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RESEARCH PAPER

Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats

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BACKGROUND AND PURPOSE
To evaluate the role of 2-arachidonoyl glycerol (2AG) in the regulation of nausea and vomiting using animal models of vomiting and of nausea-like behaviour (conditioned gaping).

EXPERIMENTAL APPROACH
Vomiting was assessed in shrews (*Suncus murinus*), pretreated with JZL184, a selective monoacylglycerol lipase (MAGL) inhibitor which elevates endogenous 2AG levels, 1 h before administering the emetogenic compound, LiCl. Regulation of nausea-like behaviour in rats by exogenous 2AG or its metabolite arachidonic acid (AA) was assessed, using the conditioned gaping model. The role of cannabinoid CB_1 receptors, CB_2 receptors and cyclooxygenase (COX) inhibition in suppression of vomiting or nausea-like behaviour was assessed.

KEY RESULTS
JZL184 dose-dependently suppressed vomiting in shrews, an effect prevented by pretreatment with the CB_1 receptor inverse agonist/antagonist, AM251. In shrew brain tissue, JZL184 inhibited MAGL activity *in vivo*. In rats, 2AG suppressed LiCl-induced conditioned gaping but this effect was not prevented by AM251 or the CB_2 receptor antagonist, AM630. Instead, the COX inhibitor, indomethacin, prevented suppression of conditioned gaping by 2AG or AA. However, when rats were pretreated with a high dose of JZL184 (40 $mg \cdot kg^{-1}$), suppression of gaping by 2AG was partially reversed by AM251. Suppression of conditioned gaping was not due to interference with learning because the same dose of 2AG did not modify the strength of conditioned freezing to a shock-paired tone.

CONCLUSIONS AND IMPLICATIONS
Our results suggest that manipulations that elevate 2AG may have anti-emetic or anti-nausea potential.

LINKED ARTICLES
This article is part of a themed section on Cannabinoids in Biology and Medicine. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-8. To view Part I of Cannabinoids in Biology and Medicine visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

Δ^9-THC, Δ^9-tetrahydrocannabinol; 2AG, 2-arachidonoyl glycerol; AA, arachidonic acid; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; AM630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone; anandamide, *N*-arachidonoylethanolamine; CB, cannabinoid; FAAH, fatty acid amide hydrolase; FP, fluorophosphonate; HPβCD, 2-hydroxypropyl-β-cyclodextrin; JZL184, 4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate; MAGL, monoacylglycerol lipase; PG-Gs, prostaglandin glycerol esters; TR, taste reactivity

Introduction

One of the earliest known therapeutic effects of marihuana was that of suppression of nausea and vomiting. There is now considerable evidence to confirm that cannabinoid agonists, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), WIN 212-2 and the highly potent HU-210, interfere with nausea-like behaviour and vomiting in animal models (see Parker *et al.*, 2011). These findings suggest that the endocannabinoid system is important for the regulation of nausea and vomiting (see Sanger, 2007).

The endogenous cannabinoids consist of the lipid messengers *N*-arachidonoylethanolamine, or anandamide (Devane *et al.*, 1992), and 2-arachidonoylglycerol (2AG; Mechoulam *et al.*, 1995), and are synthesized "on demand" from *N*-arachidonoyl phosphatidylethanolamine and 1, 2-diacylglycerol (Piomelli, 2003) respectively. Once released, endocannabinoids bind to metabotropic cannabinoid CB_1 receptors (nomenclature follows Alexander *et al.*, 2011) on pre-synaptic axon terminals, which results in inhibition of neurotransmitter release. The endocannabinoids are then inactivated via uptake and enzymatic hydrolysis in the postsynaptic cell (Deutsch and Chin, 1993; Di Marzo *et al.*, 1994). Anandamide is hydrolysed by fatty acid amide hydrolase (FAAH; Cravatt *et al.*, 1996), whereas monoacylglycerol lipase (MAGL; Dinh *et al.*, 2002) is the principal enzyme responsible for degradation of 2AG. Because FAAH and MAGL are both members of the serine hydrolase superfamily (Simon and Cravatt, 2010), their activity states in proteomes can be directly assessed by labelling with the activity-based probe fluorophosphonate rhodamine (FP-rhodamine) and visualization by fluorescence scanning following SDS-PAGE separation (Long *et al.*, 2009a). Recently, inhibitors of FAAH (PF-3845) and MAGL (JZL184) with activity *in vivo*, have been disclosed and used in a variety of animal models for studying heightened signalling by endocannabinoids (Ahn *et al.*, 2009; Long *et al.*, 2009a).

Comparable to the anti-emetic and anti-nausea properties of synthetic or plant derived cannabinoids, exogenous anandamide also suppressed vomiting in the least shrew (Darmani, 2002) and in the ferret (Van Sickle *et al.*, 2001; 2005). Similarly, prolonging the action of endogenous anandamide by inhibition of FAAH with URB597 also interfered with cisplatin- and nicotine-induced vomiting in the house musk shrew, *Suncus murinus* (Parker *et al.*, 2009) and in the ferret (Sharkey *et al.*, 2007). These effects were mediated by action at the CB_1 receptor because they were reversed by pretreatment with the CB_1 antagonist/inverse agonist, SR141716 (Parker *et al.*, 2009). Therefore, these studies suggest that the endocannabinoid system, through the action of anandamide at CB_1 receptors, regulates vomiting.

Recent evidence indicates that the endocannabinoid system not only regulates vomiting, but also regulates nausea-like behaviour, as assessed by the conditioned gaping reaction in rats. Although rats do not vomit, they display a selective distinctive reaction, gaping, in the taste reactivity (TR) test (Grill and Norgren, 1978) when exposed to cues (flavours or contexts), previously paired with emetic drugs (see Parker *et al.*, 2008 for review). Only drugs that produce vomiting in species capable of vomiting produce conditioned gaping reactions in rats, and clinically used anti-emetic drugs, such as 5-HT_3 receptor antagonists, prevent the establishment of conditioned gaping reactions in rats. These reactions are a more selective measure of nausea than is the traditionally employed conditioned taste avoidance measure (see Parker *et al.*, 2011). Cannabinoid receptor agonists (Δ^9-THC and HU-210), like 5-HT_3 antagonists, also suppress the establishment of conditioned gaping in rats, induced by LiCl (Limebeer and Parker, 1999; Parker *et al.*, 2003). Also, the FAAH inhibitor, URB597, prevented the establishment of LiCl-induced conditioned gaping in rats in a dose-dependent manner and this effect was facilitated by co-treatment with exogenous anandamide (Cross-Mellor *et al.*, 2007). These results suggest that anandamide attenuates nausea, as well as vomiting.

Considerable evidence indicates a role for anandamide in the regulation of nausea and vomiting; however, at present, only a few studies have evaluated the effects of 2AG on emesis and none have evaluated its effect on nausea-like behaviour in rats. The endocannabinoid 2AG has been reported to be both emetic (Darmani, 2002) and anti-emetic (Van Sickle *et al.*, 2005). In the least shrew, administration of 2AG (2.5, 5.0 or 10.0 $mg \cdot kg^{-1}$) dose-dependently produced vomiting responses, an effect that was partially blocked by pretreatment with anandamide (Darmani, 2002). Similarly, brain 2AG levels were increased following cisplatin-induced vomiting (Darmani *et al.*, 2005). On the other hand, Van Sickle *et al.* (2005) reported that, in ferrets, a low dose of 2AG (0.5 $mg \cdot kg^{-1}$) attenuated vomiting when combined with the cannabinoid re-uptake inhibitor, VDM11, and a higher dose (2.0 $mg \cdot kg^{-1}$) was capable of blocking vomiting alone. Although it is unclear whether doses higher than 2 $mg \cdot kg^{-1}$ would produce greater anti-vomiting effects in ferrets, these conflicting reports regarding the effects of 2AG on vomiting may point to important species differences in the function of 2AG. The effect of 2AG on vomiting in *S. murinus* and nausea-like behaviour in the rat conditioned gaping model remains unknown, and the effect of MAGL inhibition on nausea-like behaviour or vomiting has also yet to be established.

Recently, a selective inhibitor of MAGL activity, JZL184, has been described (Long *et al.*, 2009a,b), which at doses of 4 mg·kg^{-1} and 16 mg·kg^{-1} reduced MAGL activity in the mouse brain by 75% and 85%, respectively, within 0.5 h. Additionally, the lower dose of JZL184 was found to elevate 2AG to five times the normal level, while a high dose of 40 mg·kg^{-1} resulted in a 10-fold increase in brain levels of 2AG. Therefore, JZL184 offers a selective tool to investigate the biological effect of endogenous 2AG, *in vivo*.

The experiments reported here evaluated the potential of JZL184 and/or exogenous 2AG to regulate vomiting in the shrew model and nausea-like behaviour in the rat model of conditioned gaping. Experiment 1 evaluated the potential of JZL184 to modify LiCl-induced vomiting in *S. murinus*. The mechanism of action of JZL184-induced suppression of vomiting was assessed by determining if its anti-vomiting effect would be reversed by the CB_1 receptor inverse agonist/antagonist AM251. JZL184 has been shown to be less potent against rat MAGL (Long *et al.*, 2009b) and, consistent with these results, an initial experiment confirmed that systemic administration of JZL184 (16 and 40 mg·kg^{-1}, i.p.) did not modify LiCl-induced conditioned gaping reactions. Therefore, Experiment 2 evaluated the ability of exogenously administered 2AG to modify nausea-like behaviour in rats using the conditioned gaping model. Pilot experiments determined that the highest dose of 2AG used in these rat experiments did not produce conditioned gaping when explicitly paired with 0.1% saccharin solution. Experiment 3 evaluated the mechanism by which 2AG suppressed LiCl-induced conditioned gaping in rats in two subexperiments: (i) Experiment 3a assessed the potential of AM251 (CB_1 receptor antagonist/inverse agonist) and AM630 (CB_2 receptor antagonist/inverse agonist) to reverse the effect of 2AG on gaping. As well, the potential of AM251 to reverse the suppressive effect of co-treatment of a high dose of JZL184 (40 mg·kg^{-1}) and 2AG on conditioned gaping was assessed. It was reasoned that if the duration of action of 2AG was prolonged by inhibition of MAGL activity, the antinausea-like effect may be produced by its agonism of CB_1 receptors. (ii) In Experiment 3b, the role of downstream metabolites of 2AG on suppression of gaping was assessed by evaluating the potential of the cyclooxygenase (COX) inhibitor, indomethacin, to reverse the suppressive effect of 2AG. As well, the potential of AA to suppress LiCl-induced conditioned gaping was evaluated. Experiment 4 assessed the alternative hypothesis that instead of suppressing LiCl-induced nausea, 2AG may have interfered with sensory processing of saccharin because it was administered prior to the saccharin infusion. Therefore, in Experiment 4, rats were injected with 2AG or vehicle immediately after intraoral exposure to saccharin solution, but 15 min prior to an injection of LiCl or saline. Finally, the potential of 2AG to interfere with learning in an auditory conditioned fear task was assessed in rats in Experiment 5.

Methods

Animals

All animal care and experimental procedures complied with the recommendations of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Guelph. We used male Sprague-Dawley rats weighing between 250 and 300 g at the start of experiments (Charles River Lab, St Constant, QC, Canada), as well as male (30–45 g) and female (20–30 g) *S. murinus* which were bred and raised from a colony at the University of Guelph. The rats were single-housed and maintained on a reverse light/dark cycle (7:00 am lights off; 7:00 pm lights on) with free access to food (Iams rodent chow, 18% protein) and tap water except during testing, which occurred during the dark cycle. The shrews were single-housed and maintained on a diurnal light/dark cycle (7:00 am lights on; 7:00 pm lights off) with free access to food (Iams cat chow) and tap water except during testing. After all behavioural testing in each experiment, the animals were killed by CO_2.

Drug treatments

All injections were given i.p.. The selective MAGL inhibitor, JZL184 (Cayman Chemicals) was prepared in a vehicle solution (VEH) of 45% 2-hydroxypropyl-β-cyclodextrin (HPβCD) at concentrations of 8 mg·mL^{-1} (16 mg·kg^{-1} dose) or 13.33 mg·mL^{-1} (40 mg·kg^{-1} dose) and delivered at a volume of either 2 mL·kg^{-1} (16 mg·kg^{-1} dose) or 3 mL·kg^{-1} (40 mg·kg^{-1} dose). The time course and doses of JZL184 for pretreatment were selected on the basis of previous experiments performed by Long *et al.* (2009a), demonstrating a dose-dependent increase in 2AG that, within 1 h of administration, was sufficient to elevate brain 2AG to peak activity in mice. The drugs 2AG, AM251 (Tocris), AM630 (Tocris), Indomethacin (Sigma) and arachidonic acid (AA; Sigma) were prepared in a 1:1:18 solution of ethanol, Cremaphor, and physiological saline, respectively. For 2AG, the concentrations were 0.125 mg·mL^{-1} (0.5 mg·kg^{-1} dose), 0.313 mg·mL^{-1} (1.25 mg·kg^{-1} dose) or 0.5 mg·mL^{-1} (2.0 mg·kg^{-1} dose) and injected at a volume of 4 mL·kg^{-1}. For AM251, the concentrations were 1.0 mg·mL^{-1} (1.0 mg·kg^{-1} dose), 2.5 mg·mL^{-1} (2.5 mg·kg^{-1} dose) or 5.0 mg·mL^{-1} (5.0 mg·kg^{-1} dose) administered at 1 mL·kg^{-1} (1.0, 2.5 or 5.0 mg·kg^{-1}). For AM630, the concentration was 2.5 mg·mL^{-1} and injected at a volume of 1 mL·kg^{-1} (2.5 mg·kg^{-1}). For Indomethacin, the concentration was 0.83 mg·mL^{-1} administered in a volume of 3 mL·kg^{-1} (2.5 mg·kg^{-1}). For AA, the concentration was 0.5 mg·mL^{-1} administered at a volume of 4 mL·kg^{-1}. The treatment drug was 0.15 M LiCl (Sigma), which was prepared in sterile water and administered at a volume of 20 mL·kg^{-1} (127 mg·kg^{-1}) in rats and 60 mL·kg^{-1} (390 mg·kg^{-1}) in shrews. These doses were selected on the basis of their effectiveness in producing conditioned gaping (Limebeer and Parker, 2000) and vomiting (Parker *et al.*, 2004) respectively.

Preparations of brain proteomes

Brains were homogenized (Dounce homogeniser) in PBS, pH 7.5, followed by a low-speed centrifugation (1400× *g*, 5 min) to remove debris. The supernatant was then subjected to centrifugation (64 000× *g*, 45 min) to provide the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was washed and resuspended in PBS buffer by sonication. Total protein concentration in each fraction

was determined using a protein assay kit (Bio-Rad). Samples were stored at –80°C until use.

FP-rhodamine labelling of tissue proteomes

FP-rhodamine (Patricelli *et al.*, 2001) and PF-3845 (Ahn *et al.*, 2009) were synthesized as previously described. Tissue proteomes were diluted to 1 mg·mL^{-1} in PBS and FP-rhodamine was added at a final concentration of 1 μM in a 50 μL total reaction volume. After 30 min at 25°C, the reactions were quenched with 4 × SDS-PAGE loading buffer, boiled for 5 min at 90°C, subjected to SDS-PAGE and visualized in-gel using a flatbed fluorescence scanner (Hitachi). For experiments involving a pre-incubation with inhibitor, the reactions were prepared without FP-rhodamine. PF-3845 was added at the indicated concentration and incubated for 30 min at 37°C. FP-rhodamine was then added and the reaction was carried out exactly as described above.

Behavioural apparatus

The TR chamber. At each stage of the experiment, the rats were individually placed in a clear Plexiglas chamber (29 × 29 × 10 cm) that was resting on a glass plate. Two 60 W lights suspended from the apparatus illuminated the chamber. To allow optimal viewing of orofacial responses, a mirror was placed at a 45 degree angle below the glass plate. Prior to being placed in the chamber, the rats were connected to an infusion pump via a section of PE 90 tubing attached to their intra-oral cannula. During the course of the session, orofacial and somatic responses were recorded using a Sony video camera (Handy Cam) that was placed directly in front of the mirror and connected to a computer.

Shrew observation chamber. The shrews were observed in a similar apparatus as the TR chamber; however, as well as being video recorded, behavioural observations were conducted live using 'The Observer' (Noldus Information Technology, Sterling, VA, USA). Shrews were placed individually into the chamber and emetic episodes were observed via a mirror located at a 45 degree angle below the glass plate.

Fear conditioning chamber. The rats were conditioned in one of four chambers (30 × 24 × 40 cm; MED-Associates, Burlington, VT, USA) constructed of aluminum (rear and side walls) and Plexiglas (door and ceiling), which were housed in sound-attenuating cabinets. The chambers were equipped with a speaker to present discrete auditory stimuli and a solid-state grid scrambler to deliver foot shocks. Background noise (65 dB) was provided by a ventilation fan that was housed in each cabinet. The activity of each rat was continuously recorded by a near-infrared-based imaging camera that was connected to a computer. Freezing behaviour was assessed using 'Video Freeze' (MED-Associates; Burlington, VT, USA) software (30 fps, movement threshold: 150, min freeze duration: 90), and per cent freezing behaviour was calculated as the amount of time each rat was immobile per minute. The original chamber context (Context A) was used for habituation and conditioning, whereas a novel context (Context B) was created for the purpose of specifically assessing conditioned behaviour to the auditory stimulus alone. Context B consisted of chambers that were equipped with an opaque white plastic insert over the floor bars and an opaque black plastic teepee overtop. The chambers were wiped down with a 1% acetic acid solution to yield a novel odour. To further distinguish from the original context, the testing room was illuminated by a dim red light (40 W), whereas the sound-attenuating cabinets were illuminated by white ambient light.

Surgery for implantation of the intra-oral cannula

The rats were handled 3 days before the implantation. For surgery, they were anesthetized with isoflurane gas, administered the antibiotic depocillin (0.33 mg·kg^{-1}, s.c.; Pen Aqueous) and the non-steroidal anti-inflammatory/analgesic drug, carprofen (0.1 mg·kg^{-1}, i.p.; Pfizer). A 15-gauge stainless steel needle was inserted at the mid-area on the back of the neck, and guided subcutaneously below the ear and across the cheek, where it exited into the oral cavity behind the first molar. A 10 cm section of polyethylene tubing (PE 90, I.D. 0.86 mm, O.D. 1.27 mm) was inserted into the needle, which was then removed from the animal, allowing only the tubing to remain in place. Three elastomer squares (8 × 8 mm) were threaded onto the tubing and drawn all the way to the neck, securing the cannula firmly in place. The intra-oral section of the cannula was held in place by a flanged end of the tubing over a section of surgical mesh that rested flush against the skin. Twenty-four hours after surgery, rats were given a second dose of carprofen (0.1 mg·kg^{-1}) and their health was monitored for 3 days following surgery. Intra-oral cannulae were also flushed once a day, for 3 days, with chlorohexidine.

Behavioural procedures

Experiment 1: effect of MAGL inhibition on LiCl-induced vomiting in shrews. The shrews were each offered four meal-worms (*Tenebrio* sp.) in their home cage 15 min prior to receiving pretreatment injections. The pretreatment occurred 60 min prior to behavioural testing, in which animals received an injection of JZL184 (0, 16, 40 mg·kg^{-1}) and were observed in their home cage for vomiting episodes. An additional two groups were also injected with AM251 (5 mg·kg^{-1}) 5 min prior to pretreatment with 40 mg·kg^{-1} JZL184 or vehicle. No shrew vomited during the 60 min period following the pretreatment. Immediately prior to the observation period, the shrews were injected with either physiological saline (SAL) or LiCl and placed in the observation chamber for 45 min. During this time, the frequency of vomiting episodes (expulsion of fluids from stomach) and the latency (in seconds) to the first vomiting episode were measured. In cases when no shrew vomited, the latency measure consisted of the duration of the test session (2700 s). The shrews were randomly assigned to the six experimental groups with approximately equal numbers of males and females in each group: VEH-LiCl (n = 5), JZL184 16-LiCl (n = 5), JZL184 40-LiCl (n = 5), JZL184 40-SAL (n = 5), AM251-JZL184 40-LiCl (n = 6) and AM251-VEH-LiCl (n = 6). Group designation is denoted by the pretreatment and treatment injection, respectively.

To assess the effectiveness of JZL184 in inhibiting MAGL levels in the shrew brain, two additional groups of shrews were fed four meal worms 15 min prior to being injected with 40 mg·kg^{-1} JZL184 (n = 2; one male and one female) or VEH

(n = 2; one male and one female) and placed in the observation chamber for 60 min. There was no vomiting during this 60 min observation period. Shrews were then immediately decapitated, and the brains removed within 4 min and immediately frozen in dry ice. Brains were prepared and labelled with FP-rhodamine as described above.

Experiment 2: effect of exogenous 2AG pretreatment on LiCl-induced conditioned gaping in rats. The rats were handled and weighed for three consecutive days prior to intra-oral cannulation surgery. Following surgery, they were allowed 3 days to recover before starting experiments, during which time post-operative monitoring was conducted. All experiments consisted of adaptation, conditioning and a TR test. The rats received a single adaptation trial to habituate them to the chamber and infusion procedure. For this session, they were placed in the TR chamber and received a 2 min intra-oral infusion of water (reverse osmosis water infused at 1 mL·mL^{-1}). On the following day, the rats received a single conditioning trial. They received a pretreatment injection of 2AG (VEH, 0.5, 1.25 or 2.0 mg·kg^{-1}) 15 min prior to receiving a 2 min intra-oral infusion of saccharin solution (0.1%) and their orofacial and somatic reactions were recorded on video. Immediately following the infusion, the rats were injected with either 20 mL·kg^{-1} of LiCl (0.15 M) or SAL and were returned to their home cage. The 60 rats were randomly assigned to eight experimental groups, which included: VEH-LiCl (n = 9), VEH-SAL (n = 8), 0.5 mg·kg^{-1} 2AG-LiCl (n = 7), 0.5 mg·kg^{-1} 2AG-SAL (n = 7), 1.25 mg·kg^{-1} 2AG-LiCl (n = 8), 1.25 mg·kg^{-1} 2AG-SAL (n = 8), 2 mg·kg^{-1}-2AG-LiCl (n = 7) and 2 mg·kg^{-1} 2AG-SAL (n = 6), with group designation denoted by the pretreatment and treatment injection, respectively. Seventy-two hours following conditioning, the rats received a drug-free TR test. During this session, they were re-exposed to a 2 min intra-oral infusion of saccharin solution and their orofacial and somatic responses were recorded. The conditioning and testing videos were later scored for the reaction of gaping (wide opening of the mouth with bottom excisors exposed) by a rater blind to the experimental conditions using 'The Observer'.

Experiment 3: mechanism of 2AG suppression of LiCl-induced conditioned gaping in rats. Experiment 3 evaluated potential mechanisms by which 2AG suppressed conditioned gaping in Experiment 2. In Experiment 3a, the potential of AM251 (CB_1 receptor antagonist/inverse agonist) and AM630 (CB_2 receptor antagonist/inverse agonist) to reverse the effect of 2AG on gaping were evaluated. As well, the potential of AM251 to reverse the suppressive effect of co-treatment of a high dose of JZL184 (40 mg·kg^{-1}) and 2AG on conditioned gaping was assessed. Experiment 3b determined if the COX enzyme inhibitor, indomethacin, would reverse the anti-nausea-like effects of 2AG or AA. In each of Experiments 3a and 3b, the rats in Groups VEH-LiCl (n = 9) and 2 mg·kg^{-1} 2AG-LiCl (n = 7) from Experiment 2 were compared with each pretreatment group in the analyses.

Experiment 3a: role of cannabinoid receptors in 2AG induced suppression of conditioned gaping in rats. The 70 rats included in the analysis received similar treatment as in Experiment 2, except at specified. To determine the role of CB_1 and CB_2 receptors in the suppression of LiCl-induced conditioned gaping by 2AG, Groups AM251 (1 mg·kg^{-1})-2AG (n = 8), AM251 (2.5 mg·kg^{-1})-2AG (n = 9), AM251 (5.0 mg·kg^{-1})-2AG (n = 8) and AM630 (2.5 mg·kg^{-1})-2AG (n = 8) were injected with the antagonist 15 min prior to receiving an injection of 2AG (2 mg·kg^{-1}) and 15 min later received the intra-oral infusion of saccharin solution followed immediately by LiCl. Additionally, three groups were pretreated with JZL184 60 min prior to a pairing of saccharin and LiCl: Group JZL184 (n = 8) was injected with JZL184 alone; Group JZL184-2AG (n = 7) was also injected with 2AG 15 min prior to the conditioning trial; Group JZL184-AM251-2AG (n = 6) received the additional injection of AM251 (5.0 mg·kg^{-1}) 30 min following the injection of JZL184.

Experiment 3b: effect of the COX inhibitor, indomethacin, on 2AG and AA suppression of LiCl-induced conditioned gaping in rats. The 48 rats included in the analysis received treatment similar to that in Experiment 3a, except that they were injected with indomethacin (2.5 mg·kg^{-1}) 15 min prior to pretreatment with VEH, 2AG (2 mg·kg^{-1}) or AA (2 mg·kg^{-1}). Fifteen minutes later, all rats received a pairing of saccharin infusion with LiCl. In Experiment 3b, the 48 rats were randomly assigned to the following groups: VEH-LiCl (n = 9), 2AG-LiCl (n = 7), AA-LiCl (n = 9), INDO-VEH-LiCl (n = 7), INDO-2AG-LiCl (n = 8), INDO-AA-LiCl (n = 8).

Experiment 4: potential of 2AG administered following saccharin to interfere with LiCl induced conditioned gaping. Experiment 4 determined if administration of 2AG following saccharin solution rather than preceding saccharin administration would interfere with LiCl-induced conditioned gaping. Experiment 4 was conducted as Experiment 2 except as specified. During conditioning, the 31 rats were intraorally infused with saccharin solution. Immediately following the infusion, they were injected with 2 mg·kg^{-1} 2AG or VEH. Fifteen minutes later, they were injected with LiCl or saline. The groups were: Sac-VEH-Saline (n = 8), Sac-2AG-Saline (n = 7), Sac-VEH-LiCl (n = 8), Sac-2AG-LiCl (n = 8).

Experiment 5: potential of 2AG to interfere with auditory fear conditioning. Experiment 5 evaluated whether a dose of 2AG (1.25 mg·kg^{-1}) that was capable of interfering with the establishment of conditioned gaping would also interfere with the establishment of auditory fear conditioning. Fear conditioning was based on the procedures of Maren (1999). The rats received a single 10 min habituation session, followed 24 h later by a single 7 min conditioning trial. For conditioning, the 32 rats were divided into groups (balanced for average freezing during habituation) and received an injection of either vehicle (n = 16) or 1.25 mg·kg^{-1} 2AG (n = 16) 15 min prior to conditioning in the chamber (Context A). The rats received either one or three tone (85 dB, 2000 Hz, 10 s) – footshock (2 s, 0.8 mA) pairings with a 70 s intertrial interval that began 3 min after the rats were placed into the chambers. The groups were: VEH – one tone-shock pairing (n = 8), 2AG – one tone-shock pairing (n = 8), VEH – three tone-shock pairings (n = 8), 2AG – three tone-shock pairings (n = 8). Twenty-four hours later, fear conditioning was assessed with

a 10 min test that occurred in a novel context (Context B). During this test, the tone occurred after 2 min and remained on for the remainder of the 10 min session.

Data analysis

Behavioural data were evaluated by analysis of variance (ANOVA). In Experiment 1, the number of vomiting episodes and latency to first vomiting episode were analysed using a single-factor ANOVA with group (VEH-LiCl, JZL16-LiCl, JZL40-LiCl, JZL40-SAL, AM-JZL40-LiCl and AM-VEH-LiCl) as a between-subjects factor. In Experiment 2, the number of gapes for each rat was entered into an ANOVA with pretreatment (at four levels: 0.0, 0.5, 1.25 and 2.0 mg·kg^{-1} 2AG) and conditioning drug (at two levels: LiCl or saline) as between subjects factors. In Experiments 3a and 3b, the number of gapes for each rat was entered into single-factor ANOVAs. In Experiment 4, the number of gapes were evaluated in a between factors ANOVA for pretreatment (VEH, 2AG) and conditioning drug (Saline, LiCl). Finally in Experiment 5, separate mixed factors ANOVAs [between groups factor of pretreatment (VEH, 2AG), within groups factor of time period] were performed for freezing behaviour for rats that received one or three tone-shock pairings. During conditioning, the freezing during the 70 s preshock period was compared with each 70 s post-shock period. During the drug-free tone test trial, each min of testing was entered into the ANOVA; the tone began during Min 3 and continued throughout the remaining 10 min test. Group differences were evaluated using planned comparison tests ($\alpha = 0.05$).

Results

Experiment 1: effect of MAGL inhibition on LiCl-induced vomiting in shrews

JZL184 dose-dependently interfered with LiCl-induced vomiting in shrews and AM251 reversed this effect. Figure 1 presents the mean (±SEM) number of vomiting episodes and latency (second) to the first vomiting episode displayed by shrews in Experiment 1. For the frequency data, statistical analyses revealed a significant main effect of group, $F(5,26) = 10.4$, $P < 0.001$; Groups JZL184 40-SAL and JZL184 40-LiCl displayed fewer vomiting episodes than all groups except JZL184 16-LiCl. Group JZL184 16-LiCl also displayed fewer vomiting episodes than the VEH-LiCl Group ($P < 0.05$). There was no significant difference between Groups JZL184 40-LiCl and JZL184 40-SAL. For the latency data, statistical analyses revealed a significant main effect of group, $F(5,26) = 16.8$, $P < 0.001$; Group JZL184 40-LiCl displayed a longer latency to vomit than all groups treated with LiCl except JZL184 16-LiCl. All LiCl-treated groups displayed a shorter latency than Group JZL184 40-SAL. The proportion of shrews that displayed vomiting in each experimental group was as follows: JZL184 40-SAL, 0% (0/5); JZL184 40-LiCl, 60% (3/5); JZL184 16-LiCl, 100% (5/5); VEH-LiCl, 100% (5/5); AM251-JZL184 40-LiCl, 100% (6/6); AM251-VEH-LiCl, 100% (6/6).

To confirm that JZL184 inhibited MAGL in shrew tissue, brains from animals treated with vehicle or JZL184 were harvested and labelled with the serine hydrolase-directed activity probe FP-rhodamine. MAGL labelled as a ~30–32 kDa

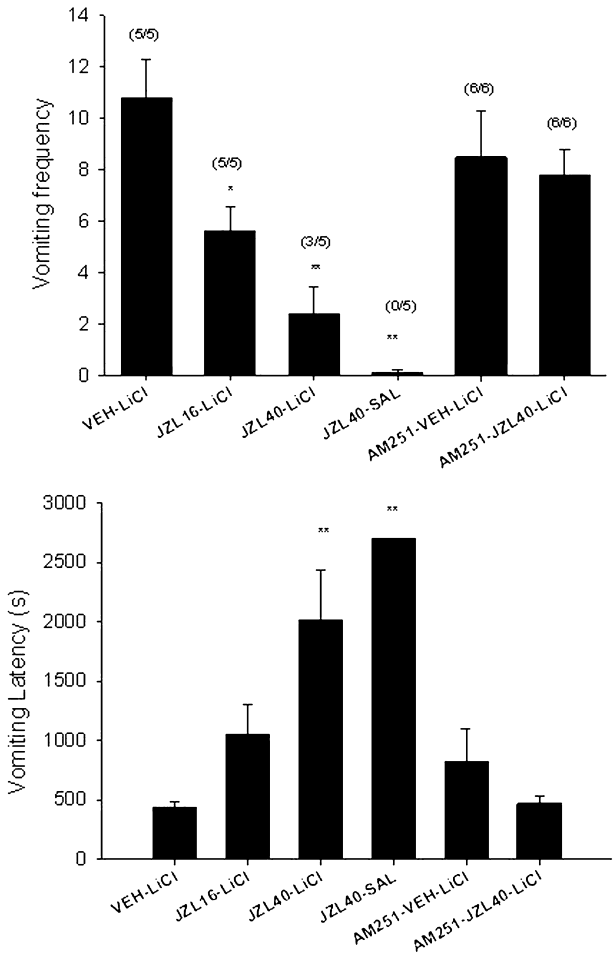

Figure 1

Mean (±SEM) number of vomiting episodes (upper section) and mean (±SEM) latency (seconds; lower section) to first vomiting episode displayed by *S. murinus* during the 45 min observation period following a treatment injection of 60 mL·kg^{-1} of 0.15 M LiCl or saline (Group JZL40-SAL) in Experiment 1. The various groups received different pretreatments prior to the treatment injections, including: VEH-LiCl ($n = 5$), JZL184 16-LiCl ($n = 5$), JZL184 40-LiCl ($n = 5$), JZL184 40-SAL ($n = 5$), AM251-JZL184 40-LiCl ($n = 6$) and AM251-VEH-LiCl ($n = 6$), with group designation denoted by the pretreatment and treatment injection respectively. *$P < 0.05$; **$P < 0.01$) indicate significant differences from VEH-LiCl. Additionally, the number of shrews that vomited in each group is presented above each bar.

doublet which was present in vehicle-treated but not in JZL184-treated shrews (Figure 2, lanes 1–4). A second off-target of JZL184 was observed at ~60 kDa. Pretreatment of brain samples with the FAAH inhibitor PF-3845 before FP-rhodamine labelling blocked labelling of the upper 60 kDa band, but not the lower 60 kDa band, demonstrating that the off-target is not FAAH (Figure 2, lanes 5–8). We suspect that this off-target is a carboxylesterase enzyme found in rodents but not humans because of its molecular weight and because JZL184 had previously been shown to have carboxylesterase off-targets in mice (Li *et al.*, 2005; Long *et al.*, 2009b). Pretreatment with JZL184 before FP-rhodamine labelling dem-

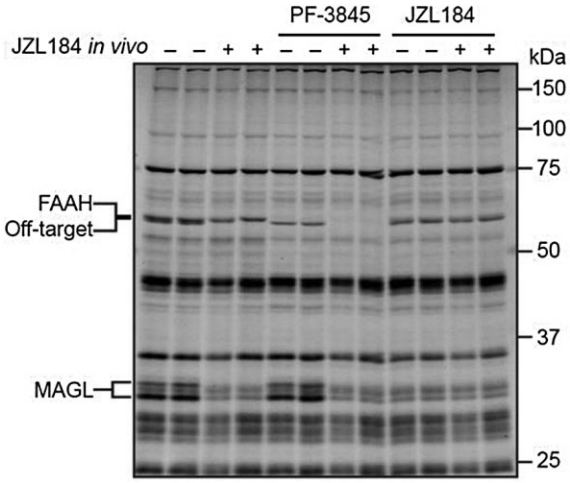

Figure 2

Labelling of brain membranes from shrews treated with vehicle (–) ($n = 2$, lanes 1 and 2) or JZL184 (+) ($n = 2$, lanes 3 and 4) using the serine hydrolase-directed activity probe FP-rhodamine (1 μM, 30 min, room temperature). Brain proteomes were also pretreated with PF-3845 (1 μM, 30 min, 37°C, lanes 5–8) or JZL184 (1 μM, 30 min, 37°C, lanes 9–12) prior to labelling by FP-rhodamine to confirm that the 60 kDa band and the 30–32 kDa doublet are FAAH and MAGL respectively.

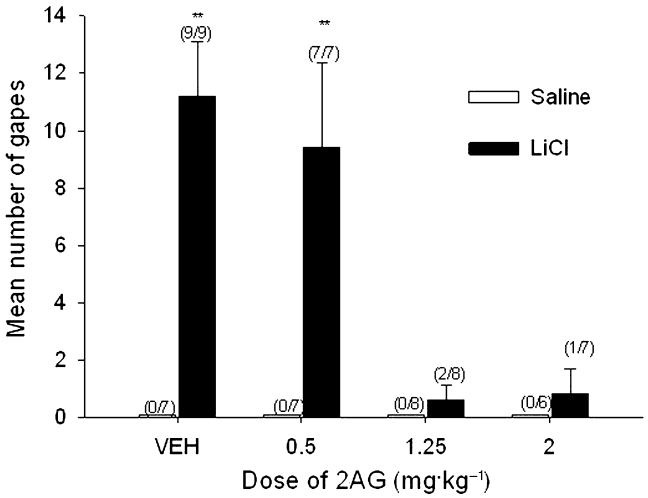

Figure 3

Mean (±SEM) number of gapes elicited by 20 mL·kg^{-1} of 0.15 M LiCl- or saline-paired 0.1% saccharin solution in rats during the drug-free TR test in Experiment 2. During conditioning, rats were pretreated with 0.0, 0.5, 1.25 or 2.0 mg·kg^{-1} of 2AG. The groups were: VEH-LiCl ($n = 9$), VEH-SAL ($n = 8$), 0.5 mg·kg^{-1} 2AG-LiCl ($n = 7$), 0.5 mg·kg^{-1} 2AG-SAL ($n = 7$), 1.25 mg·kg^{-1} 2AG-LiCl ($n = 8$), 1.25 mg·kg^{-1} 2AG-SAL ($n = 8$), 2 mg·kg^{-1}-2AG-LiCl ($n = 7$) and 2 mg·kg^{-1} 2AG-SAL ($n = 6$), with group designation denoted by the pretreatment and treatment injection respectively. **$P < 0.01$ indicates that groups VEH-LiCl and 0.5 mg·kg^{-1} 2AG-LiCl gaped significantly more than all other groups, which did not differ from one another. The number of rats that gaped in each group is presented above each bar.

onstrated that MAGL was completely inhibited *in vivo* at 40 mg·kg^{-1} (Figure 2, lanes 9–12).

Experiment 2: effect of exogenous 2AG on LiCl-induced conditioned gaping in rats

Both 1.25 mg·kg^{-1} and 2.0 mg·kg^{-1} 2AG interfered with the establishment of conditioned gaping following a single pairing of saccharin and LiCl. Figure 3 presents the mean number of gapes displayed by each group during the TR test. Statistical analyses revealed significant main effects of pretreatment drug, $F(3,52) = 9.1$, $P < 0.001$, conditioning drug, $F(1, 52) = 34.6$; $P < 0.001$, as well as a pretreatment by conditioning drug interaction, $F(3,52) = 9.1$, $P < 0.001$. Rats in groups VEH-LiCl and 0.5 mg·kg^{-1}-2AG LiCl displayed significantly more gaping than any other group on the test trial. No other groups differed from one another.

Experiment 3: mechanism of 2AG suppression of LiCl-induced conditioned gaping in rats

Neither pretreatment with the CB_1 receptor antagonist, AM251, nor the CB_2 receptor antagonist, AM630, reversed the suppressive effect of 2AG on LiCl-induced conditioned gaping in rats. However, when JZL184 and 2AG were co-administered, the suppressive effect of 2AG on conditioned gaping was partially reversed by AM251. Not only 2AG, but also AA, suppressed LiCl-induced conditioned gaping and these effects were reversed by the COX inhibitor, indomethacin.

Experiment 3a: role of cannabinoid receptors in 2AG induced suppression of conditioned gaping in rats. Figure 4 presents the mean number of gapes displayed by each group during the TR test in Experiment 3a. Statistical analyses revealed a significant main effect of pretreatment, $F(8,61) = 10.8$; $P < 0.001$; rats pretreated with vehicle alone, JZL184 alone or JZL184-AM251-2AG displayed more gapes during the infusion of LiCl-paired saccharin solution than any other group, but did not significantly differ from one another.

Experiment 3b: effect of COX inhibition on 2AG or AA suppression of LiCl-induced conditioned gaping in rats. In Experiment 3b, the COX inhibitor, indomethacin, reversed the suppressant effect of both 2AG and AA on LiCl-induced conditioned gaping. Figure 5 presents the mean number of gapes during the TR test of Experiment 3b. Statistical analyses revealed a significant main effect of pretreatment, $F(5,42) = 6.7$, $P < 0.001$; rats pretreated with 2AG alone or AA alone displayed fewer gapes than any other group.

Experiment 4: potential of 2AG administered following saccharin to interfere with LiCl-induced conditioned gaping

When delivered after an intra-oral infusion of saccharin solution, 2AG suppressed LiCl-induced conditioned gaping reactions, as in Experiment 2 when it was administered before infusion of saccharin solution. Figure 6 presents the mean number of gapes during the TR test of Experiment 4. The

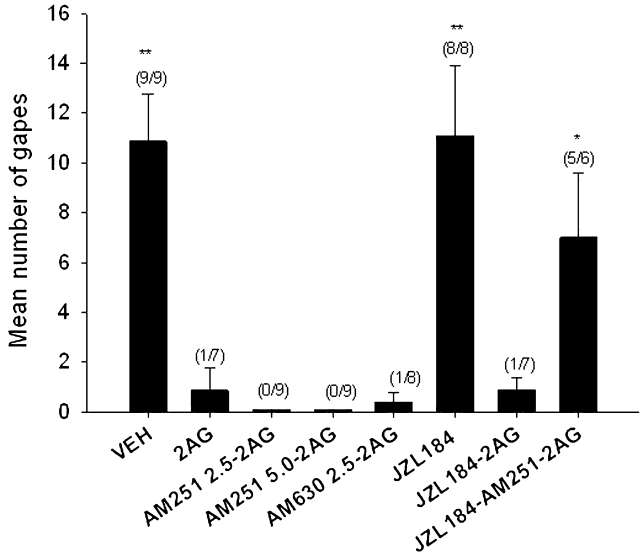

Figure 4

Mean (±SEM) number of gapes displayed by rats during a drug-free TR test in Experiment 3a. During conditioning, prior to a saccharin-LiCl pairing the rats were given the following pretreatments: VEH (n = 9), 2AG (n = 7), AM251 (1.0 mg·kg^{-1})-2AG (n = 8), AM251 (2.5 mg·kg^{-1})-2AG (n = 9), AM251 (5.0 mg·kg^{-1})-2AG (n = 8), AM630 (2.5 mg·kg^{-1})-2AG (n = 8), JZL184 (n = 8), JZL184-2AG (n = 7) or JZL184-AM251-2AG (n = 6). **P < 0.01; *P < 0.05) indicate that groups VEH, JZL184 and JZL184-AM251-2AG gaped more than any other group. The number of rats that gaped in each group is indicated above each bar.

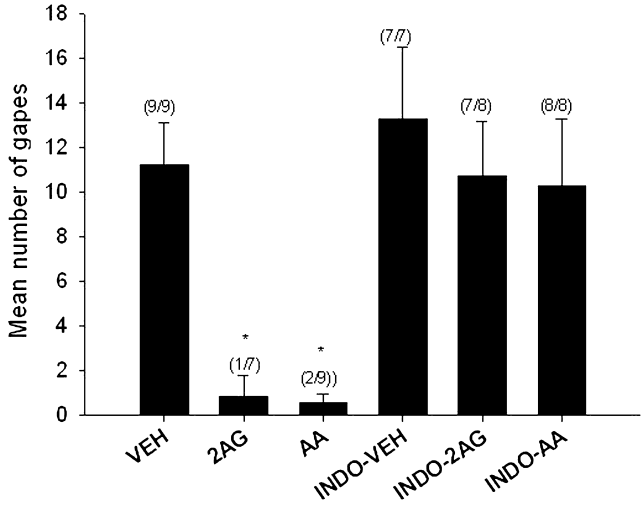

Figure 5

Mean (±SEM) number of gapes displayed by rats in the drug-free TR test in Experiment 3b. During conditioning prior to a saccharin-LiCl pairing, the rats were given the following pretreatments: VEH (n = 9), 2AG (n = 7), AA (n = 9), INDO-VEH (n = 7), INDO-2AG (n = 8), INDO-AA (n = 8). *P < 0.05 indicates that groups 2AG and AA gaped less than any other group, with no differences between other groups. The number of rats that gaped in each group is indicated above each bar.

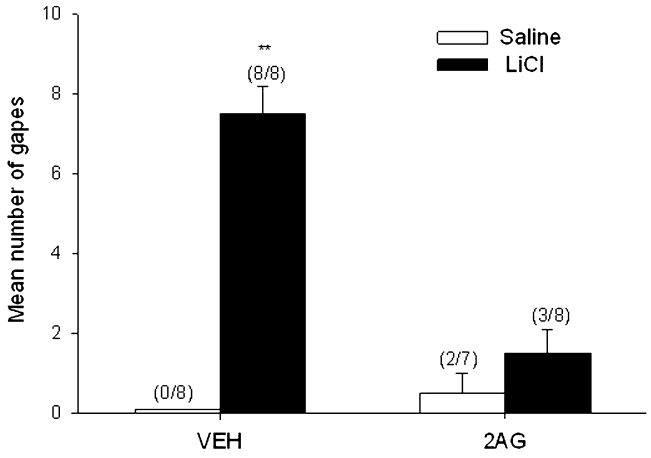

Figure 6

Mean (±SEM) number of gapes displayed by rats during the drug-free TR test in Experiment 4. Unlike Experiment 2, 2AG or VEH were injected following (rather than before) intra-oral delivery of the saccharin solution and 15 min prior to injection of LiCl with seven to eight rats per group. **P < 0.01 indicates that the group Sac-VEH-LiCl displayed significantly more gaping than any other group. The number of rats that gaped in each group is indicated above each bar.

2-by-2 ANOVA revealed significant effects of pretreatment drug, $F(1,27) = 12.6$; $P < 0.001$, conditioning drug, $F(1,27) = 42.3$; $P < 0.001$ and a pretreatment drug by conditioning drug interaction, $F(1,27) = 19.3$; $P < 0.001$. Rats in the group Sac-VEH-LiCl displayed significantly more gaping reactions than any other group.

Experiment 5: potential of 2AG to interfere with auditory fear conditioning in rats

Rats receiving either a single tone-shock pairing or three tone-shock pairings displayed conditioned freezing to the tone. However, pretreatment with 1.25 mg·kg^{-1} 2AG did not interfere with the learning of auditory fear conditioning. Figure 7 presents the mean per cent conditioned freezing behaviour during the preshock period and each post-shock period during the fear conditioning session for rats that received three pairings or one pairing of tone and shock. Among rats that received three tone-shock pairings, there was only a significant main effect of period, $F(3,42) = 32.7$, $P < 0.001$, but not a significant pretreatment by period interaction, with both groups showing a similar increase in the level of post-shock freezing across parings. Among rats that received one tone-shock pairing, there was only a significant main effect of period, $F(1,14) = 10.0$, $P < 0.01$; rats showed a greater per cent freezing during the post-shock period than in the pre-shock period.

Conditioned freezing during the tone test is presented in Figure 8. The tone was presented at the beginning of Min 3, and the repeated measures analysis of per cent freezing across Min 1–3 revealed a significant effect of period for both the rats given three tone-shock pairings, $F(2,28) = 13.1$; $P < 0.001$, and for the rats given one tone-shock pairing, $F(2,28) = 9.9$; $P < 0.001$; rats displayed greater freezing during Min 3 (presentation of the tone) than during Min 1 or Min 2, indicating

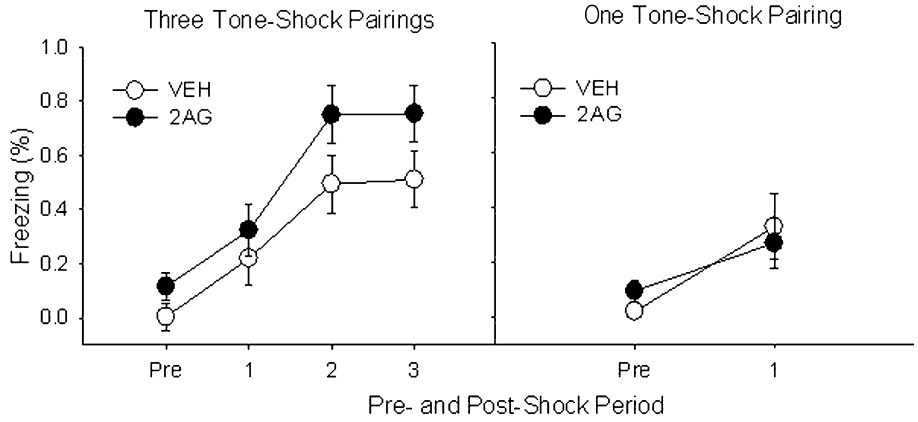

Figure 7

Mean (±SEM) per cent freezing during the pre-shock period and each post-shock period during conditioning in Experiment 5 among rats (n = 8 per group) treated with either VEH or 1.25 mg·kg^{-1} 2AG prior to receiving one or three tone-shock pairings.

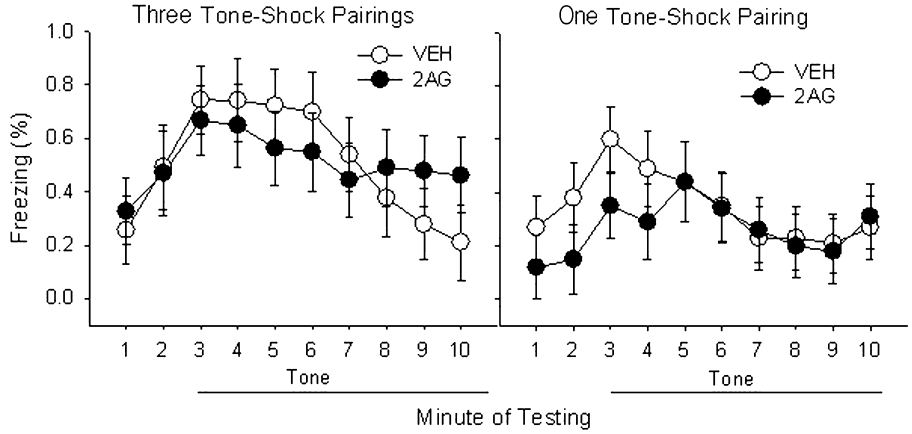

Figure 8

Mean (±SEM) per cent freezing during each minute of drug-free testing in Experiment 5 for rats (n = 8 per group) that received either VEH or 1.25 mg·kg^{-1} 2AG prior to receiving either one or three tone-shock pairings during conditioning. The tone was turned on at minute 3 and remained on throughout the test.

that they had learned the tone-shock association. There was neither a main effect of pretreatment nor a pretreatment by period interaction. Statistical analyses of per cent freezing during Min 3–10 for the rats that received three tone-shock pairings also only revealed a significant main effect of period, $F(7,98) = 6.1$, $P < 0.001$, indicating that freezing decreased across minutes during the tone presentation. The pattern of results for rats that received one tone-shock pairing during Min 3–10 was similar, such that there was a significant main effect of period, $F(7,98) = 4.5$; $P < 0.001$, with per cent freezing decreasing during the tone presentation across minutes of testing.

Discussion

Consistent with the reported anti-emetic effects of 2AG in ferrets (Van Sickle *et al.*, 2005), manipulations that elevate 2AG produced anti-vomiting/anti-nausea-like effects in the *S. murinus* and rat models, respectively. The MAGL inhibitor, JZL184, which was shown to selectively inhibit MAGL activity in shrew brain tissue, attenuated vomiting produced by LiCl in *S. murinus*. This effect appeared to be CB_1 receptor dependent, because pretreatment with AM251 reversed (or at least partially inhibited) the suppression of vomiting by 40 mg·kg^{-1} JZL184. This dose of JZL184 has been shown to produce a 10-fold increase in brain levels of 2AG in mice within 0.5 h (Long *et al.*, 2009a). Because JZL184 was relatively ineffective in rats in the conditioned gaping model of nausea-like behaviour (pilot data not shown) and in other rodent models (Long *et al.*, 2009a), the potential of 2AG itself to modify LiCl-induced gaping was assessed. At doses of 1.25 and 2.0 mg·kg^{-1}, 2AG prevented the establishment of LiCl-induced gaping. The effect of 2AG on gaping was not reversed by the CB_1 receptor antagonist/inverse agonist, AM251, or the CB_2 receptor antagonist, AM630. On the other hand, the

suppression of LiCl-induced conditioned gaping during co-administration of the MAGL inhibitor, JZL184, and 2AG (which presumably prolonged the duration of action of 2AG) was partially reversed by pretreatment with AM251. This latter effect suggests that, under conditions of prolonged action, nausea-like behaviour may have been prevented in rats by the action of 2AG on CB_1 receptors, as was vomiting in the shrews.

Downstream metabolites of 2AG also played a role in the suppression of LiCl-induced nausea-like behaviour, because like 2AG, AA also suppressed conditioned gaping. Furthermore, the COX inhibitor, indomethacin, completely reversed the suppressant effects of both 2AG and AA on conditioned gaping in rats. These effects suggest that the anti-nausea-like effects of 2AG in rats are mediated by downstream metabolites of AA. Because in the presence of MAGL inhibition, the antinausea-like behaviour produced by 2AG are CB_1 dependent, it is conceivable that co-localization of MAGL and COX may be important in the regulation of nausea and vomiting.

When released at the synapse, 2AG has a relatively short duration of action as it is rapidly degraded (e.g. Van der Steldt and Di Marzo, 2004; Fowler, 2007), which may lead to bioactive products with different physiological effects. 2AG can be hydrolysed *in vivo* by MAGL into AA and glycerol (Dinh *et al.*, 2002). AA is then metabolized by COX enzymes to generate prostaglandins such as PGE_2. In addition to these reactions, 2AG (and its metabolite AA) can be directly oxygenated by COX-2, producing prostaglandin glycerol esters (PG-Gs) such as PGE_2-G (Kozak *et al.*, 2001; Hu *et al.*, 2008; Woodward *et al.*, 2008). *In vitro* data suggest that PGE_2-G may act at different receptors from the prostanoid receptors that mediate the effects of PGE_2, and as well, PGE_2-G is rapidly metabolized to PGE_2 (Kozak *et al.*, 2001). Recent *in vivo* data also suggests that PGE_2-G is produced by oxygenation of 2AG, and plays a role in regulation of pain and immunomodulation (Hu *et al.*, 2008). These downstream metabolites of 2AG and AA may be partially responsible for the anti-nausea-like effects reflected in the suppression of conditioned gaping, because these effects were prevented by pretreatment with the COX inhibitor, indomethacin.

Given that 2AG was administered prior to the conditioning trial, it is also possible that the effects reported here were the result of 2AG interference with learning *per se* rather than a suppression of LiCl-induced nausea. To test the hypothesis that 2AG may be interfering with the sensory processing of the saccharin taste when administered prior to the intra-oral infusion of saccharin during conditioning, in Experiment 4, rats were administered 2AG or VEH immediately following the saccharin and 15 min prior to the injection of LiCl. As was evident in Experiment 2, when 2AG was administered prior to exposure to a saccharin infusion, rats displayed suppressed gaping to the LiCl-paired saccharin solution during the drug-free TR test. These results confirm that the suppressed conditioned gaping was the result of a reduction in the nausea-like behaviour produced by LiCl, not of a sensory processing failure of the saccharin solution. As well, Experiment 5 evaluated the potential of 2AG to interfere with the establishment of conditioned freezing elicited by a shock-paired tone. Rats were pretreated with Vehicle or 1.25 $mg \cdot kg^{-1}$ 2AG, a dose that was highly effective in preventing LiCl-induced conditioned gaping in Experiment 2. Then, two separate groups were given either a single tone-shock pairing or three tone-shock pairings, to ensure that ceiling effects did not mask 2AG-induced learning deficits. Rats given either one or three tone-shock pairings displayed conditioned freezing during the tone test, but 2AG did not interfere with this conditioning. Therefore, it is more likely that the suppression of conditioned gaping in Experiment 2 was the result of a 2AG-mediated interference with LiCl-induced nausea-like behaviour, rather than learning.

The present experiments suggest that, like anandamide (Van Sickle *et al.*, 2001; Cross-Mellor *et al.*, 2007) and inhibitors of FAAH (Cross-Mellor *et al.*, 2007; Parker *et al.*, 2009; Sharkey *et al.*, 2007), 2AG and MAGL inhibitors may have therapeutic potential in the treatment of nausea and vomiting.

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Conflict of interest

There is no conflict of interest on behalf of all authors.

References

Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK *et al.* (2009). Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chem Biol 16: 411–420.

Alexander SPH, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th Edition. Br J Pharmacol 164 (Suppl. 1): S1–S324.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384: 83–87.

Cross-Mellor SK, Ossenkopp KP, Piomelli D, Parker LA (2007). Effects of the FAAH inhibitor, URB597, and anandamide on lithium-induced taste reactivity responses: a measure of nausea in the rat. Psychopharmacology 190: 135–143.

Darmani NA (2002). The potent emetogenic effects of the endocannabinoid, 2AG (2-arachidonoylglycerol) are blocked by delta(9)-tetrahydrocannabinol and other cannabinoids. J Pharmacol Exp Ther 300: 34–42.

Darmani NA, McClanahan BA, Trinh C, Petrosino S, Valenti M, Di Marzo V (2005). Cisplatin increases brain 2-arachidonoylglyclerol (2AG) and concomitantly reduces intestinal 2AG and anandamide levels in the least shrew. Neuropharmacology 49: 502–513.

Deutsch DG, Chin SA (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 46: 791–796.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al*. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946–1949.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz J-C *et al*. (1994). Formation and inactivation of endogenous cannabinoid anandamde in central neurons. Nature 372: 686–691.

Dinh TP, Freund TP, Piomelli D (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121: 149–158.

Fowler CJ (2007). The contribution of cyclooxygenase-2 to endocannabinoid metabolism and action. Br J Pharmacol 152: 594–601.

Grill HJ, Norgren R (1978). The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 143: 263–279.

Hu SS, Bradshaw HB, Chen JS, Tan B, Walker JM (2008). Prostaglandin E_2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NFκB activity. Br J Pharmacol 153: 1538–1549.

Kozak KR, Crews BC, Ray JL, Tai HH, Morrow JD, Marnett LJ (2001). Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides *in vitro* and *in vivo*. J Biol Chem 276: 36993–36998.

Li B, Sedlacek M, Manoharan I, Boopathy R, Duysen E, Masson P *et al*. (2005). Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem Pharmacol 70: 1673–1684.

Limebeer CL, Parker LA (1999). Delta-9-tetrahydrocannabinol interferes with the establishment and the expression of conditioned rejection reactions produced by cyclophosphamide: a rat model of nausea. Neuroreport 10: 3769–3772.

Limebeer CL, Parker LA (2000). The antiemetic drug ondansetron interferes with lithium-induced conditioned rejection reactions, but not lithium-induced taste avoidance in rats. J Exp Psychol Anim Behav Process 26: 371–384.

Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE *et al*. (2009a). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioural effects. Nat Chem Biol 5: 37–44.

Long JZ, Nomura DK, Cravatt BF (2009b). Characterization of Monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. Chem Biol 16: 744–753.

Maren S (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. J Neurosci 19: 8696–8703.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminiski NE, Schatz AR *et al*. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50: 83–90.

Parker LA, Mechoulam R, Schlievert C, Abbott L, Fudge ML, Burton P (2003). Effects of cannabinoids on lithium-induced conditioned rejection reactions in a rat model of nausea. Psychopharmacology 166: 156–162.

Parker LA, Kwiatkowska M, Burton P, Mechoulam R (2004). Effect of cannabinoids on lithium-induced vomiting in the *Suncus murinus* (house musk shrew). Psychopharmacology 171: 156–161.

Parker LA, Rana SA, Limebeer CL (2008). Conditioned nausea in rats: assessment by conditioned disgust reactions, rather than conditioned taste avoidance. Can J Exp Psychol 62: 198–209.

Parker LA, Limebeer CL, Rock EM, Litt DL, Kwiatkowska M, Piomelli D (2009). The FAAH inhibitor URB-597 interferes with cisplatin- and nicotine-induced vomiting in the Suncus murinus (house musk shrew). Physiol Behav 97: 121–124.

Parker LA, Rock EM, Limebeer CL (2011). Regulation of nausea and vomiting by cannabinoids. Br J Pharmacol 163: 1411–1422.

Patricelli MP, Giang DK, Stamp LM, Burbaum JJ (2001). Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. Proteomics 1: 1067–1071.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4: 873–884.

Sanger GJ (2007). Endocannabinoids and the gastrointestinal tract: what are the key questions? Br J Pharmacol 152: 663–670.

Sharkey KA, Cristino L, Oland LD, Van Sickle MD, Starowicz K, Pittman QJ *et al*. (2007). Arvanil, anandamide and N-arachidonoyl-dopamine (NADA) inhibit emesis through cannabinoid CB1 and vanilloid TRPV1 receptors in the ferret. Eur J Neurosci 25: 2773–2782.

Simon GM, Cravatt BF (2010). Activity-based proteomics of enzyme superfamilies – serine hydrolases as a case study. J Biol Chem 285: 11051–11055.

Van der Steldt M, Di Marzo V (2004). Metabolic fate of endocanabinoids. Curr Neuropharmacol 2: 37–48.

Van Sickle MD, Oland LD, Ho W, Hillard CJ, Mackie K, Davison JS *et al*. (2001). Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 121: 767–774.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al*. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 310: 329–332.

Woodward DF, Liang Y, Krauss AH-P (2008). Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol 153: 410–419.