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Olvanil: A non-pungent TRPV1 activator has anti-emetic properties in the ferret

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ABSTRACT

Anti-emetic drugs such as the tachykinin NK_1 receptor antagonists are useful to control emesis induced by diverse challenges. Evidence suggests pungent capsaicin-like TRPV1 activators also have broad inhibitory anti-emetic activity. However, pungent compounds are associated with undesirable effects including adverse actions on the cardiovascular system and on temperature homeostasis. In the present investigations using the ferret, we examine if the non-pungent vanilloid, olvanil, has useful anti-emetic properties without adversely affecting behaviour, blood pressure or temperature control. Olvanil (0.05–5 mg/kg, s.c.) was compared to the pungent vanilloid, resiniferatoxin (RTX; 0.1 mg/kg, s.c.), and to the anandamide reuptake inhibitor, AM404 (10 mg/kg, s.c.), for a potential to inhibit emesis induced by apomorphine (0.25 mg/kg, s.c.), copper sulphate (50 mg/kg, intragastric), and cisplatin (10 mg/kg, i.p.). Changes in blood pressure and temperature were also recorded using radiotelemetry implants.

In peripheral administration studies, RTX caused transient hypertension, hypothermia and reduced food and water intake, but also significantly inhibited emesis induced by apomorphine, copper sulphate, or cisplatin. Olvanil did not have a similar adverse profile, and antagonised apomorphine- and cisplatin-induced emesis but not that induced by copper sulphate. AM404 reduced only emesis induced by cisplatin without affecting other parameters measured. Following intracerebral administration only olvanil antagonised cisplatin-induced emesis, but this was associated with transient hypothermia. In conclusion, olvanil demonstrated clear anti-emetic activity in the absence of overt cardiovascular, homeostatic, or behavioural effects associated with the pungent vanilloid, RTX. Our studies indicate that non-pungent vanilloids may have a useful spectrum of anti-emetic properties via central and/or peripheral mechanisms after peripheral administration.

1. Introduction

Tachykinin NK_1 receptor antagonists are a relatively new class of anti-emetic first identified as capable of preventing emesis induced via diverse challenges in ferrets, a species with proven translational value (Holmes et al., 2009), by blocking the action of substance P in the nucleus tractus solitarius (NTS) and/or closely associated brainstem structures (Tattersall et al., 1996; Watson et al., 1995). Treatments depleting or reducing the release of substance P from emetic circuits could represent an alternative approach to our control of emesis. In this regard, we have previously focused on the anti-emetic potential of resiniferatoxin (RTX) as a modulator for transient receptor potential vanilloid-1 (TRPV1) receptors, which regulate neuropeptide release from sensory afferents (Szallasi and Blumberg, 1999).

In our original studies in ferrets, RTX antagonised the emesis induced by stimuli acting by both central (loperamide) and peripheral (copper sulphate, total body X-radiation) mechanisms, but it also caused hypothermia (Andrews and Bhandari, 1993). In *Suncus murinus* (house musk shrew), RTX antagonises nicotine, copper sulphate, motion, and cisplatin-induced emesis (Andrews et al., 2000b; Rudd and Wai, 2001). Importantly, others have shown that RTX antagonises apomorphine and cisplatin-induced emesis in ferrets (Yamakuni et al., 2002). Further, the pungent TRPV1/cannabinoid CB1 receptor agonist, arvanil, has been shown to have anti-emetic activity against morphine-6-glucuronide-induced emesis in ferrets, with the studies being extended to show that vanilloid receptor antagonists are not emetic indicating that TRPV1 sites are not tonically activated under normal circumstances (Sharkey et al., 2007).

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Capsaicin and other 'pungent' vanilloids are regarded as being irritant before having subsequent anti-nociceptive properties that are clinically useful e.g. as a treatment for neuropathic pain, bladder over-activity, and urinary incontinence (Szallasi, 2001); both actions are mediated via TRPV1 channels (Szallasi and Blumberg, 1999). Olvanil is an example of an orally active non-pungent compound that has useful anti-nociceptive properties in animal models without causing hypothermia or affecting cardiovascular performance (Appendino et al., 2005; Dray and Dickenson, 1991). It is possible that such compounds have anti-emetic effects without causing other unwanted side effects, particularly since non-pungent vanilloids, including olvanil, have been described as desensitising, but not activating, sensory nerve terminals in the NTS of rodents (Geraghty and Mazzone, 2002).

In the present studies, we decided to investigate if olvanil, in comparison with RTX, has a potential to prevent the emesis induced by apomorphine, a centrally acting emetic inducing emesis via the area postrema (Knox et al., 1993), copper sulphate, a peripherally acting emetic with a mechanism predominantly involving the abdominal vagi (Andrews et al., 1990), and the chemotherapeutic drug, cisplatin, which induces emesis possibly by mixed central and peripheral mechanisms (Rudd and Andrews, 2004). However, olvanil has also been reported to inhibit the anandamide membrane transporter (AMT), albeit at concentrations ten times higher than those that are required for TRPV1 activation (Beltramo and Piomelli, 1999). Therefore, our studies were done in comparison with the AMT inhibitor/weak activator of TRPV1 receptors, AM404 (Beltramo and Piomelli, 1999). Using a behavioural video-tracking system and ferrets implanted with radiotelemetry devices, we also investigated the potential of olvanil, RTX, and AM404 to alter behaviour, cardiovascular function and temperature homeostasis, as a means of assessing the general tolerability and safety of the compounds (Szallasi and Blumberg, 1996).

2. Materials and methods

2.1. Animals

Castrated male albino or fitch ferrets weighing between 1 and 2 kg were used. They were obtained from Southland Ferrets (Invercargill, New Zealand) and housed at 24 ± 1 °C and 50 ± 5% humidity. Artificial lighting was provided between 0600 and 1800 h, and water and dry pelleted cat chow (Feline Diet 5003, PMI® Feeds, St. Louis, U.S.A.) were available *ad libitum*. All experiments were conducted in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and under a licence provided by the Government of the Hong Kong SAR, and approval from the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.2. Stereotaxic surgery and implantation of radiotelemetric devices to record blood pressure and temperature

Animals were injected with buprenorphine (0.05 mg/kg, s.c.) as a preoperative analgesic and were anaesthetised with xylazine (2 mg/kg, i.m.) and ketamine (35 mg/kg, i.m.). To cannulate the forth ventricle, the animals were placed into a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, USA) and the skin on the dorsal aspect of skull was incised and the skull surface prepared for cannulation (target site: midline 2 mm rostral to the posterior edge of the skull; see Kan et al., 2008). A burr hole was made and a guide cannula was lowered 14 mm below the surface of the skull and fixed in place using screws and dental acrylic. The incision was closed using suture. Using aseptic techniques, the bodies of the transmitters (TL11M2-C50-PXT, Data Sciences International, U.S.A.) were implanted subcutaneously on the dorsal aspect of the animal, with the pressure catheter inserted into the right carotid artery and secured with suture. All skin incisions were closed with interrupted suture and sprayed with antibiotic aerosol (Tribiotic Spray®, Riker Laboratories, UK) and silicone wound dressing (Opsite®, Smith and Nephew, UK). Animals then received a second buprenorphine (0.05 mg/kg, s.c.) injection 12 h post surgery and were allowed 7 day recovery prior to experimentation.

2.3. Behavioural observation and recording of radiotelemetric data

Ferrets were transferred to observation chambers (49 × 49 × 60 cm^3) illuminated to 15 ± 1 Lux. Recordings commenced 24 h after introduction of the animals to the experiment arena. The image of each animal was captured by an overhead camera (Panasonic WV-CP460/P; Panasonic, Yokohama, Japan) and the analog-video signal was converted to digital by a frame grabber. Calculations of movement (sensitivity, 2 cm) were made using EthoVision Color Pro software (Version 2.3; Noldus Information Technology, Costerweg, Netherlands) running on a personal computer. Emesis was characterised by rhythmic abdominal contractions that were either associated with the forceful 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 defecation and/or tenesmus or micturition was characterised by lower abdominal and pelvic contractions where the animals also had raised tails. Consecutive episodes of retching and/or vomiting or defection and/or tenesmus or micturition were considered separate when the animal changed its location in the observation cage, or when the interval between episodes exceeded 5 s. Other behaviours measured included episodes of sleeping (durations ≥ 5 min), curling up (i.e. durations ≥ 5 min), crouching/hunching (durations ≥ 1 min), eating (i.e. durations ≥ 1 min), and drinking (i.e. durations ≥ 10 s) (Lau et al., 2005). In animals that had the radiotelemetry implants, blood pressure, heart rate, and subcutaneous temperature measurements were recorded wirelessly via RMC1 receivers (Dataquest Acquisition and Analysis, Data Sciences International, U.S.A.) placed in the vicinity of cages. After 30 min of recording basal activity, the animals were injected subcutaneously with olvanil (0.05–5 mg/kg), AM404 (10 mg/kg), resiniferatoxin (0.1 mg/kg), or vehicle (0.5 ml/kg, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8). The dose of RTX was based on our previous anti-emetic studies in the ferret (Andrews and Bhandari, 1993), and the doses of olvanil and AM404 were extrapolated from investigations showing analagesic activity in rodents (Dray and Dickenson, 1991; La Rana et al., 2006). Behaviour and/or physiological measurements were then continued for a further 24 h. In other experiments involving animals that had not been implanted with radiotelemetry devices, olvanil (0.05–5 mg/kg), AM404 (10 mg/kg), resiniferatoxin (0.1 mg/kg), or vehicle was administered subcutaneously 30 min prior to the administration of apomorphine (0.25 mg/kg, s.c. in 0.5 ml/kg, 0.01% w/v sodium metabisulphite), copper sulphate.$5H_2O$ (50 mg/kg, intragastric, in 2 ml/kg distilled water), or cisplatin (10 mg/kg, i.p. in 10 ml/kg 1% mannitol in saline 0.9% w/v). In these latter experiments, the animals were observed for changes in behaviour for the next 4–24 h. In some experiments, cisplatin treated animals were injected with drugs or vehicles at the first episode of retching and/or vomiting. To facilitate this, a 30-gauge injection needle was inserted 2 mm below the tip of the guide cannula to enable injections to be made over 30 s; drug doses were selected based on our previous studies in *S. murinus* (Wan and Rudd, 2004). All treatments were randomised and administered following a Latin square design, and behavioural measurements were made by investigators who were blinded to the treatments.

2.4. Drug used

Apomorphine hydrochloride, D-mannitol and resiniferatoxin were from Sigma–Aldrich, St. Louis, USA. AM404 (*N*-(4-hydroxyphenyl) 5*Z*,8*Z*, 11*Z*, 14*Z*-eicosatetraenamide) and olvanil (*N*-vanillyloleoylamide) were from Tocris, Bristol, UK. Cisplatin was from David Bull Laboratories, Victoria, Australia. Copper sulphate pentahydrate was from British Drug Houses Laboratory Supplies, Dorset, UK. Doses are expressed as the free base.

2.5. Data analysis

The radiotelemetry data were analysed using DQ A.R.T. Gold software (Dataquest Acquisition and Analysis, Data Sciences International, U.S.A.) or Spike 2 (Cambridge Electronic Design, Ltd., Cambridge, England). Differences between behavioural and physiological data of control and drug treated animals were analysed by various techniques using GraphPad Prism 5.0a (GraphPad Software, California, U.S.A.). The Log_{10} transformed locomotor activity data, and the retching and vomiting and food and water consumption data were analysed using a one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison tests. Blood pressure and temperature data, which were sampled every 10 min, were analysed by a repeated measures 2-way ANOVA followed by Bonferroni multiple comparison tests. Latency data and all other behaviours were analysed using a Kruskal–Wallis test followed by Dunn's multiple comparison tests. When an animal failed to retch or vomit, a latency value equal to the test period observation time (e.g. 24 h) was used to perform the statistical analysis. Results are expressed as the mean ± S.E.M, unless otherwise stated. In all cases, difference between treatment groups were considered significant when $P < 0.05$.

3. Results

3.1. Potential of vanilloids and AM404 to affect behaviour, blood pressure, and temperature

The subcutaneous administration of olvanil, AM404, RTX, or vehicle was not associated with any signs of pain or irritancy. Further, none of the treatments were associated with retching or

vomiting. Prior to treatment, systolic and diastolic pressures were 126.2 ± 2.2 and 100.7 ± 1.4 mmHg, respectively; heart rate was 224.0 ± 4.9 beats per min (BPM) and body temperature was 38.2 ± 0.1 °C ($n = 24$, data averaged over 30 min prior to injections). The administration of vehicle, olvanil, or AM404 was not associated with any significant effect on the cardiac parameters or temperature when assessed over a 24 h period; only 0–6 h data were shown ($P > 0.05$; Fig. 1). Conversely, RTX caused hypertension, with systolic and diastolic pressures increasing to 268.7 ± 10.7 and 223.2 ± 6.5 mmHg, respectively, at 20 min post injection ($P < 0.001$; Fig. 1). This effect was maintained for ~20 min before decreasing rapidly; however, significant differences in diastolic pressure compared to vehicle treated animals were still evident for a further 2 h ($P < 0.01$; Fig. 1). RTX and the other compounds failed to modify heart rate, although this parameter appeared to be rather variable during the observation period ($P > 0.05$; Fig. 1).

RTX s.c. also had a marked effect on body temperature that was evident within 20 min of administration. A fall of ~4.6 °C was seen at 2.2 h post administration ($P < 0.001$), and the hypothermia was maintained for approximately 5.5 h (Fig. 1). Olvanil and AM404 did not cause hypothermia ($P > 0.05$). We decided to focus our analysis of the spontaneous behaviour over the first 0–6 and then the subsequent 7–24 h periods to coincide with the changes in physiological data obtained with RTX. Using this approach, there were no differences between the vehicle and the olvanil or AM404 treated animals ($P > 0.05$; Table 1). However, RTX produced a trend to reduce locomotor activity ($P > 0.05$) and reduced the number of episodes of eating by 60.0 and 57.7% during the 0–6 and 7–24 h periods, respectively ($P < 0.01$); the feeding frequency during the 0–6 h periods was also significantly lower compared with data recorded for olvanil and AM404 treated animals ($P < 0.05$; Table 1).

The reduction in feeding frequency by RTX was also reflected in the amount of food consumed during the entire 24 h observation period, with consumption reduced by 58.7% ($P < 0.01$); the reduction was also significantly different from consumption seen in the olvanil and AM404 treated animals ($P < 0.01$; Table 1). Compared with the control vehicle treated animals, none of the drug treatments affected significantly the number of episodes of sleeping, curling up, and crouching/hunching exhibited by the vehicle treated animals ($P > 0.05$). Similarly, compared with the controls, none of the treatments affected drinking frequency, or the amount of water consumed during the study period, although the activity in the RTX-treated animals was greatly reduced, and was significantly less than the olvanil 5 mg/kg treated animals during the 0–24 h period (a 73.0% reduction in water consumption, $P < 0.01$; Table 1).

3.2. Effect of subcutaneously administered vanilloids and AM404 on apomorphine and copper sulphate-induced emesis

None of the animals treated with olvanil, AM404, RTX, or vehicle experienced emesis during the 30 min pretreatment time. Pooling of locomotor data from the pretreatment periods of both experiments did not reveal any statistical differences in distance travelled (or velocity data) between vehicle controls and drug treatment groups (data not shown, $P > 0.05$). Apomorphine-induced emesis following a median latency of 7.1 min (25 and 75% percentiles were 5.5 and 12.2 min, respectively) and comprised 30.5 ± 5.3 retches + vomits in 5.5 ± 1.0 episodes during the 4 h observation period (Fig. 2). Olvanil antagonised the retching + vomiting induced by apomorphine dose-dependently (Fig. 2). The maximum reduction of episodes and of retches + vomits were 84.9 and 72.7%, respectively at 5 mg/kg ($P < 0.01$; 4 out of 6 animals were protected completely). Copper sulphate-induced emesis following a median latency of 3.9 min (25 and 75% percentiles were 2.8 and 5.4 min, respectively) and comprised 170.5 ± 36.1 retches + vomits in

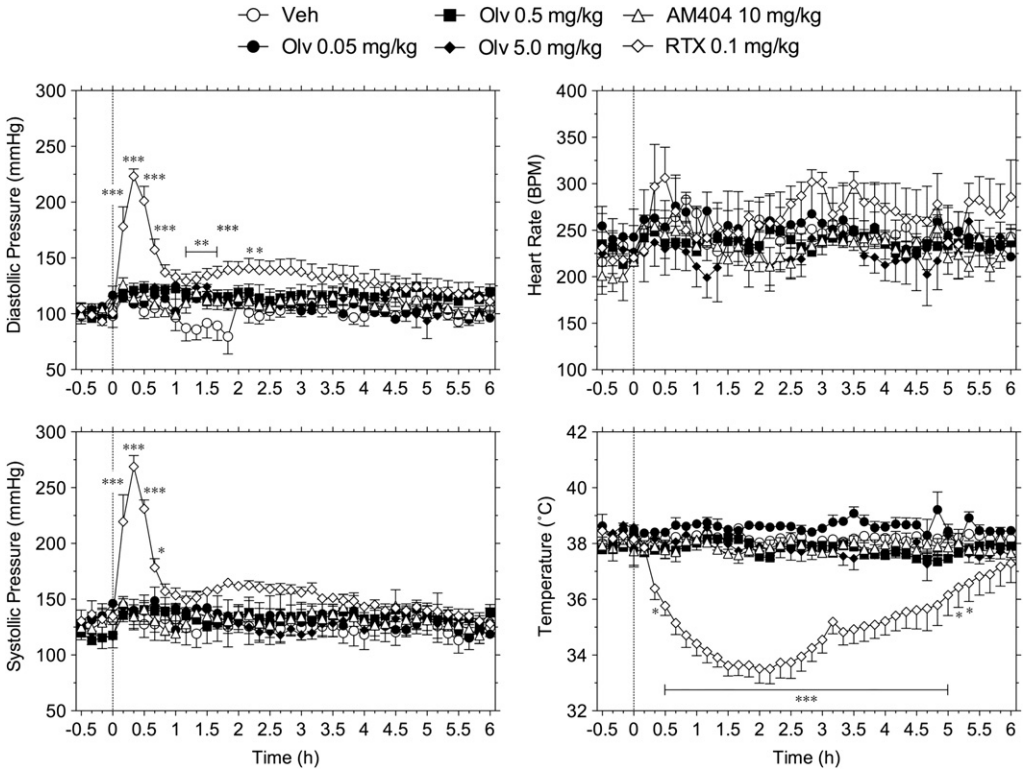

Fig. 1. The effect of resiniferatoxin (RTX; 0.1 mg/kg, s.c.), olvanil (OLV; 0.05–5 mg/kg, s.c.), AM404 (10 mg/kg, s.c.), or vehicle (0.5 ml/kg, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8, s.c.) on blood pressure, heart rate and temperature. Physiological data were acquired wirelessly every 10 min and represent the mean ± S.E.M of 4 determinations. Significant differences relative to the vehicle treated animals are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (repeated measures two-way ANOVA with Bonferroni post tests).

Table 1
Effect of olvanil (OLV), AM404, or resiniferatoxin (RTX) on spontaneous activity and feeding and drinking in the ferret.

Behaviour (episode/time period)	Time (h)	Veh	OLV (0.05)	OLV (0.5)	OLV (5)	AM404 (10)	RTX (0.1)
Sleep	0–6	8.8 ± 0.8	9.0 ± 1.4	7.8 ± 0.8	6.8 ± 0.3	9.8 ± 0.9	10.2 ± 3.3
Curl-up		10.0 ± 3.1	11.8 ± 3.5	12.3 ± 1.1	9.8 ± 2.3	18.0 ± 1.8	15.6 ± 4.1
Crouch/Hunch		8.0 ± 5.2	9.3 ± 3.7	8.8 ± 3.4	4.8 ± 1.4	3.4 ± 0.8	2.0 ± 1.5
Def/Mic		6.3 ± 0.8	7.0 ± 1.4	8.8 ± 1.3	9.0 ± 0.4	6.4 ± 1.0	6.4 ± 1.3
Eat		16.0 ± 2.5	12.5 ± 0.5††	18.3 ± 2.4††	21.0 ± 5.1†	14.4 ± 1.8†	6.4 ± 2.8**
Drink		13.5 ± 3.8	13.5 ± 3.6	11.0 ± 0.4	9.8 ± 1.5	10.8 ± 1.2	3.0 ± 2.5
Distance (m)		29.4 ± 9.1	12.2 ± 2.4	14.4 ± 3.0	15.2 ± 3.9	15.3 ± 6.1	7.0 ± 2.6
Velocity (m/s)		13.9 ± 4.6	5.7 ± 1.1	6.6 ± 1.4	7.0 ± 1.8	8.8 ± 2.5	5.2 ± 1.7
Sleep	7–24	19.3 ± 1.9	20.0 ± 3.0	21.8 ± 3.9	17.8 ± 2.2	18.2 ± 3.7	22.8 ± 2.3
Curl-up		34.0 ± 10.3	31.3 ± 6.0	35.8 ± 9.7	39.0 ± 9.3	41.2 ± 7.6	51.4 ± 12.7
Crouch/Hunch		8.0 ± 4.2	13.5 ± 10.5	7.8 ± 3.8	16.0 ± 11.0	4.8 ± 1.6	3.6 ± 0.9
Def/Mic		5.8 ± 1.0	7.0 ± 1.2	7.5 ± 1.0	8.5 ± 0.5	5.8 ± 0.9	6.0 ± 1.1
Eat		17.0 ± 2.5	12.5 ± 0.5	18.5 ± 2.4	24.3 ± 5.8	14.6 ± 1.8	7.2 ± 2.7**
Drink		19.3 ± 4.4	18.0 ± 0.7	17.3 ± 1.4	19.5 ± 2.6	14.2 ± 2.6	7.6 ± 3.7
Distance (m)		38.6 ± 7.2	98.8 ± 285	38.0 ± 8.2	53.7 ± 6.2	43.4 ± 17.8	34.0 ± 15.3
Velocity (m/s)		7.2 ± 0.8	15.4 ± 4.4	6.4 ± 0.8	8.5 ± 1.1	8.4 ± 2.3	9.1 ± 4.6
Food (g/kg)	0–24	40.7 ± 3.9	40.9 ± 4.4††	42.1 ± 3.9††	46.1 ± 2.1†††	44.5 ± 3.0†††	16.8 ± 5.8**
Water (g/kg)		90.6 ± 10.6	97.8 ± 17.7	95.5 ± 9.0	125.7 ± 16.0††	78.2 ± 19.5	33.9 ± 16.8

Doses of drugs in mg/kg are shown in parenthesis. Def/Mic = defecation/micturation. Data represents the mean ± S.E.M. of 4–5 determinations. Velocity is the average velocity during each time period Significant differences relative to vehicle (Veh) control treated animals are indicated as $^{**}P < 0.01$; significant differences relative to RTX (0.1) treated animals are indicated as $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ (one-way ANOVA with post hoc Bonferroni multiple comparisons, or Kruskal–Wallis test with Dunn's tests, as appropriate).

28.0 ± 3.4 episodes during the 4 h observation period. However, olvanil did not antagonise copper sulphate-induced emesis (Fig. 3). Conversely, RTX (0.1 mg/kg) antagonised the number of episodes and retches + vomits induced by apomorphine by 78.8 and 93.4%, respectively (Fig. 2; $P < 0.01$; 5 out of 6 animals were protected completely), and the number of episodes and retches + vomits induced by copper sulphate by 98.2 and 99.0%, respectively (Fig. 3; $P < 0.01$; 2 out of 4 animals were protected completely). AM404 (10 mg/kg) was ineffective against both emetics ($P > 0.05$; Figs. 2 and 3).

Food and water consumption was measured during the 4 h observation period where controls (vehicle plus vehicle) treated animals consumed 10.6 ± 1.2 g/kg of food and 23.7 ± 5.6 g/kg of water (pooled data, $n = 10$). The treatment of vehicle plus apomorphine, or olvanil or AM404 plus apomorphine, did not affect food and water consumption, but RTX plus apomorphine treated animals

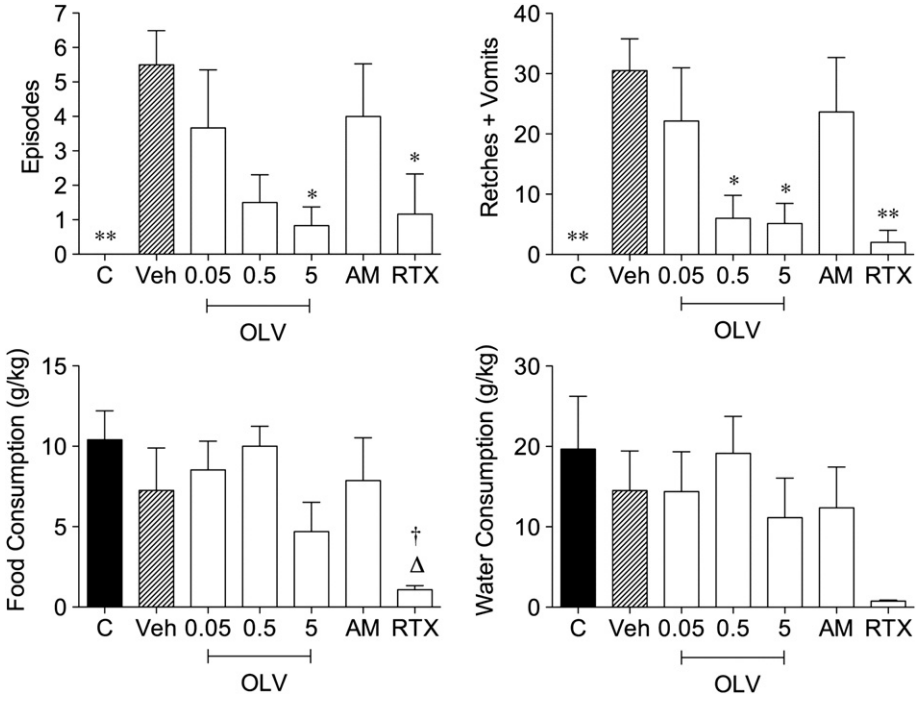

Fig. 2. The effect of resiniferatoxin (RTX; 0.1 mg/kg, s.c.), olvanil (OLV; 0.05–5 mg/kg, s.c.), AM404 (10 mg/kg, s.c.), or vehicle (0.5 ml/kg, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8, s.c.) on apomorphine (0.25 mg/kg, s.c.)-induced retching and/or vomiting. The effect of drug and/or vehicle treatment on 4 h food and water consumption and data from animals treated with the vanilloid/AM404 vehicle combined with the vehicle used for apomorphine (C; 0.01% w/v sodium metabisulphite, 0.5 ml/kg) are also shown. Results represent the mean ± S.E.M of 6 determinations. Significant differences relative to vehicle + apomorphine treated animals are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$; significant differences relative to vehicle + vehicle treated animals (C) are indicated as $^{\dagger}P < 0.05$; significant difference relative to OLV 0.5 mg/kg treated animals are indicated as $^{\Delta}P < 0.05$ (one-way ANOVA with Bonferroni post tests).

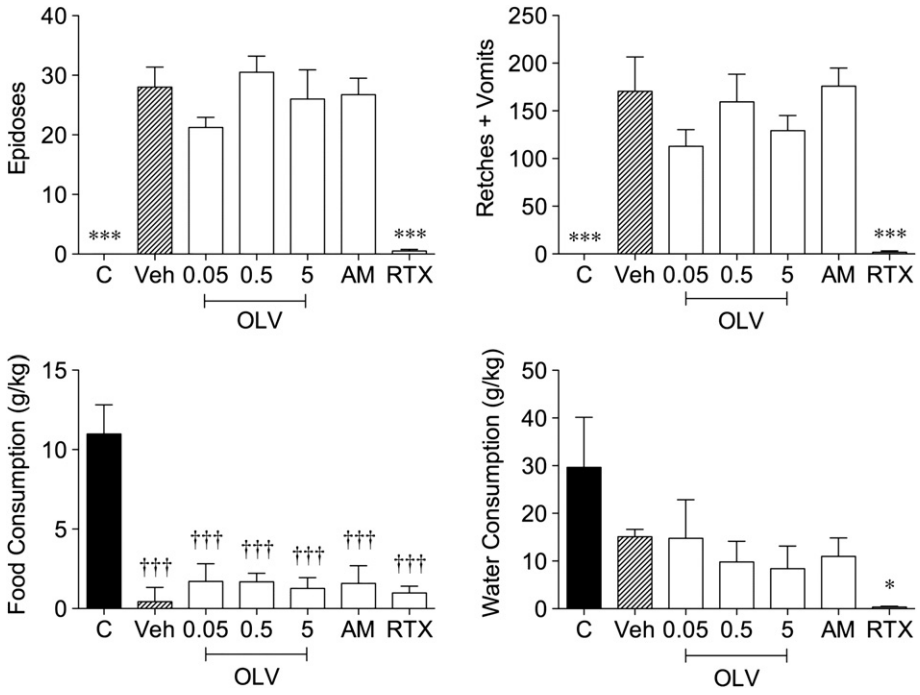

Fig. 3. The effect of resiniferatoxin (RTX; 0.1 mg/kg, s.c.), olvanil (OLV; 0.05–5 mg/kg, s.c.), AM404 (10 mg/kg, s.c.), or vehicle (0.5 ml/kg, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8, s.c.) on copper sulphate.$5H_2O$ (50 mg/kg, intragastric)-induced retching and/or vomiting. The effect of drug and/or vehicle treatment on 4 h food and water consumption and data from animals treated with the vanilloid/AM404 vehicle combined with the vehicle used for apomorphine (C; 0.01% w/v sodium metabisulphite, 0.5 ml/kg) are also shown. Results represent the mean ± S.E.M of 6 determinations. Significant differences relative to vehicle + apomorphine treated animals are indicated as $^{*}P < 0.05$, $^{***}P<0.001$; significant differences relative to vehicle + vehicle treated animals (C) are indicated as $^{\dagger\dagger\dagger}P < 0.001$ (one-way ANOVA with Bonferroni post tests).

ate significantly less food (Fig. 2; an 85.0% reduction was observed, $P < 0.05$), and water consumption was also markedly depressed ($P > 0.05$). Similarly, the RTX plus copper sulphate treated animals consumed significantly less water than the controls (a 98% reduction was observed, $P < 0.05$), with all animals treated with olvanil, AM404, or RTX, plus copper sulphate, consuming significantly less food (>70% less than controls (Fig. 3; $P < 0.001$).

3.3. Effect of vanilloids and AM404 on cisplatin-induced emesis

3.3.1. Subcutaneous studies

In vehicle treated animals, cisplatin-induced retching and vomiting with a median onset of 85.4 min (25 and 75% percentiles were 79.5 and 90.8 min, respectively). Most of the retching + vomiting response occurred in the first 6 h period in which there were 263.6 ± 17.7 retches + vomits distributed over 36.3 ± 2.9 episodes. During this period, olvanil at 0.5 and 5 mg/kg reduced the numbers of retches + vomits by 35.4% ($P < 0.05$) and 37.9% ($P < 0.01$), respectively; and RTX 0.1 mg/kg protected 3 out of 4 ferrets, with an overall 99.9% ($P < 0.001$) reduction in retching + vomiting being observed (Fig. 4). AM404 10 mg/kg also reduced the numbers of retches + vomits by 37.6% ($P < 0.05$; Fig. 4). The retching + vomiting occurring over the remainder of the observation time (7–24 h) was relatively sporadic, with none of the drug treatments impacting on the response ($P > 0.05$; Fig. 4). However, it is important to note that the antagonism provided by olvanil 5 mg/kg or RTX 0.1 mg/kg were still detected if the analysis was performed on the 0–24 h datasets (39.0 and 90.2% reductions were recorded for olvanil and RTX, respectively; $P < 0.05$).

In these experiments, the control (vehicle + vehicle) treated animals consumed 37.4 ± 9.9 and 77.0 ± 21.0 g/kg of food and water, respectively, during the entire 24 h observation time (Fig. 4). Cisplatin, alone or in combination with the vanilloids or AM404, decreased food intake significantly by approximately 78–86% ($P < 0.001$; Fig. 4) and reduced water consumption, albeit insignificantly, by 77–87% ($P > 0.05$; Fig. 4). None of the drug treatments affected the locomotor activity significantly (distance travelled or velocity) when assessed over the 24 h observation period ($P > 0.05$, data not shown).

3.3.2. Intracerebroventricular studies

These experiments were conducted in animals implanted with telemetry implants and the temperature of the animals recorded during the habituation period was 37.7 ± 0.1 °C (pooled data sampled over 30 min, $n = 30$). In these experiments, cisplatin-induced emesis with a median latency of 99.4 min (25 and 75% percentiles were 84.3 and 112.9 min, respectively); and just prior to emesis, the temperature of the animals was 37.9 ± 0.1 °C (pooled data sampled over 30 min, $n = 30$). As soon as the first episode of emesis induced by cisplatin had subsided, animals were removed and injected i.c.v. with olvanil, AM404, or RTX, or vehicle, and then returned to their cages for further observation. The control cisplatin + vehicle treated animals exhibited 123.2 ± 15.9 and 93.4 ± 41.6 retches + vomits, respectively, during the subsequent 0–6 and 7–24 h observation periods (Fig. 5). Olvanil dose-dependently reduced retching and vomiting response during the 6 h period following cisplatin administration, with the highest dose 30 μg reducing retching + vomiting by 89.1% ($P < 0.05$; Fig. 5). During this period, olvanil also reduced body temperature by up to 3.9 ± 0.7 °C (maximum fall seen with 10 μg; $P < 0.05$; vehicle caused a fall of 1.8 ± 0.2 °C; Fig. 6). The antagonism of emesis afforded by olvanil showed a trend to persist for the remainder of the 24 h observation period, although non-significantly ($P > 0.05$). RTX at 0.3 μg produced a fall in temperature of 3.3 ± 0.5 °C ($P < 0.01$; Fig. 6), which was similar to the fall produced by the highest dose of olvanil 30 μg. Conversely, RTX failed to antagonise emesis ($P > 0.05$; Fig. 5).

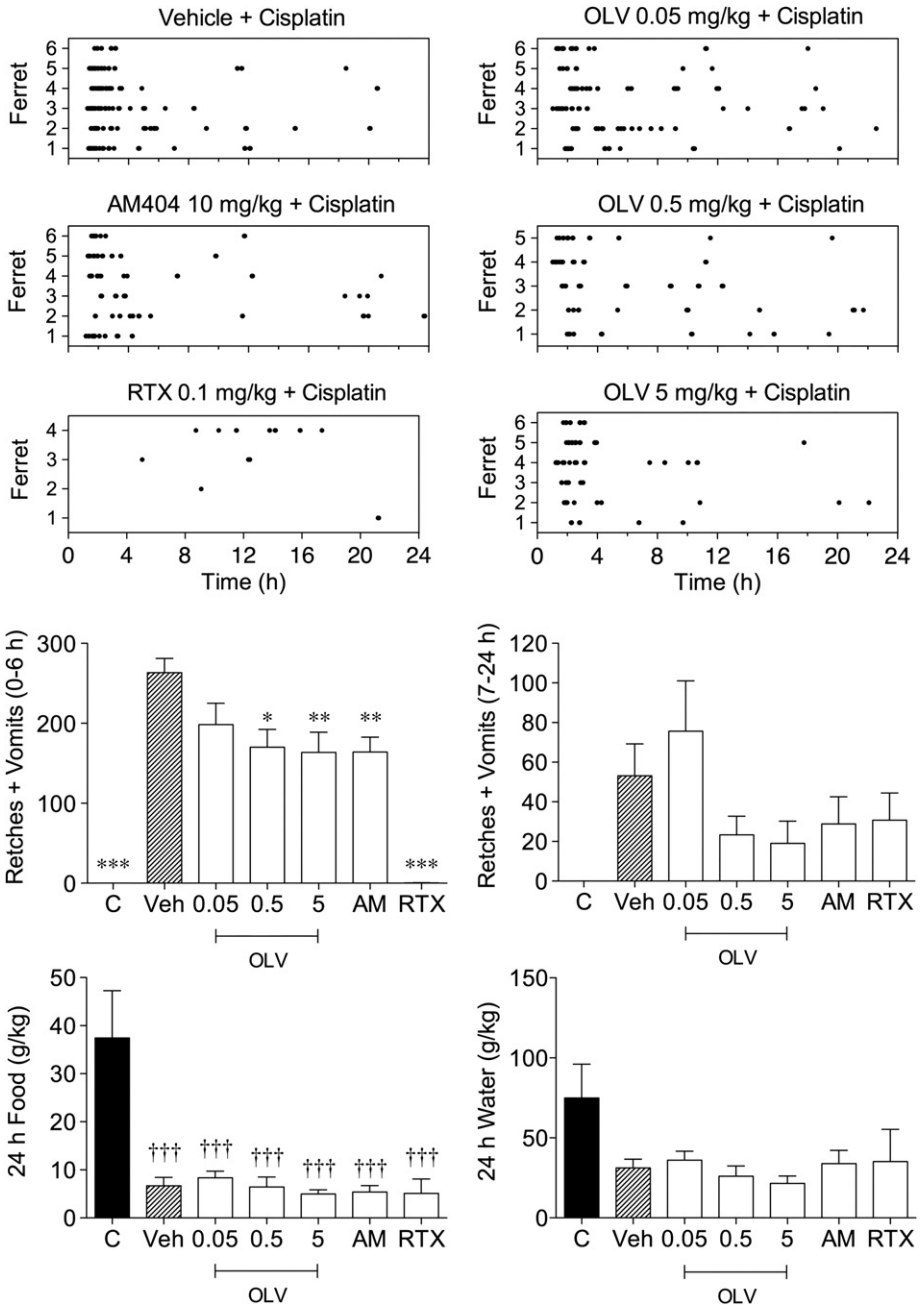

Fig. 4. The effect of resiniferatoxin (RTX; 0.1 mg/kg, s.c.), olvanil (OLV; 0.05–5 mg/kg, s.c.), AM404 (10 mg/kg, s.c.), or vehicle (0.5 ml/kg, Tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8, s.c.) on cisplatin (10 mg/kg, i.p.)-induced retching and/or vomiting; individual episodes of retching and/or vomiting are shown as filled circles. The effect of drug and/or vehicle treatment on 4 h food and water consumption and data from animals treated with the vanilloid/AM404 vehicle combined with the vehicle used for cisplatin (C; 0.1 (w/w) % mannitol in saline, 5 ml/kg) are also shown. Results represent the mean ± S.E.M of 4–6 determinations. Significant differences relative to vehicle + cisplatin treated animals are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; significant differences relative to vehicle + vehicle treated animals (C) are indicated as $^{\dagger\dagger\dagger}P < 0.001$ (one-way ANOVA with Bonferroni post tests).

AM404 60 μg did not antagonise cisplatin-induced emesis, nor did it affect body temperature significantly ($P > 0.05$; the maximum fall recorded was 1.8 ± 0.5 °C; Figs. 5 and 6).

In those animals receiving cisplatin and vehicle, food and water consumption during the 24 h observation period was 14.8 ± 1.1 and 43.3 ± 6.8 g/kg, respectively. None of the drug pretreatments altered consumption ($P > 0.05$, data not shown).

4. Discussion

Previous studies have shown that RTX has broad inhibitory anti-emetic properties in ferrets (Andrews and Bhandari, 1993) and shrews (cisplatin, copper sulphate, nicotine, motion, RTX, or capsaicin) (Andrews et al., 2000b; Rudd and Wai, 2001). Subsequently, studies by others have shown that RTX also prevents cisplatin-induced emesis in dogs and emesis induced by apomorphine and cisplatin in ferrets; the latter experiments against cisplatin in ferrets were particularly important since they demonstrated that RTX prevented the delayed phase of the cisplatin-induced emetic response (Yamakuni et al., 2002).

The importance of the present investigations was to show for the first time that a non-pungent vanilloid is capable of antagonising drug-induced emesis. Thus, olvanil produced a dose-related inhibition of apomorphine- and cisplatin-induced emesis, with the

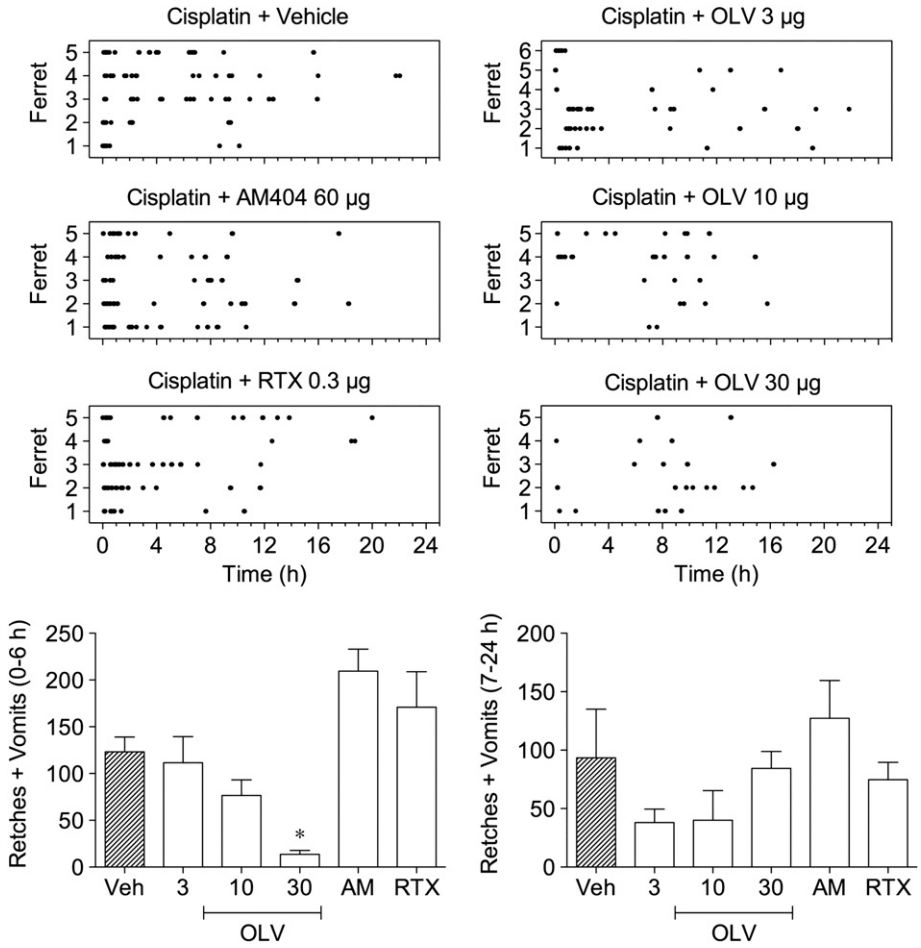

Fig. 5. The effect of i.c.v. administered resiniferatoxin (RTX; 0.3 µg), olvanil (OLV; 3–30 µg), AM404 (60 g), or vehicle (10 µl, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8) on cisplatin (10 mg/kg)-induced retching and/or vomiting. Vanilloids or AM404 or vehicle was administered after the first episode of cisplatin-induced emesis; subsequent episodes of retching and/or vomiting are shown as filled circles. Results represent the mean ± S.E.M of 5–6 determinations. Significant differences relative to vehicle + cisplatin treated animals are indicated as $^{*}P < 0.05$ (one-way ANOVA with Bonferroni post tests).

effects against cisplatin being retained following i.c.v. administration. This profile suggests a central mechanism of action and a high density of TRPV1 sites are present in the ferret NTS, with some also present in the area postrema and dorsal motor nucleus of the vagus nerve (Sharkey et al., 2007). Importantly, the anti-emetic effects of subcutaneously administered olvanil were seen at doses that had no effect on blood pressure and temperature or on feeding/drinking activity, or other spontaneous behaviours including locomotor performance. The significance of these findings, in comparison with data obtained from experiments with the pungent vanilloid, RTX, and the AMT inhibitor, AM404, is discussed below.

The anti-emetic action of subcutaneously administered RTX against copper sulphate, apomorphine, or cisplatin was confirmed in the present studies. Indeed, RTX antagonised emesis induced by all three challenges to a similar extent (~90%). But whilst the spectrum of anti-emetic action of RTX was impressive, and similar to the activity exhibited by tachykinin NK_1 receptor antagonists in both ferret and *S. murinus* models (Andrews et al., 2000b; Andrews and Rudd, 2004), the radiotelemetry studies that we performed revealed an unfavourable profile on cardiovascular performance and temperature homeostasis; and we also observed a clear reduction in feeding behaviour and food consumption. Studies with capsaicin in common laboratory animals usually show an activation of vagal reflexes leading to bradycardia and hypotension (Szallasi and Blumberg, 1999). This is clearly different from the data obtained in our studies in the ferret using radiotelemetry, and in the original anaesthetised ferret studies where one out of four animals tested also exhibited transient hypertension to RTX (Andrews and Bhandari, 1993). However, there is a study in anaesthetised rats showing that intravenous bolus doses of capsaicin can induce an initial fall and then an increase in blood pressure, with a slower infusion only resulting in a pressor response (Chahl and Lynch, 1987). The latter situation may be similar to the expected absorption kinetics following the subcutaneous administration of RTX in our studies.

In our studies, olvanil produced a dose-related inhibition of apomorphine- and cisplatin-induced emesis with the effects against cisplatin being retained following i.c.v. administration also suggesting a central site of action. There was a ~90% reduction of apomorphine-induced emesis when olvanil was given subcutaneously, but the effect against cisplatin was only between 35 and 70%. Significantly, the anti-emetic effects of olvanil were seen at doses that had no effect on blood pressure and temperature, or on feeding/drinking activity or other spontaneous behaviours including locomotor performance. Clearly, these latter observations are important to delineate that the anti-emetic effects of olvanil occur in the absence of adverse effects commonly associated with pungent TRPV1 activators seen in our studies and in the literature (Szallasi and Blumberg, 1999). Some TRPV1 ligands have affinity for dopamine D_1 but not D_2 receptors (Dekermendjian et al., 1997; Jonassohn et al., 1997),

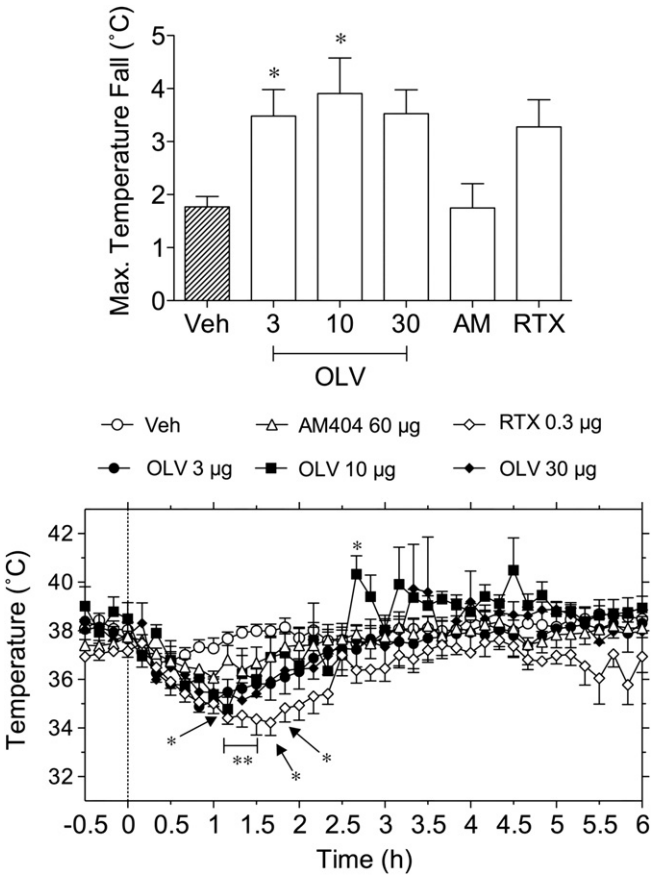

Fig. 6. The effect of i.c.v. administered resiniferatoxin (RTX; 0.3 µg), olvanil (OLV; 3–30 µg), AM404 (60 g), or vehicle (10 µl, tween 80/ethanol and saline 0.9% w/v in the ratio of 1:1:8) on body temperature in cisplatin (10 mg/kg)-treated animals. Vanilloids or AM404 or vehicle was administered after the first episode of cisplatin-induced emesis at $t = 0$ (dotted line). Results represent the mean ± S.E.M of 5–6 determinations. Significant differences relative to vehicle + cisplatin treated animals are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$ (one-way ANOVA or repeated measures 2-way ANOVA followed by Bonferroni multiple comparison tests, as appropriate.

and apomorphine-induced emesis primarily involves D_2 receptors (Yoshikawa et al., 1996). However, RTX also blocks apomorphine-induced emesis (Yamakuni et al., 2002) and is structurally dissimilar to olvanil with no report of either compound blocking dopamine receptors.

The reason why olvanil was active against apomorphine-induced emesis but inactive against copper sulphate-induced emesis in comparison with RTX is unknown. The emetic action of copper sulphate and cisplatin utilise the abdominal vagal afferent system, so it was expected that olvanil would have prevented the emesis induced by both challenges. However, whilst copper sulphate and cisplatin utilise similar pathways, they may not be identical since 5-HT_3 receptor antagonists block the acute emesis induced by cisplatin but not copper sulphate-induced emesis (Costall et al., 1990). A non-pungent compound, therefore, may not be sufficiently potent in these circumstances, where different transmitters could be released centrally in response to each emetic stimulus. Alternatively, the superior block of emesis by RTX compared with olvanil in our studies could have resulted from the severe hypertension modulating signalling through the NTS via the activation of baroreceptors (Bowser-Riley et al., 1990; Pilowsky and Goodchild, 2002). In anaesthetised *S. murinus* Uchino et al. (Uchino et al., 2006) have shown modulation by the carotid and aortic baroreceptors of the emetic response induced by vestibular and vagal (including gastric) afferents providing additional support for the above hypothesis which requires direct testing.

The central administration studies that we performed with the vanilloids also revealed further important differences between the profiles of the compounds. In these studies, the animals were allowed to develop retching and/or vomiting to cisplatin before giving olvanil or RTX. Olvanil rapidly antagonised emesis but RTX was ineffective; it neither potentiated nor inhibited retching or vomiting. However, the radiotelemetry data clearly indicated that both compounds had been used at equipotent doses, with both olvanil and RTX inducing a comparable reduction in temperature, which may also indicate that olvanil has some degree of pungency via this route. Interestingly, olvanil has been reported to cause pain in humans (intradermal administration; Movahed et al., 2005) and nociceptive behaviour in mice (into the paw, but not dermally; Iida et al., 2003) The differences in responses compared to other studies where the compound appears non-pungent has been taken as a consequence of lipophilicity and ease of access to nociceptors (Iida et al., 2003) or target TRPV1 sites (these studies following i.c.v. compared to s.c. administration).

Other studies have shown that RTX is initially emetic following i.c.v. administration (Andrews et al., 2000a; Rudd and Wai, 2001; Shiroshita et al., 1997). It is possible, therefore, that anti-emetic effects of RTX are masked by its own emetic potential and that anti-emetic effects cannot be immediately seen, particularly if emesis is ongoing. For example, i.c.v. administration studies in the dog have shown that RTX is initially emetic and the anti-emetic effects develop slowly (~40 min) (Shiroshita et al., 1997). Peripheral administration of RTX during an ongoing cisplatin-induced delayed emesis response has also revealed a slow onset of 4–8 h (Yamakuni et al., 2002); such a long latency has past the main phase of cisplatin-induced emesis in our experiments.

Prior to starting the studies, we had considered that olvanil might have some additional indirect effects on emesis as it has been reported to inhibit anandamide uptake (see Introduction). This is because AMT inhibition could elevate extracellular anandamide levels that could lead to activation of both TRPV1 and cannabinoid CB1 receptors (Ross, 2003), and that anandamide has also been reported to weakly inhibit 5-HT_{3A} receptors (Xiong et al., 2008). However, in our peripheral administration studies, AM404 had no discernable action to modify spontaneous behaviour or to modify blood pressure and temperature, but it did antagonise cisplatin-induced emesis when administered peripherally. Conversely, AM404 was unable to affect cisplatin-induced emesis when administered intracerebroventricularly. This profile against cisplatin is clearly different from the data obtained with olvanil, suggesting that olvanil is more likely to involve an inhibition of emesis via TRPV1 receptors in the central nervous system, but a role of TRPV1 and possibly CB1 receptors cannot be discounted for the action of olvanil in the periphery.

Other AMT inhibitors have been studied for a capacity to inhibit emesis but results have been inconsistent. In *Cryptotis parva* (the least shrew), peripheral administration of the AMT inhibitors, OMDM1 and VDM11, are ineffective in preventing cisplatin-induced emesis, but VDM11 antagonised apomorphine-induced emesis in this species (Darmani et al., 2005) and morphine-6-glucuronide-induced emesis in ferrets (Sharkey et al., 2007). The reasons for the conflicting findings are unknown but may relate to the selectivity of the AMT inhibitors and/or species differences. In fact, AM404 and VDM11 inhibit anandamide uptake with a similar potency, and they also reduce anandamide metabolism by inhibiting fatty acid amide hydrolase (FAAH) (Hillard et al., 2007). Yet FAAH inhibitors do not have a consistent action on emesis control. Thus, the FAAH inhibitor, URB597, antagonises morphine −6-glucuronide-induced emesis in the ferrets (Sharkey et al., 2007),

while in *C. parva*, URB597, and another FAAH inhibitor, arachidonoyl-serotonin, are emetic (Darmani et al., 2005). The anti-emetic versus emetic nature of the compounds are reasoned to represent a balance between useful actions of elevated anandamide at cannabinoid CB1 and vanilloid TRPV1 sites compared to the potential conversion of the elevated anandamide to arachidonic acid, which then acts as a precursor for emetic eicosanoids (Darmani et al., 2005; Sharkey et al., 2007). However, it also seems reasonable to assume that there may be species differences in the role of anandamide in emetic mechanisms.

In conclusion, non-pungent vanilloids may have useful anti-emetic properties in situations where central dopaminergic systems are being activated and/or against chemotherapy-induced emesis involving central and peripheral pathways for induction of emesis. However, studies in the presence of TRPV1 antagonists are required to fully elucidate the mechanisms involved.

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