

Attenuation of anticipatory nausea in a rat model of contextually elicited conditioned gaping by enhancement of the endocannabinoid system

Cheryl L. Limebeer · Rehab A. Abdullah ·
Erin M. Rock · Elizabeth Imhof · Kai Wang ·
Aron H. Lichtman · Linda A. Parker

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Abstract

Rationale Enhancement of the endocannabinoid (EC) system may reduce anticipatory nausea (AN).

Objectives The experiments evaluated the potential of the dual fatty acid amide hydrolase (FAAH)/monoacylglycerol lipase (MAGL) inhibitor, JZL195, on its own and combined with anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) to reduce contextually elicited gaping, a measure of AN in rats.

Methods Following four context lithium chloride (LiCl) pairings, rats were injected with vehicle (VEH) or JZL195 (10 mg kg⁻¹, intraperitoneally) 105 min before an injection of VEH, 2-AG (1.25 mg kg⁻¹), or AEA (5.0 mg kg⁻¹). Fifteen minutes later, all rats were placed in the LiCl-paired context for 5 min and in a different context for a 15-min locomotor test. Whole brains were extracted for EC analysis. The potential of the CB₁ antagonist, SR141716, to reverse the suppression of AN by both JZL195 and AEA and of the CB₂ antagonist, AM630, to reverse the suppression of AN by JZL195 was then evaluated.

Results JZL195 suppressed gaping and elevated AEA, palmitoylethanolamine, and oleoylethanolamide. As the suppression of gaping was reversed by SR141716, but not by AM630, the effect was CB₁ mediated. The suppressive effect of JZL195 on gaping, as well as elevation of AEA and 2-AG,

was amplified by pretreatment with either AEA or 2-AG. On its own, AEA, but not 2-AG, also suppressed gaping—an effect that was also prevented by CB₁ antagonism.

Conclusions JZL195 reduces AN primarily by acting as a FAAH inhibitor, but MAGL inhibition is also indicated.

Keywords Learning · Rat · Chemotherapy · Fatty acid amide hydrolase (FAAH) · Monoacylglycerol lipase (MAGL) · Anandamide · 2-Arachidonoyl glycerol · Nausea · Gaping · Lithium chloride

Anticipatory nausea (AN) develops as a conditional association between the contextual cues of the clinic and nausea-inducing chemotherapy treatment (Neese et al. 1980). Once AN develops, classical anti-emetic treatments, such as the 5-HT₃ antagonist ondansetron, have little effect, thus underscoring the challenge in treating this condition (Morrow et al. 1998; Foubert and Vaessen 2005). AN is reported as one of the most distressing factors of chemotherapy treatment and significantly decreases the patient's quality of life (Akechi et al. 2010).

The search for an effective treatment for AN has been limited by the lack of selective rodent preclinical models of nausea because rats and mice do not vomit in response to toxins. Recently, a rat model of AN, contextually elicited conditioned gaping reactions, has been identified (Limebeer et al. 2008). Gaping is a disgust reaction in rats that is elicited unconditionally by bitter tasting quinine (Garcia et al. 1974; Grill and Norgren 1978). Gaping consists of rapid, large amplitude openings of the mouth with simultaneous retraction at the corners of the mouth (Grill and Norgren 1978) requiring the same orofacial musculature used by other species for vomiting (Travers and Norgren 1986). Following pairings with emetic drugs, rats display conditioned gaping reactions to a sweet solution (Grill and Norgren 1978). Unlike

C. L. Limebeer · E. M. Rock · E. Imhof · K. Wang ·
L. A. Parker (✉)
Department of Psychology and Neuroscience Graduate Program,
University of Guelph, Guelph, ON N1H 2W1, Canada
e-mail: parkerl@uoguelph.ca

R. A. Abdullah · A. H. Lichtman
Department of Pharmacology and Toxicology, Virginia
Commonwealth University School of Medicine,
Richmond, VA, USA

traditional intake measures of flavor–drug associations in rats, conditioned gaping reactions are exclusively produced by treatments that produce vomiting in other species and are consistently prevented by anti-emetic treatments, including 5-HT₃ antagonists and cannabinoids (see Parker et al. 2009a for review).

Rats not only display conditioned gaping to an illness paired flavor, but they also display conditioned gaping to an illness paired context, in a dose-dependent relation (Limebeer et al. 2008). Contextually elicited conditioned gaping is analogous to the phenomenon of AN in human chemotherapy patients. In this model, rats are injected with an emetic drug, such as lithium chloride (LiCl) before they are placed in a distinctive conditioning chamber. Following three to four pairings, rats repeatedly gape when reintroduced to the context in the absence of LiCl. Interestingly, shrews which vomit in response to an emetic treatment also display a conditioned retching reaction when reintroduced to the context in which they previously vomited (Parker and Kemp 2001; Parker et al. 2006). With the development of animal models of AN, it is possible to evaluate the potential of drug treatments to reduce AN.

Exogenous cannabinoids, such as Δ^9 -tetrahydrocannabinol (THC) found in the marijuana plant, are effective in suppressing AN in both the rat conditioned gaping model (Limebeer et al. 2006) and in a shrew conditioned retching model (Parker and Kemp 2001; Parker et al. 2006). On the other hand, just as is seen with human chemotherapy patients, ondansetron is ineffective in reducing AN once it has developed (Limebeer et al. 2006; Parker et al. 2006). Currently, AN is most likely treated with nonspecific anti-anxiety drugs (benzodiazepines, such as lorazepam, Malik et al. 1995; Razavi et al. 1993). However, both benzodiazepines and THC have sedating side effects, which may diminish their therapeutic effectiveness.

The endocannabinoid (EC) system has been implicated in control of nausea and vomiting (Parker et al. 2011). Anandamide (AEA) and 2-arachidonyl glycerol (2-AG) are endogenous agonists for cannabinoid G protein-coupled receptors CB₁ and CB₂ (Devane et al. 1992; Mechoulam et al. 1995), which are rapidly degraded by fatty acid amide hydrolase (FAAH) (Deutsch and Chin 1993) and monoacylglycerol lipase (MAGL) (Dinh et al. 2002; Blankman et al. 2007), respectively. Inhibitors of AEA and 2-AG degradation offer a potentially effective alternative treatment strategy for stimulating the endocannabinoid system (Long et al. 2009a). These selective FAAH and MAGL inhibitors produce a subset of the behavioral responses seen with direct CB₁ agonists, including analgesia (Long et al. 2009b; Lichtman et al. 2004; Kinsey et al. 2009). Moreover, inhibition of MAGL, but not FAAH, produces hypomotility in mice (Long et al. 2009a). Neither FAAH nor MAGL inhibitors induce the cataleptic behavioral reactions evident with direct CB₁ agonists (Kathuria et al. 2003; Long et al. 2009b), and FAAH inhibitors are inactive in animal models of drug abuse (Gobbi et al. 2005).

Rock et al (2008) recently demonstrated that the FAAH inhibitor, URB597 (Fegley et al. 2005), dose dependently suppressed the expression of AN elicited by a LiCl-paired chamber. The suppression of AN was reversed by the CB₁ antagonist/inverse agonist, SR141716, which on its own did not modify the strength of the contextually elicited gaping reactions (Rock et al. 2008). Inhibition of FAAH not only elevates AEA, but also other fatty acids including *N*-palmitoylethanolamine (PEA) and oleoylethanolamide (OEA), which act as agonists of the peroxisome proliferator-activated receptors (PPAR; e.g., Gaetani et al. 2008). However, the finding that SR141716 reversed the suppression indicates a CB₁ mechanism of action, most likely due to an elevation of AEA. The MAGL inhibitor, JZL184, which selectively elevated 2-AG, has also been shown to reduce LiCl-induced vomiting in the *Suncus murinus* in a CB₁-dependent manner. Although JZL184 is much less effective in suppressing MAGL in rats (Long et al. 2009b) than in mice or *S. murinus* (Sticht et al. 2012), exogenous 2-AG interfered with the establishment of LiCl-induced gaping elicited by a flavor in rats, due to the action of downstream metabolites (Sticht et al. 2012). Therefore, both elevated AEA and 2-AG have been shown to reduce conditioned nausea in rats.

Recently, the dual FAAH and MAGL inhibitor, JZL195, has been reported to reduce both enzymes in mouse and rat brains (Long et al. 2009a); albeit, it is equipotent in inhibiting both enzymes in mouse brain tissue, but eightfold more potent in inhibiting FAAH than MAGL in rat brain. Recently, in vivo microdialysis of interstitial AEA and 2-AG levels from the rat nucleus accumbens revealed that JZL195 increased 2-AG and AEA levels approximately 50 and 100 %, respectively, from basal levels (requiring an ionic pulse; Wiskerke et al. 2012). In mice, in vivo studies demonstrated that, when compared with selective inhibitors of FAAH or MAGL per se, the dual inhibitor produced a dramatic CB₁ receptor-mediated antinociceptive effect, as well as generalized to the THC discriminative cue in the drug discrimination paradigm and elicited catalepsy (Long et al. 2009a). The following experiments evaluated the potential of JZL195 alone and in combination with AEA and 2-AG to interfere with AN in the contextually elicited conditioned gaping model in rats and to modify activity level per se. The potential of the CB₁ inverse agonist/antagonist SR141716 to reverse the suppression of AN was evaluated as well. Finally, whole brains were removed following the behavioral testing and analyzed for levels of AEA, 2-AG, PEA, OEA, and the metabolic product, arachidonic acid (AA).

Materials and methods

Animals

Animal procedures complied with the Canadian Council on Animal Care, and the protocols were approved by the

Institutional Animal Care Committee at University of Guelph. A total of 73 naïve male Sprague–Dawley rats, obtained from Charles River Laboratories (St. Constant, Quebec), were used for assessment of AN. They were individually housed in shoebox cages, subjected to an ambient temperature of 21 °C and a 12/12-h light–dark schedule (lights off at 7 am), and maintained on food (Highland Rat Chow [8640]) and water ad libitum. Their body weights ranged from 300 to 440 g on the day of testing for AN.

Drugs

All injections were administered intraperitoneally (ip). Lithium chloride (Sigma-Aldrich) was prepared in a 0.15 M solution with sterile water and was administered at a volume of 20 ml kg⁻¹ (127.2 mg kg⁻¹ dose). The drugs JZL195, AEA, 2-AG (Cayman Chemicals), SR141716 (Sequoia Chemicals, UK), and AM630 (Tocris) were prepared in a 1:1:18 solution of ethanol/Tween 80/physiological saline. The drugs were first dissolved in ethanol and then Tween 80 was added to the solution, and the ethanol was evaporated off with a nitrogen stream after which the saline was added. The final vehicle (VEH) consisted of 1:9 (Tween/saline). At test, all drugs were administered at a volume of 1 ml kg⁻¹. The concentration of JZL195 was 10 mg ml⁻¹, the concentration of AEA was 5 mg ml⁻¹, the concentration of 2-AG was 1.25 mg ml⁻¹, the concentration of SR141716 was 2.5 mg ml⁻¹, and the concentration of AM630 was 3.0 mg ml⁻¹. The dose of JZL195 was selected on the basis of a body surface area dose translation (Reagan-Shaw et al. 2007) from effective mouse doses (Long et al. 2009b). The doses of AEA (Cross-Mellor et al. 2007) and 2-AG (Sticht et al. 2012) were selected on the basis of their potential to interfere with the acute nausea produced by LiCl in rats. The dose of SR141716 was selected on the basis of its potential to reverse the suppression of AN by URB597, without producing an effect on AN on its own (Rock et al. 2008). The dose of AM630 was selected on the basis of its potential to block the anti-allodynic effects of FAAH and MAGL inhibition (Guindon et al. 2013).

Biochemical analyses

Extraction and quantification of endocannabinoids by liquid chromatography-tandem mass spectrometry

Rats were sacrificed 2.5 h following vehicle or JZL195 (10, 20, or 40 mg kg⁻¹ ip). Additionally, subjects were given an injection of VEH, AEA (5 mg kg⁻¹ ip), or 2-AG (1.25 mg kg⁻¹ ip) 105 min after vehicle or JZL195. Rats were euthanized by rapid decapitation (restrained in a decapicorne—Braintree Scientific, MA, USA), and their brains were harvested, snap frozen in dry ice-cooled isopentane, and stored in a -80 °C freezer until being shipped to Virginia Commonwealth

University under dry ice and stored at -80 °C until the time of processing. Tissues were further processed according to methods described previously (Ramesh et al. 2011; Kinsey et al. 2013)

On the day of processing, the pre-weighed rat brains were homogenized with 5 ml chloroform/methanol (2:1 v/v containing 0.0348 g phenylmethylsulfonyl fluoride/ml). One quarter of the homogenate (1.25 ml) was taken and diluted to 1.4 ml with the chloroform/methanol used for homogenization. Internal standards (2 pmol AEA-d8, 1 nmol 2-AG-d8, 3.3 nmol PEA-d4, 3 nmol OEA-d4, and 1 nmol AA-d8) were added to each sample. Homogenates were mixed with 0.3 ml of 0.73 % w/v NaCl, vortexed, and centrifuged for 10 min at 3,220×g (4 °C). The aqueous phase and debris were collected and extracted again twice with 0.8 ml chloroform. The organic phases from the three extractions were pooled, and the organic solvents were evaporated under nitrogen gas. Dried samples were reconstituted with 0.1 ml chloroform and mixed with 1 ml cold acetone. The mixtures were centrifuged for 5 min at 1,811×g (4 °C) to precipitate protein. The upper layer of each sample was collected and evaporated under nitrogen. Dried samples were reconstituted with 0.1 ml methanol and placed in autosample vials for analysis.

LC/MS/MS was used to quantify AEA, 2-AG, PEA, OEA, and AA. The mobile phase consisted of methanol (90:10)/0.1 % ammonium acetate and 0.1 % formic acid. The column used was a Discovery® HS C18, 4.6×15 cm, 3 μm (Supelco, USA). Ions were analyzed in multiple reaction monitoring mode and the following transitions were monitored in positive mode: (348>62) and (348>91) for AEA; (356>62) for AEA-d8; (379>287) and (279>269) for 2-AG; (387>96) for 2-AG-d8; (300>62) and (300>283) for PEA; (304>62) for PEA-d4; (326>62) and (326>309) for OEA; and (330>66) for OEA-d4; in negative mode: (303>259) and (303>59) for AA and (311>267) for AA-d8.

A calibration curve was constructed for each assay based on linear regression using the peak area ratios of the calibrators. The extracted standard curves ranged from 1.25 to 400 pmol for AEA, from 1 to 64 nmol for 2-AG, from 0.078 to 1.25 nmol for PEA and OEA, and from 2 to 32 nmol for AA.

Apparatus

The distinctive context utilized for conditioning varied the dimensions of location, visual, and tactile cues from the home cage environment. The room was dark with two 40-W lights on either side of the conditioning chamber. The conditioning chamber was made of black Plexiglas sides (22.5×26×20 cm) with an opaque lid. The chamber was placed on a table with a clear Plexiglas top. A mirror beneath the chamber on a 45° angle facilitated viewing of the ventral surface of the rat. A Sony videocamera (Handycam, Henry's Camera, Waterloo, ON, Canada) was used to videotape the rats from the mirror

beneath the chamber. The videotapes were later scored using “The Observer” event recording software (Noldus, Inc., Netherlands).

The activity chamber was constructed of white Plexiglas with the dimensions of 60×25×25 cm and located in a different room than the AN chamber illuminated with a red light. A videocamera mounted on an extension pole above the chamber captured the activity of the rat which was sent to a computer for analysis of distance (centimeter) traveled using the Ethovision software program (Noldus, Inc., Netherlands).

Behavioral procedures

Effect of JZL195 alone and co-administered with AEA and 2-AG on AN, locomotor activity, and EC levels

All rats received four conditioning trials, with 72 h between trials. On each conditioning trial, each rat was injected with LiCl and immediately placed in the distinctive context for 30 min. The AN test trial occurred 72 h after the final conditioning trial. On the test trial, the rats were randomly assigned to one of six groups on the basis of treatment 1 (VEH or JZL195) and treatment 2 (VEH, AEA, or 2-AG), with $n=8-9$ /group. The rats received treatment 1 (VEH or JZL195) 105 min prior to receiving treatment 2 (VEH, AEA, or 2-AG) 15 min prior to placement in the context. The rats remained in the context for 5 min and their orofacial reactions were videotaped from the mirror beneath the chamber. VEH-injected rats display the most contextually elicited gaping reactions during the first 5 min of a longer test trial (Limebeer et al 2008; Rock et al 2008). The videotapes were later scored for the number of gapes (large amplitude openings of the mouth with simultaneous retractions of the corners of the mouth exposing incisors). Immediately following the AN test trial, the rats were placed in the novel activity chamber for 15 min, and their locomotor activity was automatically videotracked. Immediately following the activity trial, the rat brains were removed as previously described.

Role of CB₁ receptor: reversal by SR141716

In order to determine the role of the CB₁ receptor in the suppression of conditioned gaping, three additional groups were conditioned and tested. Groups JZL195-SR ($n=8$) and VEH-SR ($n=8$) were injected with JZL195 (10 mg/kg) or VEH 90 min before receiving SR141716 (2.5 mg/kg) and 30 min later were placed in the AN chamber for 5 min. Group SR-AEA ($n=8$) was injected with SR141716 (2.5 mg/kg) 15 min prior to receiving AEA (5 mg/kg) and 15 min later was placed in the AN chamber for 5 min. Immediately following the AN test, the rats were placed in the novel activity chamber for 15 min and their locomotor activity was videotracked. For statistical comparison, the

number of gapes and distance (centimeter) traveled displayed by each of these groups was compared with groups VEH-VEH, VEH-AEA, and JZL195-VEH.

In experiment 2b, the potential of CB₂ antagonism to reverse the suppressive effect of JZL195 on contextually elicited gaping was assessed in a separate group that was administered with JZL195 (10 mg kg⁻¹) 90 min before AM630 (3 mg kg⁻¹) and was placed in the AN chamber 30 min later for 5 min. Immediately following the AN test, they were given the 15-min locomotor test. This group was compared with groups VEH-VEH and JZL195-VEH.

Data analyses

In experiment 1, to determine the potential of the treatments to modify AN, activity, and EC levels, the number of gapes, distance (centimeter) traveled in activity chambers, and amount of whole brain 2-AG, AEA, PEA, OEA, and AA was entered into a 2 (treatment 1: VEH, JZL195) × 3 (treatment 2: VEH, 2-AG, AEA) between-groups analysis of variance (ANOVA). Significant interactions were analyzed: (1) as one-way ANOVAs for each of VEH and JZL195 (treatment 1) across VEH, 2-AG, and AEA (treatment 2) with subsequent least significant difference (LSD) pairwise comparisons indicated by * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ and (2) as planned pairwise comparisons of VEH and JZL195 (treatment 1) for each of VEH, 2-AG, and AEA (treatment 2) with significant effects depicted as # $p<0.05$, ## $p<0.01$, and ### $p<0.001$. In experiment 2a, to determine the role of the CB₁ receptor antagonism, the number of gapes and distance traveled were entered into a single factor ANOVA (groups: VEH-VEH, VEH-SR, VEH-AEA, JZL195-VEH, SR-AEA, and JZL195-SR) with subsequent LSD pairwise comparison tests. As well, in experiment 2b, to determine the effect of CB₂ antagonism, the number of gapes and distance traveled were entered into a single factor ANOVA (groups: VEH-VEH, JZL-VEH, and JZL-195-VEH).

Results

Effect of JZL195, alone and co-administered with AEA and 2-AG, on AN, locomotor activity, and EC levels

AN test Systemic administration of JZL195 suppressed conditioned gaping elicited by a LiCl-paired context, an effect that was enhanced by co-administration of AEA or 2-AG. Systemic administration of AEA alone, but not 2-AG alone, also suppressed conditioned gaping. Figure 1 presents the mean (±SEM) number of gapes elicited by the LiCl-paired context for each group. The upper section represents the rats given VEH as treatment 1, the lower section represents the rats given JZL195 as the treatment 1, and the abscissa represents

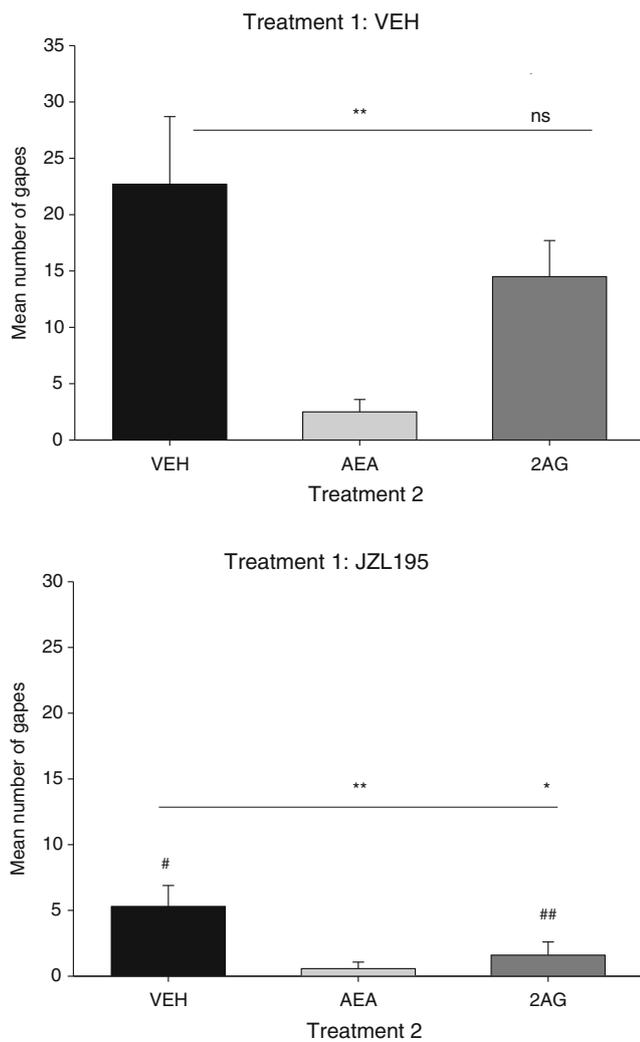


Fig. 1 Mean (\pm SEM) number of gapes elicited by the LiCl-paired context during the 5-min test by rats in the various groups. The *top half* of the figure presents the groups treated with VEH 2 h prior to the AN test, and the *bottom half* of the figure presents the groups treated with JZL195 2 h prior to the AN test. The *abscissa* depicts the groups treated with VEH, AEA, or 2-AG 15 min prior to the AN test. Asterisks (*) signify significant differences when the VEH pretreatment groups and the JZL195 pretreatment groups are analyzed separately. The number signs (#) signify significant differences between VEH and JZL195 when each treatment 2 group (VEH, AEA, and 2-AG) is analyzed separately. *, # $p < 0.05$; **, ## $p < 0.01$; ***, ### $p < 0.001$

the rats given VEH, 2-AG, or AEA as treatment 2. The analysis revealed main effects of treatment 1 ($F(1, 43) = 17.96$, $p < 0.001$), treatment 2 ($F(2, 43) = 8.19$, $p = 0.001$), and a treatment 1 \times treatment 2 interaction ($F(2, 43) = 3.29$; $p = 0.047$). Separate one-way ANOVAs revealed significant effects for both the VEH-treated groups ($F(2, 22) = 4.83$; $p = 0.018$) and the JZL195-treated groups ($F(2, 22) = 4.83$; $p = 0.012$). Among the VEH-treated groups, the rats in group VEH-AEA, but not VEH-2-AG, gaped less than rats in group VEH-VEH (** $p < 0.01$). Among the JZL195-treated groups, rats in both groups JZL195-AEA (** $p = 0.008$) and JZL195-

2-AG (* $p = 0.032$) gaped less than group JZL195-VEH. Additionally, group JZL195-VEH showed suppressed gaping relative to VEH-VEH (# $p = 0.016$), and group JZL195-2-AG showed less gaping than VEH-2-AG (### $p = 0.002$).

Locomotor test Anandamide increased locomotor activity in the 15-min test that followed the AN test. Figure 2 presents the mean distance (centimeter) traveled during the 15-min locomotor activity test that immediately followed the AN test. The overall analysis revealed a significant effect of treatment 2 ($F(2, 43) = 15.23$; $p < 0.001$) and a treatment 1 \times treatment 2

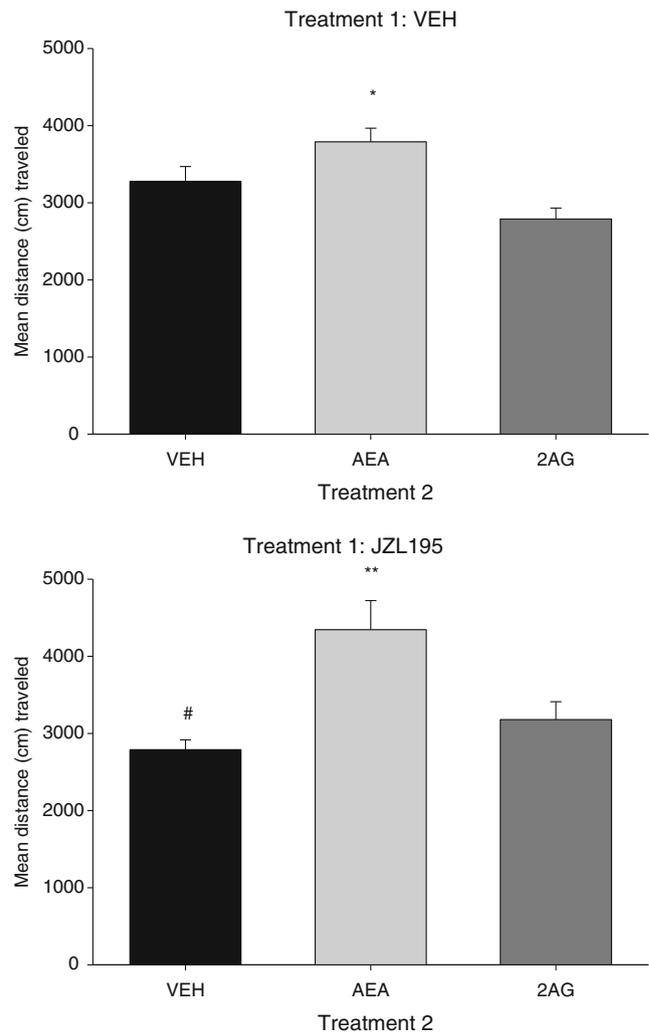


Fig. 2 Mean (\pm SEM) distance (centimeter) traveled during the 15-min locomotor activity test that immediately followed the AN test. The *top half* of the figure presents the groups treated with VEH 2 h prior to the AN test, and the *bottom half* of the figure presents the groups treated with JZL195 2 h prior to the AN test. The *abscissa* depicts the groups treated with VEH, AEA, or 2-AG 15 min prior to the AN test. Asterisks (*) signify significant differences when the VEH pretreatment groups and the JZL195 pretreatment groups are analyzed separately. The number signs (#) signify significant differences between VEH and JZL195 when each treatment 2 group (VEH, AEA, and 2-AG) is analyzed separately. *, # $p < 0.05$; **, ## $p < 0.01$; ***, ### $p < 0.001$

interaction ($F(2, 43)=3.29$; $p=0.047$). Separate one-way ANOVAS revealed significant effects for both the VEH-treated groups ($F(2, 21)=8.59$; $p=0.002$) and the JZL195-treated groups ($F(2, 22)=9.86$; $p<0.001$). Among both the VEH- ($*p<0.05$) and JZL195- ($**p<0.01$) treated groups, rats subsequently treated with AEA were more active than any other group. The rats in group JZL195-VEH displayed reduced locomotion relative to rats in group VEH-VEH ($^{\#}p=0.046$) in agreement with Long et al. (2009a, b) as well.

Biochemical analysis The results of the analyses of whole brains collected immediately after the 20-min behavioral testing for 2-AG, AEA, PEA, OEA, and AA are presented in Table 1.

2-AG 2-AG levels were not elevated by JZL195 alone, but were elevated when JZL was combined with either 2-AG or AEA. Neither exogenous 2-AG nor AEA alone produced elevated 2-AG levels. The overall analysis revealed a significant main effect of treatment 1 ($F(1, 43)=34.47$; $p<0.001$), treatment 2 ($F(2, 43)=4.45$; $p=0.18$), and a treatment 1 \times treatment 2 interaction ($F(2, 43)=6.15$; $p=0.004$). Separate one-way ANOVAS revealed that only the JZL195-treated groups differed significantly ($F(2, 22)=5.71$; $p=0.01$), which was the result of group JZL195-AEA showing enhanced 2-AG relative to JZL195-VEH ($***p<0.001$) or JZL195-2-AG ($*p=0.018$). Groups JZL195-2-AG and JZL195-AEA had higher levels of 2-AG than groups VEH-2-AG ($^{\#}p<0.010$) and VEH-AEA ($^{\#\#\#}p<0.001$), respectively. However, the 2-AG levels of group JZL195-VEH did not significantly differ from group VEH-VEH ($p=0.093$). This latter finding prompted us to evaluate the potential of higher doses (20 and 40 mg kg⁻¹) of JZL195 to elevate 2-AG levels under similar temporal conditions as the experiments above. With an $n=2$ /group, the mean level of 2-AG (nanomoles per gram)

produced by the higher doses of JZL195 was VEH (7.322), 20 mg kg⁻¹ JZL195 (8.112), and 40 mg kg⁻¹ (9.135), indicating that even these higher doses administered in vivo to rats did not elevate 2-AG whole brain levels.

AEA JZL195 alone elevated the AEA levels, and this effect was dramatically enhanced by its combination with exogenous AEA. The overall analysis revealed a significant main effect of treatment 1 ($F(1, 43)=23.78$; $p<0.001$), treatment 2 ($F(2, 43)=16.30$; $p<0.001$), and a treatment 1 \times treatment 2 interaction ($F(2, 43)=16.06$; $p<0.001$). Separate one-way ANOVAS revealed a significant effect among VEH ($F(2, 21)=4.36$; $p=0.026$) and JZL195 ($F(2, 22)=16.73$; $p<0.001$) treatment 1 groups; exogenous administration of AEA elevated brain AEA in both VEH-AEA ($*p<0.05$) and JZL195-AEA ($***p<0.001$) relative to other groups. As well, comparison tests of VEH and JZL195 treated rats showed that JZL195 significantly elevated AEA levels in rats subsequently treated with VEH, 2-AG, or AEA ($^{\#\#\#}ps<0.001$).

PEA and OEA JZL195 elevated levels of both PEA and OEA overall, an effect that did not vary by treatment 2. The overall analysis revealed only a significant main effect of treatment 1 for levels of both PEA ($F(1, 43)=219.23$; $p<0.001$) and OEA ($F(1, 43)=204.3$; $p<0.001$).

AA Among the JZL195-treated animals, but not the VEH-treated animals, subsequent treatment with either AEA or 2-AG reduced levels of AA, but JZL195 treatment alone did not modify levels of AA. The overall analysis revealed a significant main effect of treatment 2 ($F(2, 43)=10.7$; $p<0.001$) and an interaction of treatment 1 \times treatment 2 that approached statistical significance ($F(2, 43)=2.9$; $p=0.06$). Separate one-way ANOVAS revealed that the JZL195-treated groups differed ($F(2, 22)=9.3$; $p<0.001$), following pretreatment with

Table 1 Mean levels of endocannabinoids/fatty acids measured from whole brains collected immediately following behavioral testing

Treatment 1–treatment 2	2-AG (nmol/g)	AEA (pmol/g)	PEA (pmol/g)	OEA (pmol/g)	AA (pmol/g)
VEH-VEH ($n=8$)	7.894 (± 1.542)	3.554 (± 0.292)	0.201 (± 0.010)	0.0678 (± 0.003)	69.494 (± 4.500)
VEH-2-AG ($n=8$)	5.402 (± 0.328)	3.236 (± 0.207)	0.201 (± 0.004)	0.070 (± 0.003)	63.676 (± 1.605)
VEH-AEA ($n=8$)	5.783 (± 0.328)	4.546 [*] (± 0.400)	0.209 (± 0.012)	0.072 (± 0.006)	61.716 (± 1.443)
JZL195-VEH ($n=9$)	12.928 (± 2.259)	25.801 (± 2.372) ^{###}	1.296 (± 0.076) ^{###}	0.605 (± 0.028) ^{###}	76.093 (± 5.917)
JZL195-2-AG ($n=8$)	15.820 (± 3.325) ^{##}	24.352 (± 2.827) ^{###}	1.470 (± 0.041) ^{###}	0.141 (± 0.500) ^{###}	56.533 ^{**} (± 2.254) [#]
JZL195-AEA ($n=8$)	29.288 ^{***} (± 5.072) ^{###}	334.187 ^{***} (± 78.020) ^{###}	1.309 (± 0.218) ^{###}	0.662 (± 0.115) ^{###}	52.381 ^{***} (± 2.868) [#]

Values represent mean quantity of lipid/gram brain weight \pm SEM. Asterisks depict significant differences in comparisons of VEH, 2-AG, and AEA separately for rats pretreated with VEH or JZL195. Number signs depict differences between VEH and JZL195 for each of VEH, 2-AG, and AEA treatment 2 groups

^{*}, [#] $p<0.05$, ^{**}, ^{##} $p<0.01$, ^{***}, ^{###} $p<0.001$

JZL195, exogenous administration of AEA ($p=0.001$), or 2-AG ($p=0.003$) reduced AA levels relative to VEH. As well, groups JZL195-AEA ($p=0.011$) and JZL195-2-AG ($p=0.022$) had lower levels of AA than groups VEH-AEA and VEH-2-AG, respectively.

Role of CB₁ receptor: reversal by SR141716

The CB₁ inverse agonist/antagonist, SR141716, reversed the suppressive effect of both JZL 195 and AEA on AN in the gaping model and the enhancement of locomotor activity produced by AEA in the activity test. A single factor ANOVA for the gaping measure revealed a significant main effect of treatment ($F(5, 43)=3.6$; $p=0.009$). As is evident in Fig. 3, groups VEH-AEA and JZL195-VEH (* $p<0.025$) displayed significantly less gaping than any other group. A single factor ANOVA for the distance traveled measure also revealed a significant main effect of treatment ($F(5, 43)=4.6$; $p=0.002$); as shown in Fig. 4, group VEH-AEA was more active than all other groups ($p<0.05$), including SR-AEA ($p<0.001$).

CB₂ antagonism did not reverse the suppressive effect of JZL195. The single factor ANOVA for gaping revealed a significant main effect of treatment ($F(2, 22)=6.7$; $p=0.005$); Group VEH-VEH (mean=22.7±6.6) displayed significantly ($p<0.01$) more gaping than either group JZL195-VEH (mean=5.3±1.5) or JZL195-AM630 (mean=4.6±1.8). There were no significant differences in the locomotor test.

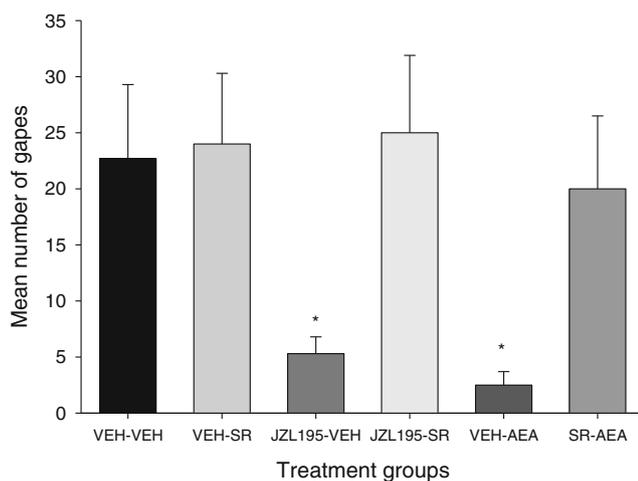


Fig. 3 Mean (\pm SEM) number of gapes elicited by the LiCl-paired context during the 5-min test by rats in the various groups. Both groups VEH-AEA ($n=8$) and JZL195-VEH ($n=9$) gaped significantly less than groups VEH-VEH ($n=8$), VEH-SR ($n=8$), SR-AEA ($n=8$), or JZL195-SR ($n=8$). * $p<0.05$

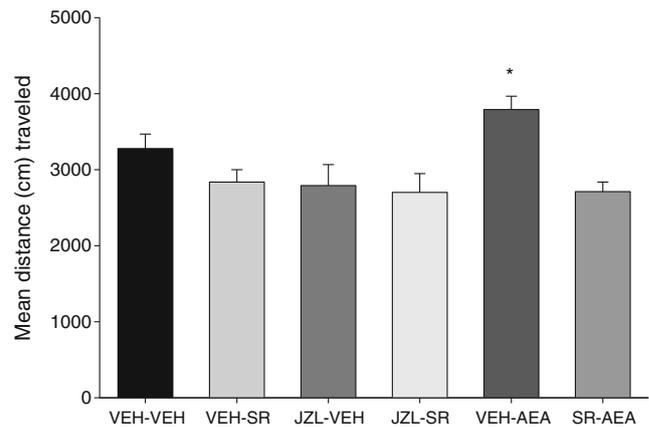


Fig. 4 Mean (\pm SEM) distance (centimeter) traveled during the 15-min locomotor activity test that immediately followed the AN test. Group VEH-AEA displayed elevated activity relative to all other groups ($p<0.05$), including SR-AEA

Discussion

As has previously been demonstrated with the FAAH inhibitor, URB597 (Rock et al 2008), pretreatment with the dual FAAH/MAGL inhibitor, JZL195 (10 mg kg⁻¹), reduced the contextually elicited LiCl-induced conditioned gaping in rats. This effect was CB₁ dependent because pretreatment with the CB₁ antagonist, SR141716, but not the CB₂ antagonist, AM630, reversed the suppressive effect of JZL195. Pretreatment with exogenous doses of AEA (5 mg kg⁻¹) and 2-AG (1.25 mg kg⁻¹), which have previously been shown to interfere with the establishment of nausea-induced gaping to a flavor cue (Cross-Mellor et al. 2007; Sticht et al. 2012), revealed that only AEA reduced AN. This effect was also reversed by pretreatment with SR141716. This finding suggests that the primary suppressive effect on contextually elicited gaping may be mediated by the inhibition of FAAH, rather than MAGL. However, the action of MAGL is also indicated because when combined with either AEA or 2-AG, the suppressive effect of JZL195 on AN was enhanced.

Evaluation of whole brain endocannabinoid levels collected following the AN and activity tests revealed that JZL195 on its own elevated the fatty acids, AEA, OEA, and PEA, but not 2-AG. The effect was most likely the result of AEA and not OEA or PEA which act as agonists of the α -PPAR receptor (e.g., Gaetani et al. 2008) because the suppressive effect of JZL195 on AN was reversed by CB₁ antagonism. Even when the dose of JZL195 was increased to 40 mg kg⁻¹, it did not elevate 2-AG relative to VEH. However, whole brain levels of AEA and 2-AG measured in the present study may not reflect the concentrations of these endogenous cannabinoids at key CB₁ receptors that mediate the blockade of conditional gaping produced by JZL195. For instance, Wiskerke et al (2012) found that JZL195 elevated 2-AG and AEA levels in the nucleus accumbens about 50 and 100 %, respectively.

When co-administered with either AEA or 2-AG, JZL195 elevated the respective EC. The suppression of LiCl-induced conditioned gaping by JZL195 was enhanced when it was combined with either AEA or 2-AG as well. Indeed, group JZL195-AEA displayed approximately a 75-fold increase in AEA relative to group VEH-AEA. Curiously, administration of exogenous anandamide to JZL195-treated rats also resulted in about a twofold increase in 2-AG brain levels. These findings suggest that JZL195 prevented the catabolism of the exogenously delivered AEA and concomitantly elevated 2-AG levels by possibly enhancing synthesis or interfering with catabolism of this endogenous cannabinoid. Similarly, group JZL195-2-AG elevated 2-AG by threefold relative to group VEH-2-AG; however, group JZL195-VEH did not differ from JZL195-2-AG. Collectively, this pattern of findings suggests that JZL195 may be more effective in inhibiting FAAH in the whole rat brain, but MAGL inhibition is also indicated. High bulk levels of 2-AG could mask detection of local changes in 2-AG when endocannabinoids are measured in whole brain samples. Therefore, a role for MAGL cannot be discounted on the basis of measurement of endocannabinoid content alone.

The locomotor activity test revealed that exogenously administered AEA produced slightly elevated activity level relative to VEH or 2-AG, regardless of JZL195 or VEH pretreatment. Since the effects of AEA on motor activity are biphasic, with low doses elevating activity and high doses suppressing activity (e.g., Sulcova et al. 1998), these results suggest that at least by the time of the locomotor test (approximately 20 min post-AEA injection), the brain concentration of AEA was similar to that of rats experiencing a low dose of AEA. These results may also reflect an anxiolytic effect of anandamide, indicated by increased exploration of an open field. The suppressed gaping produced by AEA was therefore not merely a function of decreased sensorimotor responding. The elevated locomotor activity by AEA was reversed by SR141716, indicating that it was a CB₁-mediated effect. As well, in experiment 1, group JZL195-VEH displayed slightly suppressed locomotor activity relative to VEH-VEH consistent with the findings of Long et al. (2009a).

The present findings confirm previous results indicating that FAAH inhibition reduces contextually elicited conditioned gaping (Rock et al. 2008) in rats as it also reduces acute nausea (Cross-Mellor et al. 2007) and vomiting in shrews (Parker et al. 2009b) and ferrets (van Sickle et al. 2005; Sharkey et al. 2007). Although inhibition of FAAH elevates multiple endocannabinoid-like molecules that show activity at multiple target receptors, the anti-emetic effects (similar to the anti-nausea effects reported here) were reversed by pretreatment with SR141716, indicating a CB₁ mechanism of action (e.g., see Parker et al. 2011).

The suppression of contextually elicited conditioned gaping by JZL195 is not likely simply the result of impaired retention of a previously learned response. FAAH inhibition by OL-135

and genetic deletion of FAAH in mice enhanced both acquisition and extinction of spatial learning in a fixed platform water maze task, and this enhancement was blocked by SR141716 (Varvel et al. 2007). The FAAH inhibitor URB597 also enhanced passive avoidance learning (Mazzola et al. 2009) when given prior to conditioning, but not when given prior to testing (as is the case in the present studies). The enhancement of learning by FAAH deletion appears to be selective to aversive tasks; in rewarding tasks, no effects on learning are detected (Wise et al. 2009). Although Wise et al. (2012) found that JZL195 disrupted short-term spatial memory in a water maze in mice, they also demonstrated elevation of both 2-AG and AEA, unlike our findings in rats. Thus, it is unlikely that the suppression of gaping elicited by the LiCl-paired context among the JZL195-treated animals was the result of a drug-induced deficit in reference memory.

As new inhibitors are developed that are more effective in suppression of MAGL in rats, there may be the opportunity to better evaluate the role of 2-AG in regulation of nausea in these rat models. We have previously shown that exogenous 2-AG suppresses acute nausea in rats (Sticht et al. 2012), but the effect was mediated by downstream metabolites of 2-AG, because (1) the suppression of LiCl-induced gaping was not reversed by the CB₁ antagonist AM251, and (2) the effect was reversed by the COX inhibitor, indomethacin. The MAGL inhibitor, JZL184, which is relatively ineffective in suppressing MAGL in rat tissue (Long et al. 2009b), was highly effective in suppressing vomiting by a CB₁ mechanism of action and in suppressing MAGL in the tissue of the *S. murinus* (Sticht et al. 2012). Indeed, when the catabolism of exogenous 2-AG was prevented by co-administration of the MAGL inhibitor, JZL184, the suppression of acute nausea in rats by 2-AG was reversed by a CB₁ antagonist. Although 2-AG has anti-emetic effects in ferrets (Van Sickle et al. 2005) and house musk shrews (Sticht et al. 2012, 2013), it appears to be pro-emetic in the least shrew (Darmani 2002; Darmani et al. 2005), indicating species differences in the role of the endocannabinoid system in the regulation of nausea and vomiting.

The development of treatments which boost the activity of the “on demand” endocannabinoid system may have promise in the regulation of both acute and anticipatory nausea in chemotherapy treatment of cancer. The treatment of AN is of particular interest, given that no selective treatments are currently available for this debilitating side effect of cancer treatment.

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