Research

Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-α nuclear receptors

Carmen Mazzola,[1,2] Julie Medalie,[1] Maria Scherma,[1] Leigh V. Panlilio,[1] Marcello Solinas,[3] Gianluigi Tanda,[4] Filippo Drago,[2] Jean Lud Cadet,[5] Steven R. Goldberg,[1] and Sevil Yasar[5,6,7]

[1]*Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, NIDA, NIH, DHHS, Baltimore, Maryland 21224, USA;* [2]*Department of Experimental and Clinical Pharmacology, Medical School, University of Catania, Catania 86022, Italy;* [3]*Department Laboratoire de Biologie et Physiologie Cellulaires, CNRS-6187, Université de Poitiers, 86022 Poitiers, France;* [4]*Psychobiology Section, Medications Discovery Research Branch, Intramural Research Program, NIDA, NIH, DHHS, Baltimore, Maryland 21224, USA;* [5]*Molecular Neuropsychiatry Branch, Intramural Research Program, NIDA, NIH, DHHS, Baltimore, Maryland 21224, USA;* [6]*Division of Geriatric Medicine and Gerontology, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland 21224, USA*

Inhibitors of fatty acid amide hydrolase (FAAH) increase endogenous levels of anandamide (a cannabinoid CB_1-receptor ligand) and oleoylethanolamide and palmitoylethanolamide (OEA and PEA, ligands for α-type peroxisome proliferator-activated nuclear receptors, PPAR-α) when and where they are naturally released in the brain. Using a passive-avoidance task in rats, we found that memory acquisition was enhanced by the FAAH inhibitor URB597 or by the PPAR-α agonist WY14643, and these enhancements were blocked by the PPAR-α antagonist MK886. These findings demonstrate novel mechanisms for memory enhancement by activation of PPAR-α, either directly by administering a PPAR-α agonist or indirectly by administering a FAAH inhibitor.

Peroxisome proliferator-activated receptor-α (PPAR-α) is a ligand-activated transcriptional factor that regulates the expression of genes involved in lipid utilization, fatty acid oxidation, and inflammation (van Raalte et al. 2004; LoVerme et al. 2006). Immunolocalization studies of PPAR-α in the adult rat brain suggest that this nuclear receptor might have specific functions in regulating expression of genes involved in cholinergic neurotransmission and learning and memory processes (Moreno et al. 2004; Cimini et al. 2005). For example, there are high concentrations of PPAR-α receptors in the hippocampus and amygdala (Moreno et al. 2004). However, the potential involvement of PPAR-α in learning and memory processes has not been systematically investigated.

Endogenous ligands for PPAR-α include the lipid mediators *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA). In addition, anandamide (*N*-arachidonoylethanolamine), which has primarily been studied as an endogenous ligand for G-protein-coupled cannabinoid CB_1 receptors that mediate the behavioral effects of cannabis and its active constituent Δ^9-tetrahydrocannabinol (THC) (Devane et al. 1992; Solinas et al. 2008), has recently begun to receive attention as a potential endogenous PPAR-α ligand (O'Sullivan 2000; Mackie and Stella 2006; Sun et al. 2007). OEA has primarily been studied as a satiety factor (Rodriguez de Fonseca et al. 2001; Fu et al. 2003) and PEA as an anti-inflammatory factor (Kuehl et al. 1957; Calignano et al. 1998; Jaggar et al. 1998). OEA and PEA are structurally similar to anandamide but do not bind to or activate cannabinoid CB_1 receptors. Anandamide, OEA, and PEA are all inactivated primarily by the intracellular serine enzyme, fatty acid amide hydrolase (FAAH) (Cravatt and Lichtman 2002; Fegley et al. 2005). Consequently, selective FAAH-inhibiting drugs such as cyclohexyl carbamic acid 3′-carbamoyl-3-yl ester (URB597) increase endogenous levels of anandamide, OEA, and PEA in the brain (Fegley et al. 2005; Piomelli et al. 2006).

In the present experiments, the effects of FAAH inhibition on learning and memory processes and the involvement of cannabinoid CB_1 receptors and PPAR-α nuclear receptors in those effects were studied using a passive-avoidance procedure in rats. FAAH inhibition was accomplished by administering URB597. The effects of URB597 were studied alone and after pretreatment with the selective cannabinoid CB_1 receptor antagonist/inverse agonist rimonabant (SR141716) and the selective PPAR-α antagonist MK886 (Kehrer et al. 2001). In addition, the effects of direct activation of PPAR-α receptors were studied by administering [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid (WY14643), a selective PPAR-α agonist (Forman et al. 1997; Krey et al. 1997), and the effects of direct activation of cannabinoid CB_1 receptors was studied by administering THC.

Male Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 250–280 g were housed two per cage with food and water available ad libitum in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Experimental procedures were conducted during the light phase. Each animal was adapted to daily handling for 1 wk before the start of experiments. All experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse (NIDA) and the Guide for Care and Use of Laboratory Animals (National Research Council 2003).

[7]**Corresponding author.**
E-mail syasar@jhmi.edu; fax (410) 550-2513.
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The procedure used to study learning and memory was adapted from Mazzola et al. (2003). The two-compartment step-through apparatus (MED Associates model ENV-010MC) consisted of an illuminated compartment and a dark compartment, separated by a guillotine door. The illumination (measured with a Sekonic light meter, model L-308-B) was ~172 lux in the center of the light compartment and ~5 lux in the dark compartment facing the doorway. In the first session, an adaptation trial was conducted by placing the rat into the illuminated compartment facing away from the dark compartment; after 60 sec the guillotine door was opened, allowing the rat to enter the dark compartment. The latency for the rat to fully enter the dark compartment was recorded. The guillotine door was closed once the dark compartment was entered, then the rat was returned to its home cage.

During the learning trial, conducted 24 h after the habituation trial, rats were again placed in the illuminated compartment and the guillotine door was opened after 60 sec; when the rat entered the dark compartment, the door was closed and a scrambled foot shock (0.5 mA and 2 sec) was delivered to the grid floor. The rat was then returned to its home cage. Several rats that returned to the light compartment before the door finished closing during this learning trial were dropped from the study. Twenty-four hours after the learning trial, a retention test was performed by placing the rat in the illuminated compartment with the door open, then measuring the latency for complete entry into the dark compartment. No shock was delivered during the test. The test session ended after 300 sec if the rat did not enter the dark compartment.

Drugs were administered either before or immediately after the learning trial (to assess effects on memory acquisition and consolidation, respectively), or before the retention test (to assess effects on memory retrieval). URB597 (Kadmus) and MK886 (Tocris) were dissolved in 20% DMSO and sterile water. WY14643 (Tocris) was dissolved in 70% DMSO and sterile water. THC and rimonabant (SR141716) (NIDA, NIH) were dissolved in 2% Tween 80, 2% ethanol, and sterile water. Scopolamine (Tocris) was dissolved in sterile water. All drugs were injected intraperitoneally (i.p.) in a volume of 1 mL/kg.

During the adaptation and learning trials, all vehicle control and drug groups entered the dark compartment rapidly, and latencies to enter the dark compartment did not differ significantly between any of the groups and their vehicle control groups during these phases (Figs. 1A and 2A). When scopolamine (0.5 mg/kg) was given 30 min before the learning trial, latencies to enter the dark compartment during the retention test 24 h later were markedly decreased (t-test $t_{(13)} = 2.65$, $P < 0.05$), confirming that passive-avoidance learning in this procedure was sensitive to impairment by an amnesic agent (Fig. 1C).

The FAAH inhibitor URB597 (0.1–1.0 mg/kg), injected 40 min before the learning trial, had a significant enhancing effect on memory acquisition, increasing the latency to enter the dark compartment during the retention test 24 h later (Fig. 1C; ANOVA $F_{(3,79)} = 5.15$, $P < 0.003$). Similarly, the PPAR-α synthetic agonist WY14643 (10–40 mg/kg), injected 10 min before the learning trial, also had a significant enhancing effect on memory acquisition (Fig. 1C; ANOVA $F_{(3,51)} = 4.82$, $P < 0.005$). These enhancing effects of URB597 and WY 14643 were only seen when they were given before the learning trial, not when they were given immediately after the learning trial (to test for effects on memory consolidation; Fig. 1D) or when they were given 40 min (URB597) or 10 min (WY14643) before the retention test (to test for effects on memory retention; Fig. 1E). In contrast, the CB1 receptor agonist

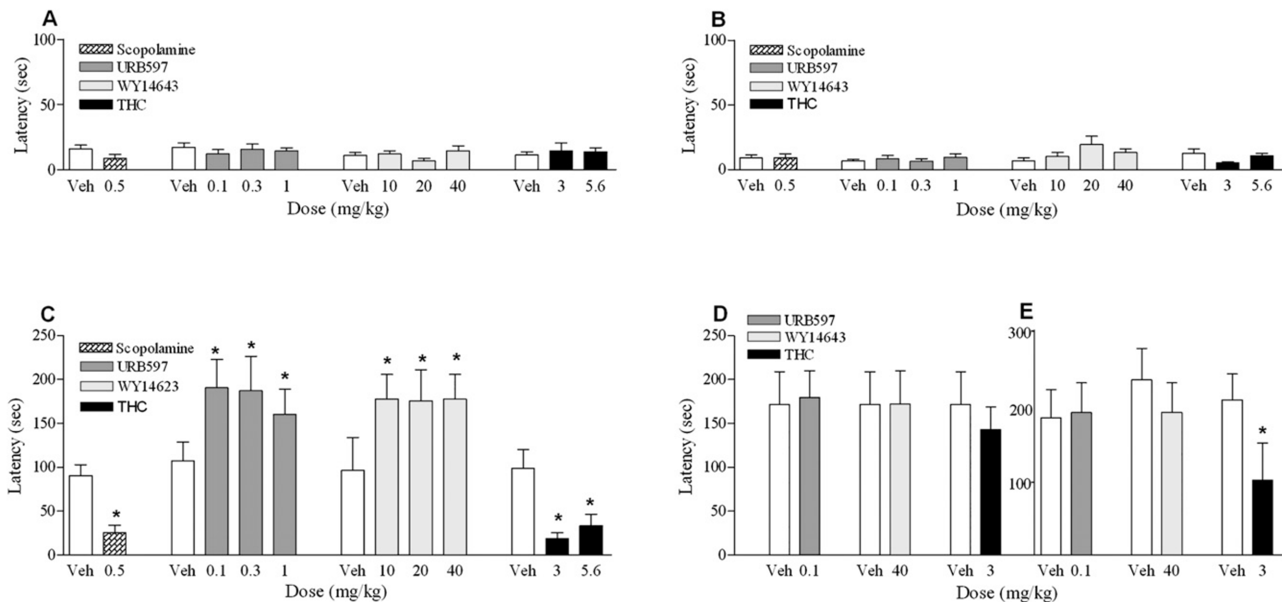

Figure 1. Effects of drugs on memory acquisition, consolidation, and retrieval. Data are expressed as mean latency (sec) ± SEM to enter the dark compartment. (*A*–*C*) Show latencies during the adaptation trial (*A*), learning trial (*B*), and retention test (*C*) for rats that received a drug or vehicle injection only before the learning trial. None of the groups differed from vehicle controls during the adaptation or learning trials. During the retention test, performed 24 h after the learning trial, rats that had received scopolamine or THC before the learning trial had significantly shorter latencies than control rats, indicating that these drugs impaired memory acquisition. In contrast, latencies were significantly higher than controls in rats that had received URB597 or WY14643 before the learning trial, indicating that these drugs enhanced memory acquisition. (*D*) Panel shows that neither URB597 nor WY14643 had a significant effect on latencies during the test in groups that received these drugs immediately after the learning trial or (*E*) 20 or 40 min, respectively, before the test, indicating that these drugs did not alter memory consolidation or retention. Latencies during the habituation and learning trials for the rats in *D* are not shown, but were similar to those seen in *A* and *B*. From *left* to *right*, *N*s for the bars in *A*, *B*, and *C* were: 7, 8, 10, 10, 11, 7, 10, 8, 8, and 7; in *D*: 10, 10, 10, 10, 10, and 9; and in *E*: 10, 9, 10, 8, 10, and 10. (*) $P < 0.05$ compared with vehicle control (VEH), paired comparisons performed with Tukey procedure.

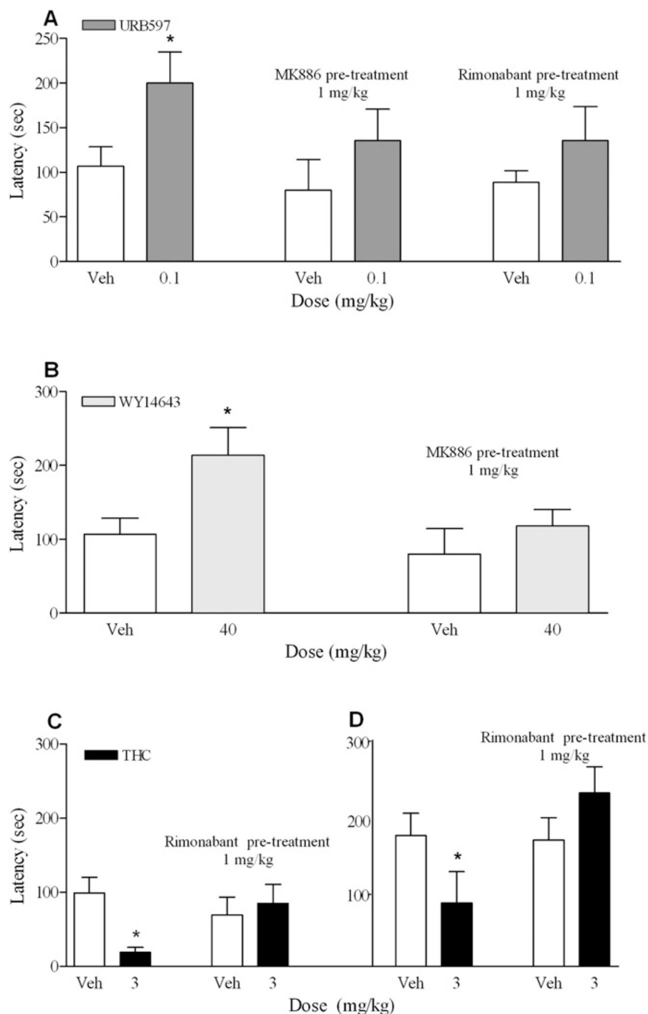

Figure 2. Blockade of URB597-, WY14643-, and THC-induced effects on memory acquisition. Data are expressed as mean latency (sec) ± SEM to enter the dark compartment during the retention test. Blockade of PPAR-α by MK886 (1 mg/kg) reversed the enhancement of memory acquisition by URB597 (0.1 mg/kg; *A*) and WY14643 (40 mg/kg; *B*). Blockade of CB_1 reversed the enhancement produced by URB597 and also reversed the deficits induced by THC (3 mg/kg) when given before the learning trial (*C*) or before the retention test (*D*). From *left* to *right*, *N*s for the bars in *A* were: 10, 10, 8, 8, 8, and 10; in *B*: 10, 7, 8, and 8; in *C*: 10, 7, 8, and 10; and in *D*: 9, 8, 9, and 9. (*) $P < 0.05$ compared with vehicle control (VEH), paired comparisons performed with Tukey procedure.

THC (3 and 5.6 mg/kg) injected 30 min before the learning trial significantly impaired memory acquisition (Fig. 1C; $F_{(2,22)} = 20.85$, $P < 0.05$), and this impairment (THC 3 mg/kg) was reversed by pretreatment with 1 mg/kg rimonabant (Fig. 2C; ANOVA, interaction of pretreatment and treatment, $F_{(1,36)} = 7.65$, $P < 0.05$). THC (3 mg/kg) also impaired retention when given 30 min before the test (*t*-test $t_{(18)} = 2.2$, $P < 0.05$; Fig. 1E), and this impairment was reversed by 1 mg/kg rimonabant (Fig. 2D; ANOVA, interaction of pretreatment and treatment, $F_{(2,22)} = 5.51$, $P < 0.05$).

Further testing demonstrated that the memory-enhancing effects of URB597 were blocked when rats were pretreated with either 1.0 mg/kg of the PPAR-α antagonist MK886 (ANOVA, interaction of pretreatment and treatment, $F_{(1,32)} = 6.29$, $P < 0.05$) or 1.0 mg/kg of the CB1-receptor antagonist rimonabant (ANOVA, interaction of pretreatment and treatment, $F_{(1,34)} = 8.76$, $P < 0.05$) 60 min before the learning trial (Fig. 2A). The enhancements produced by giving WY14643 before the learning trial were also blocked by 1.0 mg/kg MK886 (Fig. 2B; ANOVA, interaction of pretreatment and treatment, $F_{(1,29)} = 5.36$, $P < 0.05$). Neither 1.0 mg/kg of MK886 nor 1.0 mg/kg of rimonabant affected learning when given with the vehicles for URB597 or WY14643 before the learning trial (Fig. 2A,B).

In a second set of experiments, designed to assess the possibility that URB597, WY14643, or THC might induce motor or emotional effects that could influence the acquisition or expression of the passive-avoidance response, we also investigated the effects of these drugs on locomotor activity and anxiety-related behavior of naïve male Sprague-Dawley rats in an open-field test (Prut and Belzung 2003) and a light/dark test (Scherma et al. 2008). Open-field arenas (Med Associates) were enclosed in sound-attenuation chambers, with two arenas in each chamber and a small light on the wall of the chamber providing illumination of ~2.6 lux. The open-field arenas (41 × 41 × 32 cm) were composed of clear acrylic and had sawdust bedding on the floor. Activity was measured during 5-min sessions (a duration similar to that used in the learning trial and retention test of the passive-avoidance procedure) with a 16 × 16 array of photobeams using Med Associates Open Field Activity Software.

The measures analyzed for the open-field test were: distance traveled, number of ambulatory episodes, average speed within ambulatory episodes, number of stereotypy counts, number of vertical counts, number of jump counts, number of entries into a center zone (defined as a square covering 1/9th of the field), and time spent within 5 cm of the walls of the field (thigmotaxis), as shown in Figure 3. At the doses tested, URB597, WY14643, and THC had little or no effect on general activity (Fig. 3A–F) or anxiety-related behavior (i.e., center-zone entries and thigmotaxis) (Fig. 3G,H; Prut and Belzung 2003) in the open field. URB597 (0.1 mg/kg) produced a significant increase in jumping (Fig. 3G; $F_{(3,24)} = 7.59$, $P < 0.001$). WY14643 (20 mg/kg) produced a marginal decrease in distance traveled (Fig. 3A; ANOVA $F_{(3,30)} = 8.34$, $P < 0.056$). All other measures were unaffected.

The light/dark test utilized the same two-compartment step-through apparatus (MED Associates model ENV-010MC) used for the passive avoidance studies, with the same levels of illumination. To parallel the procedure used to test learning and memory in the present study, there was a 5-min adaptation trial the day before the test trial; this differed from the procedure used in our previous study, in which URB597 had significant anxiolytic effects, in that no adaptation trial was conducted in the earlier study (Scherma et al. 2008). During both the adaptation and test trials, rats were placed in the illuminated compartment facing away from the dark compartment; after 60 sec, the guillotine door was opened, allowing the rat to enter the dark compartment. During the 5-min test, time spent (seconds) in the light compartment and the level of activity (counts/minute) were measured. At the doses tested, neither URB597 nor WY 14643 had a significant effect on time spent in the light compartment or on the level of activity compared with vehicle-treated controls. The mean ± SEM numbers of seconds spent in the light compartment were 129.9 ± 7.2 for URB597, 131 ± 14.9 for URB597's vehicle, 102.2 ± 11.1 for WY 14643, and 128.6 for WY 14643's vehicle. The mean ± SEM numbers of activity counts per minute were 49.8 ± 2.7 for URB597, 50.7 ± 5.4 for URB597's vehicle, 41.7 ± 4.0 for WY 14643, and 50.0 ± 3.8 for WY 14643's vehicle (all *P*s > 0.13). The failure to see significant anxiolytic effects of URB597, as we did in previous experiments using the light/dark test (Scherma et al. 2008), may be due to the procedural change of allowing an adaptation trial prior to the test trial, which would minimize stress during testing in the present experiments.

The results of this study indicate that manipulations of PPAR-α activity can have positive effects on memory acquisition

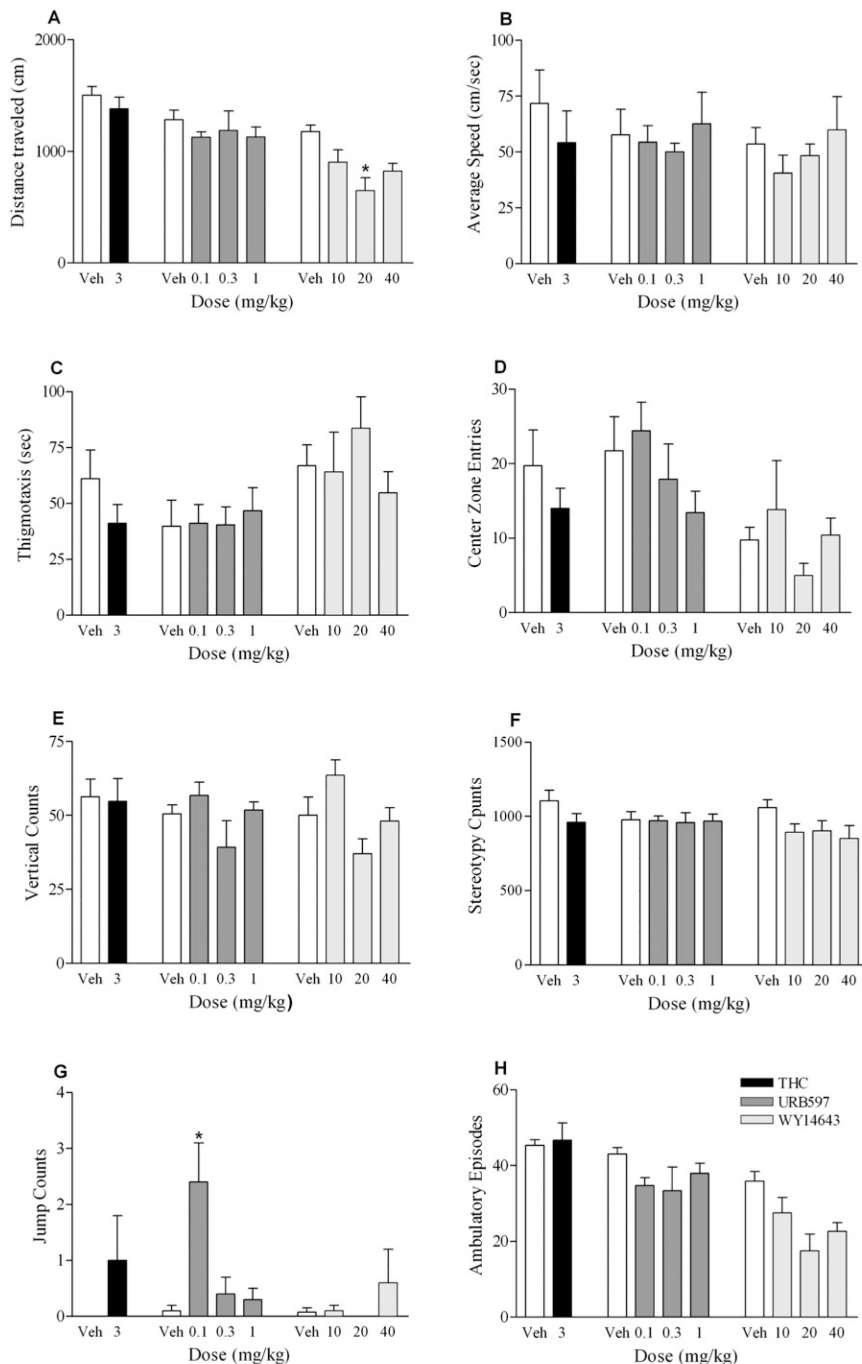

Figure 3. Effects of drugs on open-field behavior. Data are expressed as mean ± SEM. (*A*) Distance traveled, (*B*) average speed, (*C*) time spent within 5 cm of a wall (thigmotaxis), (*D*) center-zone entries, (*E*) vertical counts, (*F*) stereotypy counts, (*G*) jump counts, and (*H*) ambulatory episodes. Although URB597 (0.1 mg/kg) produced a small but significant increase in jumping (*G*) ($P < 0.05$) and WY14643 (20 mg/kg) produced a marginal decrease in distance traveled (*A*) ($P < 0.056$) compared with vehicle control (VEH), none of the other treatments had a significant effect on any of these measures of general activity (*A*,*B*,*E*,*H*) or anxiety-related behavior (*C*,*D*). From *left* to *right*, *N*s for the bars in all panels were: 7, 7, 7, 7, 7, 7, 13, 6, 6, and 7. All paired comparisons performed with Tukey procedure.

in a passive-avoidance procedure. These effects were obtained when a synthetic PPAR-α agonist (WY14643) was administered, or when levels of endogenous lipid amides that act at CB_1 and PPAR-α receptors were increased by the FAAH inhibitor URB597. In both of these cases, the memory-enhancing effects were blocked by pretreatment with the selective PPAR-α antagonist, MK886. These results are consistent with the recent finding that FAAH inhibition can enhance place-memory acquisition in the water-maze procedure (Varvel et al. 2007) and extend them by demonstrating the role of PPAR-α in such effects.

URB597 and WY14643 did not affect memory consolidation or retrieval when given immediately after the learning trial or before the retention test, respectively. The possibility remains that they could affect consolidation under dosing, training, or testing parameters other than the ones used. It seems unlikely that the onset of action of URB597 was outside the window of consolidation, since systemically administered URB597 can almost completely block FAAH activity within 15 min (Kathuria et al. 2003). In addition, administration of WY14643, which is likely to produce a more rapid and direct activation of PPAR-α within the window of consolidation, was also ineffective.

Neither the enhanced memory acquisition produced by URB597 and WY14643 nor the impaired memory acquisition produced by THC can be attributed to changes in general activity, exploration, arousal, or anxiety, since URB597, WY14643, and THC had little or no effect on any open-field activity measure, and URB597 and WY14643 had no significant effect on anxiety or activity in the light–dark box. Furthermore, none of these drugs altered latencies to enter the dark compartment during the learning trial of the passive-avoidance procedure. The fact that we did not observe significant anxiolytic effects of URB597 like those we obtained in previous experiments using the light/dark test (Scherma et al. 2008) may be due to the procedural change of allowing an adaptation trial prior to the test trial, which would minimize stress during testing (Haller et al. 2009). Some of the endogenous fatty acids affected by FAAH inhibition (i.e., OEA and anandamide) also affect signaling at the TRPV1 receptor (Ahern 2003; Starowicz et al. 2007; Rubino et al. 2008). TRPV1 knock out mice show reduced anxiety and fear-based learning compared with wild-type littermates (Marsch et al. 2007), and systemic administration of high doses of anandamide may induce TRPV1-mediated anxiety-like effects or disruptions in behavior when given in combination with a FAAH inhibitor (Scherma et al. 2008; Panlilio et al. 2009). However, systemic administration of a FAAH inhibitor alone has not been shown to produce such effects (Scherma et al. 2008; Panlilio et al. 2009).

Systemically administered cannabinoid CB_1-receptor agonists such as THC can cause learning and memory impairments in passive-avoidance tests in rodents (present experiments) (Castellano et al. 1997, 2003; Mishima et al. 2001; Niyuhire et al. 2007). This suggests that indirect cannabinergic effects of URB597 (i.e., increased anandamide levels) could produce a learning impairment. However, URB597 only increases anandamide levels at those neuronal sites and brain areas where anandamide is synthesized and released, producing a neuron-specific activation of CB_1 receptors in those areas, unlike the global activation of all CB_1 receptors everywhere in the brain produced by systemic administration of CB_1 agonists such as THC. Thus, it is possible that activation of CB_1 receptors at specific neuronal sites in selective areas of the brain, as would be expected with URB597, does not impair learning and memory. In contrast, although this hypothesis could not be tested in the present study, the fact that the effects of URB597 were reversed by the CB_1 antagonist/inverse agonist rimonabant may indicate that selective activation of CB_1 receptors can produce synergistic effects with PPAR-α. These results are consistent with recent findings that joint stimulation of PPAR-α and CB_1 receptors produces synergistic antinociceptive effects on peripheral pain, that these effects are reversed by rimonabant (Russo et al. 2007), and that antinociceptive effects of URB597 on peripheral pain can be blocked by PPAR-α antagonism (Sagar et al. 2008). Further studies are needed to determine whether synergistic enhancement of learning and memory can be achieved with local coadministration of naturally occurring endogenous ligands for CB_1 receptors (anandamide) and PPAR-α (OEA and PEA), whose brain levels are all increased by FAAH inhibition.

It is well known that PPAR-α receptors are intimately involved in inflammatory processes (e.g., Combs et al. 2001) and in disruptions in glucose metabolism (e.g., Guerre-Millo et al. 2000) that may contribute to cognitive decline with Alzheimer's disease. PPAR-α activation reduces elevated glucose levels by improving insulin sensitivity (Guerre-Millo et al. 2000) and lowers levels of proinflammatory cytokines in aged animals (Poynter and Daynes 1998). PPAR-α receptors are also involved in regulation of the biosynthesis of acetylcholine (de la Monte and Wands 2006), and they are present in relatively large numbers in memory-related brain areas (Moreno et al. 2004). Polymorphisms in the PPAR-α gene are associated with increased risk for Alzheimer's disease (Brune et al. 2003). In the present experiments we show that the FAAH inhibitor URB597 and the PPAR-α agonist WY14643 can acutely enhance memory acquisition through actions involving PPAR-α. These findings provide novel mechanisms for cognitive enhancement either by synergistic activation of PPAR-α and cannabinoid CB_1 receptors by FAAH inhibition or by direct activation of PPAR-α by administration of selective PPAR-α agonists. The findings with FAAH inhibition further suggest a new approach for developing medications that work indirectly by enhancing the actions of endogenous lipid amide mediators where they are synthesized and released.

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