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Genital grooming and emesis induced by vanilloids in *Suncus murinus*, the house musk shrew

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Abstract

The potential of resiniferatoxin and capsaicin to modulate emesis and genital grooming was investigated in *Suncus murinus*. Resinifertoxin (3–30 nmol, i.c.v.), *E*-capsaicin (10–100 nmol, i.c.v.) and *Z*-capsaicin (100 nmol, i.c.v.) induced emesis ($P < 0.05$) and subsequently antagonised the emetic response induced by intragastric copper sulphate (480.6 μmol/kg; $P < 0.05$). However, resiniferatoxin failed to affect nicotine-induced (30.7 mol/kg, s.c.) emesis ($P > 0.05$). Only resiniferatoxin induced genital grooming that was antagonised ($P < 0.05$) by capsazepine (300–600 nmol, i.c.v.) and ruthenium red (3 nmol, i.c.v.). *E*-capsaicin-induced emesis was antagonised by capsazepine (300–600 nmol, i.c.v.; $P < 0.05$) and ruthenium red (3 nmol, i.c.v.; $P < 0.05$) but resiniferatoxin-induced emesis was resistant to capsazepine (30–600 nmol, i.c.v.; $P > 0.05$). The emetic action of resiniferatoxin but not *E*-capsaicin was subject to tachyphylaxis. In cross-tachyphylaxis experiments, *E*-capsaicin reduced the genital grooming induced by resiniferatoxin ($P < 0.05$). The data are discussed in relation to the classification of vanilloid receptors and mechanisms involved in emesis and genital grooming.

Keywords: Vanilloid; Emesis; Genital-grooming; *Suncus murinus*; (Shrew)

1. Introduction

There has been considerable interest in the development of broad-spectrum antiemetic drugs. Such drugs are capable of inhibiting emesis induced by diverse stimuli and appear useful to suppress vomiting induced by unknown etiology (e.g. chemotherapy-induced delayed emesis; Rizk and Hesketh, 1999; Rudd et al., 1996) or where the cause of vomiting is multi-factorial (e.g. following surgery with anaesthesia; Gardner and Perren, 1998). The tachykinin NK_1 receptor antagonists represent an important class of broad-spectrum antiemetic drugs that probably reduce emesis by antagonising the action of substance P at tachykinin NK_1 receptors in the nucleus tractus solitarius and/or closely associated structures (Tattersall et al., 1996). Drug treatments capable of modulating substance P function in the brain by alternative mechanisms may prove useful to modulate the emetic reflex.

Resiniferatoxin, an ultrapotent capsaicin analog, has a broad inhibitory action to reduce emesis in the ferret (Andrews and Bhandari, 1993). The mechanism to reduce emesis probably involves the activation of vanilloid receptors and a reduced function of substance P and/or calcitonin gene related peptide (CGRP) in the emetic reflex (Andrews and Bhandari, 1993). Recent experiments in the dog have shown that capsaicin and resiniferatoxin administered into the fourth ventricle can enhance the firing of medial solitary neurones to culminate in fictive retching responses (Shiroshita et al., 1997). The mechanism was hypothesized to involve a release of excitatory transmitter(s) from closely situated vagal afferents in the brainstem. The initial facilitatory action was subsequently followed by a refractory period where retching could not be induced by electrical stimulation of the vagus (Shiroshita et al., 1997).

We conducted preliminary experiments in *Suncus murinus* to investigate the antiemetic action of subcutaneously administered resiniferatoxin (Rudd and Naylor, 1995). In our studies, resiniferatoxin was associated with emesis and a subsequent antagonism of nicotine-induced emesis was not dose-related. The emetic action of resiniferatoxin in *S. murinus* has been confirmed and further detailed studies conducted to investigate its dual mechanism of action on the emetic reflex (Andrews et al., 2000).

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Our previous studies confirmed that *S. murinus* tachykinin NK_1 receptors are different from human or rodent type tachykinin NK_1 receptors and that such differences may be important when considering the use of the animal in emesis research (Rudd et al., 1999). There are also reports of species differences in the pharmacology of vanilloid receptors in common laboratory animals (Szallasi, 1994). In the present study, therefore, we have used an intraventricular injection technique and several ligands to characterize the role of central vanilloid receptors in the emetic reflex of *S. murinus*. The present study also reports the central vanilloid receptor involvement in the mechanisms controlling genital grooming. The action of resiniferatoxin to induce ano-genital grooming has been documented in *S. murinus* but the precise mechanism is unknown (Andrews et al., 2000). The present studies may have relevance to the development of novel drugs to prevent emesis and/or treat sexual dysfunction.

2. Materials and methods

2.1. Animals

The experiments were performed on male *S. murinus* (60–85 g), bred at the Chinese University of Hong Kong. Prior to the experiments, they were housed in a temperature-controlled room at 24 ± 1°C under artificial lighting, with lights on between 0700 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. Animals were not used more than once.

2.2. Stereotaxic surgery

The methods used to cannulate the lateral ventricle are similar to those described by Kakimoto et al. (1997). Animals were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.) and placed into a stereotaxic frame equipped with custom-made ear-bars and mouthpieces (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 cleared of connective tissue. A burr hole was made in the skull, 8.2 mm rostral to the posterior edge and −0.9 mm lateral to the crest. A guide cannula (23 gauge) was lowered into the brain to a 1.2-mm depth below the surface of the dura and fixed with dental cement to a brass anchor screw that was secured to the

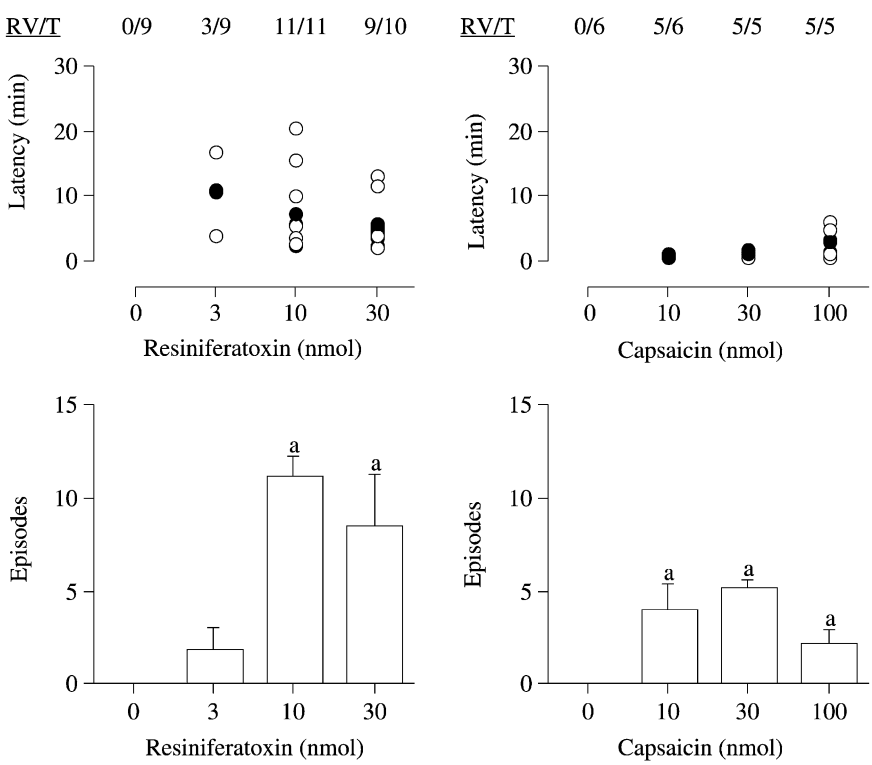

Fig. 1. The emetic action of intraventricularly administered resiniferatoxin and *E*-capsaicin in *S. murinus*. Open circles represent the individual latencies to the first episode of retching and/or vomiting. Filled circles represent the mean latencies of the respective treatment groups. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting is also shown. Significant differences from vehicle-treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

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 behavioural studies.

2.3. Measurement of emesis and genital grooming

On the day of the experiment, animals were transferred to clear Perspex observation chambers (21 × 14 × 13 cm) for the assessment of behaviour by a trained observer. Episodes of emesis were characterized 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). Episodes of retching and/or vomiting 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.

Animals were also observed for episodes of genital grooming. This behaviour was characterized as a focussed active licking of the genital prepuce region and/or penis and was sometimes accompanied by penile erection. An episode of grooming was considered separate when an animal changed its location in the observation chamber, or when the interval between licking exceeded 2 s.

2.4. Administration of drugs

A 30-gauge injection needle was inserted intracerebroventricularly (i.c.v.) into the lateral cerebral ventricle via the previously implanted guide cannula (the needle extended 0.5 mm below the implanted tip of the guide cannula). Resiniferatoxin (3–30 nmol), *E*-capsaicin (10–100 nmol) or *Z*-capsaicin (100 nmol), or their respective vehicles, were then injected (dosing volume: 10 μl, administered over 2 s). The needle was removed and animals monitored for changes in behaviour occurring during the subsequent 90-min observation period. Nicotine (30.7 μmol/kg, s.c.) or copper sulphate pentahydrate (480.6 μmol/kg, intragastric) were administered 3 h after the initial i.c.v. infusion to assess any potential antiemetic action induced by the vanilloids (animals were observed for 30 min in these tests).

In other experiments, capsazepine (30–600 nmol), ruthenium red (0.1–3 nmol) or their respective vehicles were injected i.c.v. (dosing volume, 10 μl) 15 min prior to the i.c.v. injection of resiniferatoxin (10 nmol) or *E*-capsaicin (100 nmol).

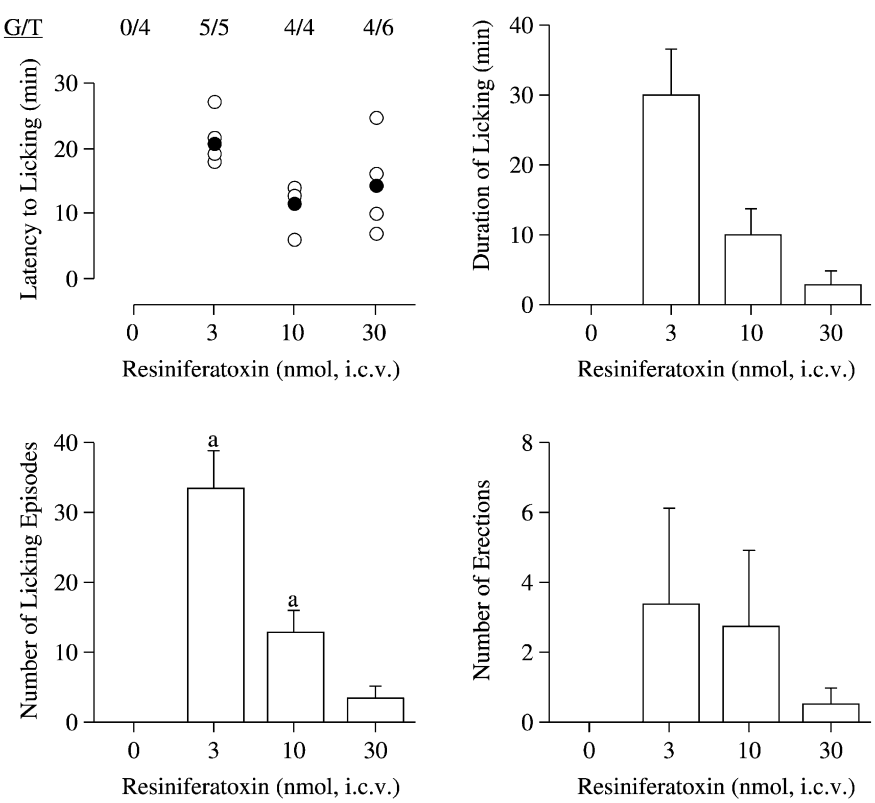

Fig. 2. The potential of resiniferatoxin to induce genital grooming in *S. murinus*. Open circles represent the individual latencies to the first episode of grooming. Filled circles represent the mean latencies of the respective treatment groups. The number of animals exhibiting genital grooming out of the number of animals tested (G/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of grooming and erections are also shown. Significant differences from vehicle-treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

A set of experiments were also designed to investigate a potential tachyphylaxis and cross-tachyphylaxis of the effects of resiniferatoxin (10 nmol, i.c.v.) and *E*-capsaicin (100 nmol, i.c.v.). In these experiments, each agonist was administered twice, with a 3-h interval between injections.

At the end of the experiments, the animals were killed with an overdose of pentobarbitone. Methylene blue dye (10 μl) was infused i.c.v. and the brains removed to confirm the site of injection.

2.5. Statistical analysis

The total number of episodes of retches and/or vomits and episodes of genital grooming were recorded in each animal following drug or vehicle administration. The latencies to onset of the respective behaviours were also recorded. Results represent the mean ± S.E.M. In general, the significance of difference between treatments were assessed by an unpaired Student's *t*-test or one-way analysis of variance (ANOVA) followed by a Fisher's Protected Least Significant Difference (PLSD) test, as appropriate (Statsview®, Abacus Concepts, USA). However, in the tachyphylaxis experiments, the significance of difference between treatments was assessed by a repeated two-factor ANOVA with comparisons of specified means by preplanned contrasts (SuperANOVA®, Abacus Concepts). Differences were considered significant when $P < 0.05$. The dose of vanilloid producing five episodes of retching and/or vomiting or the dose of vanilloid inhibiting emesis by 60% (ID_{60}) was determined on the mean data by nonlinear regression analysis (Kailidagraph™ Synergy Software, USA) The doses producing five episodes and inhibiting emesis by 60% were selected, as they were in the linear portion of the nonlinear fits.

2.6. Drug formulation

Resiniferatoxin (Sigma, USA), *E*-capsaicin ((*E*)-*N*-[4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide) (Tocris, UK) and *Z*-capsaicin ((*Z*)-*N*-[4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide) (Tocris) were dissolved in Tween 80/ethanol/saline (0.9% w/v) in a ratio of 1:1:8. Capsazepine (Research Biochemicals International, USA) was dissolved in 50% dimethylsulfoxide (made up in saline; 0.9% w/v). Ruthenium red (Sigma) was dissolved in saline (0.9% w/v). Both (−)-nicotine di-D-tartrate (Research Biochemicals Interna-

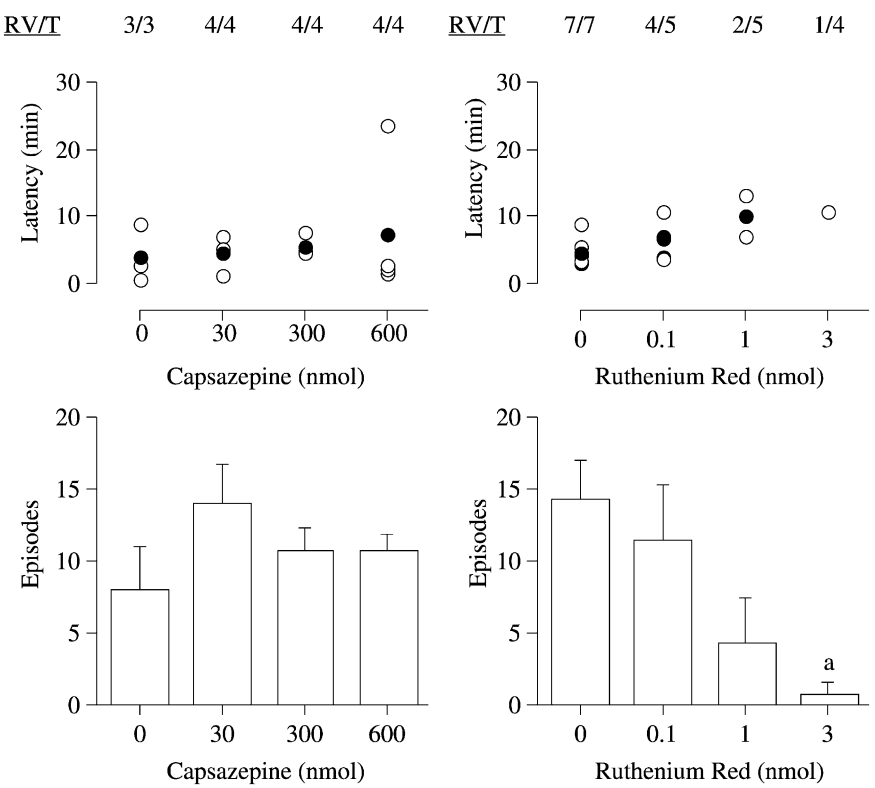

Fig. 3. The antiemetic potential of capsazepine and ruthenium red to antagonise resiniferatoxin-induced (10 nmol, i.c.v.) emesis in *S. murinus*. Open circles represent the individual latencies to the first episode of retching and/or vomiting. Filled circles represent the mean latencies of the respective treatment groups. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting are also shown. Significant differences from vehicle-treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

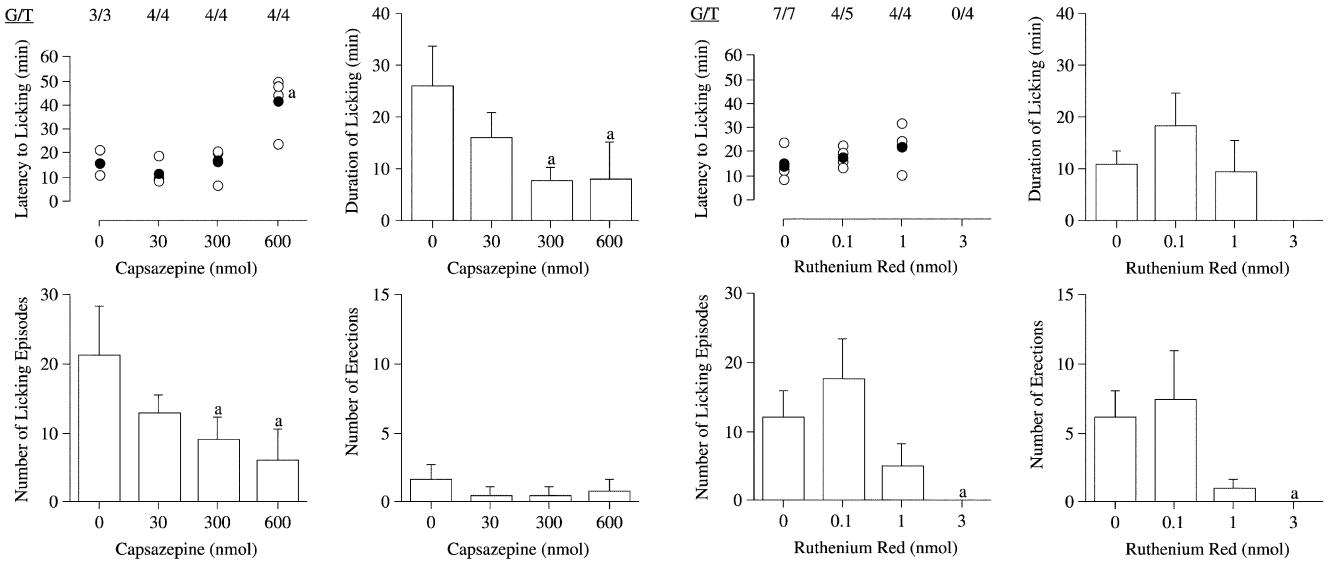

Fig. 4. The potential of capsazepine and ruthenium red to antagonise resiniferatoxin-induced (10 nmol, i.c.v.) genital grooming in *S. murinus*. Open circles represent the individual latencies to the first episode of grooming. Filled circles represent the mean latencies of the respective treatment groups. The number of animals exhibiting genital grooming out of the number of animals tested (G/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of grooming and erections are also shown. Significant differences from respective vehicle treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

tional) and copper sulphate pentahydrate (British Drug Houses, UK) were dissolved in distilled water and administered in a volume of 2 ml/kg; other drug administration volumes are indicated in the text.

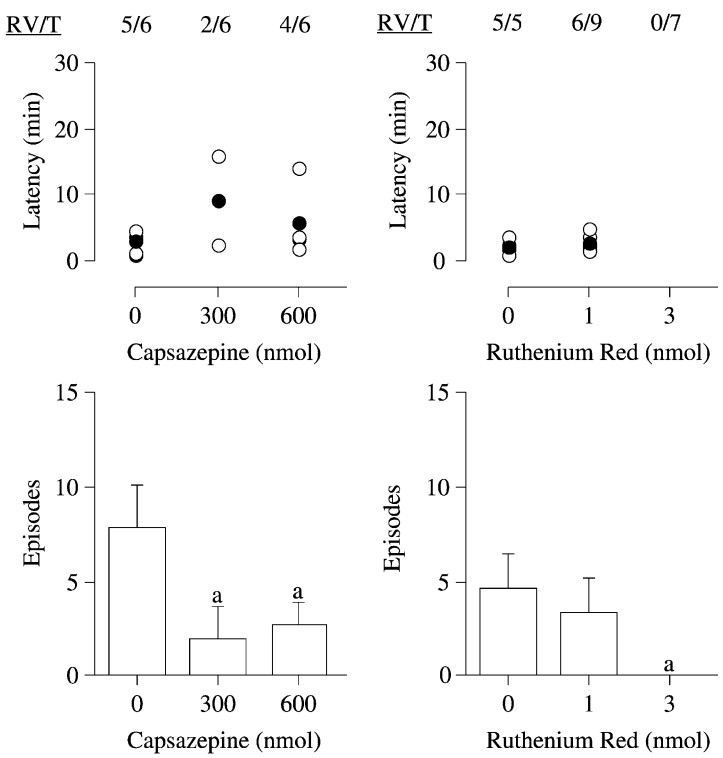

Fig. 5. The antiemetic potential of capsazepine and ruthenium red to antagonise *E*-capsaicin-induced (100 nmol, i.c.v.) emesis in *S. murinus*. Open circles represent the individual latencies to the first episode of retching and/or vomiting. Filled circles represent the mean latencies of the respective treatment groups. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting are also shown. Significant differences from vehicle-treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

3. Results

3.1. Resiniferatoxin- and capsaicin-induced emesis and genital grooming

Resiniferatoxin, 3 nmol, i.c.v., induced 1.9 ± 1.1 episodes of retching and/or vomiting in three out of nine animals (latency = 10.7 ± 4.6 min). Higher doses of resiniferatoxin were more consistent in inducing episodes of retching and/or vomiting and 30 nmol induced 8.5 ± 2.8 episodes in 9 out of 10 animals following a latency of 5.7 ± 1.4 min (Fig. 1). The estimated dose of resiniferatoxin producing five episodes of retching and/or vomiting was 3.3 nmol. Resiniferatoxin also induced genital grooming at all doses tested (Fig. 2). However, we did not determine the minimum effective dose in these studies. At 3 nmol, i.c.v., 33.6 ± 5.3 episodes of genital grooming occurred following a latency of 21.0 ± 2.0 min; the duration from the first to the last recorded episode was 29.0 ± 6.0 min. Penile erection was not consistently observed (e.g. three out of five animals at 3 nmol, one out of six animals at 30 nmol) and both the intensity of genital grooming and the incidence of erection decreased in a dose-related manner (Fig. 2).

The lowest dose of *E*-capsaicin (10 nmol) i.c.v. induced 4.0 ± 1.4 episodes of retching and/or vomiting in five out of six animals ($P < 0.05$), following a latency of 0.9 ± 1.5 min. At 100 nmol, i.c.v. there were 6.7 ± 1.2 episodes of retching and/or vomiting after a latency of 3.0 ± 1.0 min (Fig. 1). The estimated dose of *E*-capsaicin producing five episodes of retching and/or vomiting was 25.4 nmol. *Z*-capsaicin at 100 nmol, i.c.v., induced 4.6 ± 1.6 episodes of retching and/or vomiting after a latency of 4.5 ± 1.8 min ($P < 0.05$; six of eight animals responded). *E*-capsaicin and *Z*-capsaicin failed to induce genital grooming or penile erection ($n = 5–8$).

Capsazepine (30–600 nmol, i.c.v.) and ruthenium red (0.1–3 nmol, i.c.v.) were used to characterize the emesis and genital grooming induced by resiniferatoxin (10 nmol, i.c.v.) and emesis induced by capsaicin (100 nmol, i.c.v.). Capsazepine failed to significantly antagonise resiniferatoxin-induced emesis ($P > 0.05$; Fig. 3) but significantly antagonised resiniferatoxin-induced genital grooming by 71.9% at 600 nmol ($P < 0.05$; Fig. 4); this dose delayed the onset of grooming (compared to the mean onset in control animals) by up to 25.5 min ($P < 0.05$; Fig. 4). Penile erection was not significantly affected ($P > 0.05$; Fig. 4). Ruthenium red also antagonised resiniferatoxin-induced emesis and grooming but only at 3 nmol, i.c.v. ($P < 0.05$; Figs. 3 and 4). Capsazepine at 300 and 600 nmol, i.c.v., significantly antagonised ($P < 0.05$) capsaicin-induced episodes of retching and/or vomiting (max-

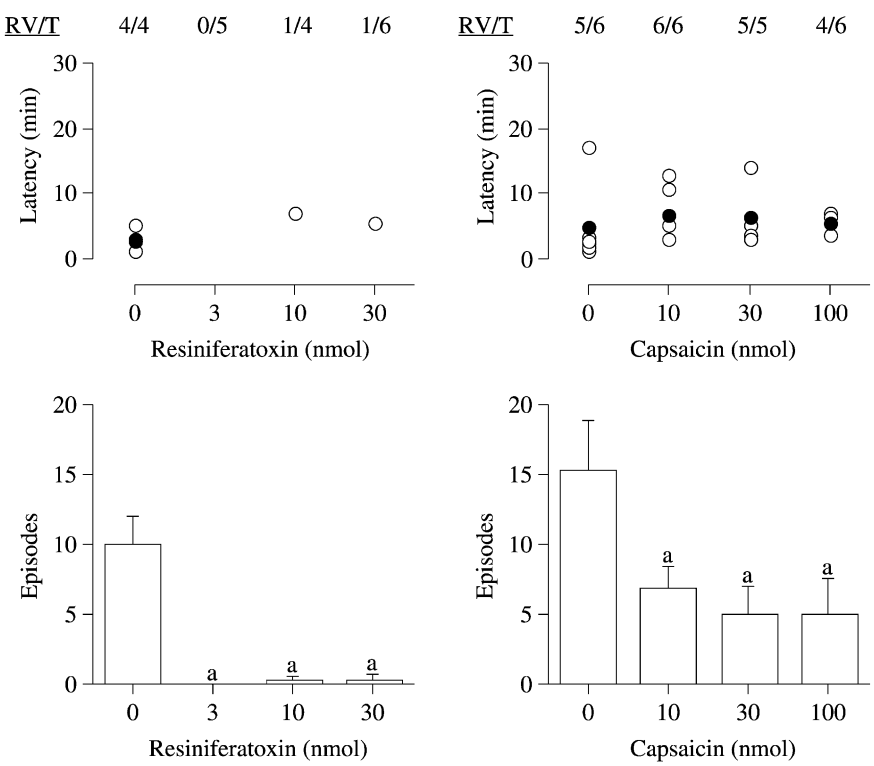

Fig. 6. The antiemetic potential of resiniferatoxin and *E*-capsaicin to antagonise intragastric copper sulphate pentahydrate-induced (480.6 μmol) emesis in *S. murinus*. Open circles represent the individual latencies to the first episode of retching and/or vomiting. Filled circles represent the mean latencies of the respective treatment groups. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting are also shown. Significant differences from vehicle-treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA, followed by a post hoc Fisher's PLSD test).

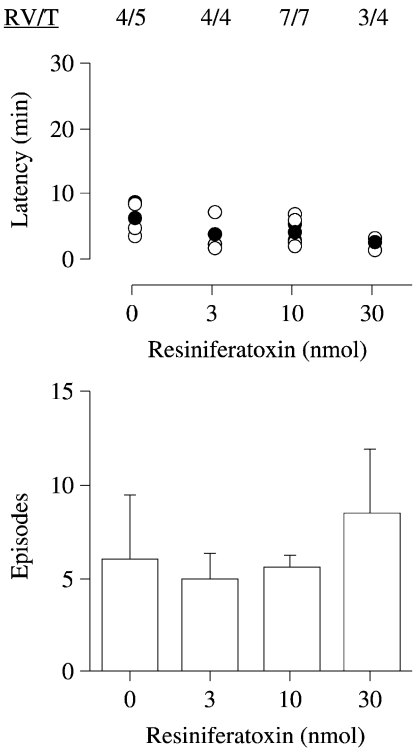

Fig. 7. Failure of resiniferatoxin to antagonise nicotine-induced (30.7 μmol, s.c.) emesis in *S. murinus*. Open circles represent the individual latencies to the first episode of retching and/or vomiting. Filled circles represent the mean latencies of the respective treatment groups. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a 'fraction'. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting are also shown. There were no significant differences from vehicle-treated animals ($P > 0.05$, one-way ANOVA).

imum reduction was 66.0% at 300 nmol, i.c.v.; Fig. 5). Ruthenium red prevented ($P < 0.05$) capsaicin-induced emesis in seven out of seven animals at 3 nmol, i.c.v. (Fig. 5).

3.2. Antiemetic action of vanilloids

A 3-h pretreatment with resiniferatoxin (3–30 nmol, i.c.v.) was highly effective ($ID_{60} < 3$ nmol, i.c.v.) to significantly antagonise (> 96% reduction at all doses) intragastric copper sulphate-induced emesis ($P < 0.05$; Fig. 6) but failed to modify nicotine-induced emesis ($P > 0.05$; Fig. 7). The capsaicinoids were not tested for the potential to reduce nicotine-induced emesis. However, *E*-Capsaicin (10–100 nmol) significantly antagonised ($ID_{60} = 10.3$ nmol, i.c.v.) intragastric copper sulphate-induced emesis by 55.5–67.4% ($P < 0.05$; Fig. 6). *Z*-capsaicin at 100 nmol, i.c.v., significantly antagonised copper sulphate-induced emesis by 55.9% (controls: 17.0 ± 4.6 episodes, *Z*-capsaicin-treated animals: 7.2 ± 2.2 episodes; $P < 0.05$, $n = 5–8$). The latency of copper sulphate to induce emesis was not significantly affected ($P > 0.05$) by *E*-capsaicin (Fig. 6) or *Z*-capsaicin (data not shown).

3.3. Repeat dose studies with resiniferatoxin and E-capsaicin

In these studies, the interval between drug administrations was 3 h. Following this protocol, resiniferatoxin (10 nmol, i.c.v.) initially induced emesis ($P < 0.05$), then prevented the emesis induced by a subsequent administration of resiniferatoxin (10 nmol, i.c.v.; $P < 0.05$) or *E*-capsaicin (100 nmol, i.c.v.; $P < 0.05$; see Fig. 8). Conversely, *E*-capsaicin (100 nmol, i.c.v.) was initially emetic ($P <$

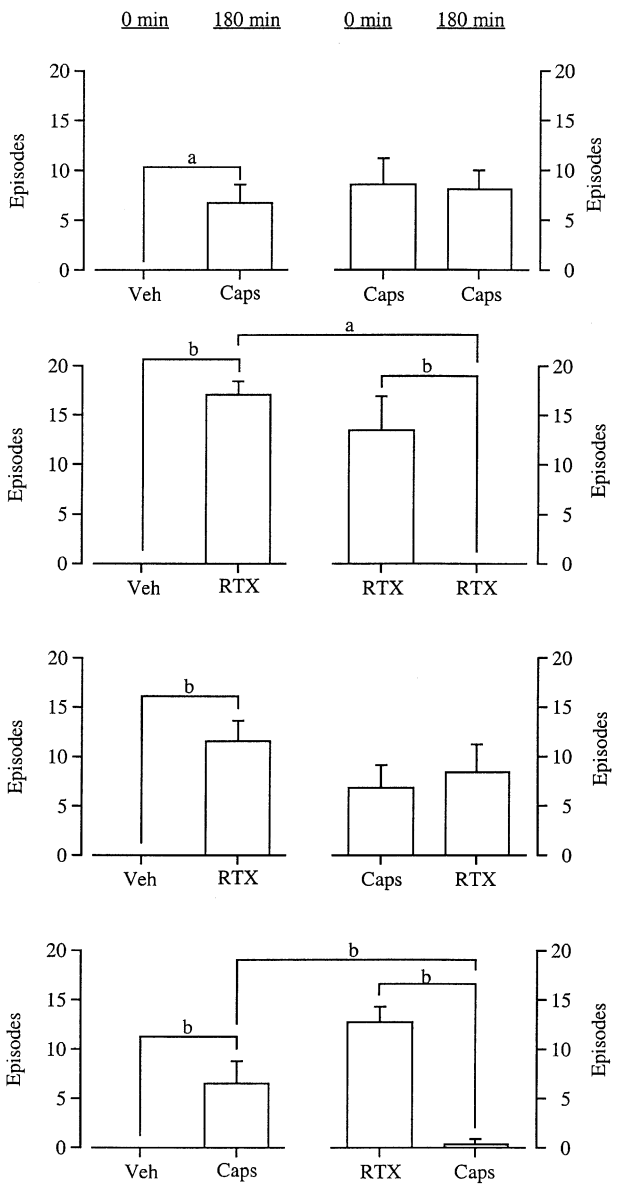

Fig. 8. Potential tachyphylaxis and cross-tachyphylaxis of the emetic action of resiniferatoxin (RTX; 10 nmol, i.c.v.) and *E*-capsaicin (Caps; 100 nmol, i.c.v.) in *S. murinus*. The mean ± S.E.M. of the total number of episodes of retching and/or vomiting are shown. Significant differences between respective treatment groups (including the respective vehicle (Veh) treatments) are indicated as $^{a}P < 0.05$ and $^{b}P < 0.01$ (repeated two-factor ANOVA with comparisons of specified means by preplanned contrasts).

0.05) but did not prevent the emesis induced by a subsequent administration of *E*-capsaicin (100 nmol, i.c.v.; $P > 0.05$) or resiniferatoxin (10 nmol, i.c.v.; $P > 0.05$; see Fig. 8).

Resiniferatoxin (10 nmol, i.c.v.) initially induced genital grooming ($P < 0.05$) but prevented the genital grooming induced by a subsequent administration of resiniferatoxin (10 nmol, i.c.v.; $P < 0.05$) and had no action in modifying the inactivity of *E*-capsaicin (Fig. 9). However, while *E*-capsaicin (100 nmol, i.c.v.) was inactive in inducing genital grooming, it did reduce the number of genital grooming episodes induced by a subsequent injection of resiniferatoxin (10 nmol, i.c.v.; a 41.8% reduction was observed, $P < 0.05$; see Fig. 9).

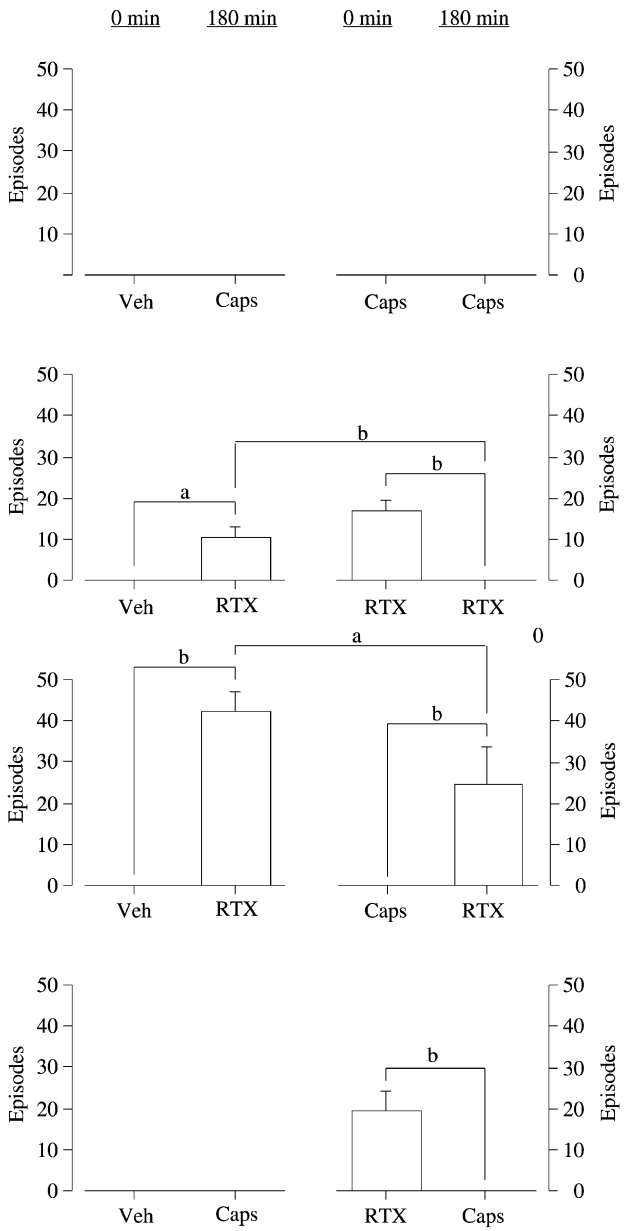

Fig. 9. Potential tachyphylaxis and cross-tachyphylaxis of the genital grooming potential of resiniferatoxin (RTX; 10 nmol, i.c.v.) and *E*-capsaicin (Caps; 100 nmol, i.c.v.) in *S. murinus*. The mean ± S.E.M. of the total number of episodes of genital grooming are shown. Significant differences between respective treatment groups (including the respective vehicle (Veh) treatments) are indicated as $^{a}P < 0.05$ and $^{b}P < 0.01$ (repeated two-factor ANOVA with comparisons of specified means by preplanned contrasts).

4. Discussion

4.1. General pharmacology of vanilloid receptors

There is biological and electrophysiological evidence for the existence of vanilloid receptor subtypes (Szallasi and Blumberg, 1999). There are also species differences in the pharmacology of vanilloid receptors (Szallasi, 1994; Szallasi and Blumberg, 1999). However, to date, only one vanilloid receptor has been cloned (from the rat) and has been termed the vanilloid VR1 receptor (Caterina et al., 1997). Vanilloids like resiniferatoxin and capsaicin behave as agonists at the rat vanilloid VR1 receptor and facilitate the opening of a nonselective cation channel that subsequently leads to calcium influx and transmitter release (Jerman et al., 2000). The action of resiniferatoxin and capsaicin at vanilloid VR1 receptors is antagonised by capsazepine and ruthenium red (Jerman et al., 2000; Szallasi and Blumberg, 1999). Such a mechanism has been proposed to explain the emesis induced by the vanilloids (see Section 1).

The antiemetic action of resiniferatoxin and capsaicin may involve the inhibition of voltage-gated calcium channels to prevent further transmitter release although the depletion of transmitters, down regulation of vanilloid VR1 receptors and neurodegeneration may also occur in the emetic circuits (Andrews et al., 2000; Shiroshita et al., 1997; Szallasi and Blumberg, 1999). The site of antiemetic action of vanilloids is probably in the dorsal vagal complex (see Andrews et al., 2000).

4.2. Emetic action

In the rat (a species incapable of vomiting), resiniferatoxin appears to be orders of magnitude more potent than capsaicin in inducing hypothermia (Szallasi et al., 1989), inhibiting the electrically evoked twitch response of the vas deferens (Maggi et al., 1990) and to reduce xylene-induced Evan's blue extravasation (Szallasi et al., 1989). However, in other tissue/systems, resiniferatoxin is only as potent (e.g. to contract the bladder; Maggi et al., 1990), or is only slightly more potent (e.g. to induce eye wipings; Szallasi et al., 1989) than capsaicin. In our studies, resiniferatoxin was only approximately seven times more potent (based on doses producing five episodes of retching and/or vomiting) than *E*-capsaicin to induce emesis following i.c.v. administration. The emetic action of resiniferatoxin and *E*-capsaicin was antagonised by ruthenium red to initially confirm an action at vanilloid receptors (Amann

and Maggi, 1991; Szallasi and Blumberg, 1999). However, capsazepine failed to block the action of resiniferatoxin at doses that antagonised capsaicin-induced emesis. The failure of capsazepine to antagonise resiniferatoxin-induced emesis was unexpected given its known ability to compete with [^{3}H]resiniferatoxin for vanilloid receptor binding sites and to act as a competitive receptor antagonist in functional assays in tissues of other species (Jerman et al., 2000). The data may indicate that resiniferatoxin and *E*-capsaicin bind to different sites on *S. murinus* vanilloid receptors involved in emesis.

Phorbol, 12-phenylacetate 13 acetate 20-homovanillate (PPAHV), a phorboid vanilloid, is structurally very similar to resiniferatoxin. PPAHV and capsaicin are excitatory on rat trigeminal ganglion neurones (Liu et al., 1998). However, capsazepine blocks the action of capsaicin but is only partially effective on some neurones, or ineffective on other neurones, to block the action of PPAHV (Liu et al., 1998). It was suggested that there are distinct subtypes of vanilloid receptors in rat trigeminal ganglion neurones. The data are similar with the mechanism of resiniferatoxin and *E*-capsaicin to induce emesis in *S. murinus* and it is tempting to speculate a similarity in the pharmacological profile of the receptor types between the species. Unfortunately, we do not know the exact involvement of the trigeminal neurones in the emetic mechanism of the vanilloids and we have not tested the emetic potential of PPAHV. However, the trigeminal system is involved in pain pathways and possibly the mechanisms involved in migraine-induced emesis (Dahlof and Hargreaves, 1998).

In the present study, we also provided evidence of the emetic activity of *Z*-capsaicin. One dose of *Z*-capsaicin was used and was approximately equipotent with *E*-capsaicin in inducing emesis. This was predicted as the capsaicinoids studied only differed in the configuration of the end aliphatic chain attached to the substituted benzene ring. The aliphatic chain of the molecule is thought to be of minor importance in terms of the biological activity of capsaicin and essentially contributes to lipophilicity (Klopman and Li, 1995).

4.3. Antiemetic action

The present studies identified the differential activity of resiniferatoxin to inhibit copper sulphate- but not nicotine-induced emesis following i.c.v. administration. Unfortunately, we did not examine the potential of the capsaicinoids to antagonise nicotine-induced emesis, but both *E*- and *Z*-capsaicin antagonised copper-sulphate-induced emesis by approximately 55–68%. The potential of resiniferatoxin to inhibit copper sulphate-induced emesis occurred at all doses and the threshold antiemetic dose is probably below the dose required to induce emesis. As the threshold dose was not precisely determined, it is difficult to calculate the relative antiemetic potency of the vanilloids. However, we can approximate that resiniferatoxin is at least 3.4 times more potent than *E*-capsaicin to reduce emesis (based on ID_{60} values and the lowest dose of resiniferatoxin). The data may indicate the possibility of dissociating the emetic effects of resiniferatoxin from its useful antiemetic potential.

At present, it is not known why resiniferatoxin is inconsistent in reducing nicotine-induced emesis (these studies and Rudd and Naylor, 1995). In an earlier study, a shorter 1-h subcutaneous pretreatment of resiniferatoxin was highly effective in reducing nicotine-induced emesis (Andrews et al., 2000). However, the precise mechanism of action of nicotine in inducing emesis is unknown. In other species, nicotine has been proposed to involve an action at the area postrema (Beleslin and Krstic, 1987) and/or the vestibular apparatus (Money and Cheung, 1983). However, it is not known if there is an additional peripheral mechanism.

If there is an additional peripheral mechanism, which nicotine activates to induce emesis, then it may explain why resiniferatoxin is more active when administered subcutaneously. It is also possible that the antiemetic action of resiniferatoxin to reduce nicotine-induced emesis fades after 1 h.

The tachyphylaxis experiments conducted were important to reveal the action of resiniferatoxin in preventing its own emesis and the emesis induced by *E*-capsaicin following an i.c.v. administration. Conversely, *E*-capsaicin was not active in reducing its own emesis or the emesis induced by resiniferatoxin. The failure of *E*-capsaicin to antagonise subsequent vanilloid receptor-mediated emesis was unexpected, since a 3-h pretreatment of capsaicin was effective in reducing copper sulphate-induced emesis. The data are difficult to explain, as we do not know if there is differential resiniferatoxin- and *E*-capsaicin-induced desensitization of vanilloid receptors (Szallasi and Blumberg, 1999). Further studies are therefore required to resolve the mechanism.

However, the failure of resiniferatoxin to prevent nicotine-induced emesis and the failure of *E*-capsaicin to prevent its own emesis was relevant in the interpretation of data in the present studies. This demonstrates that animals are still responsive to emetic challenges, even following pretreatment with drugs which are emetic. The data, therefore supports the specificity of the vanilloids to antagonise emesis (Andrews et al., 2000).

4.4. Genital grooming action

A major finding of the present study was the activity of resiniferatoxin administered centrally to induce genital grooming at low doses. Unfortunately, we did not determine the minimum dose of resiniferatoxin to induce grooming as the primary aim of the study was to examine the effect of vanilloids on the emetic reflex. Nevertheless, the minimum dose to induce genital grooming appears lower than required to evoke emesis (the reverse situation

apparently occurs following subcutaneous administration; Andrews et al., 2000). Importantly, the latency of resiniferatoxin to induce grooming was much longer than required to induce emesis and this may indicate that resiniferatoxin takes longer to penetrate the brain areas involved in sexual behaviour than the areas regulating emesis, or that the mechanism to induce grooming is indirect.

The vanilloid receptor mechanisms mediating genital grooming appear strikingly different from those involved in emesis. In particular, resiniferatoxin appears more potent in inducing grooming, and the grooming is antagonised by both ruthenium red and capsazepine. Further, *E*-capsaicin and *Z*-capsaicin did not induce grooming, in contrast to their action on the emetic reflex. However, we must be cautious in concluding that the vanilloid receptor involvement in genital grooming is insensitive to *E*-capsaicin because of data generated from the cross-tachyphylaxis experiments. Thus, *E*-capsaicin reduced the activity of resiniferatoxin to induce grooming by approximately 42%, which may suggest that both vanilloids interact at the same receptor. If this is the case, the receptors may have a similar profile to the known vanilloid VR1 rat receptor, where resiniferatoxin is ultrapotent (relative to capsaicin) and the actions are antagonised by both capsazepine and ruthenium red (Szallasi and Blumberg, 1999).

The major brain areas involved in sexual behaviour include the hypothalamus and medial preoptic area and associated limbic system structures (Dornan and Malsbury, 1989a). [^{3}H]resiniferatoxin binding sites are present in these brain areas in other species (Acs et al., 1996; Mezey et al., 2000). If vanilloid receptor recognition sites are present in the same brain areas in *S. murinus*, they would represent logical targets to modulate sexual arousal and genital grooming and may explain the behaviour observed in the present studies.

Substance P and CGRP are found in the circuits involved in sexual behaviour and could be expected to be involved in the genital grooming induced by the vanilloids. For example, there are reports that substance P, injected into the medial preoptic area, can reduce the latency of ejaculation in the male rat (Dornan and Malsbury, 1989b) and an injection into the mesencephalic central grey induces lordosis in the female rat (Dornan et al., 1987).

4.5. Conclusions

In conclusion, we have demonstrated the role of vanilloid receptors in the emetic reflex and central mechanisms involved in genital grooming of *S. murinus*. The vanilloid receptors involved in emesis are activated by capsaicinoids and resiniferatoxin but capsazepine is inactive to block the action of resiniferatoxin. The vanilloid receptor mechanisms involved in genital grooming may be relatively insensitive to capsaicinoids (or rapidly desensitizes?) but is activated by low doses of resiniferatoxin; the genital grooming induced by resiniferatoxin is antagonised by both capsazepine and ruthenium red. The present study may therefore provide the first evidence for the existence of vanilloid receptor subtypes in *S. murinus*.

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