intraperitoneal (ID_{50} values ranged from 2.1–2.3 mg/kg) administration to suggest that these compounds have similar penetration into the brain and similar affinity for *S. murinus* tachykinin NK_1 receptors. Both compounds were also as effective, in terms of ID_{50} values (range = 2.2–3.0 mg/kg, i.p.) to antagonise copper sulphate-induced emesis. Though not directly comparable, the data generated from the nicotine and copper sulphate studies suggest that CP-99,994 is approximately 10 times less active in the *S. murinus* than in the ferret to prevent emesis (Bountra et al., 1993; Watson et al., 1995). Consistent with the difference in anti-emetic potency, radioligand binding studies have revealed that CP-99,994 has six times less affinity for the *S. murinus* tachykinin NK_1 receptor ($IC_{50} = 12$ nM; Tattersall et al., 1995) compared to the affinity for the ferret tachykinin NK_1 receptor ($IC_{50} = 1.97$ nM; Watson et al., 1995). No data are available to indicate the affinity of CP-122,721 for either ferret or *S. murinus* receptors but this compound is a non-competitive antagonist at human tachykinin NK_1 receptors expressed in IM-9 cells (approximate IC_{50} value = 0.2 nM) and has a similar

affinity to CP-99,994 (McLean et al., 1996). Like CP-99,994, CP-122,721 was also 2.5–10 times less active to inhibit emesis in *S. murinus* than in the ferret following a peripheral administration.

An antagonism of motion-induced emesis was also produced by CP-99,994 which is consistent with the use of the racemic compound in *S. murinus* (Gardner et al., 1995b). The anti-emetic action was clearly evident at 10 mg/kg, i.p. but a complete inhibition of emesis was also seen at 1 mg/kg ($P > 0.05$) which is much lower than required to prevent nicotine- or copper sulphate-induced emesis. CP-99,994 appears more potent in this test possibly because provocative motion is the weakest emetic stimulus used in the studies. However, CP-100,263, the enantiomer of CP-99,994, was less active against motion-induced emesis even when used at the high dose of 30 mg/kg, i.p. The data provide evidence that tachykinin NK_1 receptor antagonism is probably the mechanism of anti-emetic action of CP-99,994; such stereoselective effects are also reported in the cat (Lucot et al., 1997). CP-100,263 was also inactive to prevent nicotine-induced emesis to confirm the stereoselective action (present studies; Tattersall et al., 1995) and we also observed no effect with this compound against copper sulphate-induced emesis.

Some of our studies used FK 888, a peptide based antagonist that has high affinity for human-like tachykinin NK_1 receptors ($K_i = 1.2–3.6$ nM; Goso et al., 1994). FK 888 failed to antagonise copper sulphate-induced emesis but did show a trend to reduce nicotine-induced emesis. The antagonism of the emesis was evident following either a central ($ID_{50} = 58$ μg/dorsal vagal complex) or peripheral administration ($ID_{50} = 19.2$ mg/kg). The peripheral activity of the compound was initially unexpected given the reports of a failure of intravenously administered FK 888 (1 mg/kg) to prevent cisplatin-induced emesis in the ferret (Rupniak et al., 1997). However, following an intravenous administration, FK 888 is able to antagonise (ID_{50} value = 3.7 mg/kg) the foot-tapping induced by the central administration of the tachykinin NK_1 receptor agonist, GR 73632 ((+)-Ava [L-Pro^9, Me–Leu^{10}]substance P-(7–11); Rupniak et al., 1997) to suggest that this compound does cross the blood brain barrier. In our studies, the intra-dorsal vagal complex administration of FK 888 was approximately 13 times less active than CP-99,994 to reduce nicotine-induced emesis but was only eight times less active following the peripheral administration. FK 888 is known to have approximately four times lower affinity for human tachykinin NK_1 receptors ($K_i = 1.2–3.6$ nM; Goso et al., 1994) than CP-99,994 ($K_i = 0.3$ nM; Tattersall et al., 1996) but since CP-99,994 is also slightly less active at *S. murinus* tachykinin NK_1 receptors, the small difference in potency of FK 888 may be expected. We can only speculate that the lack of activity of FK 888 in the ferret model may relate to the low dose used and/or a metabolism of the compound during the 4 h assessment period; the present studies assessed anti-emetic activity over a shorter 30 min observation time.

As we have reported, some of our studies used copper sulphate to induce emesis to assess the anti-emetic activity of the antagonists. The emesis induced by copper sulphate probably results from gastric irritation and can be reduced by bilateral abdominal vagotomy and splanchnectomy in other species (Wang and Borison, 1951; Andrews et al., 1990). The model may therefore be useful to mimic situations in man where gastrointestinal disturbance could contribute to the development of emesis. However, the antagonists that we studied only produced a 76–80% reduction of the number of the emetic episodes to suggest that mechanisms other than those involving tachykinin NK_1 receptors could be involved in mediating the emesis. An examination of the literature reveals that tachykinin NK_1 receptor antagonists can reliably prevent low-dose (6–12.5 mg/kg, p.o.) copper sulphate-induced emesis in the ferret and dog (Watson et al., 1995; Gonsalves et al., 1996). However, when copper sulphate is used at a higher dose (40 mg/kg, p.o.) there is a small tachykinin NK_1 receptor antagonist resistant component of the response (Bountra et al., 1993; Gardner et al., 1995a). It is also very interesting that tachykinin NK_1 receptor antagonists are also ineffective to prevent the emesis induced by mechanical distention of the oesophagus in *S. murinus* (Andrews et al., 1996). It is tempting to speculate that the tachykinin NK_1 resistant phase of copper sulphate- and mechanical distension-induced emesis may involve an activation of similar pathways from the gastrointestinal tract. The clinical significance, if any, of the tachykinin NK_1 resistant emesis remains to be determined. The ability of tachykinin NK_1 receptor antagonists to prevent emesis induced via different mechanisms is supported by the present studies and is likely to occur at a point pivotal to the emetic reflex such as the dorsal vagal complex. We used GR 82334 as an antagonist to help characterize the tachykinin NK_1 receptors in *S. murinus* since this compound has already been shown to block emesis in the ferret following an injection into the nucleus tractus solitarius (Gardner et al., 1994). In the ferret, GR 82334 appears less potent than CP-99,994 to inhibit emesis following an injection into the brainstem (Gardner et al., 1994; Tattersall et al., 1996) but in our studies, GR 82334 was surprisingly potent to inhibit emesis. In fact, in terms of ID_{50} values, GR 82334 was slightly more potent than both CP-122,721 and CP-99,994 to antagonise nicotine-induced emesis but could only provide a 63% reduction of emesis whereas CP-99,994 was completely effective. It is not clear why GR 82334 was unable to produce a more effective block of emesis since it dose dependently inhibits emesis in the ferret (Gardner et al., 1994). However, we were surprised at the activity of GR 82334 to inhibit emesis since in functional studies in the rat the compound is 8 and 400 times less potent than CP-99,994 and RP 67580, respectively, to inhibit outward K^+ currents in NG 108-15 (Phenna et al., 1996). GR

82334 also has approximately 60 times lower affinity for human tachykinin NK_1 receptors than CP-99,994 (R. Hagan, personal communication).

Certainly, the rank order of potency of GR 83224 > CP-122,721 ≥ CP-99,994 > FK 888 > RP 67580 to inhibit nicotine-induced emesis is not consistent with a known action of the compounds at human- or rat-like tachykinin NK_1 receptors. This suggests that the *S. murinus* tachykinin NK_1 receptor has a unique pharmacological profile. However, there was a basic difference between our central administration studies and those conducted in the ferret (Gardner et al., 1994; Tattersall et al., 1996). We administered the antagonists into the dorsal vagal complex while in the ferret studies, the drugs were discretely administered to the vicinity of the nucleus tractus solitarius and it is not certain if this could contribute to the observed differences.

In conclusion, we have found that CP-122,721, CP-99,994, FK 888 and GR 82334 but not RP 76580 are capable of differentially antagonizing emesis in *S. murinus* by a mechanism that is likely to involve tachykinin NK_1 receptors located in the dorsal vagal complex. These compounds are all known to have reasonable affinity for the human-like tachykinin NK_1 receptor. However, the rank order of potency to inhibit emesis in *S. murinus* is not consistent with an action at either the human- or rat-like tachykinin NK_1 receptors since GR 82334 was surprisingly active and RP 67580 was virtually without effect following an intra-dorsal vagal complex injection. The data confirm the contention that the *S. murinus* tachykinin NK_1 receptor has a unique pharmacological profile (Tattersall et al., 1995). Taken together, we suggest that the *S. murinus* is not a useful species to use to screen for tachykinin NK_1 receptor antagonists to prevent emesis in man. This view is supported by the recent report of the low anti-emetic potency of GR 203040 in *S. murinus*. GR 203040, a novel phenylpiperidine, has high affinity for the human-like tachykinin NK_1 receptor ($K_i = 0.03–0.08$ nM; Beattie et al., 1995) and is an extremely potent anti-emetic in the ferret but is required in relatively high-doses to produce a significant reduction of emesis in *S. murinus* (Gardner et al., 1995a). It is clear that the clinical potential of this compound would have been missed had studies only been carried out using *S. murinus*.

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