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Evaluation of the anti-emetic potential of anti-migraine drugs to prevent resiniferatoxin-induced emesis in *Suncus murinus* (house musk shrew)

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Abstract

Activation of vanilloid receptors has commonly been used to facilitate neurogenic inflammation and plasma exudation to model components of the pathogenesis of migraine; however, these studies have been performed mainly in species lacking the emetic reflex. In the present studies, therefore, we used *Suncus murinus*, a species of insectivore capable of emesis, to investigate if the vanilloid receptor agonist resiniferatoxin is capable of modeling the emesis associated with migraine. Resiniferatoxin (100 nmol/kg, s.c.) induced an emetic response that was antagonized significantly ($P<0.05$) by ruthenium red (1–3 μmol), (2*R-trans*)-4-[1-[3,5-bis(trifluromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (*S*)-hydroxybutanedioate (R116301; 10–100 μmol/kg), and scopolamine (1 μmol/kg), but not by dihydroergotamine (0.3–3 μmol/kg), sumatriptan (1–10 μmol/kg), methysergide (1–10 μmol/kg), tropanyl 3,5-dichlorobenzoate (MDL72222; 3–30 μmol/kg), ondansetron (0.3–3 μmol/kg), metoclopramide (3–30 μmol/kg), domperidone (3–30 μmol/kg), diphenhydramine (1–10 μmol/kg), or indomethacin (3–30 μmol/kg). The failure of a wide range of representative anti-migraine drugs to reduce retching and vomiting limits the use of this model to identify/investigate novel treatments for the emesis (and nausea) associated with migraine attacks in humans. However, the results provide further evidence for the involvement of a novel vanilloid receptor in resiniferatoxin-induced emesis and implicate both tachykinins and acetylcholine in the pathway(s) activated by resiniferatoxin in *S. murinus*.

Keywords: Emesis; Nausea; Migraine; Resiniferatoxin; Vanilloid; Sumatriptan; Dihydroergotamine; *Suncus murinus*

1. Introduction

Migraine is a debilitating condition that is characterized by a severe and painful–often pulsatile-type–headache, which can be accompanied by photophobia, phonophobia, and nausea and vomiting (Ramussen and Olesen, 2000). The mechanism of the headache is thought to be initiated in the brain and involves activation of the trigeminal nerve (by unknown mechanisms) that subsequently causes neuropeptide release to mediate vasodilation of the cranial vasculature with associated plasma extravasation (Beattie et al., 1995; Moskowitz, 1984; Moskowitz and Buzzi, 1991). The neuropeptides involved probably include substance P and calcitonin gene related peptide, although other mediators such as histamine and 5-hydroxytryptamine (5-HT), coming from other sources, may also play a role (Beattie et al., 1995; Buzzi et al., 1995). It is probably the distension of the large cerebral blood vessels, venous sinuses, pial vessels, and those in the dura mater, and the accompanying oedema, that contribute to activation of nociceptive C-fibre trigeminal afferents and thus the sensation of pain (Edvinsson, 2000).

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Neurogenic models of migraine have become popular for screening drugs with the potential to alleviate migraine (Buzzi et al., 1995; Tassorelli and Buzzi, 1996). The models generally involve electrically stimulating the trigeminal ganglia and establishing if test compounds can prevent the subsequent plasma extravasation that can occur in extracranial tissues; transmitter release and histochemical studies may also be run in parallel (Reuter et al., 2000). This type of model clearly identifies the potential of sumatriptan and dihydroergotamine to reduce plasma extravasation and these agents are particularly useful for aborting migraine attacks in humans (Buzzi et al., 1995; Buzzi and Moskowitz, 1990, 1991).

Over half of adult migraineurs are reported to have nausea accompanied by vomiting, and nausea occurs in 90% of patients with migraine (Davidoff, 1995). It has been reported that in patients with severe, prolonged migraine attacks, "vomiting constitutes the most potentially harmful aspect of the attack" because of the fluid and electrolyte loss that can cause collapse, requiring intravenous hydration (Davidoff, 1995). The mechanisms involved in migraine-induced nausea and vomiting are unknown and have been little investigated, but could involve several of the above mediators acting on afferents (cranial, cerebral, and visceral) projecting to the brainstem nuclei responsible for integration of the emetic reflex and rostral projection of signals for nausea (Dahlof and Hargreaves, 1998; De Ponti, 2000). Certainly, the neurogenic models of migraine reveal an increase in c-*fos* expression in brainstem areas such as the area postrema and nucleus tractus solitarius (Cutrer et al., 1995; Ter Horst et al., 2001), which are implicated in the emetic reflex (Dahlof and Hargreaves, 1998). However, it is possible that abdominal vagal afferents could be implicated in the pathogenesis of migraine in view of their sensitivity to 5-HT (Bley et al., 1994), and 5-HT_3 receptor antagonists reduce migraine associated emesis (Couturier et al., 1991; Loisy et al., 1985).

It is well known that migraine and the associated symptoms can be aborted by sumatriptan and dihydroergotamine (mechanism involving activation of 5-$HT_{1B/1D}$ and possibly 5-HT_{1F} and other receptors; Kumar, 1994; Mitsikostas et al., 2002), or reduced by prophylactic treatment with methysergide (a classical nonselective 5-HT_2 receptor antagonist; Tfelt-Hansen and Saxena, 2000). However, there are also studies reporting the use of anti-emetic drugs in the treatment of migraine including tropanyl 3,5-dichlorobenzoate (MDL 72222; Loisy et al., 1985) and granisetron (Couturier et al., 1991) (5-HT_3 receptor antagonists), metoclopramide (an anti-dopaminergic and 5-HT_3 receptor blocker with some 5-HT_4 receptor agonist activity; Dahlof and Hargreaves, 1998), domperidone (a dopamine D_2 receptor antagonist; Jaecques et al., 1979), scopolamine (an anti-muscarinic drug; Dahlof and Hargreaves, 1998), and diphenhydramine (an anti-histaminergic drug; Dahlof and Hargreaves, 1998). Unfortunately, however, the benefit of many of these anti-emetics against migraine-induced emesis is not known precisely since the studies have been performed in a limited number of patients, and/or nausea and emesis were not the major focus of the studies.

Since most plasma extravasation-type studies have been performed under anaesthesia, or have used rodents (or other species incapable of vomiting), it has not been possible to simultaneously study mechanisms involved in nausea and emesis. However, it is interesting that capsaicin injected into the dura mater or intracisternally has been used as an alternative means of mimicking neurogenic inflammation involving plasma exudation for assessing the anti-migraine potential of compounds (Buzzi et al., 1995; Tassorelli and Buzzi, 1996) and that previous studies have detailed the emetic action of intra-arterially or subcutaneously administered resiniferatoxin (an ultrapotent capsaicin-like compound) in *Suncus murinus* (Andrews et al., 2000; Smith et al., 2002). There are also studies in this species detailing the emetic action of capsaicin and resiniferatoxin following intraventricular administration (Andrews et al., 2000; Rudd and Wai, 2001) and studies in the dog showing that capsaicin and resiniferatoxin applied to the fourth ventricle induces firing of neurons in the medial nucleus tractus solitarius and transient fictive retching (Shiroshita et al., 1997).

There are no animal models of migraine-induced emesis. However, it is possible that either capsaicin or resiniferatoxin could be used to develop a model of migraine-induced emesis. In the present studies, therefore, we investigated if resiniferatoxin-induced emesis in *S. murinus* is prevented by drugs known to reduce migraine-induced nausea and emesis in humans (see above). The presence of a very large nucleus tractus spinalis and nucleus trigemini in *S. murinus* also makes it suitable for the study of migraine mechanisms (Sugiura and Kitoh, 1984). We also investigated the role of tachykinin NK_1 receptors in the emetic response using (2*R*-*trans*)-4-[1-[3,5-bis(trifluromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (*S*)-hydroxybutanedioate (R116301) as a representative tachykinin NK_1 receptor antagonist (Megens et al., 2002), since substance P is expected to be released from sensory nerves by vanilloids (Szallasi and Blumberg, 1999). In other studies, the potential involvement of prostanoids in resiniferatoxin-induced emesis was investigated using indomethacin, a nonselective cyclooxygenase inhibitor, since analgesic drugs have some benefits in the treatment of migraine (Limmroth and Przywara, 2000). In the process of validating the model, we also examined the potential of the vanilloid receptor antagonists, ruthenium red (Amann and Maggi, 1991) and capsazepine (Bevan et al., 1992; Szallasi, 1994), to antagonize the emetic response.

2. Methods

2.1. Animals

The experiments were performed on female *S. murinus* (25–50 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±5%. Animals were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, St. Louis, USA). All experiments were conducted under licence 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. Induction and measurement of emesis

On the day of experiment, the animals were transferred to clear Perspex observation chambers (21 cm×14 cm×13 cm) for the assessment of emetic behaviour. They were allowed 30 min to adapt before any further experimental procedure. The effect of resiniferatoxin (1–100 nmol/kg, s.c.) was first investigated over a 90 min observation time to ascertain the optimum dose to use in the anti-emetic studies. In subsequent experiments, ruthenium red (0.03–10 μmol/kg), capsazepine (30–100 μmol/kg), sumatriptan (1–10 μmol/kg), methysergide (1–10 μmol/kg), dihydroergotamine (0.3–3 μmol/kg), ondansetron (0.3–3 μmol/kg), MDL72222 (3–30 μmol/kg), metoclopramide (3–30 μmol/kg), domperidone (3–30 μmol/kg), diphenhydramine (1–10 μmol/kg), scopolamine (1–10 μmol/kg), R116301 (10–100 μmol/kg), indomethacin (3–30 μmol/kg), or their respective vehicles were administered subcutaneously 30 min prior to the injection of resiniferatoxin (100 nmol/kg; dose determined in the preliminary studies). Animals were then observed for 90 min for the production of emesis.

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. At the end of the observation period, animals were killed by an intraperitoneal injection of pentobarbital sodium (60 mg/kg). No animal was used more than once.

2.3. Formulation of drugs

Resiniferatoxin (Sigma Chemical) was dissolved in Tween 80:ethanol:saline (0.9% wt/vol) in the ratio 1:1:8. Capsazepine (Research Biochemicals International, USA) and dihydroergotamine mesylate (Tocris, UK) were dissolved in 50% dimethylsulphoxide (made up in saline, 0.9% wt/vol). Domperidone (Sigma-Aldrich, St. Louis, USA), tropanyl 3,5-dichlorobenzoate (MDL72222; Sigma-Aldrich, St. Louis, USA), and (2*R*-*trans*)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (*S*)-hydroxybutanedioate (R116301; Johnson and Johnson Pharmaceutical Research and Development, Belgium) were freshly dissolved in 100% dimethylsulphoxide. Sumatriptan succinate (GlaxoSmithKline, Greenford, UK), ondansetron hydrochloride dihydrate (GlaxoSmithKline, Barnard Castle, UK), metoclopramide hydrochloride (Sigma-Aldrich, St. Louis, USA), scopolamine hydrochloride (Sigma-Aldrich, St. Louis, USA), ruthenium red (Sigma Chemical, USA), and diphenhydramine hydrochloride were formulated in saline (0.9% wt/vol). Methysergide maleate (Sigma Chemical, USA) was dissolved in distilled water and indomethacin (Sigma Chemical, USA) was dissolved in 10% (wt/vol) sodium hydrogen carbonate. All drugs or vehicles were administered in a volume of 2 ml/kg.

2.4. Statistical analysis

The latency data were analyzed using a Kruskal–Wallis test followed by a Dunn's multiple comparison test (GraphPad Prism version 4.0b, GraphPad Software, San Diego, CA, USA). When an animal failed to retch or

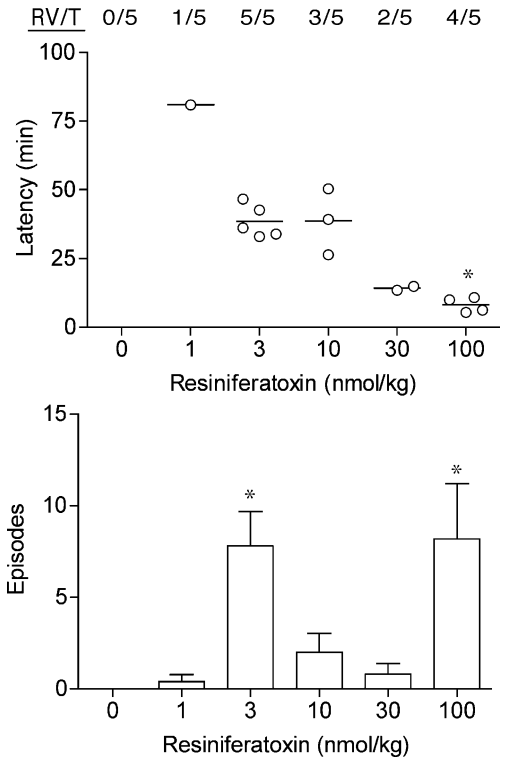

Fig. 1. The emetic potential of resiniferatoxin in *Suncus murinus*. Individual latencies to the first episode of retching and/or vomiting are indicated as open circles (horizontal lines represent the mean latencies of the respective treatment groups). The mean±S.E.M. of the total number of episodes of retching and/or vomiting out of the number of animals (RV/T) tested is also shown. Significant differences relative to the vehicle treated animals are indicated as *P<0.01 (one-way ANOVA followed by Dunnett's multiple comparison tests, or by a Kruskal–Wallis test, followed by a Dunn's multiple comparison test as appropriate).

vomit, a latency value equal to the test period observation time (i.e., 90 min) was used to perform the statistical analysis. The retching+vomiting episodes data were analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's tests (GraphPad Prism version 4.0b). Where appropriate, ID_{50} values were calculated by nonlinear regression analysis (GraphPad Prism version 4.0b). Results are expressed as the mean±S.E.M. unless otherwise stated. In all cases, differences between treatment groups were considered significant when $P<0.05$.

3. Results

3.1. Resiniferatoxin-induced emesis

Resiniferatoxin induced emesis in one of five animals at 1 nmol/kg, following a latency of approximately 80 min. The latency to induce emesis appeared to decrease in a dose-related manner, with 100 nmol/kg inducing emesis in 8.2±1.3 min in four of five animals (Fig. 1). However, the number of emetic episodes induced by resiniferatoxin was not dose related, with both 3 and 100 nmol/kg inducing seven to eight episodes and 1, 3, and 10 nmol/kg inducing 0.4 to two episodes (Fig. 1). Resiniferatoxin, 100 nmol/kg, was selected as the dose to use in the drug antagonism studies based on it having a reasonable number of emetic episodes combined with a shorter latency to induce emesis.

3.2. Potential of ruthenium red, capsazepine, and R116301 to antagonize resiniferatoxin (100 nmol/kg, s.c.)-induced emesis

In these experiments, resiniferatoxin (100 nmol/kg, s.c.) induced 0–17 episodes of retching and/or vomiting (i.e., one animal failed to respond) following a latency of 4.5–34.6 min (Fig. 2). Ruthenium red antagonized resiniferatoxin-induced emesis in a dose related manner, preventing emesis in all four animals at 3 μmol/kg ($P<0.05$); the ID_{50} dose was approximately 0.5 μmol. Capsaicin was ineffective against resiniferatoxin-induced emesis (i.e., no effect on the number of episodes, or on the latency; $P>0.05$). The tachykinin NK_1 receptor antagonist R116301 antagonized significantly the number of episodes at doses as low as 10 μmol/kg (a 56% reduction was observed; $P<0.05$) and prevented emesis in three of six animals at 100 μmol/kg (consequently reducing emesis by approximately 86%; $P<0.01$). However, R116301 failed to modify the latency to either the first retch or vomit in the animals that were not completely protected ($P>0.05$).

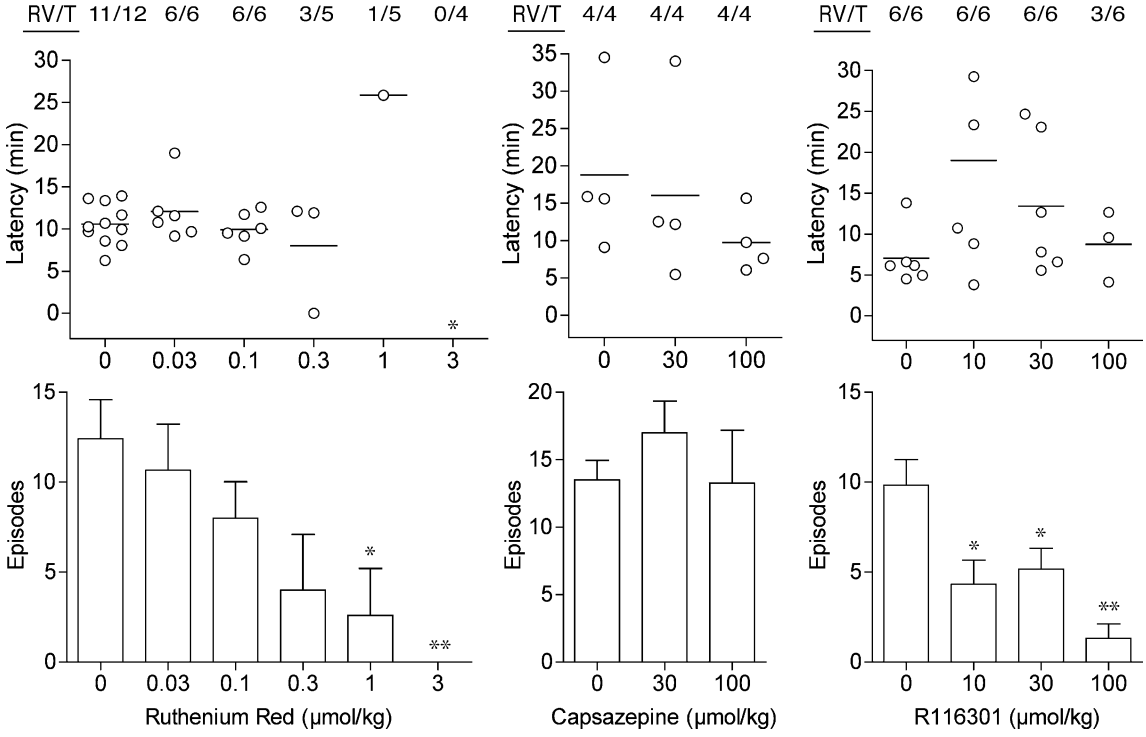

Fig. 2. The effect of ruthenium red, capsazepine, or R116301 on resiniferatoxin (100 nmol/kg, s.c.)-induced emesis in *Suncus murinus*. Individual latencies to the first episode of retching and/or vomiting are indicated as open circles (horizontal lines represent the mean latencies of the respective treatment groups). The mean±S.E.M. of the total number of episodes of retching and/or vomiting out of the number of animals (RV/T) tested is also shown. Significant differences relative to the control treated animals are indicated as *$P<0.05$ or **$P<0.01$ (one-way ANOVA followed by Dunnett's multiple comparison tests, or by a Kruskal–Wallis test, followed by a Dunn's multiple comparison test as appropriate).

3.3. Potential of 5-HT receptor ligands to modify resiniferatoxin (100 nmol/kg, s.c.)-induced emesis in S. murinus

In these experiments, resiniferatoxin (100 nmol/kg, s.c.) induced approximately 6–21 episodes of retching and/or vomiting following a latency of approximately 3–19 min (Table 1). Dihydroergotamine (0.3–3 μmol), sumatriptan (1–10 μmol/kg), MDL72222 (3–30 μmol/kg), ondansetron (0.3–3 μmol/kg), and methysergide (1–10 μmol/kg) all failed to modify significantly either the latency of resiniferatoxin to induce emesis ($P>0.05$), or the total number of episodes of retching and/or vomiting ($P>0.05$).

3.4. Potential of a cyclooxygenase inhibitor and anti-dopaminergic, anti-histaminergic, and anti-cholinergic drugs on resiniferatoxin (100 nmol/kg, s.c.)-induced emesis in S. murinus

In these experiments, resiniferatoxin (100 nmol/kg, s.c.) induced 6–13 episodes of retching and/or vomiting following a latency of 7–18 min (Table 2). Indomethacin (3–30 μmol/kg), metoclopramide (3–30 μmol/kg), domperidone (3–30 μmol/kg), and diphenhydramine (1–10 μmol/kg) all failed to modify significantly either the latency of resiniferatoxin to induce emesis ($P>0.05$), or the total number of episodes of retching and/or vomiting ($P>0.05$). However, scopolamine reduced significantly the number of retching and/or vomiting episodes induced by resiniferatoxin by approximately 37% at 1 μmol/kg ($P<0.05$); a higher dose of 10 μmol had no significant action ($P>0.05$).

Table 1
The effect of 5-HT receptor ligands on resiniferatoxin (RTX; 100 nmol/kg, s.c.)-induced emesis in *Suncus murinus*

Treatment (mg/kg)	Latency (min)	Number of episodes	Responders
RTX+Veh	3.9±6.8	12.7±2.4	6/6
RTX+DHE 0.3 μmol/kg	6.1±3.0	17.8±3.2	6/6
RTX+DHE 1.0 μmol/kg	7.1±2.0	12.2±1.8	6/6
RTX+DHE 3.0 μmol/kg	9.4±2.0	14.0±1.5	6/6
RTX+Veh	8.3±2.3	7.8±3.0	4/6
RTX+SUMA 1.0 μmol/kg	6.8±1.0	8.2±0.6	6/6
RTX+SUMA 3.0 μmol/kg	6.1±1.0	13.2±1.3	6/6
RTX+SUMA 10.0 μmol/kg	5.4±1.3	4.5±1.6	5/6
RTX+Veh	10.0±2.0	6.3±2.2	6/8
RTX+MDL 3.0 μmol/kg	18.7±3.7	8.0±3.0	6/8
RTX+MDL 30.0 μmol/kg	12.4±2.0	7.3±1.3	8/8
RTX+Veh	2.9±0.7	21.0±4.8	6/6
RTX+OND 0.3 μmol/kg	2.7±0.8	24.8±2.8	5/6
RTX+OND 3.0 μmol/kg	2.3±0.6	24.8±1.9	6/6
RTX+Veh	18.6±5.9	6.0±4.4	5/6
RTX+METH 1.0 μmol/kg	14.9±2.9	7.0±2.0	5/6
RTX+METH 3.0 μmol/kg	11.8±4.1	9.3±3.3	5/6
RTX+METH 10.0 μmol/kg	12.6±3.3	6.2±1.5	5/6

Dihydroergotamine (DHE), sumatriptan (SUMA), MDL72222 (MDL), ondansetron (OND), methysergide (METH), or their respective vehicles (Veh) were administered intraperitoneally 30 min prior to the administration of RTX. Latency data are expressed as the mean time (mean±S.E.M) of only the animals that had episodes. There were no significant differences relative to the respective control RTX+Veh treated animals ($P>0.05$, one-way ANOVA or Kruskal–Wallis test, as appropriate).

Table 2
The effect of a cyclooxygenase inhibitor and anti-dopaminergic, anti-histaminergic, and anti-cholinergic drugs on resiniferatoxin (RTX; 100 nmol/kg, s.c.)-induced emesis in *Suncus murinus*

Treatment (mg/kg)	Latency (min)	Number of episodes	Responders
RTX+Veh	11.0±0.8	11.1±2.8	6/6
RTX+INDO 3.0 μmol/kg	11.4±1.0	9.1±3.0	4/6
RTX+INDO 30.0 μmol/kg	8.7±1.5	9.2±3.5	4/6
RTX+Veh	17.1±4.7	11.2±3.6	5/6
RTX+MCP 3.0 μmol/kg	14.0±1.8	12.7±2.0	6/6
RTX+MCP 30.0 μmol/kg	16.4±3.1	8.0±3.0	4/6
RTX+Veh	8.3±2.3	10.8±2.5	6/6
RTX+DOM 3.0 μmol/kg	6.8±1.0	11.0±1.8	6/6
RTX+DOM 30.0 μmol/kg	6.1±1.0	9.3±3.0	6/6
RTX+Veh	10.8±1.4	6.8±2.0	7/8
RTX+DIPH 1.0 μmol/kg	10.6±1.2	9.4±2.1	7/8
RTX+DIPH 10.0 μmol/kg	10.2±0.9	8.5±2.4	7/8
RTX+Veh	7.7±1.3	13.2±1.8	6/6
RTX+SCOP 1.0 μmol/kg	12.0±1.0	8.3±1.8*	5/6
RTX+SCOP 10.0 μmol/kg	5.5±0.9	8.6±1.3	6/6

Indomethacin (INDO), metoclopramide (MCP), domperidone (DOM), diphenhydramine (DIPH), scopolamine (SCOP), or their respective vehicles (Veh) were administered intraperitoneally 30 min prior to the administration of RTX. Latency data are expressed as the mean time (mean±S.E.M) of only the animals that had episodes. For the episodes data, significant differences relative to the respective control RTX+Veh treated animals are indicated at *$P<0.05$ (one-way ANOVA followed by Dunnett's multiple comparison tests, or by a Kruskal–Wallis test, as appropriate). For the latency data, there were no significant differences relative to the respective control RTX+Veh treated animals ($P>0.05$, Kruskal–Wallis test).

4. Discussion

The major purpose of the present studies was to explore the use of resiniferatoxin to model the emesis experienced by patients suffering from a migraine attack. We found that resiniferatoxin did not induce a 'clean' dose–response curve, since two peaks of retching and vomiting activity were evident at 3 and 100 nmol/kg. The reason for this is unknown but may relate to a differential activity of resiniferatoxin to affect central and peripheral pathways to induce and subsequently inhibit emesis (Andrews et al., 2000; Rudd and Wai, 2001). Thus, the lower doses (<10 nmol) may be more representative of an activation and subsequent inhibition of peripheral emetic pathways, since the drug was administered subcutaneously. The higher dose (100 nmol/kg) may be representative of an additional activation of central pathways in conjunction with the peripheral one(s), since the effect on latency appeared dose related and is partly consistent with our previous work, where bilateral abdominal vagotomy also antagonizes, but does not abolish the emesis induced by resiniferatoxin at 160 nmol/kg, s.c. (Andrews et al., 2000; Rudd and Wai, 2001). Certainly, the intracerebroventricular administration of resiniferatoxin (3–30 nmol) produces a 'standard' shaped

dose–response curve, where resiniferatoxin is approximately seven times more potent than capsaicin to induce emesis (Rudd and Wai, 2001). As our studies were designed to mimic the effect of a migraine attack, we used the higher dose of resiniferatoxin, 100 nmol/kg, for the drug antagonism studies.

Clearly, however, none of the drugs used in the treatment of migraine, or migraine-associated symptoms, except perhaps for scopolamine, was effective in reducing resiniferatoxin-induced emesis (see Section 1). This was exemplified by a failure of dihydroergotamine and sumatriptan, effective migraine-abortive agents, to reduce emesis (Perry and Markham, 1998). However, we did not expect a complete block of emesis since these drugs fail to prevent capsaicin-induced c-*fos* expression in the area postrema and solitary tract (Cutrer et al., 1995; Mitsikostas et al., 2002; Nozaki et al., 1992). Whilst it is not known if *S. murinus* possesses 5-$HT_{1B/1D}$ receptors, the failure of these major therapeutic drugs to antagonize emesis is inescapable and must not be overlooked when considering the suitability of resiniferatoxin to model migraine-induced emesis. Nevertheless, *S. murinus* is known to have 5-HT_3 receptors, given that 5-HT_3 receptor agonists are emetic and 5-HT_3 receptor antagonists have anti-emetic actions (Sam et al., 2003; Torii et al., 1991a, b); but consistent with our previous studies with 5-HT_3 receptor antagonists (Andrews et al., 2000), ondansetron and MDL72222 were inactive against resiniferatoxin (160 nmol/kg)-induced emesis.

We were initially unsure if methysergide would have actions to antagonize resiniferatoxin-induced emesis given that it is rather nonselective for certain 5-HT receptor subtypes that have also been reported to mediate effects in the emetic reflex. Thus, methysergide is a relatively nonselective 5-HT_2 and 5-HT_7 receptor antagonist, with weak agonist activity at some 5-HT_1 receptor subtypes (Tfelt-Hansen and Saxena, 2000). 5-HT_2 (particularly 5-HT_{2C}) receptors mediate the contraction of *S. murinus* intestine (Javid and Naylor, 1999a, b) but DOI ((±)-1,(2,5-dimethyoxy-4-idophenyl)-2-aminopropane)), a 5-$HT_{2A/2C}$ receptor agonist, has anti-emetic effects against motion and cisplatin in this species that can be reversed by ketanserin (another 5-HT_2 receptor antagonist; Okada et al., 1995). However, we did expect a weak anti-emetic action via 5-HT_{1A} receptors, since this receptor subtype is heavily involved in the emetic reflex of *S. murinus*, with several studies showing the broad inhibitory action of 8-OH-DPAT ((±)-8-hydroxy-dipropylaminotetralin)) to reduce emesis including that induced by resiniferatoxin, 160 nmol/kg (Andrews et al., 1996; Andrews et al., 2000; Okada et al., 1994; Rudd et al., 1999). However, methysergide is a more effective treatment of migraine if used prophylactically (Tfelt-Hansen and Saxena, 2000); therefore its effects may be more related to a potential down-regulation/upregulation of receptor types in the clinical setting that were not revealed by its acute use in the present studies.

A role for dopamine receptors in the emetic action of resiniferatoxin has not been examined before and a failure of metoclopramide and domperidone to prevent emesis indicates that it is unlikely that resiniferatoxin releases dopamine to act at D_2 receptors to mediate its emetic action. Similarly, it is unlikely that histamine is released by resiniferatoxin to induce emesis given the failure of diphenhydramine to antagonize the response. Whilst metoclopramide and histamine H_1 receptor antagonists have anti-emetic actions against some emetic stimuli in *S. murinus* (Matsuki et al., 1988; Ueno et al., 1988) and have some efficacy to reduce migraine-induced nausea and emesis (see Section 1), their inactivity in the present study demonstrates a further deficiency of resiniferatoxin to model the clinical situation of migraine-induced emesis. This is particularly important given that dopamine and histamine are implicated in migraine (Mascia et al., 1998; Tfelt-Hansen, 1996), with intravenously administered histamine being used in humans to induce headache (Iversen, 1995). The usefulness of metoclopramide and domperidone in some studies may relate to their action to stimulate gastrointestinal motility and thus facilitate the absorption of other drugs to treat migraine (Dahlof and Hargreaves, 1998; De Ponti, 2000), or alleviate gastric stasis associated with nausea, but this was not simulated in the present studies.

Several prostaglandins induce headache in humans (Iversen, 1995) and we have demonstrated the emetic action of prostanoids in *S. murinus* (Kan et al., 2003), but indomethacin was inactive in reducing resiniferatoxin-induced emesis. This represents a new finding and is important considering that prostanoids may lower the threshold for activation of vanilloid receptors (Linhart et al., 2003), and there are reports that vanilloids stimulate prostaglandin synthesis (Someya et al., 2002). Certainly, cyclooxygenase inhibitors such as diclofenac are reported to be effective in treating the pain associated with migraine and have some benefits against the accompanying nausea and vomiting (McNeely and Goa, 1999). There are also reports of the ability of vanilloids to stimulate acetylcholine release in the gastrointestinal tract (Bartho and Vizi, 1985) which may explain the minor anti-emetic action that we observed with scopolamine, but it is unknown if potential motility changes contribute to emetic/anti-emetic effects in *S. murinus*. However, scopolamine is mainly considered to be a centrally acting anti-emetic (Yates et al., 1998) and further studies are therefore required to fully resolve the mechanisms involved, but anti-cholinergics may have a place in the treatment of migraine (Nicolodi and Sicuteri, 1999). Yet we must be cautious in our interpretation of the data, since a higher dose of scopolamine was inactive.

The emetic action of resiniferatoxin was predictably prevented by ruthenium red to confirm an action at vanilloid receptors (Amann and Maggi, 1991; Szallasi and Blumberg, 1999). However, resiniferatoxin was resistant to pretreatment with capsazepine, a functional antagonist also used to define vanilloid receptors (Jerman et al., 2000). It is possible

that *S. murinus* vanilloid receptors are unique, since there are known differences in the pharmacology of vanilloid receptors in other species (Jordt and Julius, 2002; Szallasi, 1994). However, we have also observed a similar differential action of ruthenium red and capsazepine to antagonize resiniferatoxin (10 nmol, i.c.v.)-induced emesis following intraventricular administration, but capsaicin-induced emesis was blocked by both antagonists (Rudd and Wai, 2001). Certainly, there is evidence of capsazepine-insensitive vanilloid-induced responses in other species (Capasso et al., 2002; Liu et al., 1998; Rinder et al., 1996), so our studies may also indicate a novel subtype of vanilloid receptor.

There was initial excitement that tachykinin NK_1 receptor antagonists would prove useful to treat migraine (Beattie et al., 1995) but clinical results have been less promising (Williamson and Hargreaves, 2001). Nevertheless, the present studies confirm the involvement of tachykinin NK_1 receptors in the emetic mechanism of action of resiniferatoxin. In our studies, R116301 (K_i values at human, gerbil, ferret, and guinea pig tachykinin NK_1 receptors are 0.4, 6.4, 8.3, and 13 nM, respectively; Megens et al., 2002) was potent to antagonize emesis and is consistent with its broad inhibitory action to inhibit emesis in ferrets, cats, and dogs (Megens et al., 2002), supporting the contention that substance P may be released by resiniferatoxin to induce emesis (Andrews et al., 2000).

In conclusion, we investigated the potential of subcutaneously administered resiniferatoxin to model the emesis experienced by patients suffering from migraine. However, none of the standard treatments for migraine could antagonize emesis, with only scopolamine providing a modest reduction of retching and vomiting. These results indicate that subcutaneously administered resiniferatoxin in *S. murinus* is unlikely to provide a model of migraine-induced emesis. However, the studies revealed a differential ability of ruthenium red and capsazepine to antagonize emesis, suggesting the involvement of a novel vanilloid receptor in resiniferatoxin-induced emesis, and the antiemetic action of R116301 confirms a role for tachykinin NK_1 receptors in emesis. Together, these results suggest that *S. murinus* is a suitable model in which to study the relationships between vanilloid and tachykinin NK_1 receptors in emesis.

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