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Emetic action of the prostanoid TP receptor agonist, U46619, in *Suncus murinus* (house musk shrew)

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Abstract

The emetic action of the prostanoid TP receptor agonist, 11α,9α-epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid (U46619; 300 μg/kg, i.p.), was investigated in *Suncus murinus*. The emetic response was reduced by 76% following bilateral abdominal vagotomy ($P<0.001$) and by reserpine (5 mg/kg, i.p., 24 h pretreatment; $P<0.05$) but U46619 administered i.c.v. (30–300 ng) was not emetic, suggesting a peripheral mechanism involving monoamines. However, fenfluramine (5 mg/kg, repeated treatment) and *para*-chlorophenylalanine (100–400 mg/kg) and ondansetron (0.3–3 mg/kg) were inactive ($P>0.05$) to reduce U46619-induced emesis precluding a role of 5-HT and 5-HT_3 receptors in the mechanism. Similarly, phentolamine (0.3–3 mg/kg), propranolol (3 mg/kg), and their combination, and metoclopramide (0.3–3 mg/kg), domperidone (0.3–3 mg/kg), droperidol (0.3–3 mg/kg), scopolamine (0.3–3 mg/kg) and promethazine (0.3–3 mg/kg) were inactive ($P>0.05$) to reduce the retching and vomiting response. However, the tachykinin NK_1 receptor antagonist, (+)-2*S*,3*S*(-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine) (CP-122,721; 1–10 mg/kg) antagonized emesis ($P<0.01$). In conclusion, U46619-induced emesis appears to be mediated via a predominant peripheral mechanism sensitive to reserpine and is not likely to involve adrenoceptors, dopamine, 5-HT_3, muscarinic or histamine (H_1) receptors. The action of CP-122,721 to reduce U46619-induced emesis extends the spectrum of anti-emetic action tachykinin NK_1 receptor antagonists to mechanisms involving TP receptors.

Keywords: U46619; Prostanoid TP receptor; Tachykinin NK_1 receptor; Emetic; Anti-emetic; Prostanoid; (*Suncus murinus*)

1. Introduction

The anti-emetic action of glucocorticoids to reduce chemotherapy-induced emesis has been hypothesized to involve partly a reduction of prostaglandin synthesis via inhibition of phospholipase A_2 and by preventing cyclo-oxygenase-2 expression (Sam et al., 2001). The hypothesis is supported by studies in the piglet showing the anti-emetic activity of cyclo-oxygenase inhibitors to reduce cisplatin-induced emesis (Girod et al., 2002); there are also reports of a reduction of radiation-induced emesis in the dog (Carpenter et al., 1986).

Cyclo-oxygenase enzymes metabolize arachidonic acid to form prostaglandin G_2. A peroxidase enzyme then converts prostaglandin G_2 to prostaglandin H_2 and from here several synthase enzymes form prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 (prostacyclin) and thromboxane A_2 (Coleman et al., 1994). It is now well established that these prostaglandins and thromboxane A_2 exert their biological actions by interacting with five major prostanoid receptors, namely DP, EP, FP, IP and TP receptors (Coleman et al., 1994). We previously investigated the role of each prostanoid receptor in the emetic reflex of the ferret (Kan et al., 2002) and *Suncus murinus* (Kan et al., in press). Our studies in the ferret revealed the emetic activity of EP (prostaglandin E_2, 17-phenyl-ω-trinor prostaglandin E_2, misoprostol and sulprostone), DP, (5-(6-Carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin: BW245C) and TP (11α,9α-epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid: U46619) receptor agonists (Kan et al., 2002). A different situation occurs in *S. murinus* where only the TP receptor agonist U46619 was potent to induce emesis, although the DP receptor agonist BW245C also induced emesis at high dose (possibly through TP receptors; Kan et al., in press).

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Little is known about the mechanism of action of prostanoids to induce emesis. However, a consistent feature that interests us from data in both the ferret and *S. murinus* is the potent emetic activity of the TP receptor agonist, U46619. This may indicate the importance of the TP receptor in the emetic reflex and it is possible that it represents a site for anti-emetic development. In the present studies, therefore, we decided to investigate the emetic mechanism of action of U46619 in more detail using *S. murinus*. Central administration and nerve lesion studies were conducted in an attempt to determine a site of action of U46619 to induce emesis. To investigate the role of monoaminergic and 5-hydroxytryptaminergic systems to the mechanism of emetic action, we used reserpine to deplete monoamine stores (Giachetti and Shore, 1978) and *para*-chlorophenylalanine and fenfluramine to reduce 5-hydroxytryptamine levels (Jequier et al., 1967; Kleven and Seiden, 1989). Detailed studies also examined the role of classical anti-emetic drugs (antagonists at dopamine, acetylcholine, adrenergic, histamine H_1 and 5-hydroxytryptamine receptors; Marin et al., 1990) and the tachykinin NK_1 receptor antagonist, (+)-2*S*,3*S*(-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine) (CP-122,721; Gonsalves et al., 1996), to determine the contribution of various receptor systems to the mechanism of emetic action.

2. Methods and materials

2.1. Animals

The experiments were performed on male or female *S. murinus* (30–80 g), bred at the Chinese University of Hong Kong. They were maintained in temperature-controlled room at 24 ± 1 °C under artificial lighting, with lights on between 0700 and 1730 h. Artificial humidity was maintained at $50 \pm 5\%$. Animals were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, St. Louis, MO, USA). All experiments were conducted under license from the Government of the Hong Kong SAR and the Animal Research Ethics Committee, The Chinese University of Hong Kong. No animal was used more than once.

2.2. Central administration studies

The technique used to cannulate the lateral ventricle was similar to that described by Rudd and Wai (2001). Male animals (50–80 g) were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) and placed into a stereotaxic frame equipped with custom-made ear bars and mouthpieces (level 0 mm on the inter-aural line; Model 900, 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 according to the following coordinates: 8.2 mm rostral to the cross-suture and 0.9 mm to the right of the midline. A guide cannula (23 gauge, 13 mm long, Cooper's Needle Works, UK) was lowered into the brain to a 1.2 mm depth below the surface of the dura (i.e. 2 mm above the lateral ventricle) and fixed with dental acrylic (Procare Dental, Bradford, UK) 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 (2/0 braided silk suture, Mersilk, Ethicon, UK). The skin wound was sprayed with antibiotic aerosol (Tribiotic Spray®, Riker Laboratories, UK) and silicone wound dressing (OpSite®, Smith and Nephew, UK). Animals were allowed 72 h to recover from the operative procedure prior to the administration of drugs.

2.3. Nerve lesion studies

The surgical techniques to lesion the vagi have been described previously (Mutoh et al., 1992). Briefly, the animals were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) and the ventral abdominal surface shaved from the costal margin to the inguinal ligament. The skin was subsequently disinfected with 0.5% chlorhexidine in 70% alcohol. A midline 1.5 cm laparotomy incision was then made and the ventral and dorsal trunks running along the esophagus were located by blunt dissection and at least 0.5 cm of each nerve removed (the serosa of the oesophagus was slightly incised to facilitate the procedure). Braided silk suture (2/0, Mersilk, Ethicon) was used to ligate the cut ends of the vagi. The abdominal contents were moistened with sterile saline and the peritoneum and skin layers closed separately with 2/0 braided silk sutures using interrupted stitches. Skin wounds were sterilized with 0.5% chlorhexidine in 70% alcohol and sprayed with antibiotic aerosol (Tribiotic Spray®, Riker Laboratories) and then silicone wound dressing (Opsite®, Smith and Nephew). Sham operation was performed using similar procedures except the nerves were not lesioned. All animals were allowed 7 days to recover from the operative procedures.

2.4. Induction and measurement of emesis

On the day of experiment, the animals were transferred to clear Perspex observation chambers ($21 \times 14 \times 13$ cm) for the assessment of emetic behaviour. They were allowed 30 min to adapt before any further experimental procedure.

In one set of experiments, a 30-gauge injection needle (Cooper's Needle Works) was inserted intracerebroventricularly (i.c.v.) via the previously implanted guide cannula (the needle extended 2.5 mm below the implanted tip of the guide cannula) to facilitate the administration of U46619 or vehicle (3% v/v ethanol in saline; dosing volume:10 μl). Animals were then observed for 60 min and then 10 μl of

Evans blue dye was injected i.c.v. and the animals were killed with pentobarbital sodium (60 mg/kg, i.p.). The brains were dissected and inspected: only data from animals showing blue staining of the walls of the lateral and 4th ventricles were included for data analysis.

In another set of experiments, the previously vagotomized or sham-operated animals were injected intraperitoneally with U46619 (300 μg/kg) and observed for 60 min for behavioural change. After 24 h, the animals were administered copper sulphate (50 mg/kg, intragastrically) and animals observed for 30 min for the production of retching and/or vomiting. This approach was used to confirm the success of the surgical lesion (see Section 2.3) as emesis induced by copper sulphate is inhibited by vagotomy (Andrews et al., 1990).

In a final set of experiments, phentolamine (0.3–3 mg/kg), propranolol (3 mg/kg), metoclopramide (0.3–3 mg/kg), domperidone (0.3–3 mg/kg), droperidol (0.3–3 mg/kg), ondansetron (0.3–3 mg/kg), scopolamine (0.3–3 mg/kg), promethazine (0.3–3 mg/kg) and (+)-2*S*,3*S*(-3-(2-methoxy-5-trifluoromethoxybenzyl)amino-2-phenylpiperidine) (CP-122,721; 1–10 mg/kg), or their respective vehicles, were injected subcutaneously as a 30-min pretreatment prior to the administration of U46619 (300 μg/kg, i.p.). The effect of reserpine (5 mg/kg, i.p., once at −24 h,) and *para*-chlorophenylalanine (100–400 mg/kg, i.p., administered every 24 h, for 4 days) on U46619 (300 μg/kg, i.p.)-induced emesis was also investigated.

The intraperitoneal dose of U46619 300 μg/kg was selected to produce a reliable emetic response based on our previous investigations (Kan et al., in press). All animals were observed for 60 min following U46619 administration. An episode of emesis was characterised by rhythmic abdominal contractions that were 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). An episode of retching and/or vomiting was considered separate when an animal changed its location in the observation chamber, or when the interval between retches and/or vomits exceeded 2 s.

2.5. Formulation of drugs

11α,9α-Epoxymethano-15*S*-hydroxyprosta-5*Z*,13*E*-dienoic acid (U46619; Cayman Chemical, USA) was prepared in absolute ethanol at 1 mg/ml and stored at −20 °C. It was diluted using distilled water immediately prior to use for the peripheral administration studies (final ethanol concentration, 15% v/v), or with saline (0.9% w/v) for the central administration studies (final ethanol concentration, 3% v/v); the vehicles for U46619 were not associated with emesis (ethanol concentrations used are below the thresholds to induce emesis; see Chen et al., 1997). Domperidone (Sigma-Aldrich, St. Louis, MO, USA) and droperidol (Sigma-Aldrich) were freshly dissolved in dimethylsulphoxide. Metoclopramide hydrochloride (Sigma-Aldrich), scopolamine hydrochloride (Sigma-Aldrich), promethazine hydrochloride (Sigma-Aldrich), CP-122,721 hydrochloride (Pfizer, Groton, USA), phentolamine hydrochloride (Sigma-Aldrich), propranolol hydrochloride (Sigma-Aldrich) and copper sulphate pentahydrate (Riedel-DeHaën, Germany) were dissolved in distilled water. Ondansetron hydrochloride dihydrate (GlaxoSmithKline, Barnard Castle, UK) and fenfluramine hydrochloride (Sigma-Aldrich) were formulated in saline (0.9% w/v). Reserpine (Sigma-Aldrich) and *para*-chlorophenylalanine (Sigma-Aldrich) were prepared as suspensions in Tween 80 (5% v/v). Drug doses (excepting copper sulphate pentahydrate) are indicated as the free acid or base. *para*-Chlorophenylalanine or its vehicle was administered as 4 ml/kg; all drugs or vehicles injected intraperitoneally were administered in a volume of 2 ml/kg.

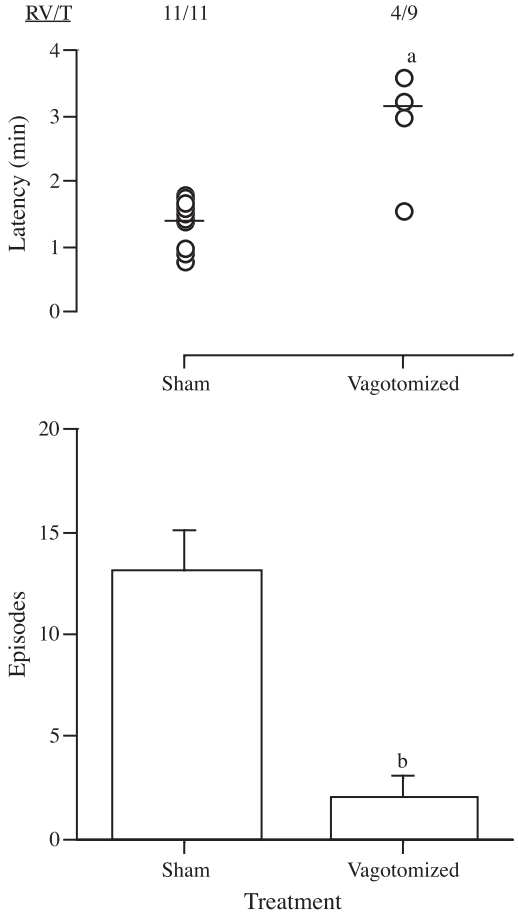

Fig. 1. The effect of bilateral abdominal vagotomy on U46619 (300 μg/kg, i.p.)-induced emesis in *S. murinus*. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines represent the mean latencies of the respective treatment group). The mean ± S.E.M. of the total number of episodes of retching and/or vomiting and the number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Significant differences relative to the respective vehicle treated animals are indicated as $^{a}P<0.05$, $^{b}P<0.001$ (unpaired Student's *t*-test).

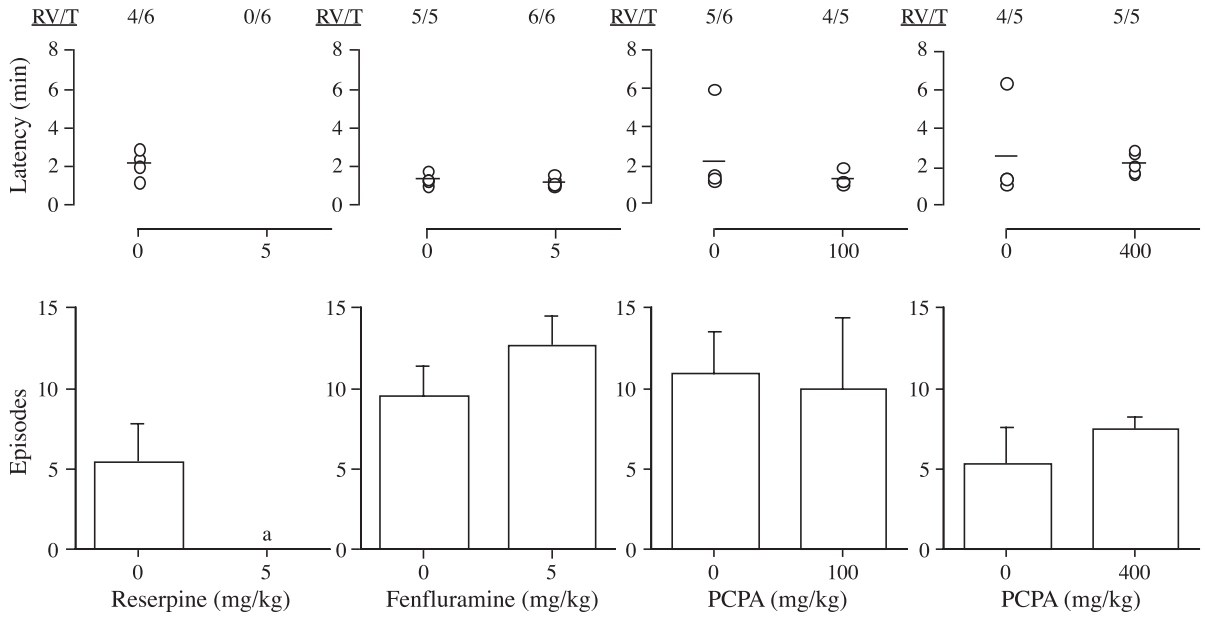

Fig. 2. The effect of reserpine, fenfluramine and *para*-chlorophenylalanine (PCPA) on U46619 (300 μg/kg, i.p.)-induced emesis in *S. murinus*. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines represent the mean latencies of the respective treatment group). The mean ± S.E.M. of the total number of episodes of retching and/or vomiting and the number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Significant differences relative to the respective vehicle treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA followed by a Fisher's PLSD test).

2.6. Statistical analysis

In each animal, the following parameters were recorded: (1) the latency to first retch/vomit and (2) the number of episodes of retches and/or vomits. In general, the significance of difference between treatments was assessed either by a Student's unpaired *t*-test or by one-way analysis of variance (ANOVA) followed by a Fisher's protected least significant difference (PLSD) test (Statsview®, Abacus Concepts, USA), as appropriate. However, in the nerve lesion studies, the episode data from the sham-operated animals were also fitted to a Poisson distribution and an "episode value" for "successful surgery" determined by calculating the value corresponding to the 1% lower tail (Microsoft Excel 2001, Microsoft©, USA). If copper sulphate induced a greater number of episodes than calculated from the 1% lower tail, it was assumed the surgery was unsuccessful and the data were not included in the subsequent analysis. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effect of intracerebroventricularly administered U46619

Intracerebroventricular administration of U46619 at 30, 100 and 300 ng failed to induce emesis ($n = 3$). The vehicle (10 μl of 3% ethanol in saline) for U46619 also failed to induce emesis ($n = 3$) and failed to prevent nicotine (5 mg/kg, s.c.)-induced emesis (data not shown).

3.2. Effect of bilateral abdominal vagotomy on U46619-induced emesis

U46619 300 μg/kg induced 13.2 ± 2.0 episodes of retching and/or vomiting with a latency of 1.4 ± 0.1 min in sham-operated animals. After 24 h, the animals were administered copper sulphate (50 mg/kg, intragastric). In these experiments, copper sulphate induced 10.6 ± 1.3

Table 1
Effect of phentolamine and/or propranolol on U46619 (300 μg/kg, i.p.)-induced emesis

Treatment (mg/kg)	Latency (min)	No. of episodes	Responders
U46619 + Veh	1.7 ± 0.3	4.0 ± 2.4	3/4
U46619 + Phent 0.3	1.8 ± 0.3	7.3 ± 1.2	4/4
U44619 + Phent 3.0	1.6 ± 0.1	8.3 ± 1.7	4/4
U46619 + Veh	1.4 ± 0.2	10.5 ± 2.6	5/6
U46619 + Prop 0.3	2.6 ± 1.0	6.7 ± 2.6	5/6
U46619 + Veh	2.6 ± 1.0	7.9 ± 2.5	6/9
U46619 + Phent 3 + Prop 3	1.6 ± 0.2	9.9 ± 2.1	9/9

Phentolamine (Phent), propranolol (Prop), or vehicle (Veh) was injected subcutaneously 30 min prior to the administration of U46619 (300 μg/kg, i.p.). Latency data are expressed as the mean ± S.E.M. time of only the animals that had episodes; all other data are expressed as the means ± S.E.M. There were no significant differences relative to the respective vehicle treated animals ($P > 0.05$, one-way ANOVA or unpaired Student's *t*-test, as appropriate).

Table 2
Effect of dopamine, 5-HT_3, muscarinic and histamine receptor antagonists on U46619 (300 μg/kg, i.p.)-induced emesis

Treatment (mg/kg)	Latency (min)	No. of episodes	Responders
U46619 + Veh	1.7 ± 0.1	8.0 ± 2.2	7/8
U46619 + MCP 0.3	2.2 ± 0.4	6.0 ± 1.8	7/8
U46619 + MCP 3.0	3.0 ± 0.8	4.4 ± 1.0	7/8
U46619 + Veh	1.5 ± 0.2	6.1 ± 1.6	6/8
U46619 + Dom 0.3	1.5 ± 0.1	9.0 ± 1.7	7/8
U46619 + Dom 3.0	2.0 ± 0.2	6.1 ± 1.8	6/8
U46619 + Veh	3.4 ± 1.5	9.5 ± 2.0	4/4
U46619 + Drop 0.3	2.6 ± 0.4	3.6 ± 1.4	4/4
U46619 + Drop 3.0	2.2 ± 0.2	10.9 ± 2.5	4/4
U46619 + Veh	1.5 ± 0.3	8.5 ± 2.7	4/4
U46619 + Ond 0.3	1.8 ± 0.2	7.3 ± 2.9	4/4
U46619 + Ond 3.0	1.9 ± 0.3	8.8 ± 3.1	4/4
U46619 + Veh	1.6 ± 0.3	10.0 ± 2.5	6/8
U46619 + Scop 0.3	1.1 ± 0.2	4.3 ± 1.5	5/8
U46619 + Scop 3.0	3.9 ± 2.8	6.1 ± 1.7	6/8
U46619 + Veh	1.5 ± 0.2	11.3 ± 1.4	6/6
U46619 + Prom 0.3	1.6 ± 0.1	8.8 ± 1.0	6/6
U46619 + Prom 3.0	1.2 ± 0.1	12.2 ± 1.4	6/6

Metoclopramide (MCP), domperidone (Dom), droperidol (Drop), ondansetron (Ond), scopolamine (Scop), promethazine (Prom), or their respective vehicles (Veh) were injected subcutaneously 30 min prior to the administration of U46619 (300 μg/kg, i.p.). Latency data are expressed as the mean ± S.E.M. time of only the animals that had episodes; all other data are expressed as the means ± S.E.M. There were no significant differences relative to the respective vehicle treated animals ($P>0.05$, one-way ANOVA).

episodes following 4.0 ± 0.6 min. The data was fitted to a Poisson distribution and the episode value corresponding to the lower 1% tail was 3. Copper sulphate inducing >3 episodes in the vagotomized animals was taken as indicating failure of surgery and data involving the use of U46619 was rejected. However, vagotomy was successful to reduce significantly the number of episodes induced by copper sulphate by 99% ($P<0.001$, data not shown).

Compared to the sham-operated animals, bilateral abdominal vagotomy reduced significantly U46619-induced emesis by 76% ($P<0.001$) and significantly delayed the latency to onset of emesis by approximately 5 min ($P<0.05$; Fig. 1).

3.3. Effect of reserpine, fenfluramine and para-chlorophenylalanine on U46619-induced emesis

Reserpine (5 mg/kg), fenfluramine (5 mg/kg) and *para*-chlorophenylalanine (100 and 400 mg/kg) were used as known monoamine depleting drugs in order to investigate the effects of noradrenaline, 5-HT and dopamine in U46619 (300 μg/kg, s.c.)-induced emesis. Reserpine (5 mg/kg) abolished U46619 (300 μg/kg)-induced emesis ($P<0.05$; Fig. 2) but fenfluramine (5 mg/kg, repeated treatment) and *para*-chlorophenylalanine (100 and 400 mg/kg, repeated treatment) were ineffective to modify the retching and vomiting response ($P>0.05$; Fig. 2).

3.4. Effect of phentolamine and/or propranolol on U-46619-induced emesis

Phentolamine (0.3–3 mg/kg) and propranolol (3 mg/kg) were inactive to modify U46619 (300 μg/kg, s.c.)-induced emesis ($P>0.05$). The combination of phentolamine (3 mg/kg) with propranolol (3 mg/kg) was also inactive to modify the retching and vomiting response ($P>0.05$; Table 1).

3.5. Effect of dopamine, 5-HT_3, muscarinic and histamine receptor antagonists on U46619-induced emesis

Metoclopramide (0.3–3 mg/kg), domperidone (03.3–3 mg/kg), droperidol (0.3–3 mg/kg), ondansetron (0.3–3 mg/kg), scopolamine (0.3–3 mg/kg) and promethazine (0.3–3 mg/kg) failed to modify U46619 (300 μg/kg, s.c.)-induced emesis ($P>0.05$; Table 2).

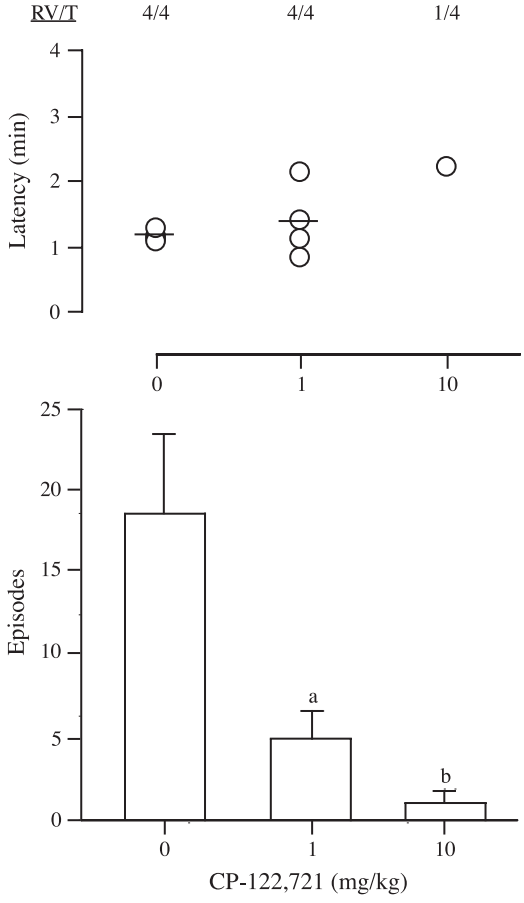

Fig. 3. The effect of CP-122,721 on U46619 (300 μg/kg, i.p.)-induced emesis in *S. murinus*. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines represent the mean latencies of the respective treatment group). The mean ± S.E.M. of the total number of episodes of retching and/or vomiting and the number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Significant differences relative to the respective vehicle treated animals are indicated as $^{a}P<0.05$, $^{b}P<0.01$ (one-way ANOVA followed by a Fisher's PLSD test).

3.6. Effect of the tachykinin NK_1 receptor antagonist, CP-122,721, on U46619-induced emesis

U46619 (300 μg/kg, s.c.) induced 18.5 ± 4.9 episodes of retching and/or vomiting following a latency of 1.2 ± 0.0 min. CP-122,721 at 1 and 10 mg/kg reduced significantly the number of episodes by 73% ($P < 0.01$) and 96% ($P < 0.001$), respectively, preventing emesis in one out of four animals at the highest dose (Fig. 3).

4. Discussion

Our previous studies in the ferret and *S. murinus* revealed the potent emetic activity of U46619. The emetic action in both species was relatively rapid in onset following intraperitoneal administration and was blocked by the TP receptor antagonist vapiprost. This suggested the involvement of peripheral TP receptors in the emetic mechanism of action (Kan et al., 2002; Kan et al., in press).

In the present studies, we confirmed the emetic action of peripherally administered U46619 and the effect of bilateral abdominal vagotomy to reduce significantly emesis supports the hypothesis of a peripheral mechanism. However, emesis was only reduced by approximately 76%, suggesting that U46619 may act at other sites in addition to those involving the vagus nerves. One potential site could be the gastrointestinal tract relaying information to the brainstem via the splanchnic nerves that are known to play a role in the vomiting reflex (Andrews et al., 1990). However, it is also possible that vagal afferents above the level of the gastrointestinal tract are involved, or that other indirect mechanisms play a role.

We also considered that U46619 might partly induce emesis via rapid absorption to act in the central nervous system. However, U46619 was not emetic following intracerebroventricular (i.c.v.) administration at doses up to 300 ng. We therefore do not believe that U46619 has a central action to induce emesis, particularly since the i.c.v. dose we used is slightly higher than the peripheral threshold dose to induce emesis (Kan et al., 2002; Kan et al., in press).

At the start of the studies, there was no information available to indicate the transmitter systems involved in U46619-induced emesis. We decided to focus on transmitter systems known to be important in the mechanisms of emesis induced by other treatments (Barnes et al., 1988; Marin et al., 1990). Thus we used reserpine as a tool well known to deplete monoamines from neuronal stores (Giachetti and Shore, 1978).

Reserpine has been reported to induce transient emesis in other species that may be related to 5-HT, dopamine or noradrenaline release (Barnes et al., 1988; Dhawan et al., 1968). However, reserpine did not induce emesis (observed for 2 h post administration) in *S. murinus* but instead prevented U46619-induced emesis. Given the anti-emetic action of reserpine, we decided to investigate the anti-emetic potential of drugs known to reduce 5-HT levels. Fenfluramine is known to transiently increase 5-HT release (Bonanno et al., 1994) before subsequently decreases neuronal 5-HT levels (Kleven and Seiden, 1989) and *para*-chlorophenylalanine is a tyrosine hydroxylase inhibitor that reduces the synthesis of 5-HT, decreasing neuronal 5-HT levels on repeated treatment (Jequier et al., 1967). Yet both fenfluramine and *para*-chlorophenylalanine were ineffective to modify U46619-induced emesis, suggesting that 5-HT and, possibly, 5-HT receptors are not involved in the emetic mechanism of action of U46619. Certainly, our other studies using ondansetron imply that 5-HT_3 receptors are not involved in U46619-induced emesis. It is an interesting possibility, therefore, that 5-HT_3 antagonist resistant phases of emesis in the clinic (e.g. the cisplatin-induced delayed phase of emesis that is sensitive to glucocorticoids (Gralla et al., 1996)) could be mediated by TP receptors.

Our attention then focused on the anti-emetic potential of adrenoceptor receptor antagonists to resolve the mechanism of anti-emetic action of reserpine. However, the nonselective α-adrenoceptor antagonist, phentolamine (Bylund et al., 1994), and the nonselective β-adrenoceptor antagonist, propranolol (Bylund et al., 1994), were ineffective to antagonize the retching and vomiting response. Indeed, even the combination of phentolamine and propranolol failed to prevent the emesis. It seems unlikely, therefore, that U46619 induces emesis via liberation of endogenous noradrenaline in *S. murinus*.

To investigate the potential role of dopamine in the emetic mechanism of action of U46619, we used several antagonists: metoclopramide, domperidone and droperidol (Jackson and Westlind-Danielsson, 1994). However, none of the compounds had activity to prevent the emetic response. Further, U46619-induced emesis was also resistant to pretreatment with the muscarinic receptor antagonist, scopolamine (Caulfield, 1993), and the histamine H_1 receptor antagonist, promethazine (Haaksma et al., 1990).

The lack of correlation between the anti-emetic activity of reserpine with inhibition of 5-HT, adrenergic, dopaminergic, cholinergic and histaminergic systems prompted us to reconsider the mechanism of action of the compound to antagonize emesis. An examination of the literature reveals that the anti-emetic action of reserpine reported in the present studies also extends to an action to reduce the emesis induced by muscarinic and nicotinic agonists (Beleslin and Krstic, 1987; Beleslin et al., 1989), α- and β-adrenoceptor agonists (Hikasa et al., 1992), dopamine receptor agonists (Jovanovic-Micic et al., 1995) and the chemotherapeutic agent cisplatin (Barnes et al., 1988; Tanihata et al., 2000). Clearly, reserpine appears to have broad inhibitory anti-emetic property that has not been fully realized.

The classes of drug that have well-known broad anti-emetic profiles include μ-opioid receptor agonists (Rudd et al., 1999a), 5-HT_{1A} (Lucot and Crampton, 1989) and 5-HT_2 receptor agonists (Okada et al., 1995), vanilloids (Andrews

et al., 2000) and tachykinin NK_1 receptor antagonists (Bountra et al., 1993). We have already negated an action of reserpine to antagonize emesis by mechanism involving 5-HT systems and there is no known action of reserpine to facilitate opioid transmission. However, there is a report of reserpine decreasing substance P levels in sensory nerves (Gilbert et al., 1981) and both vanilloids and tachykinin NK_1 receptor antagonists may be considered to reduce emesis by decreasing substance P function (Andrews et al., 2000; Rudd and Wai, 2001). Perhaps a reserpine-induced decrease in substance P levels in emetic circuits could explain the anti-emetic action. However, other potential mechanisms may also exist to inhibit emesis.

We tested the potential of the tachykinin NK_1 receptor antagonist, CP-122,721, to reduce U46619-induced emesis. Emesis was reduced by approximately 96% at 10 mg/kg and comparable reductions against nicotine- and copper sulphate-induced emesis have been reported in this species (Rudd et al., 1999b). Tachykinin NK_1 receptor antagonists are now in clinical trial (Gralla, 2002) and our finding suggests that they may provide a better control of emesis than the conventional anti-emetics that we tested in the present studies. However, we were not surprised that CP122,721 antagonized U46619-induced emesis, since tachykinin NK_1 receptors are thought to play a role downstream at a convergence point in the emetic reflex, rather than by selectively blocking a single afferent input mechanism (Rudd et al., 1999b; Tattersall et al., 1996).

In conclusion, U46619 induces emesis predominantly via a peripheral mechanism that directly/indirectly involves the abdominal vagus nerves. The emetic mechanism of action is not blocked by conventional anti-emetic drugs, or by drugs reducing 5-HT function, but is antagonized by the tachykinin NK_1 receptor antagonist CP-122,721. The anti-emetic mechanism of action of reserpine to antagonize U46619-induced emesis is unknown and requires further investigation. The potential of prostanoid TP receptor antagonists as novel anti-emetic agents should also be explored.

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