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Inhibitors of monoacylglycerol lipase, fatty-acid amide hydrolase and endocannabinoid transport differentially suppress capsaicin-induced behavioral sensitization through peripheral endocannabinoid mechanisms

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Abstract

Monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH) degrade the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), respectively. Pharmacological inhibition of these enzymes in the periphery may elucidate the role of endocannabinoids in controlling nociceptive transmission. We compared effects of the MGL inhibitor JZL184, the FAAH inhibitor URB597, and the endocannabinoid uptake inhibitor VDM11, administered locally in the paw, on behavioral hypersensitivities produced by capsaicin, the pungent ingredient in hot chili peppers. Intradermal capsaicin (10 μg i.pl.) produced nocifensive behavior, thermal hyperalgesia, and mechanical allodynia in rats. JZL184 (100 μg i.pl.) suppressed capsaicin-induced nocifensive behavior and thermal hyperalgesia without altering capsaicin-evoked mechanical allodynia. Effects of JZL184 were blocked by either the CB_1 antagonist AM251 (80 μg i.pl.) or the CB_2 antagonist AM630 (25 μg i.pl.). URB597 (75 μg i.pl.) suppressed capsaicin-induced mechanical allodynia without altering capsaicin-evoked thermal hyperalgesia or nocifensive behavior. Effects of URB597 were blocked by AM251 (80 μg i.pl.), but not by AM630 (25 μg i.pl.). VDM11 (100 μg i.pl.) suppressed capsaicin-evoked hypersensitivity for all three dependent measures (nocifensive behavior, thermal hyperalgesia, and mechanical allodynia), suggesting an additive effect following putative elevation of both AEA and 2-AG. The VDM11-induced suppression of capsaicin-evoked nocifensive behavior and thermal hyperalgesia was blocked by either AM251 (80 μg i.pl.) or AM630 (25 μg i.pl.), as observed with JZL184. The VDM11-induced suppression of capsaicin-evoked mechanical allodynia was blocked by AM251 (25 μg i.pl.) only, as observed with URB597. Thus, peripheral inhibition of enzymes hydrolyzing 2-AG and AEA suppresses capsaicin-evoked behavioral sensitization with distinct patterns of pharmacological specificity and in a non-overlapping and modality-specific manner. Modulation of endocannabinoids in the periphery suppressed capsaicin-evoked nocifensive behavior and thermal hyperalgesia through either CB_1 or CB_2 receptor mechanisms but suppressed capsaicin-evoked mechanical allodynia through CB_1 mechanisms only. Inhibition of endocannabinoid transport was more effective in suppressing capsaicin-induced sensitization compared to inhibition of either FAAH or MGL alone. These studies are the first to unveil the effects of pharmacologically increasing peripheral endocannabinoid levels on capsaicin-induced behavioral hypersensitivities. Our data suggest that 2-AG, the putative product

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Conflict of Interest
The authors declare that there is no conflict of interest.

of MGL inhibition, and AEA, the putative product of FAAH inhibition, differentially suppress capsaicin-induced nociception through peripheral cannabinoid mechanisms.

Keywords

endocannabinoid; monoacylglycerol lipase; fatty acid amide hydrolase; capsaicin; pain; endocannabinoid transport

1. INTRODUCTION

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the main pungent component of hot chili peppers, causes a burning sensation, pain, and inflammation. These characteristics make it a useful tool for studying inflammatory pain. When injected intradermally in rats, capsaicin produces hyperalgesia, defined as a decrease in pain threshold and/or an increase in pain levels in response to a normally painful stimulus [1]. Intradermal administration of capsaicin also produces nocifensive behavior in rats, characterized by licking, lifting, and guarding the injected paw [1]. Capsaicin-induced hyperalgesia is present in response to both radiant heat and mechanical stimulation. Primary hyperalgesia occurs at the site of injury and is mediated by peripheral C-fiber polymodal mechanoheat nociceptors [2] [3] [4] [5]. Secondary hyperalgesia is elicited in response to mechanical stimulation in regions surrounding the injury, and is mediated by central nervous system sensitization [4] [5] [6] as well as peripheral mechano-insensitive C-fibers [7]. Capsaicin produces hyperalgesia primarily through activation of the Transient Receptor Potential Vanilloid 1 (TRPV1) ion channel [8]. TRPV1 is a ligand-gated non-selective cation channel expressed in sensory neurons (for review see [9]). Ligands for TRPV1 such as exogenous capsaicin or protons (producing an acidic environment) decrease the temperature threshold for TRPV1 activation, producing a sensation of noxious heat, even at room temperature [10]. TRPV1 is required for inflammatory sensitization to noxious thermal stimuli; TRPV1 knockout mice failed to develop carrageenan-induced thermal hyperalgesia [11].

Cannabis has been used for centuries for its pain-relieving properties. The main active ingredient of cannabis, Δ^9-tetrahydrocannabinol, produces antinociception by binding to G protein-coupled CB_1 [12] [13] [14] and CB_2 [15] receptors. Cannabinoids produce antinociception in animal models of both acute and chronic pain (for review see [16]). Anandamide (AEA, arachidonylethanolamide) and 2-arachidonoylglycerol (2-AG) are endogenous ligands for the cannabinoid receptors. Activation of cannabinoid receptors by endocannabinoids produces antinociception [17] (for review see [18]). Endocannabinoid deactivation is controlled by distinct enzymes, although these enzymes are not selective for the endocannabinoid system. The enzyme fatty-acid amide hydrolase (FAAH) is responsible for hydrolysis of AEA into arachidonic acid and ethanolamine [19]. By contrast, the enzyme monoacylglycerol lipase (MAGL or MGL) is responsible for hydrolysis of 2-AG into fatty acid and glycerol [20]. AEA, but not 2-AG, is also an endogenous ligand for TRPV1 [21] [22]. Thus, AEA may act as an endocannabinoid to activate cannabinoid receptors to produce antinociception or as an endovanilloid at TRPV1 to produce hyperalgesia. Indeed, elevated AEA levels in the periaqueductal gray have been found to either suppress or enhance thermal nociception through TRPV1 and CB_1 mechanisms, depending on the dose [23]. In the periphery, exogenous AEA either reduces nocifensive behavior produced by capsaicin [24] or induces nocifensive behavior in the absence of capsaicin via TRPV1 activation [25].

Peripheral cannabinoid antinociceptive mechanisms involve CB_1 and CB_2 receptor activation [26] [27] [28] [29] [30] [31]. Less is known about the roles of peripheral CB_1 and CB_2 receptors in modulating capsaicin-induced sensitization. Local hindpaw injections of the CB_2-selective

agonist AM1241 reduce capsaicin-induced mechanical allodynia [32], as well as nocifensive behavior and thermal hyperalgesia [32] [33]. Intraplantar injections of the mixed CB_1/CB_2 agonist WIN55,212-2 attenuates thermal hyperalgesia, but not mechanical hyperalgesia or nocifensive behavior [34]. Similarly, intraplantar injections of WIN55,212-2 reduced mechanical and thermal hypersensitivities in response to heat injury (but not capsaicin) via CB_1- and CB_2-dependent mechanisms [35]. However, the impact of elevating endocannabinoids in the periphery on a TRPV1-activated model of pain initiation remains unknown.

Pharmacological inhibition of FAAH and MGL exhibits therapeutic potential in inflammatory pain models (for review see [36]) [31] [37] [38] [39]. The recent development of JZL184, a potent selective MGL inhibitor, offers the potential to elucidate the role of peripheral 2-AG in controlling nociceptive transmission [40]. When injected systemically, the MGL inhibitor JZL184 and the FAAH inhibitor URB597 decreased nerve injury-induced mechanical and cold allodynia via CB_1- and/or CB_2-dependent mechanisms [41]. Pharmacological inhibitors of the endocannabinoid degrading enzymes MGL and FAAH preferentially increase accumulation of distinct endocannabinoids but also other lipid mediators that do not bind to cannabinoid receptors [19] [23] [40] [42]. Thus, inhibitors of endocannabinoid deactivation are not specific for endocannabinoids. Mediation by cannabinoid receptors requires the demonstration that such effects are blocked by cannabinoid receptor antagonists, and cannot be accounted for by actions of other fatty-acid amides or monoacylglycerols that do not bind to cannabinoid receptors. For example, inhibition of FAAH also elevates levels of the fatty-acid amide palmitoylethanolamine which suppress nociception independently of CB_1 receptors through activation of proliferator peroxisome receptor-α [43].

In the present study, we compared effects of pharmacological inhibition of MGL (with JZL184), FAAH (with URB597) and endocannabinoid uptake (with VDM11), at the peripheral level, on behavioral sensitization evoked by intradermal administration of capsaicin. Nocifensive behavior, thermal hyperalgesia, and mechanical allodynia were quantified in response to drug- or vehicle-pretreatment and capsaicin administration. The CB_1-selective antagonist AM251 and the CB_2-selective antagonist AM630 were coadministered with the agonists to evaluate the specific receptor subtypes underlying antihyperalgesic and antiallodynic effects of endocannabinoid modulators. The present studies are the first to unveil the effects of pharmacologically increasing endocannabinoid levels in the periphery on nociception produced by intradermal capsaicin administration.

2. MATERIALS AND METHODS

2.1. Subjects

Two hundred and nineteen male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 260-350 g were used in behavioral experiments. Rats were allowed unlimited access to food and water, and were housed on a 12 h light/dark cycle. The experimental research protocols were approved by The University of Georgia Animal Care and Use Committee. All procedures followed the guidelines for the treatment of animals according to the International Association for the Study of Pain [44].

2.2. Drugs and Chemicals

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved (1 mg/mL) in a vehicle of 7% Tween 80 in 0.9% saline, sonicated, and filtered as described previously [1]. VDM11 [N-(4-hydroxy-2-methylphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide], URB597 [(3′-(aminocarbonyl)[1,1′-biphenyl]-3-yl)-cyclohexyl carbamate], JZL184 [4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxyl)methyl)

piperidine-1-carboxylate], AM251 [1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide] and AM630 [[6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)-methanone] were purchased from Cayman Chemical (Ann Arbor, MI). Doses of AM251 and AM630 were those used by Guindon et al. [31]. AM251, AM630, VDM11, and URB597 were dissolved in a 1:1:1:17 ratio of DMSO:ethanol:cremophor:saline. JZL184 was dissolved in a 4:1 ratio of polyethylene glycol 300:Tween 80, as described previously [40]. The vehicles employed were those used previously [31] [40] [45]. In order to evaluate pharmacological specificity, the highest doses of VDM11, JZL184, and URB597 were coadministered in cocktails with AM251 or AM630. The volume of drug or vehicle administered in the paw (i.e. either alone or in combination with antagonists) was 50 μL in all studies.

2.3. Behavioral Testing

Responsiveness to thermal and mechanical stimulation was examined in separate groups of rats to prevent development of behavioral sensitization to the stimuli. The same animals were used to evaluate thermal hyperalgesia and nocifensive behavior.

Thermal hyperalgesia was determined using the radiant heat method [46]. Rats were placed on an elevated glass platform in individual plastic cages. Radiant heat was applied through the glass to the midplantar surface of the right and left hindpaws. Rats were allowed to habituate to the apparatus for at least 15 minutes until exploratory behavior was no longer observed. Stable baseline latencies (about 12 seconds) were obtained prior to drug or vehicle administration. A ceiling latency of 20 seconds was employed to prevent tissue damage to the hindpaws. Fifteen minutes prior to capsaicin administration, rats received an intraplantar injection in the right (ipsilateral to capsaicin injection) hindpaw of one of the following: VDM11 (100 μg i.pl.; n = 8), JZL184 (1, 10, or 100 μg i.pl.; n = 6 – 8 per group), URB597 (5 or 75 μg i.pl.; n = 6 per group), AM251 (80 μg i.pl.; n = 6), AM630 (25 μg i.pl.; n = 6), or an agonist/antagonist cocktail consisting of VDM11 (100 μg i.pl.) coadministered with AM251 (80 μg i.pl.), VDM11 coadministered with AM630 (25 μg i.pl.) (n = 6 per group), JZL184 (100 μg i.pl.) coadministered with AM251 (80 μg i.pl.), or JZL184 (100 μg i.pl.) coadministered with AM630 (25 μg) (n = 6 per group). Separate groups received vehicle consisting of either a 1:1:1:17 ratio of DMSO:ethanol:cremophor:saline (n = 8) or a 4:1 ratio of polyethylene glycol 300:Tween 80 (n = 8). Separate groups of rats received an injection in the left hindpaw (contralateral to capsaicin injection) of one of the following: VDM11 (100 μg i.pl.), JZL184 (100 μg i.pl.), or 4:1 vehicle (50 μL) (n = 5 - 6 per group). At time 0, capsaicin (10 μg) was injected (10 μL) into the plantar surface of the right (ipsilateral) hindpaw. The amount of time (in seconds) that rats spent displaying nocifensive behavior (i.e. guarding, licking, or lifting the injected paw) was quantified for 5 minutes beginning immediately after capsaicin injection [1]. Thermal withdrawal latencies were recorded at 5, 15, 30, 45, and 60 minutes after capsaicin injection. The fifteen minute time interval between drug pretreatment and capsaicin injection was selected based upon previous work documenting peripheral antinociceptive effects of FAAH and MGL inhibitors at the same time point [31] [47].

Paw withdrawal thresholds to mechanical stimulation were measured using an electronic Von Frey device (IITC Life Sciences, Woodland Hills, CA). Rats were placed in individual plastic cages on an elevated wire mesh platform, and were allowed to habituate for at least 15 minutes until exploratory behavior was no longer observed. A rigid tip was applied in duplicate to the midplantar region of the left and right hindpaws before and after capsaicin administration. Mechanical stimulation was terminated when the paw was withdrawn. Stable baselines were obtained prior to experimental testing. Fifteen minutes prior to capsaicin administration, rats received an intraplantar injection (50 μL) in the right (ipsilateral) hindpaw of one of the following: VDM11 (100 μg i.pl.; n = 8), JZL184 (1, 10 or 100 μg i.pl.; n = 6 – 8 per group),

URB597 (5 or 75 μg i.pl.; n = 6 – 8 per group), AM251 (80 μg i.pl.; n = 6), AM630 (25 μg i.pl.; n = 6), or drug/antagonist cocktails (n = 6 per group) consisting of VDM11 (100 μg i.pl.) coadministered with AM251 (80 μg i.pl.), VDM11 (100 μg i.pl.) coadministered with AM630 (25 μg i.pl.), URB597 (75 μg i.pl.) coadministered with AM251 (80 μg i.pl.), or URB597 (75 μg i.pl.) coadministered with AM630 (25 μg i.pl.). Separate groups of rats received either vehicle consisting of a 1:1:1:17 ratio of DMSO:ethanol:cremophor:saline (n = 12) or vehicle consisting of a 4:1 ratio of polyethylene glycol 300:Tween 80 (n = 8). Separate groups of rats received an injection in the left (contralateral) hindpaw of either VDM11 (100 μg i.pl.; n = 6), or URB597 (75 μg i.pl.; n = 6). Capsaicin (10 μg/10 μL) was subsequently injected into the plantar surface of the right (ipsilateral) hindpaw. Mechanical withdrawal thresholds were assessed at 5, 30, 60, and 120 minutes after capsaicin injections.

2.4. Statistical Analysis

Mechanical paw withdrawal thresholds and thermal paw withdrawal latencies were determined in duplicate at each time point and averaged for each paw separately. Thermal paw withdrawal latencies and mechanical paw withdrawal thresholds were analyzed separately in the ipsilateral and contralateral hindpaws. Data obtained from thermal and Von Frey testing were analyzed by repeated measures analysis of variance (ANOVA). When sphericity determined by Mauchly's sphericity test was not assumed, the Greenhouse-Geisser correction factor was applied to all repeated factors. The sources of significant interactions were further evaluated by peforming one-way ANOVAs at each individual time point, followed by Tukey post hoc tests. Planned comparisons were performed using independent samples t-tests (one-tailed). Nocifensive behavior was analyzed using univariate ANOVA and planned comparison independent samples t-tests, one- or two-tailed as appropriate. Tukey post hocs were performed on all Univariate ANOVAs. $P \leq 0.05$ was considered statistically significant.

3. RESULTS

3.1. Control Conditions

Thermal paw withdrawal latencies and mechanical paw withdrawal thresholds were similar between groups prior to capsaicin treatment. Intradermal capsaicin produced nocifensive behavior in animals receiving vehicle (lasting 181.25 ± 11.3 s in response to 1:1:1:17 vehicle pretreatment and 244.25 ± 12 s in response to 4:1 vehicle pretreatment). Capsaicin also decreased thermal paw withdrawal latencies (by 54.2% (1:1:1:17 vehicle) and 65.4% (4:1 vehicle)), relative to baseline, at the time of maximal thermal hyperalgesia. Likewise, capsaicin lowered mechanical paw withdrawal thresholds, relative to baseline, in vehicle-treated animals (by 59.0% (1:1:1:17) and 64.2% (4:1 vehicle)). In all studies, pharmacological manipulations did not alter thermal paw withdrawal latencies or mechanical paw withdrawal thresholds, relative to vehicle, in the hindpaw contralateral to capsaicin treatment ($P > 0.05$), unless stated.

3.2. MGL inhibition via JZL184

The highest dose of JZL184 (100 μg i.pl.) suppressed capsaicin-evoked nocifensive behavior (by 19.9%) compared to vehicle ($F_{3,24} = 4.637$, $P = 0.011$; Fig. 1a), whereas lower doses were without effect. JZL184 suppressed the magnitude ($F_{3,24} = 9.996$, $P = 0.0002$; Fig. 1b) and time course ($F_{15,120} = 1.864$, $P = 0.034$) of thermal hyperalgesia. The antihyperalgesic effects produced by the high dose of JZL184 (100 μg i.pl.) outlasted that of the middle dose of JZL184 (10 μg i.pl.) ($P = 0.032$). However, intraplantar administration of JZL184 did not alter mechanical paw withdrawal thresholds, relative to vehicle, at any dose ($P = 0.64$; Fig. 1c).

A behaviorally active dose of JZL184 (100 μg i.pl.), administered to the contralateral paw, did not alter capsaicin-evoked nocifensive behavior relative to contralateral paw injections of vehicle ($P = 0.4005$; Fig. 2a). However, the vehicle itself reliably increased capsaicin-evoked

nocifensive behavior ($P = 0.0052$, two-tailed t-test); nocifensive behavior was higher in groups receiving the 4:1 vehicle in the ipsilateral compared to the contralateral paw ($F_{3,23} = 3.299$, $P = 0.038$; Tukey post hoc). Capsaicin-evoked nocifensive behavior was also similar in groups receiving ipsilateral paw injections of JZL184 and contralateral paw injections of JZL184 or vehicle. JZL184 (100 μg i.pl.), administered to the capsaicin-injected paw, suppressed the magnitude ($F_{2,19} = 12.077$, $P = 0.0004$) and time course ($F_{10,95} = 3.349$, $P = 0.001$; Fig. 2b) of capsaicin-evoked thermal hyperalgesia relative to either vehicle ($P = 0.0001$, Tukey post hoc) or the same dose of JZL184 administered to the contralateral paw ($P = 0.017$, Tukey post hoc).

To establish pharmacological specificity, the CB_1 antagonist AM251 (80 μg i.pl.) and the CB_2 antagonist AM630 (25 μg i.pl.), were coadministered with the highest effective dose of JZL184 (100 μg i.pl). The JZL184-induced attenuation of nocifensive behavior ($F_{3,24} = 5.994$, $P = 0.003$) and thermal hyperalgesia ($F_{3,24} = 8.869$, $P = 0.0004$) was blocked by either AM251 ($P = 0.011$; Tukey post hoc) or AM630 ($P = 0.004$; Tukey post hoc). This blockade was also time-dependent ($F_{15,120} = 2.26$, $P = 0.008$; Fig. 3a and 3b). Intraplantar injections of AM251 or AM630 alone had no effect on either nocifensive behavior ($P = 0.64$; Fig. 3c) or thermal paw withdrawal latencies ($P = 0.78$; Fig. 3d) relative to vehicle. However, AM251 produced a modest decrease in thermal paw withdrawal latencies, relative to vehicle, in the hindpaw contralateral to capsaicin injection ($P = 0.033$ versus vehicle, Tukey post hoc; data not shown).

3.3. FAAH Inhibition via URB597

The FAAH inhibitor URB597 did not alter capsaicin-evoked nocifensive behavior at any dose ($P = 0.35$; Fig. 4a). Similarly, URB597 did not alter the magnitude ($P = 0.488$) or time course ($P = 0.096$; Fig. 4b) of capsaicin-evoked thermal hyperalgesia, relative to vehicle. By contrast, URB597 (75 μg i.pl.) reliably suppressed capsaicin-evoked mechanical hypersensitivity throughout the observation interval; this suppression ($F_{2,21} = 8.915$, $P = 0.002$; Fig. 4c) was observed relative to either vehicle ($P = 0.001$; Tukey post hoc) or the low dose (5 μg i.pl.) of URB597 ($P = 0.036$; Tukey post hoc).

The anti-allodynic effects of URB597 (75 μg i.pl.) were mediated by a local site of action. URB597, administered to the capsaicin-injected paw, suppressed capsaicin-evoked mechanical allodynia ($F_{2,21} = 12.136$, $P = 0.0003$; Fig. 5a) relative to either vehicle ($P = 0.002$; Tukey post hoc), administered to the same paw, or URB597 ($P = 0.0001$; Tukey post hoc), administered to the opposite paw.

The URB597-induced suppression of capsaicin-evoked mechanical allodynia was blocked ($F_{3,26} = 6.291$, $P = 0.002$; Fig. 5b) by a CB_1 but not a CB_2 antagonist. URB597 suppressed mechanical allodynia relative to either vehicle ($P = 0.003$; Tukey post hoc) or URB597 coadministered with AM251 ($P = 0.006$; Tukey post hoc). By contrast, the CB_2 antagonist failed to block the anti-allodynic effects of URB597 ($P = 0.184$). When administered alone, neither AM251 nor AM630 significantly altered capsaicin-evoked mechanical allodynia relative to vehicle ($P = 0.24$; Fig. 5c).

3.4. Endocannabinoid Uptake Inhibition via VDM11

VDM11 (100 μg i.pl.), administered to the capsaicin-injected (ipsilateral) paw, decreased ($F_{2,19} = 56.41$, $P = 0.0001$) capsaicin-evoked nocifensive behavior (by 73.5%) compared to either vehicle ($P = 0.0001$ Tukey post hoc; Fig. 6a) or the same dose administered to the contralateral paw ($P = 0.0001$ Tukey post hoc). VDM11 suppressed both the magnitude ($F_{2,19} = 5.334$, $P = 0.015$) and time course ($F_{10,95} = 5.421$, $P = 0.0001$; Fig. 6b) of thermal hyperalgesia. The suppressive effect of VDM11 was maximal from 5 – 15 minutes post-capsaicin. VDM11 normalized thermal withdrawal latencies relative to baseline at five minutes

post-capsaicin ($P = 0.57$; paired two-tailed t-test). At 30 min post-capsaicin, thermal hyperalgesia was lower following VDM11 administration ipsilateral, as opposed to contralateral, to the capsaicin-injected paw ($P < 0.05$; Tukey post hoc).

VDM11 (100 μg i.pl.), administered to the capsaicin-injected paw ($F_{2,23}$=19.076, $P = 0.0001$; Fig. 6c), also suppressed mechanical allodynia; this suppression was observed relative to either vehicle ($P = 0.0001$; Tukey post hoc) or the same dose of VDM11 administered to the contralateral paw ($P = 0.001$; Tukey post hoc). This suppression was also time-dependent ($F_{8,92} = 3.837$, $P = 0.001$); VDM11 robustly suppressed mechanical allodynia at 5 ($F_{2,25} = 20.641$, $P = 0.0001$) and 30 ($F_{2,25} = 9.105$, $P = 0.001$) minutes post-capsaicin (Fig. 6c).

The VDM11-induced suppression of capsaicin-evoked nocifensive behavior ($F_{3,24} = 19.511$, $P = 0.0001$; Fig. 7a) was blocked by either the CB_1 antagonist AM251 ($P = 0.0001$; Tukey post hoc) or the CB_2 antagonist AM630 ($P = 0.0001$; Tukey post hoc). Similarly, both AM251 and AM630 blocked the VDM11-induced suppression of thermal hyperalgesia ($F_{3,24} = 5.851$, $P = 0.004$). This blockade was maximal between 5 and 30 min post-capsaicin ($P < 0.05$ for all time points; Fig. 7b). The VDM11-induced suppression of capsaicin-evoked mechanical allodynia ($F_{3,28} = 8.205$, $P = 0.0004$) was blocked by a CB_1 ($P = 0.026$; Tukey post hoc) but not a CB_2 antagonist ($P = 0.796$; Tukey post hoc; Fig. 7c). All groups exhibited a time-dependent blockade of capsaicin-evoked mechanical allodynia ($F_{3,31}$=13.322, $P = 0.0001$) that was maximal at 5 min post capsaicin (Fig. 7c). At 30 min post injection, VDM11 coadministered with AM630 produced a transient but reliable ($P = 0.02$; Tukey post hoc) decrease in thermal withdrawal latencies in the contralateral paw ($F_{12,112} = 1.399$, $P = 0.02$; data not shown).

4. DISCUSSION

4.1. Overview

Local injection of the MGL inhibitor JZL184, the FAAH inhibitor URB597, and the endocannabinoid uptake inhibitor VDM11 differentially suppressed capsaicin-evoked behavioral sensitization through peripheral cannabinoid mechanisms. These suppressions occurred in a modality-specific manner and were mediated by distinct cannabinoid receptor subtypes. Our results provide indirect support for the hypothesis that endocannabinoids in the periphery differentially suppress pain transmission initiated by intradermal capsaicin administration, effects likely to be dependent upon the specific endocannabinoid elevated (see Table 1).

In our study, intraplantar injections of capsaicin produced nocifensive behavior, thermal hyperalgesia, and mechanical allodynia in the injected paw, as described previously [1]. Local injections of endocannabinoid modulators ipsilateral, but not contralateral, to the site of injury suppressed capsaicin-evoked behavioral hypersensitivities compared to control conditions. Thus, effects of the drug manipulations employed here were mediated by a peripheral mechanism. On the whole, responses to mechanical and thermal stimulation in the paws contralateral to capsaicin were rarely different between groups. Minor exceptions may be attributed to changes in weight-bearing resulting from capsaicin injection, or by normal variation between rats. These data are in agreement with previous studies demonstrating the importance of peripheral mechanisms of cannabinoid antihyperalgesic action in other pain models [26] [27] [28] [29] [30] [31] [48] [49].

4.2. Effects of the MGL inhibitor JZL184

The MGL inhibitor JZL184 suppressed capsaicin-evoked thermal hyperalgesia and nocifensive behavior, presumably by elevating endogenous levels of 2-AG. These data are in agreement

with previous studies demonstrating antihyperalgesic effects of exogenous 2-AG and MGL inhibitors, administered locally in the paw [47]. However, mechanical allodynia is also suppressed by MGL inhibitors, administered systemically, in neuropathic pain models [41]. Neuropathic pain may elevate levels of endocannabinoid tone [50] [51] and produce regulatory changes in endocannabinoid hydrolyzing enzymes and their receptors [52]; such changes may facilitate cannabinoid-mediated attenuations of nerve injury-induced mechanical allodynia. However, in the present study, JZL184 failed to suppress capsaicin-evoked mechanical allodynia. It is noteworthy that the vehicle used here to dissolve JZL184 produced edema and enhanced capsaicin-induced nocifensive behavior (see [40] for vehicle description). Edema in conjunction with capsaicin may mask the magnitude of the JZL184-induced suppression of nocifensive behavior. However, it is important to emphasize that any pronociceptive effects produced by this vehicle did not prevent detection of JZL184-induced suppression of capsaicin-evoked nocifensive behavior. Moreover, mechanical paw withdrawal thresholds and thermal paw withdrawal latencies were similar in capsaicin-treated animals receiving the vehicle for either JZL184 or URB597; thus, the choice of vehicle employed for JZL184 is unlikely to confound interpretation of antihyperalgesic or antiallodynic effects of MGL inhibition. Doses of JZL184 employed here were selected based upon dose-response studies performed in the formalin test (unpublished data). Further dose escalation was prevented by limitations in drug solubility. It is, nonetheless, unlikely that a higher dose of JZL184 was required to observe suppression of mechanical allodynia because thermal hyperalgesia was profoundly suppressed by JZL184 at the same time points.

The exact anatomical localization of MGL in the periphery remains unknown. MGL is localized exclusively to presynaptic sites in brain [53] and has also been localized to microglial cells [54]. Thus, both neuronal and nonneuronal cells may be targeted by local injection of JZL184 into the paw. Our studies suggest that under our conditions, 2-AG, the putative product of MGL inhibition, is unlikely to target primary afferents that contribute to capsaicin-evoked mechanical allodynia at the site of injury. Following peripheral nerve damage, FAAH is known to transition from small- to large-sized cells of dorsal root ganglia [52]. It is, therefore, possible that MGL undergoes similar phenotypic switches in response to long term injury. Phenotypic switches in MGL expression following nerve injury could contribute to differences observed between effects of MGL inhibitors on mechanical allodynia in neuropathic pain models [41] [47] and the present capsaicin model. However, the development of capsaicin-induced hypersensitivity follows a rapid time course which likely precludes such downstream changes from contributing to the pattern of results observed here. Our studies indicate that endocannabinoid modulators produce a modality-specific suppression of capsaicin-evoked nocifensive behavior and thermal hyperalgesia through activation of peripheral CB_1 and CB_2 receptors. These observations are consistent with the ability of 2-AG, a product of MGL inhibition, to preferentially bind to CB_2 receptors, relative to AEA, a product of FAAH inhibition [55].

4.3. Effects of the FAAH inhibitor URB597

The FAAH inhibitor URB597 suppressed capsaicin-evoked mechanical allodynia, presumably by elevating levels of endogenous AEA. However, URB597, administered locally in the paw, did not alter capsaicin-evoked thermal hyperalgesia or nocifensive behavior under identical conditions. Local inhibition of FAAH in the periphery also suppresses mechanical allodynia in a neuropathic pain model [47]. Systemically-administered URB597 suppresses thermal hyperalgesia in a model of neuropathic pain [47] but suppresses peripheral edema without altering thermal hyperalgesia in the carrageenan model of inflammatory pain [37]. Transition of FAAH from smaller to larger cell sizes in dorsal root ganglia following peripheral nerve injury [52] may explain the differences observed between neuropathic pain and capsaicin/carrageenan models. Exogenous AEA both suppresses and induces nociception in a variety of

pain models via CB_1 and TRPV1 activation, respectively [24] [25] [56]. The failure of peripheral FAAH inhibition to suppress capsaicin-evoked thermal hyperalgesia or nocifensive behavior may perhaps be accounted for by the dual role of AEA as an endocannabinoid and endovanilloid [21] [22]. TRPV1 activation is required for thermal hyperalgesia [11]. In naive mice, 68% of FAAH expressing cells colabel with antibodies to TRPV1 [52]. Capsaicin-induced TRPV1 activation may prevent suppression of thermal hyperalgesia by endogenous AEA, the putative product of FAAH inhibition. Activation of TRPV1 receptors by capsaicin may render agonist effects of AEA at TRPV1 insignificant; thus, only agonism at CB_1 receptors is observed, resulting in suppression of mechanical allodynia. Previous studies have observed endocannabinoid-modulating activity with similar doses of URB597; thus, the local dose employed here is unlikely to be too low to effectively suppress thermal hypersensitivity and nocifensive behavior [47] [57]. Doses of URB597 higher than 75 μg i.pl. were not evaluated due to limits in drug solubility.

The URB597-induced suppression of mechanical allodynia was mediated by a peripheral CB_1 mechanism. The lower affinity of AEA, relative to 2-AG, for CB_2 [55] may account for the observation. CB_1 receptors are synthesized in dorsal root ganglion cells of heterogeneous size [51] [58] [59] and are transported to peripheral terminals [60]. Colocalization of CB_2 with TRPV1 on small cells of the dorsal root ganglion has also been reported [61]. It is also possible that endocannabinoid-induced activation of CB_2 receptors is masked by capsaicin-induced activation of TRPV1 on small cells. By contrast, AEA mobilization produced by FAAH inhibition may preferentially activate CB_1 receptors localized to medium- and larger-diameter (i.e. myelinated) fibers, independently of TRPV1 activation. Biological conditions (e.g. severe tissue acidosis) exist where TRPV1 is tonically activated (for review see [62]). Our studies suggest that in situations where TRPV1 is selectively activated, peripheral AEA may reduce mechanical allodynia via CB_1 receptor activation only. Of course, endocannabinoid modulators may produce different effects in other inflammatory or neuropathic pain models.

4.4. Effects of the endocannabinoid transport inhibitor VDM11

The existence of an endocannabinoid membrane transporter has remained controversial [63]. Endocannabinoid uptake inhibitors produce pharmacological effects in FAAH knockout mice, suggesting that endocannabinoid uptake is not dependent on FAAH [64]. The recent identification of fatty acid binding proteins that transport AEA across cell membranes [65] provide further evidence that transport inhibitors block endocannabinoid uptake. VDM11 was specifically employed in these experiments because it has very little agonist activity at TRPV1 [66], unlike other uptake inhibitors such as AM404 [67]. Moreover, VDM11 may be employed *in vivo* to increase levels of both AEA and 2-AG [42]. In the present studies, VDM11 suppressed capsaicin-evoked nocifensive behavior and thermal hyperalgesia as well as mechanical allodynia. VDM11 suppressed nocifensive behavior and thermal hyperalgesia via a CB_1/CB_2-mediated mechanism, similar to that observed following local administration of the MGL inhibitor in the paw. Moreover, VDM11 additionally suppressed capsaicin-evoked mechanical allodynia via a peripheral CB_1-mediated mechanism, similar to that observed following local administration of the FAAH inhibitor. It is reasonable to speculate that MGL inhibition preferentially increases 2-AG levels whereas FAAH inhibition preferentially elevates AEA levels, and that inhibition of endocannabinoid transport presumably suppresses all three dependent measures by elevating levels of both AEA and 2-AG. Thus, VDM11, administered locally in the paw, produces a pattern of pharmacological effects that is mimicked by those induced by inhibitors of both MGL and FAAH. Further biochemical studies are required to confirm this hypothesis. Elevation of each endocannabinoid is thus likely to produce distinct effects in modulating the behavioral response to capsaicin. Our studies also suggest that capsaicin-induced mechanical allodynia is more effectively suppressed by VDM11 than by URB597. Additional dose-response studies are required to definitively test this hypothesis.

The ability of VDM11 to block AEA's ability to cross the membrane and bind TRPV1 at its intracellular site [68] may also contribute to the pattern of effects observed herein.

4.5. Conclusion

In conclusion, pharmacological elevation of endocannabinoids in the periphery suppresses capsaicin-induced behavioral hypersensitivity via distinct cannabinoid receptor mechanisms (see Table 1). Inhibition of MGL suppresses capsaicin-induced nocifensive behavior and thermal hyperalgesia presumably by increasing accumulation of 2-AG. By contrast, inhibition of FAAH suppresses capsaicin-induced mechanical allodynia only, presumably by increasing accumulation of AEA. Inhibition of endocannabinoid uptake displays an additive effect, mimicking actions of both the FAAH and MGL inhibitor in combination, presumably by increasing accumulation of both 2-AG and AEA. Of course, it is important to emphasize that FAAH and MGL inhibitors are not selective for the endocannabinoid system. Nonetheless, the present studies identify specific roles for peripheral CB_1 and CB_2 receptors in mediating effects of FAAH and MGL inhibitors on pain initiation. Inhibition of endocannabinoid uptake suppresses capsaicin-evoked behavioral sensitization with a profile of pharmacological specificity that is, again, mimicked by inhibition of both MGL and FAAH. Future studies are necessary to further elucidate the localization of endocannabinoid degrading enzymes (i.e. FAAH, MGL), their lipid substrates (i.e. AEA, fatty-acid amides, 2-AG, monoacylglycerols) and receptors (i.e. CB_1, CB_2) in the periphery, as well as their possible modulation by pathological pain. These studies are the first to document that pharmacological inhibition of endocannabinoid uptake and degradation suppresses capsaicin-induced behavioral sensitization. Moreover, inhibition of endocannabinoid transport at the site of injury in the periphery may prove to be more therapeutically beneficial than targeting inhibition of either FAAH or MGL alone.

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Abbreviations

2-AG	2-arachidonoylglycerol
AEA	anandamide
MAGL	monoacylglycerol lipase
MGL	monoacylglycerol lipase
FAAH	fatty acid amide hydrolase
i.pl.	intraplantar
CB_1	type 1 cannabinoid receptor
CB_2	type 2 cannabinoid receptor
TRPV1	Transient Receptor Potential Vanilloid 1
ANOVA	analysis of variance

REFERENCES

1. Gilchrist HD, Allard BL, Simone DA. Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. Pain 1996;67:179–88. [PubMed: 8895246]

2. Kenins P. Responses of single nerve fibres to capsaicin applied to the skin. Neurosci Lett 1982;29:83–8. [PubMed: 7070716]

3. Szolcsanyi J, Anton F, Reeh PW, Handwerker HO. Selective excitation by capsaicin of mechano-heat sensitive nociceptors in rat skin. Brain Res 1988;446:262–8. [PubMed: 3370489]

4. Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J Neurophysiol 1991;66:212–27. [PubMed: 1919668]

5. LaMotte RH, Lundberg LE, Torebjork HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol 1992;448:749–64. [PubMed: 1593488]

6. Torebjork HE, Lundberg LE, LaMotte RH. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol 1992;448:765–80. [PubMed: 1593489]

7. Serra J, Campero M, Bostock H, Ochoa J. Two types of C nociceptors in human skin and their behavior in areas of capsaicin-induced secondary hyperalgesia. J Neurophysiol 2004;91:2770–81. [PubMed: 14762154]

8. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389:816–24. [PubMed: 9349813]

9. Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev 1991;51:159–212. [PubMed: 10353985]

10. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998;21:531–43. [PubMed: 9768840]

11. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, et al. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 2000;405:183–7. [PubMed: 10821274]

12. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–4. [PubMed: 2165569]

13. Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 1999;283:401–4. [PubMed: 9888857]

14. Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci USA 1999;96:5780–5. [PubMed: 10318961]

15. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5. [PubMed: 7689702]

16. Walker JM, Hohmann AG. Cannabinoid mechanisms of pain suppression. Handb Exp Pharmacol 2005;168:509–54. [PubMed: 16596786]

17. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9. [PubMed: 1470919]

18. Piomelli D. The molecular logic of endocannabinoid signaling. Nat Rev Neurosci 2003;4:873–84. [PubMed: 14595399]

19. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83–7. [PubMed: 8900284]

20. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoacylglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 2002;99:10819–24. [PubMed: 12136125]

21. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999;400:452–7. [PubMed: 10440374]

22. Al-Hayani A, Wease KN, Ross RA, Pertwee RG, Davis SN. The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. Neuropharmacology 2001;41:1000–5. [PubMed: 11747904]

23. Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M, et al. Elevation of endocannabinoid levels in the ventrolateral periaqueductal gray through inhibition of fatty acid amide

hydrolase affects descending nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors. J Pharmacol Exp Ther 2006;316:969–82. [PubMed: 16284279]

24. Calignano A, La Rana G, Piomelli D. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. Eur J Pharmacol 2001;419:191–8. [PubMed: 11426841]
25. Potenzieri C, Brink TS, Simone DA. Excitation of cutaneous C nociceptors by intraplantar administration of anandamide. Brain Res 2009;1268:38–47. [PubMed: 19285051]
26. Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, et al. Cannabinoids mediate analgesia largely via peripheral type I cannabinoid receptors in nociceptors. Nat Neurosci 2007;10:870–9. [PubMed: 17558404]
27. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–81. [PubMed: 9685157]
28. Richardson JD, Kilo S, Hargreaves KM. Cannabinoid reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 1998;75:111–9. [PubMed: 9539680]
29. Guindon J, LoVerme J, De Lean A, Piomelli D, Beaulieu P. Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: a role for endogenous fatty-acid ethanolamides? Eur J Pharmacol 2006;550:68–77. [PubMed: 17027744]
30. Guindon J, De Lean A, Beaulieu P. Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. Pain 2006;121:85–93. [PubMed: 16480822]
31. Guindon J, Desroches J, Beaulieu P. The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors. Br J Pharmacol 2007;150:693–701. [PubMed: 17179944]
32. Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. J Pharmacol Exp Ther 2004;308:446–53. [PubMed: 14610224]
33. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, et al. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. Anesthesiology 2003;99:955–60. [PubMed: 14508331]
34. Johanek LM, Heitmiller DR, Turner M, Nader N, Hodges J, Simone DA. Cannabinoids attenuate capsaicin-evoked hyperalgesia through spinal and peripheral mechanisms. Pain 2001;93:303–15. [PubMed: 11514089]
35. Johanek LM, Simone DA. Activation of peripheral cannabinoid receptors attenuates cutaneous hyperalgesia produced by a heat injury. Pain 2004;109:432–42. [PubMed: 15157704]
36. Jhaveri MD, Richardson D, Chapman V. Endocannabinoid metabolism and uptake: novel targets for neuropathic and inflammatory pain. Br J Pharmacol 2007;152:624–32. [PubMed: 17704819]
37. Costa B, Bettoni I, Petrosino S, Comelli F, Giagnoni G, Di Marzo V. The dual fatty acid amide hydrolase/TRPV1 blocker, N-arachidonoyl-serotonin, relieves carrageenan-induced inflammation and hyperalgesia in mice. Pharmacol Res. 2010 Epub ahead of print.
38. Maione S, De Petrocellis L, de Novellis V, Moriello AS, Petrosino S, Palazzo E, et al. Analgesic actions of N-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. Br J Pharmacol 2007;150:766–81. [PubMed: 17279090]
39. Jhaveri MD, Richardson D, Robinson I, Garle MJ, Patel A, Sun Y, et al. Inhibition of fatty acid amide hydrolase and cyclooxygenase-2 increases levels of endocannabinoid related molecules and produces analgesia via peroxisome proliferator-activated receptor-alpha in a model of inflammatory pain. Neuropharmacology 2008;55:85–93. [PubMed: 18534634]
40. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol 2009;5:37–44. [PubMed: 19029917]
41. Kinsey SG, Long JZ, O'Neal ST, Abdullah RA, Poklis JL, Boger DL, et al. Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. J Pharmacol Exp Ther 2009;330:902–10. [PubMed: 19502530]

42. de Lago E, Petrosino S, Valenti M, Morera E, Ortega-Gutierrez S, Fernandez-Ruiz J, et al. Effect of repeated systemic administration of selective inhibitors of endocannabinoid inactivation on rat brain endocannabinoid levels. Biochem Pharmacol 2005;70:446–52. [PubMed: 15963472]
43. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:227–81. [PubMed: 9685154]
44. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10. [PubMed: 6877845]
45. Moezi L, Gaskari SA, Liu H, Baik SK, Dehpour AR, Lee SS. Anandamide mediates hyperdynamic circulation in cirrhotic rats via CB(1) and VR(1) receptors. Br J Pharmacol 2006;149:898–908. [PubMed: 17043671]
46. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–88. [PubMed: 3340425]
47. Desroches J, Guindon J, Lambert C, Beaulieu P. Modulation of the anti-nociceptive effects of 2-arachidonoyl glycerol by peripherally administered FAAH and MGL inhibitors in a neuropathic pain model. Br J Pharmacol 2008;155:913–24. [PubMed: 18695638]
48. Nackley AG, Suplita RL 2nd, Hohmann AG. A peripheral cannabinoid mechanism suppresses spinal Fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 2003;117:659–70. [PubMed: 12617970]
49. Nackley AG, Zvonok AM, Makriyannis A, Hohmann AG. Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. J Neurophysiol 2004;92:3562–74. [PubMed: 15317842]
50. Lim G, Sung B, Ji RR, Mao J. Upregulation of spinal cannabinoid-1 receptors following nerve injury enhances the effects of Win55,212-2 on neuropathic pain behaviors in rats. Pain 2003;105:275–83. [PubMed: 14499445]
51. Mitrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H, et al. Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. Pain 2006;126:102–14. [PubMed: 16844297]
52. Lever IJ, Robinson M, Cibelli M, Paule C, Santa P, Yee L, et al. Localization of the endocannabinoid-degrading enzyme fatty acid amide hydrolase in rat dorsal root ganglion cells and its regulation after peripheral nerve injury. J Neurosci 2009;29:3766–80. [PubMed: 19321773]
53. Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 2004;20:441–58. [PubMed: 15233753]
54. Muccioli GG, Xu C, Odah E, Cudaback E, Cisneros JA, Lambert DM, et al. Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells. J Neurosci 2007;27:2883–9. [PubMed: 17360910]
55. Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW. Endocannabinoid 2-arachidonoyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Mol Pharmacol 2000;57:1045–50. [PubMed: 10779390]
56. Ahluwalia J, Urban L, Bevan S, Nagy I. Anandamide regulates neuropeptide release from capsaicin-sensitive primary sensory neurons by activating both the cannabinoid 1 receptor and the vanilloid receptor 1 in vitro. Eur J Neurosci 2003;17:2611–8. [PubMed: 12823468]
57. Jhaveri MD, Richardson D, Kendall DA, Barrett DA, Chapman V. Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. J Neurosci 2006;26:13318–27. [PubMed: 17182782]
58. Hohmann AG, Herkenham M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 1999;90:923–31. [PubMed: 10218792]
59. Bridges D, Rice AS, Egertova M, Elphick MR, Winter J, Michael GJ. Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience 2003;119:803–12. [PubMed: 12809701]
60. Hohmann AG, Herkenham M. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience 1999;92:1171–5. [PubMed: 10426476]

61. Anand U, Otto WR, Sanchez-Herrera D, Facer D, Yiangou Y, Korchev Y, et al. Cannabinoid receptor CB2 localisation and agonist-mediated inhibition of capsaicin responses in human sensory neurons. Pain 2008;138:667–80. [PubMed: 18692962]

62. Holzer P. Acid-sensitive ion channels and receptors. Handb Exp Pharmacol 2009;194:283–332. [PubMed: 19655111]

63. Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG. Evidence against the presence of an anandamide transporter. Proc Natl Acad Sci USA 2003;100:4269–74. [PubMed: 12655057]

64. Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A, et al. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc Natl Acad Sci USA 2004;101:8756–61. [PubMed: 15138300]

65. Kaczocha M, Glaser ST, Deutsch DG. Identification of intracellular carriers for the endocannabinoid anandamide. Proc Natl Acad Sci USA 2009;106:6375–80. [PubMed: 19307565]

66. De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide transport with negligible capsaicin-like activity. FEBS Lett 2000;483:52–6. [PubMed: 11033355]

67. Zygmunt PM, Chuang H, Movahed P, Julius D, Hogestatt ED. The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur J Pharmacol 2000;396:39–42. [PubMed: 10822052]

68. De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, Di Marzo V. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 2001;276:12856–63. [PubMed: 11278420]

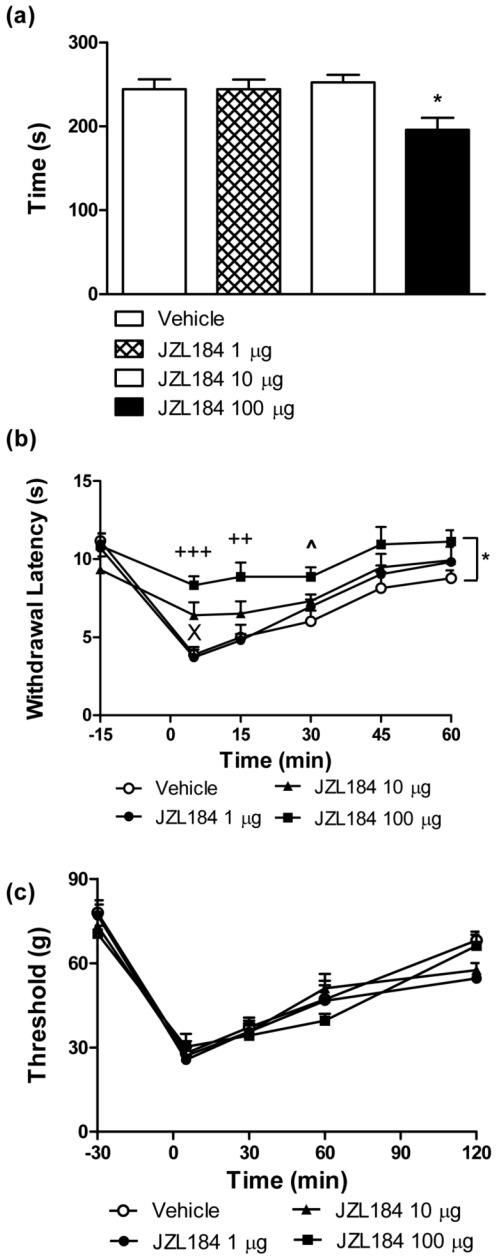

Fig. 1.

Local injection of the MGL inhibitor JZL184 in the paw suppresses capsaicin-induced nocifensive behavior and thermal hypersensitivity. (a) Only the highest dose of JZL184 (100 μg i.pl.) suppressed capsaicin-evoked nocifensive behavior compared to vehicle. (b) Suppression of thermal hyperalgesia induced by the high dose of JZL184 (100 μg i.pl.) outlasted that of the middle dose (10 μg i.pl.). (c) JZL184 (1, 10, and 100 μg i.pl.) did not alter capsaicin-induced mechanical hypersensitivity. Data are expressed as mean + SEM. +++ $P < 0.001$, ++ $P < 0.01$, versus vehicle and low dose (1 μg i.pl.) (ANOVA, Tukey post hoc); X $P < 0.05$ versus all treatments except the highest dose (100 μg i.pl.) (ANOVA, Tukey post hoc); ^ $P < 0.05$ versus vehicle (ANOVA, Tukey post hoc); * $P \leq 0.05$ JZL184 (100 μg i.pl.) versus all other groups (ANOVA, Tukey post hoc).

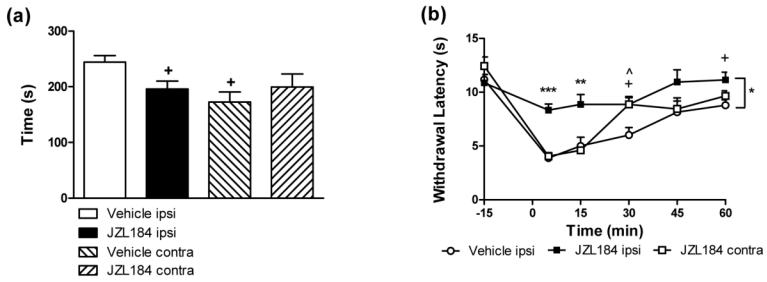

Fig. 2.

The MGL inhibitor JZL184 suppresses capsaicin-evoked nocifensive behavior and thermal hyperalgesia through a local site of action. A local injection of JZL184 (100 μg i.pl.) ipsilateral but not contralateral to the capsaicin-injected paw suppressed (a) nocifensive behavior and (b) thermal hyperalgesia. Nocifensive behavior was similar following injection of either vehicle or the active dose of JZL184 to the contralateral paw. Data are expressed as mean + SEM. *** $P \leq 0.001$, ** $P < 0.01$, * $P < 0.05$ versus all other groups (ANOVA, Tukey post hoc); + $P < 0.05$ versus vehicle ipsi (t-test, ANOVA, Tukey post hoc); ^ $P < 0.05$ JZL184 contra versus vehicle (ANOVA, Tukey post hoc).

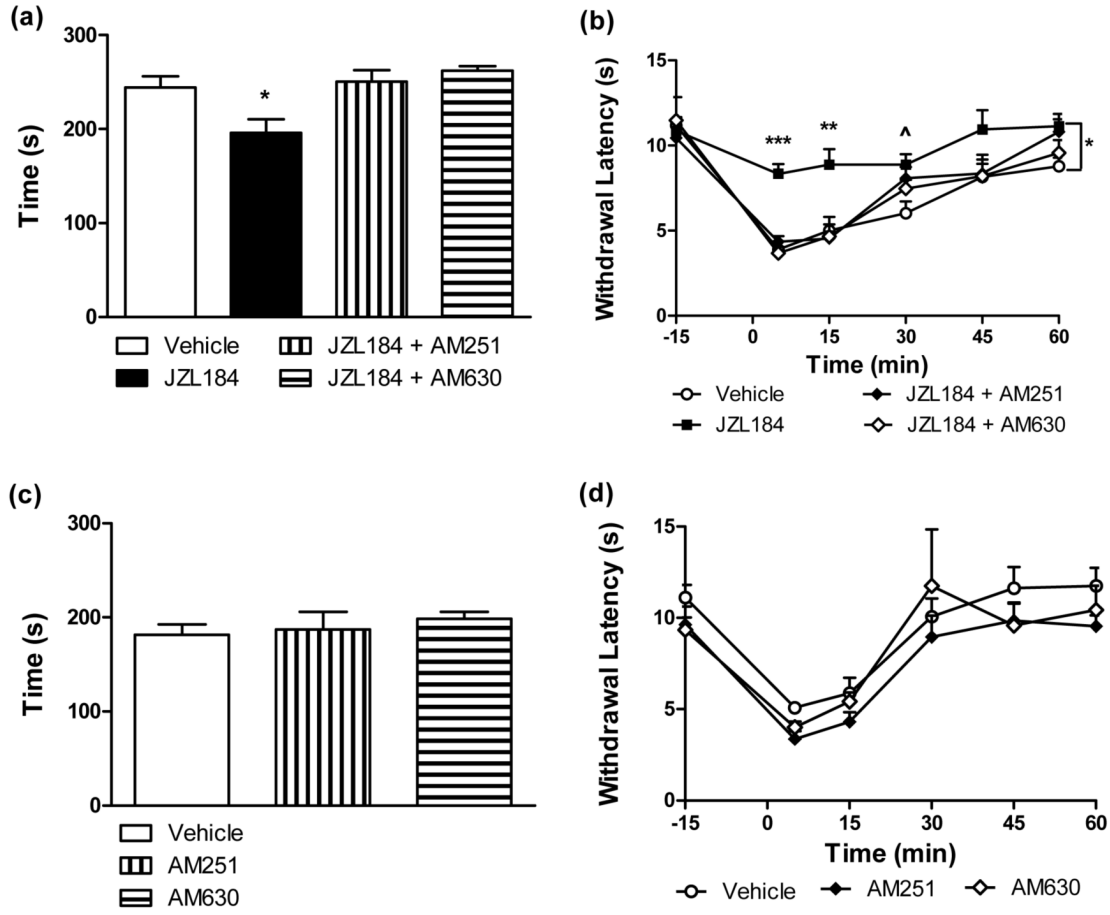

Fig. 3.

JZL184 suppresses capsaicin-evoked nocifensive behavior and thermal hyperalgesia through CB_1- and CB_2-specific mechanisms. The JZL184 (100 μg i.pl.)-induced suppressions of capsaicin-evoked (a) nocifensive behavior and (b) thermal hyperalgesia was blocked by either the CB_1 antagonist AM251 (80 μg i.pl.) or the CB_2 antagonist AM630 (25 μg i.pl.). (c, d) Effects of AM251 (80 μg i.pl.) and AM630 (25 μg i.pl.) alone did not differ from vehicle. Data are expressed as mean + SEM. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus all other groups (ANOVA, Tukey post hoc); ^ $P < 0.05$ versus vehicle (ANOVA, Tukey post hoc).

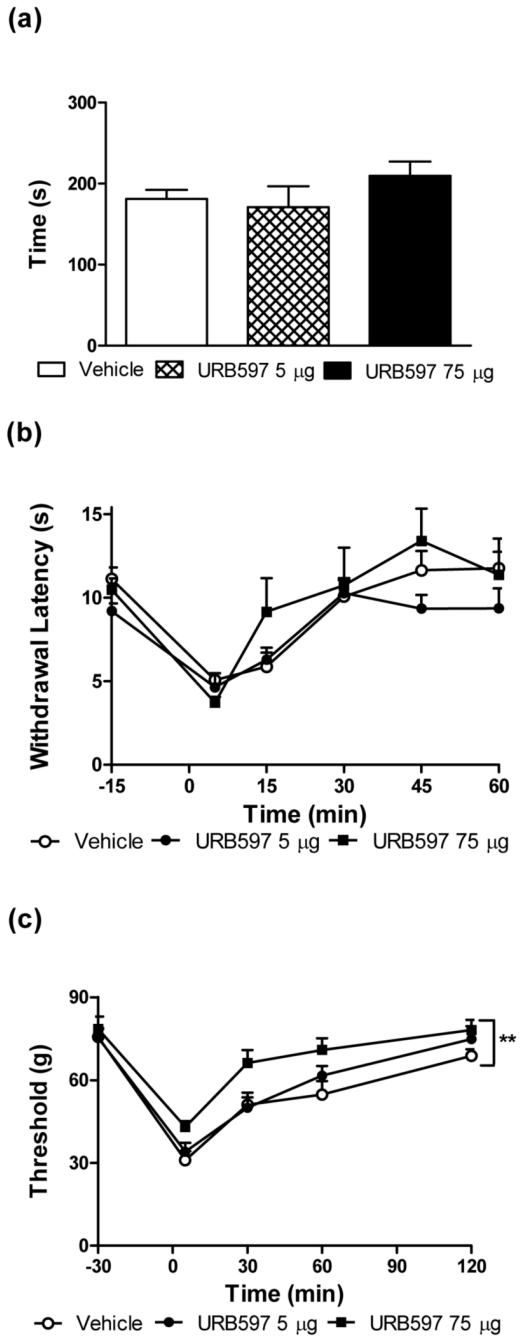

Fig. 4.

The FAAH inhibitor URB597 attenuates capsaicin-evoked mechanical hypersensitivity. URB597 (5 or 75 μg i.pl.) did not suppress capsaicin-evoked (a) nocifensive behavior or (b) thermal hyperalgesia. The highest dose of URB597 (75 μg i.pl.) suppressed capsaicin-evoked (c) mechanical allodynia. Data are expressed as mean + SEM. ** $P < 0.01$ URB597 (75 μg i.pl.) versus all other groups (ANOVA, Tukey post hoc).

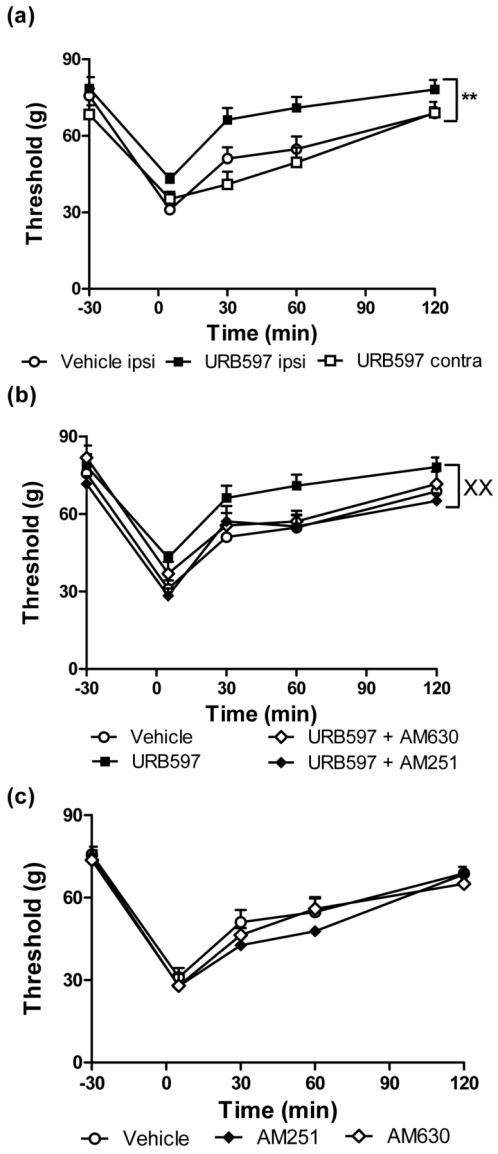

Fig. 5.

The FAAH inhibitor URB597 suppresses capsaicin-evoked mechanical hypersensitivity through a peripheral site of action. (a) A local injection of URB597 (75 μg i.pl.) ipsilateral, but not contralateral, to the capsaicin-injected paw attenuated capsaicin-evoked mechanical allodynia. (b) The URB597-induced suppression of capsaicin-induced mechanical hypersensitivity was blocked by the CB_1 antagonist AM251 (80 μg i.pl.) but not the CB_2 antagonist AM630 (25 μg i.pl.). (c) Local injection of AM251 (80 μg i.pl.) or AM630 (25 μg i.pl.) did not alter capsaicin-evoked mechanical hypersensitivity relative to vehicle. Data are expressed as mean + SEM. ** $P < 0.01$ URB597 ipsi versus all other groups (ANOVA, Tukey post hoc); XX $P < 0.01$ URB597 versus all groups except URB597 + AM630 (ANOVA, Tukey post hoc).

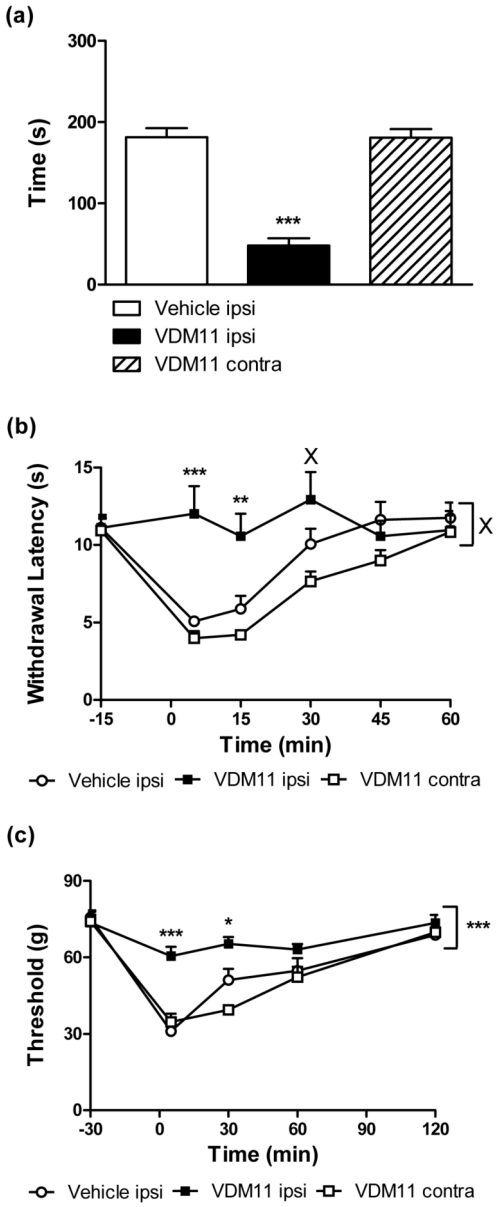

Fig. 6.

Intraplantar injection of the endocannabinoid uptake inhibitor VDM11 suppresses capsaicin-evoked nocifensive behavior, thermal hyperalgesia, and mechanical allodynia via a peripheral mechanism. Local injection of VDM11 (100 μg i.pl.) in the hindpaw ipsilateral (VMD11 ipsi), but not contralateral (VDM11 contra), to capsaicin injection suppressed (a) nocifensive behavior, (b) thermal hyperalgesia, and (c) mechanical allodynia. Data are expressed as mean + SEM. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P < 0.05$ VDM11 ipsi (100 μg i.pl.) versus all other groups (ANOVA, Tukey post hoc); X $P < 0.05$ VDM11 ipsi (100 μg i.pl.) versus VDM11 contra (100 μg i.pl.) (ANOVA, Tukey post hoc).

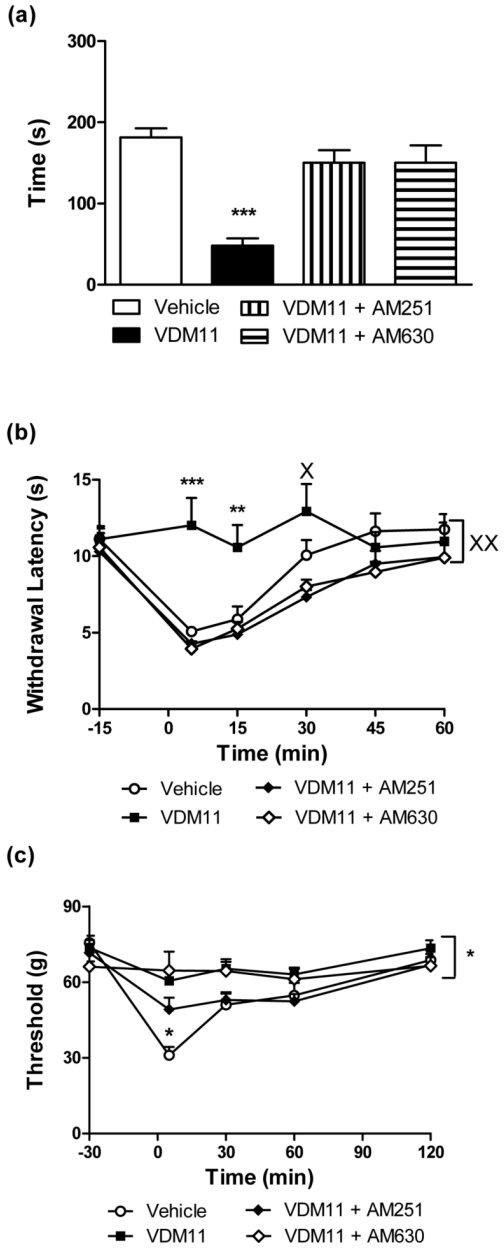

Fig. 7.

The endocannabinoid uptake inhibitor VDM11 suppresses capsaicin-evoked behavioral sensitization through modality- and cannabinoid receptor subtype-specific mechanisms. The VDM11-induced suppression of capsaicin-evoked (a) nocifensive behavior and (b) thermal hyperalgesia is blocked by either the CB_1 antagonist AM251 (80 μg i.pl.) or the CB_2 antagonist AM630 (25 μg i.pl.). (c) The VDM11 (100 μg i.pl.)-induced suppression of capsaicin-evoked mechanical allodynia was blocked by AM251 (80 μg i.pl.) but not AM630 (25 μg i.pl.). Data are expressed as mean + SEM. *** $P<0.001$, ** $P < 0.01$, * $P < 0.05$ versus all other groups (ANOVA, Tukey post hoc); XX $P < 0.01$, X $P < 0.05$ VDM11 versus all groups except vehicle (ANOVA, Tukey post hoc).

Table 1

Inhibitors of fatty-acid amide hydrolase (URB597), monoacylglycerol lipase (JZL184), and endocannabinoid transport (VDM11) differentially suppress capsaicin-evoked behavioral hypersensitivities through peripheral cannabinoid mechanisms

	Compounds		
	URB597	**JZL184**	**VDM11**
Nocifensive Behavior	—	CB_1/CB_2	CB_1/CB_2
Thermal Hyperalgesia	—	CB_1/CB_2	CB_1/CB_2
Mechanical Allodynia	CB_1	—	CB_1
Putative Effect on Endocannabinoids	↑ AEA	↑ 2-AG	↑ AEA, ↑ 2-AG