Differential Role of Anandamide and 2-Arachidonoylglycerol in Memory and Anxiety-like Responses

Arnau Busquets-Garcia, Emma Puighermanal, Antoni Pastor, Rafael de la Torre, Rafael Maldonado, and Andrés Ozaita

Background: Cannabinoid agonists are potential therapeutic agents because of their antinociceptive and anxiolytic-like effects, although an important caveat to their use is the possible adverse responses related to memory impairment. An alternative approach to circumvent this limitation consists of enhancing the concentration of the endocannabinoids anandamide and 2-arachidonoylglycerol.

Methods: Using low doses of the specific inhibitors of the endocannabinoid metabolizing enzymes fatty acid amide hydrolase, URB597, and monoacylglycerol lipase, JZL184, we analyzed their acute and chronic effects on memory consolidation, anxiolytic-like effects, and nociception in mice ($n = 6–12$ per experimental group).

Results: We show that anandamide is a central component in the modulation of memory consolidation, whereas 2-arachidonoylglycerol is not involved in this process. Interestingly, both URB597 and JZL184 induce anxiolytic-like effects through different cannabinoid receptors. In addition, the results show that the antinociceptive and anxiolytic-like responses of both inhibitors, as well as their acute effects on memory consolidation, are maintained after chronic treatment.

Conclusions: These results dissociate the role of anandamide and 2-arachidonoylglycerol in memory consolidation and anxiety and reveal the interest of cannabinoid receptor 2 as a novel target for the treatment of anxiety-related disorders.

Key Words: Anxiety, cannabinoid receptor 2 (CB2), endocannabinoid, memory, nociception, tolerance

The endocannabinoid system, composed of endogenous ligands (endocannabinoids), enzymes regulating their biosynthesis and degradation, and at least two different cannabinoid receptors (cannabinoid receptor [CB] 1 and CB2), plays a key neuromodulatory role in the central nervous system (1). The best characterized endocannabinoids are arachidonoylethanolamide (AEA), also known as anandamide (2), and 2-arachidonoylglycerol (2-AG) (3). Arachidonoylethanolamide and 2-AG are metabolized by specific enzymes. The principal AEA-hydrolyzing enzyme is fatty acid amide hydrolase (FAAH) (4), while monoacylglycerol lipase (MAGL) is the main 2-AG hydrolase (5). Therefore, the action of AEA can be prolonged by inhibiting its degradation through FAAH enzyme inhibitors, such as URB597 (6). On the other hand, endogenous 2-AG concentrations can be enhanced by the administration of the selective MAGL inhibitor, JZL184 (7).

The endocannabinoid system regulates a range of physiological processes, including memory (8), anxiety (9), and nociception (10). Thus, endocannabinoids facilitate extinction of aversive memories (8), and naturally occurring cannabinoids, such as delta-9-tetrahydrocannabinol (THC), impair learning and memory in humans (11) and in several animal models (12). However, the specific role of each endocannabinoid in memory consolidation still remains unclear. In addition, although the acute pharmacologic inhibition of FAAH has been reported to induce anxiolytic-like effects in a CB1-dependent fashion (13), it remains unclear the specific effect of 2-AG in anxiety-like responses. Moreover, FAAH and MAGL inhibition produces CB1-mediated antinociception (7,14), but the consequences of the chronic inhibition of FAAH and MAGL on these two important responses have been only partially addressed using high doses of JZL184 (15).

The aim of our study was to investigate the specific role of AEA and 2-AG in the modulation of memory, anxiety-like responses, and antinociception. We found that URB597-mediated modulation of AEA promotes memory deficits through similar mechanisms to those described for THC, while JZL184-mediated modulation of 2-AG did not alter memory consolidation. Moreover, URB597 and JZL184 administration induced anxiolytic-like effects through the activation of different cannabinoid receptors pointing to the CB2 receptor as a novel target for the treatment of anxiety-related disorders. Finally, the acute effects in memory consolidation, anxiety, and antinociception for both inhibitors were maintained after chronic administration, altogether pointing to MAGL as a promising target for therapeutic purposes.

Methods and Materials

Animals

Male Swiss albino and C57BL/6J mice (Charles River, Lyon, France) and CB1 (16) and CB2 (Jackson Laboratory, Bar Harbor, Massachusetts) constitutive knockout mice (8–10 weeks of age), weighing 26 g to 30 g at the beginning of the experiments were used. Mice were housed five per cage in a temperature (21 ± 1°C) and humidity (55 ± 10%) controlled environment. Food and water were available ad libitum. All the experiments were performed during the light phase of a 12-hour light/dark cycle (lights on at 8:00 AM and off at 8:00 PM). Animals were habituated to the environment

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conditions and handled for 1 week before starting the experiments. All animal procedures were conducted in accordance with the standard ethical guidelines (European Communities Directive 86/609/-European Economic Community) and approved by the local ethical committee (Comitè Ètic d'Experimentació Animal-Parc de Recerca Biomèdica de Barcelona). Our institution has the Animal Welfare Assurance (#A5388-01, Institutional Animal Care and Use Committee Approval Date 06/08/2009) granted by the Office of Laboratory Animal Welfare of the National Institutes of Health. All behavioral experiments were performed under blind conditions.

Drugs and Treatments

URB597 was obtained from Biomol-International (Exeter, United Kingdom); JZL184 from Cayman Chemical (Ann Arbor, Michigan); THC from THC Pharm GmbH (Frankfurt, Germany); rapamycin from Tecoland Corporation (Edison, New Jersey); MK-801 from Sigma-Aldrich (Madrid, Spain); rimonabant and SR144528 from Sanofi-Aventis (Sanofi-Aventis Recherche, Montpellier, France); and AM630 and JWH-133 from Tocris BioScience (Bristol, United Kingdom). MK-801 was dissolved in .9% sodium chloride (saline). URB597 and JZL184 were dissolved either in dimethyl sulfoxide (DMSO; Scharlau Chemie, Barcelona, Spain) for single acute administration or in 15% DMSO: 4.25% polyethylene glycol 400 (Sigma-Aldrich): 4.25% Tween-80 (Sigma-Aldrich): 76.5% saline for chronic administration. Delta-9-tetrahydrocannabinol, rimonabant, SR144528, AM630, and JWH-133 were diluted in 5% ethanol: 5% Cremophor-EL (Sigma-Aldrich): 90% saline. Rapamycin was dissolved in DMSO and administered for 5 days (17). All compounds were administered intraperitoneally in a volume of 10 mL/kg, except when dissolved in DMSO, which were injected in a volume of 2 mL/kg.

Endocannabinoid Quantification

Endocannabinoid levels were measured in brain homogenates as described in Supplement 1.

Immunoblot Analysis

Hippocampal tissues were extracted 2 hours after JZL184 (8 mg/kg), URB597 (1 mg/kg), or THC (10 mg/kg) administration and were processed as previously described (18). The antibodies used for immunoblot were antiphospho-p70S6K (T389) (1:800), anti-p70S6K (1:500) (Cell Signaling Technology, Beverly, Massachusetts), anti-CB1 receptor (1:1000) (Frontier Science, Ishikari, Japan), and antiglyceraldehyde-3-phosphate dehydrogenase (1:5000) (Santa Cruz Biotechnology, Santa Cruz, California). Optical densities of relevant immunoreactive bands were quantified after acquisition on a ChemiDoc XRS System (Bio-Rad, Hercules, California) controlled by the Quantity One software version 4.6.3 (Bio-Rad).

Context-Recognition Task

In the context-recognition task, animals learn to fear a new environment because of its temporal association with an aversive stimulus (foot shock). When exposed to the same context, conditioned animals show freezing behavior, defined as complete lack of movement except for respiration (19). Mice were trained and tested in conditioning chambers (Panlab, Barcelona, Spain) that had a stainless steel grid floor through which electric foot shocks could be delivered. On the training day, a mouse was placed in the conditioning chamber for 150 seconds before the onset of the unconditioned stimulus (.7 mA foot shock for 2 seconds) and then remained in the chamber for 30 seconds. Mice received an injection of URB597 (1 mg/kg), JZL184 (8 mg/kg), or its vehicle 20 minutes after training. Testing for contextual fear conditioning was performed 24 hours after training by measuring during 3-minute freezing behavior in the same conditioning chamber. The results were expressed as percentage of freezing time.

Object-Recognition Task

Object-recognition memory was assayed in the V-maze (Panlab) made out of black Plexiglas with two corridors (30 cm long × 4.5 cm wide, and 15 cm high walls) set at a 90° angle. The acute test was performed as previously described (20). All the drugs were injected after the training session, except rapamycin, which was administered during the 5 days before training (20). Short-term memory was tested 3 hours after the training session, while long-term memory was tested after 24 hours in independent groups of mice.

The effect of the chronic administration of URB597 and JZL184 on recognition memory was also evaluated in the V-maze during 6 days (Figure S1 in Supplement 1). A discrimination index was calculated as previously described (20) for each test session.

Anxiolytic-like Responses

Elevated Zero Maze Test. The elevated zero maze test was performed in a circular black Plexiglas apparatus (5 cm wide, inner diameter of 35 cm) with two open and two closed sections (walls are 15 cm high) elevated 40 cm above the floor. Five-minute test sessions were performed 120 minutes after URB597, JZL184, or vehicle administration, and the percentage of time spent in the open area was determined.

Elevated Plus Maze Test. The elevated plus maze test was performed in a black Plexiglas apparatus with four arms (29 cm long × 5 cm wide) set in cross from a neutral central square (5 cm × 5 cm) elevated 40 cm above the floor. Five-minute test sessions were performed 120 minutes after URB597, JZL184, JWH-133, or vehicle administration, and the percentage of time spent in the open arms was determined. Rimonabant (1 mg/kg), SR144528 (1 mg/kg), or AM630 (1 mg/kg) were administered 30 minutes before URB597, JZL184, or JWH-133 injection.

Antinociception (Acetic Acid Test)

This test was performed 120 min after drug administration. Mice received an intraperitoneal administration of acetic acid (.8% solution) injected in a volume of 10 mL/kg of body weight. Immediately after, mice were placed in individual transparent cylinders (16 cm high, 16 cm diameter) for 20 minutes. The number of writhes was counted during 15 minutes, starting 5 minutes after acetic acid administration.

Statistical Analysis

The data were analyzed using one-way analysis of variance followed by the Dunnett's post hoc test. Two-way analysis of variance (rapamycin, MK-801, rimonabant, or SR144528 pretreatment vs. URB597; rimonabant, SR144528, or AM630 vs. JZL184; rimonabant, SR144528 vs. JWH-133; wild-type, CB1, or CB2 knockout mice vs. JZL184 treatment, as factors of variation) was used when required. Comparisons were considered statistically significant when $p < .05$.

Results

Differential Effects of URB597 and JZL184 in Hippocampal Memory and Hippocampal Mammalian Target of Rapamycin Regulation

We used the object-recognition and context-recognition tasks to evaluate the effects of URB597 and JZL184 on memory consolidation. Using these paradigms, robust hippocampal-dependent learning can be achieved in a single training session (19,21); therefore, memory consolidation can be modified pharmacologically afterward, precluding the possible influence of the acute pharma-

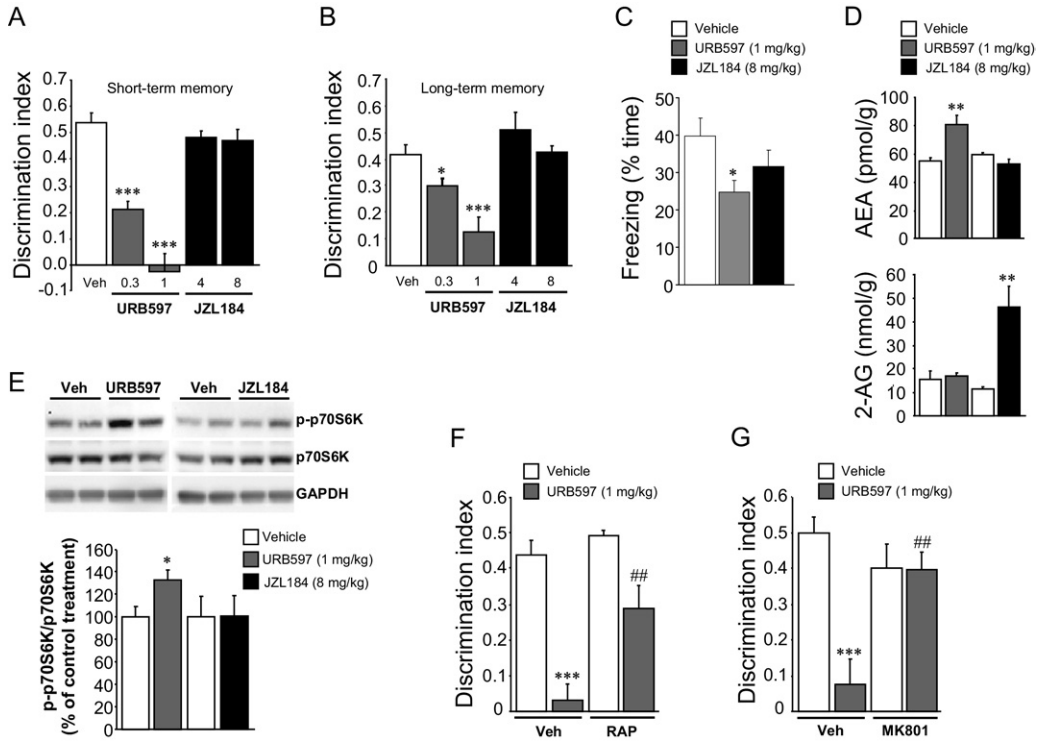

Figure 1. Differential effects of URB597 and JZL184 in memory and hippocampal mammalian target of rapamycin regulation. **(A, B)** Discrimination index in short-term **(A)** and long-term **(B)** memory for the object-recognition test after mice received URB597, JZL184, or vehicle ($n = 6–8$ per group). **(C)** Percentage of freezing time in the context-recognition test of mice ($n = 9–10$ per group) treated with URB597 (1 mg/kg), JZL184 (8 mg/kg), or vehicle. **(D)** Brain concentrations of the endocannabinoids arachidonoylethanolamide and 2-arachidonoylglycerol after systemic administration of URB597 (1 hour later) ($n = 5$) or JZL184 (2 hours later) ($n = 5$). **(E)** Representative immunoblot of hippocampal samples and optical density quantification of p70S6K (T389) phosphorylation 2 hours after URB597 ($n = 5$) or JZL184 ($n = 5$) administration. **(F, G)** Discrimination index for long-term memory in the object-recognition test following URB597 administration after pretreatment with rapamycin (1 mg/kg, 5 days) or MK-801 (.1 mg/kg) ($n = 6–8$ per group). Data are expressed as mean ± SEM. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ compared with vehicle treatment; $^{\#\#}p < .01$ compared with URB597 treatment, analysis of variance followed by Dunnett's test. AEA, arachidonoylethanolamide; 2-AG, 2-arachidonoylglycerol; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RAP, rapamycin; Veh, vehicle.

cologic effects of the drugs on locomotion or exploration during the memory acquisition or retrieval periods. Systemic URB597 administration (.3 and 1 mg/kg) produced a reduction in the discrimination index in the object-recognition task in both short-term (Figure 1A) and long-term memory (Figure 1B), while no changes were observed in the total exploration time during the test (Figure S2 in Supplement 1). This amnesic-like effect of URB597 is dependent on CB1 receptors, as it can be prevented by rimonabant pretreatment (20). Interestingly, JZL184 administration (4 and 8 mg/kg) did not impair memory in the object-recognition test (Figure 1A,B), although the levels of 2-AG were increased in the hippocampus when administered at the dose of 8 mg/kg (8.2 nmol/g wet tissue in the vehicle group vs. 58.6 nmol/g wet tissue in JZL184 group, $p < .001$). Likewise, URB597 administration (1 mg/kg) after context-recognition training produced a significant reduction in context-associated freezing behavior tested 24 hours after training, while the administration of JZL184 (8 mg/kg) only produced a slight nonsignificant decrease in the freezing behavior (Figure 1C). In agreement with previous studies (22), URB597 (1 mg/kg) and JZL184 (8 mg/kg) selectively modified the brain content of AEA and 2-AG (Figure 1D), respectively.

The hippocampal activation of mammalian target of rapamycin (mTOR)/p70S6K signaling has been associated with the cognitive deficits produced by cannabinoids (20). We observed an increase in hippocampal T389-p70S6K phosphorylation after systemic administration of URB597, whereas JZL184 administration did not modify the phosphorylation of this mTOR-dependent site (Figure 1E). The amnesic-like effects of URB597 in the object-recognition memory test were prevented by a pretreatment with the mTOR signaling inhibitor rapamycin (Figure 1F). Moreover, pretreatment with the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 also abolished the cognitive deficiency promoted by URB597 (Figure 1G), indicating that the molecular and cellular mechanisms underlying the memory impairment produced by URB597 are similar to those previously reported for THC (20).

Differential Underlying Mechanisms in the Anxiolytic-like Effects of URB597 and JZL184

The effectiveness of the endocannabinoid system modulation by URB597 and JZL184 in anxiolytic-like responses was studied using the elevated zero maze and the elevated plus maze (EPM). Acute URB597 and JZL184 administration produced anxiolytic-like responses in both paradigms (Figure 2A,B). Moreover, pharmacologic experiments using CB1 (rimonabant) and CB2 (SR144528) antagonists revealed that the anxiolytic-like effect of URB597 was mediated through CB1 receptors (Figure 2C), as previously reported (13,23), and independent of CB2 receptors (Figure 2C). In contrast, the anxiolytic-like effect of JZL184 was not sensitive to rimonabant but was abolished by pretreatment with the CB2 antagonists SR144528 and AM630 (Figure 2D).

The crucial role of CB2 receptors in the anxiolytic-like effects of JZL184 was also revealed in the EPM by the abolishment of these

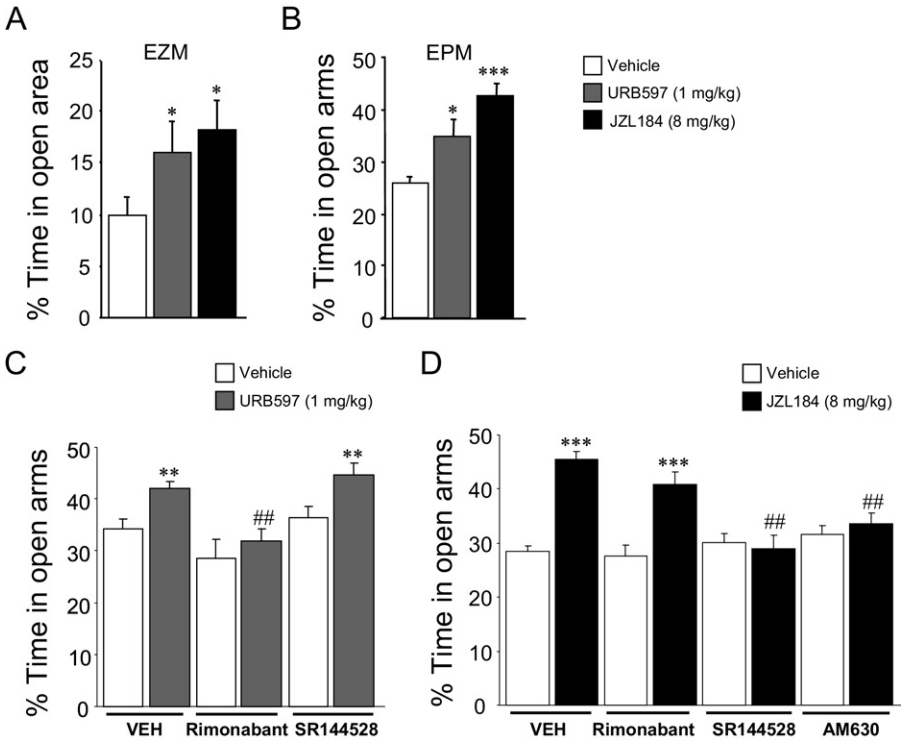

Figure 2. The anxiolytic-like effects of URB597 or JZL184 are mediated by different cannabinoid receptors. **(A, B)** Anxiolytic-like effects in the elevated zero maze or the elevated plus maze after a single administration of URB597, JZL184, or vehicle ($n = 10–15$ per group). The percentage of time spent in the open areas of the maze is represented. **(C)** Anxiolytic-like effect of URB597 was blocked after pretreatment with the cannabinoid receptor (CB) 1 receptor antagonist rimonabant (1 mg/kg, intraperitoneal [IP]). Instead, pretreatment with the CB2 receptor antagonist SR144528 (1 mg/kg, IP) did not prevent the anxiolytic-like effect of URB597. **(D)** Mice pretreated with the CB1 receptor antagonist rimonabant (1 mg/kg, IP) did show the anxiolytic-like effects of JZL184. Instead, a pretreatment with the CB2 receptor antagonists, SR144528 (1 mg/kg, IP) and AM630 (1 mg/kg, IP), prevented the anxiolytic-like effects of JZL184. Data are expressed as mean ± SEM. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ compared with vehicle treatment; $^{\#\#}p < .01$ compared with acute URB597 **(C)** or acute JZL184 **(D)**, analysis of variance followed by Dunnett's test. EPM, elevated plus maze; EZM, elevated zero maze; VEH, vehicle.

effects in CB2 knockout mice (Figure 3B), while JZL184 produced anxiolytic-like responses in CB1 knockout mice similar to those induced in their wild-type littermates (Figure 3A). All these anxiolytic-like effects were independent of changes in total activity as measured by the total number of entries (Figure S3 in Supplement 1).

We further studied the participation of the CB2 receptors in the modulation of anxiety-like responses using the selective CB2 receptor agonist JWH-133. We observed a dose-dependent anxiolytic-like effect of this compound in the EPM (Figure 3C; Figure S4 in Supplement 1) that was sensitive to CB2 receptor blockade but insensitive to CB1 receptor activation (Figure S5 in Supplement 1). Altogether, these results demonstrate the dichotomy between AEA and 2-AG on their anxiolytic-like effects, modulated by URB597 and JZL184, respectively, and uncover a new interesting role of CB2 receptors in anxiety-like responses.

JZL184 and URB597 Exhibited No Tolerance After Chronic Administration

We then addressed the possible development of tolerance to the pharmacologic effects of URB597 and JZL184 following chronic treatment. For this purpose, we investigated the effects of a chronic administration of URB597 or JZL184 (6 days, once a day) on memory, anxiety-like responses, and nociception.

To study the effect on cognitive function of both inhibitors, we used a modified protocol of the memory paradigm that allows testing object-recognition memory for 6 consecutive days in the

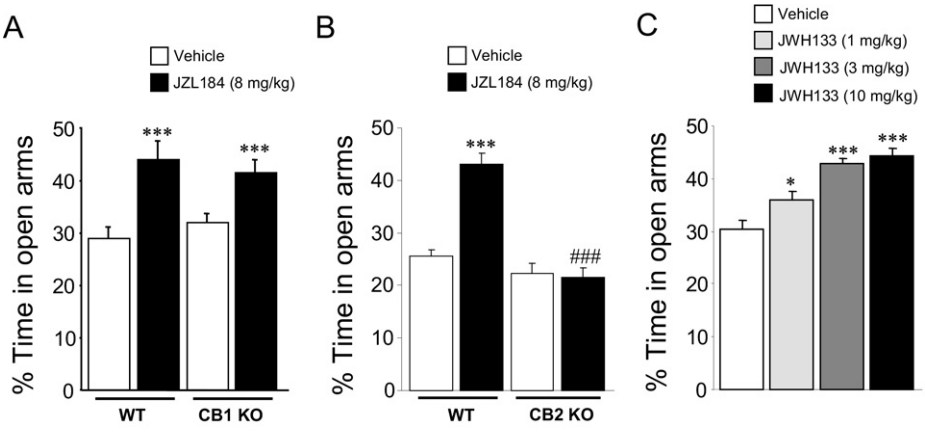

Figure 3. JZL184 (8 mg/kg) exhibited anxiolytic-like effects through a cannabinoid receptor (CB) 2 receptor mechanism. **(A)** Knockout mice for cannabinoid receptor 1 receptors did show the anxiolytic-like effects of JZL184 ($n = 8–10$). **(B)** Knockout mice for the CB2 receptors did not show the anxiolytic-like effects of JZL184 ($n = 8–10$). **(C)** The administration of a specific CB2 receptor agonist, JWH-133, produced an anxiolytic-like effect in a dose-dependent manner ($n = 8–10$ per group). Data are expressed as mean ± SEM. $^{*}p < .05$, $^{***}p < .001$ compared with vehicle treatment; $^{\#\#\#}p < .001$ compared with acute JZL184, analysis of variance followed by Dunnett's test. KO, knockout; WT, wild-type.

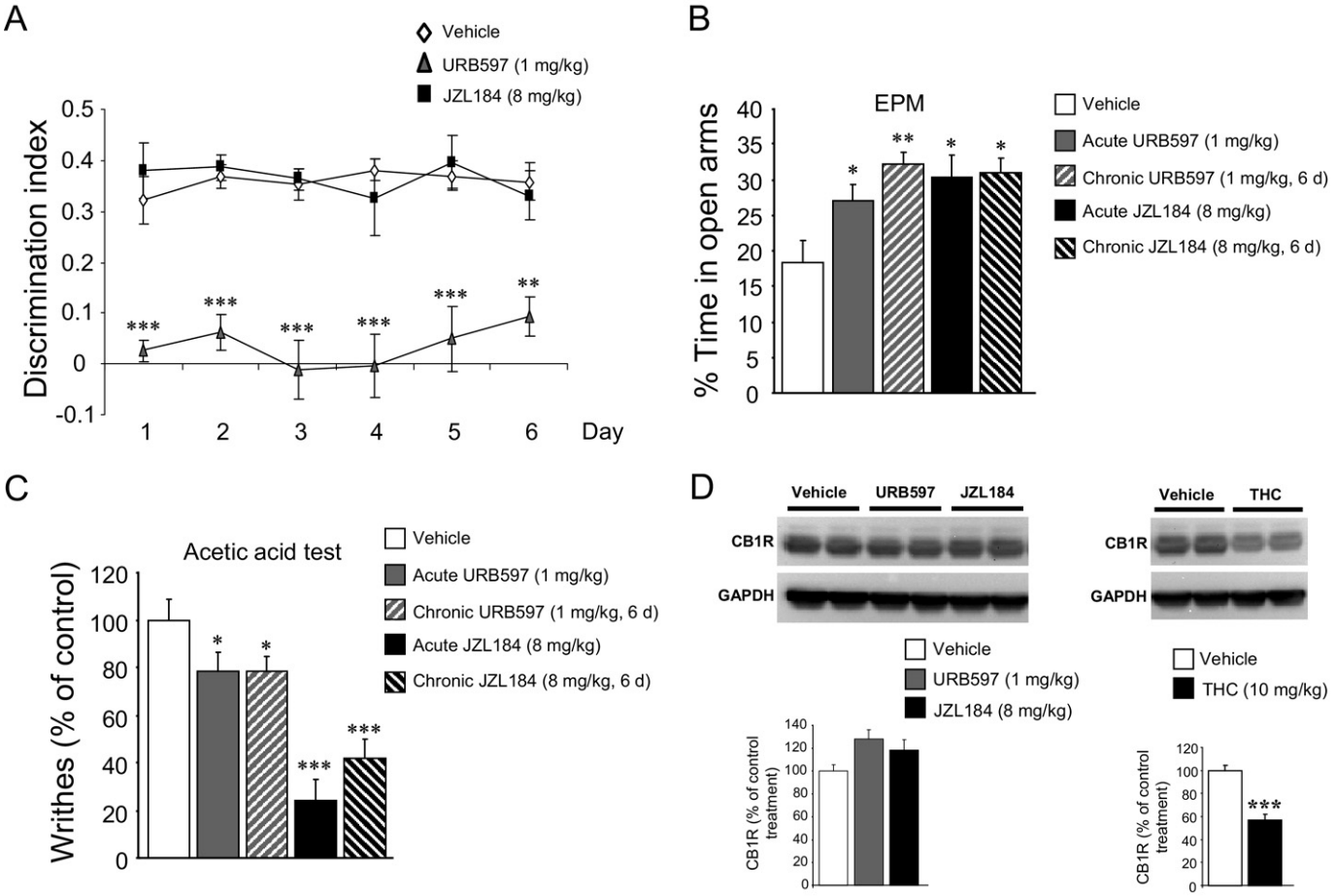

Figure 4. Chronic URB597 (1 mg/kg) or JZL184 (8 mg/kg) administration did not produce tolerance to their amnesic-like, anxiolytic-like, and antinociceptive effects. **(A)** Discrimination index during the 6 days that mice received URB597, JZL184, or vehicle ($n = 6–8$ per group) for the object-recognition memory test were represented. **(B)** Anxiolytic-like responses induced by a chronic (dashed bars) treatment of URB597, JZL184, or vehicle in the elevated plus maze. Control groups were treated with vehicle (5 days) and they received URB597, JZL184, or vehicle (solid bars) on the sixth day ($n = 8–10$ per group). **(C)** Antinociceptive response after chronic administration of URB597 or JZL184 in the acetic acid test. Mice were treated with URB597 or JZL184 for 6 days (dashed bars). Control groups were treated with vehicle (5 days) and on the sixth day they received URB597, JZL184, or vehicle (solid bars). The percentage of writhes is represented ($n = 15$ per group). **(D)** Representative immunoblot and optical density quantification of cannabinoid receptor 1 receptor expression after URB597 (1 mg/kg), JZL184 (8 mg/kg), and delta-9-tetrahydrocannabinol (10 mg/kg) administration. Data are expressed as mean ± SEM. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ compared with vehicle treatment, analysis of variance followed by Dunnett's test. CB, cannabinoid receptor; EPM, elevated plus maze; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; THC, delta-9-tetrahydrocannabinol.

same animal (Figure S1 in Supplement 1). As observed after URB597 acute administration (Figure 1B), chronic administration produced a significant amnesic-like effect during the entire chronic treatment (6 days) (Figure 4A). Moreover, mice treated chronically with JZL184 did not show such memory impairment (Figure 4A). No significant differences were observed in the total exploration time between chronic treatments (Figure S6F in Supplement 1). These results established the absence of tolerance to the amnesic-like effects of URB597 and confirmed the dissociation between AEA and 2-AG in memory consolidation.

We then addressed the possible development of tolerance to the anxiolytic-like effects of URB597 and JZL184 following chronic treatment in the EPM. Interestingly, both inhibitors showed similar anxiolytic-like effects after acute and chronic administration (Figure 4B). These effects were independent of changes in total activity (Figure S7 in Supplement 1). These results demonstrate that JZL184 has anxiolytic-like effects, similar to URB597, and the effects of both are maintained after chronic treatment.

Finally, the development of tolerance to the antinociceptive effects of these compounds was evaluated in the acetic acid test after chronic exposure to URB597 and JZL184. After chronic treatment, both drugs produced significant antinociceptive effects that were similar to those observed after acute administration (Figure 4C). Acute and chronic JZL184 (8 mg/kg) administration induced stronger antinociceptive effects than URB597 (1 mg/kg) (Figure 4C). These results support the interest of chronic protection of endocannabinoids for antinociceptive purposes given the lack of tolerance.

In agreement with the behavioral measurements for memory, anxiety, and nociception, biochemical analysis of CB1 receptors in hippocampal samples of mice chronically administered URB597 (1 mg/kg), JZL184 (8 mg/kg), or their vehicle did not show changes in protein expression (Figure 4D). In contrast, chronic THC (10 mg/kg), following the same administration schedule, did markedly decrease the expression of CB1 receptors in this brain area (Figure 4D), which is particularly sensitive to CB1 receptor downregulation after the administration of exogenous cannabinoid agonists (24).

Discussion

The modulation of the endocannabinoid system is an emerging therapeutic approach, based on the well-demonstrated medicinal properties of compounds acting on this system (25). Selective and efficacious inhibitors of the two main enzymes involved in the catabolism of AEA and 2-AG, FAAH and MAGL, respectively, have been developed, allowing the selective increase of endocannabinoid concentrations in vivo (6,7). We took advantage of these pharmacologic tools to modulate the endogenous levels of AEA and 2-AG, although the possible modulation of other bioactive lipids cannot be disregarded when using these tools. We provide compelling data showing differential effects of FAAH and MAGL inhibition on specific behavioral processes that could have important clinical implications, such as memory consolidation, anxiolytic-like responses, and antinociception.

We have previously reported that THC produces amnesic-like effects through the activation of CB1 receptors expressed on gamma-aminobutyric acidergic interneurons, the indirect activation of mTOR/p70S6K signaling cascade and NMDA receptors (20). Mammalian target of rapamycin is a kinase involved in the regulation of several cellular functions and has been related to synaptic plasticity processes (26). We previously observed that URB597 produced memory deficits in the object-recognition memory test through CB1 receptor activation (20). Here, we show that URB597, but not JZL184, administration activates hippocampal p70S6K(T389), an mTOR target that is commonly found overactivated in animal models of pathologies that run with cognitive deficiencies, such as tuberous sclerosis (17), fragile × syndrome (27), or Alzheimer's disease (28). Moreover, the chronic inhibition of FAAH produced a similar amnesic-like effect to that observed after an acute intervention. Interestingly, the MAGL inhibitor JZL184, which selectively increased brain 2-AG but not AEA content at the dose used (8 mg/kg), did not affect hippocampal mTOR signaling and did not significantly impair memory consolidation either after acute or chronic administration. Based on the immunohistochemical localization of the enzymes involved in the synthesis and degradation of 2-AG (29,30), we can speculate that the machinery involved in 2-AG processing is mainly associated with the functional activity of CB1 receptors located in glutamatergic synapses in the hippocampus, which are not involved in the memory impairment produced by cannabinoids (20). On the other hand, AEA could be mainly involved in the homeostasis of hippocampal gamma-aminobutyric acidergic terminals (31), where CB1 receptors are heavily expressed (32) and their activation plays a paramount role in the amnesic-like properties of THC (20). In line with this effect of THC, the mTOR inhibitor rapamycin or the NMDA antagonist MK-801 blocked the cognitive impairment produced by URB597, pointing to a similar mechanism on memory modulation.

Although these results provided robust evidence for a functional dissociation of each endocannabinoid-metabolizing enzyme in memory consolidation in two different paradigms, the object-recognition and the context-recognition, other memory stages, such as acquisition or retrieval, might be differentially modulated because they are sustained by different neurobiological substrates (33). In this regard, our study focused on the effects of URB597 and JZL184 in memory consolidation, while other previous studies have focused on the acquisition phase in a different paradigm, the passive-avoidance in rats (34). Interestingly, the enhancing effect on memory acquisition revealed by URB597 in those studies contrasted with the lack of effect on the consolidation phase using a low dose in the same paradigm (34). Moreover, the enhancing effects of URB597 on memory acquisition were reported to be mediated mainly by another bioactive lipid, oleoylethanolamide, through alpha-type peroxisome proliferator-activated nuclear receptors activation but not by cannabinoid receptors (34). This bioactive lipid has an important role in the modulation of the basolateral amygdala (35). In this regard, endogenous levels of AEA and 2-AG in this brain area can be modulated by emotional stimuli (8,36), which may modify the enhancing effects of the local endocannabinoid levels produced by the specific metabolizing enzyme inhibitors.

Although the role of AEA in the control of anxiety-like responses has been well documented (37), the involvement of 2-AG in such responses has not been previously addressed. Using doses of URB597 and JZL184 that selectively modulated the concentrations of each endocannabinoid and had differential effects in memory modulation, we found that both drugs had similar anxiolytic-like effects in two behavioral paradigms. These results confirm the previous findings reported for URB597 (37) and point to the 2-AG/MAGL pathway as an innovative target to obtain anxiolytic-like effects. The present and previous studies (23) have shown that the anxiolytic-like effects of URB597 are mediated through a CB1-dependent mechanism. Interestingly, we found that the anxiolytic-like effects of JZL184 were not modified by either the genetic disruption of the CB1 receptors or the pharmacologic blockade of these receptors with rimonabant. In contrast, these anxiolytic-like effects of JZL184 were absent in CB2 knockout mice and were prevented by pretreatment with the selective CB2 antagonists SR144528 or AM630. Moreover, the administration of JWH-133, a selective CB2 receptor agonist, mimicked the anxiolytic-like effects of JZL184 on the EPM and this response was also blocked by SR144528. These data reveal the crucial role of CB2 receptor activation on the modulation of anxiety and agree with the results previously reported in transgenic mice overexpressing CB2 receptors (38). Altogether, these results point to the CB2 receptor as a novel target to modulate anxiety-like responses.

Pharmacologic studies using inhibitors of AEA or 2-AG degradation showed antinociceptive effects in a variety of tests, including acute and chronic pain models (7,39). We evaluated the effects of the inhibitors of FAAH and MAGL in a model of visceral pain, the acetic acid test. Both URB597 and JZL184 induced antinociceptive effects in this test after single acute administration, consistent with the previous findings (7,15,23). At the tested dose, JZL184 showed a higher efficacy than URB597, which could be because 2-AG-mediated antinociception is mediated by both CB1 and CB2 receptors (40), while AEA-mediated antinociception depends almost exclusively on CB1 receptors (41). Moreover, the expression of the principal synthesizing enzyme for 2-AG in the central nervous system, diacylglycerol lipase alpha (42), is abundantly expressed in the nociceptive synapses on the dorsal horn of the spinal cord (43), pointing to this pronounced role of 2-AG in antinociception.

Moreover, chronic exposure to these inhibitors did not result in a reduction of their antinociceptive and anxiolytic-like effects, highlighting their potential utility as a new therapeutic strategy. In contrast, repeated administration of exogenous cannabinoids, such as THC, WIN55212 or CP55940, results in tolerance to the antinociceptive or anxiety-like effects (44,45). This lack of tolerance to the antinociceptive effects of chronic URB597 and JZL184 administration is in agreement with the absence of tolerance to nonopioid stress-induced analgesia, an effect mediated by the endocannabinoid system, where both AEA and 2-AG seem to be involved (46). Interestingly, we show that the doses of URB597 and JZL184 used in our chronic treatment did not reduce the expression of CB1 receptors in the hippocampus, whereas this receptor population was extremely sensitive to a similar treatment with THC. In agree-

ment, chronic administration of AEA produces less cellular adaptations, as well as less tolerance to antinociception, catalepsy, and hypothermia than chronic THC administration in FAAH knockout mice (47). In contrast, the antinociceptive effects induced by high doses of JZL184 were reduced after chronic treatment, resulting in cross-tolerance to CB1 receptor agonists (15). This discrepancy with our findings could be explained by the use of a very high dose of JZL184 in the previous study (40 mg/kg compared with 8 mg/kg in this study), which results in a much higher increase in the levels of 2-AG than in our study (about 10 times the control levels compared with 5 times in this study). The responses observed after the inhibition of the enzymatic degradation of endocannabinoids are reminiscent of those reported after protecting the catabolism of endogenous opioids. Indeed, a lack of tolerance to the antinociceptive properties of the peptidase inhibitors preventing the degradation of enkephalins has been previously reported (48).

Our data show that pharmacologic modulation of AEA concentrations results in memory deficits similar to those observed with THC. In contrast, pharmacologic modulation of 2-AG concentrations did not significantly affect memory consolidation. The fact that the MAGL inhibitor JZL184 had anxiolytic-like effects and promoted antinociceptive responses without affecting memory consolidation points to 2-AG modulation as a crucial pharmacologic target for the development of new therapeutic tools to activate the endocannabinoid system. The absence of tolerance after chronic treatment with a low dose of JZL184 highlights the potential interest of this new therapeutic target. Moreover, the anxiolytic-like effects produced by JZL184 are CB2-dependent and point to this receptor as an interesting alternative to treat anxiety-like disorders.

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Supplementary material cited in this article is available online.

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