The Endogenous Cannabinoid Anandamide Produces δ-9-Tetrahydrocannabinol-Like Discriminative and Neurochemical Effects That Are Enhanced by Inhibition of Fatty Acid Amide Hydrolase but Not by Inhibition of Anandamide Transport

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ABSTRACT

Anandamide is an endogenous ligand for brain cannabinoid CB_1 receptors, but its behavioral effects are difficult to measure due to rapid inactivation. Here we used a drug-discrimination procedure to test the hypothesis that anandamide, given i.v. or i.p., would produce discriminative effects like those of δ-9-tetrahydrocannabinol (THC) in rats when its metabolic inactivation was inhibited. We also used an in vivo microdialysis procedure to investigate the effects of anandamide, given i.v. or i.p., on dopamine levels in the nucleus accumbens shell in rats. When injected i.v., methanandamide (AM-356), a metabolically stable anandamide analog, produced clear dose-related THC-like discriminative effects, but anandamide produced THC-like discriminative effects only at a high 10-mg/kg dose that almost eliminated lever-press responding. Cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB-597), an inhibitor of fatty acid amide hydrolase (FAAH), the main enzyme responsible for metabolic inactivation of anandamide, produced no THC-like discriminative effects alone but dra-matically potentiated discriminative effects of anandamide, with 3 mg/kg anandamide completely substituting for the THC training dose. URB-597 also potentiated the ability of anandamide to increase dopamine levels in the accumbens shell. The THC-like discriminative-stimulus effects of anandamide after URB-597 and methanandamide were blocked by the CB_1 receptor antagonist rimonabant, but not the vanilloid VR_1 receptor antagonist capsazepine. Surprisingly, the anandamide transport inhibitors N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide (AM-404) and N-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide (UCM-707) did not potentiate THC-like discriminative effects of anandamide or its dopamine-elevating effects. Thus, anandamide has THC-like discriminative and neurochemical effects that are enhanced after treatment with a FAAH inhibitor but not after treatment with transport inhibitors, suggesting brain area specificity for FAAH versus transport/FAAH inactivation of anandamide.

The endogenous cannabinoid (CB) system, which is targeted by the psychoactive ingredient in cannabis, δ-9-tetrahydrocannabinol (THC), comprises receptors termed CB_1 and CB_2 (and others not yet identified) endogenous compounds that activate these receptors and enzymes involved in the synthesis and degradation of these endogenous cannabinoids

ABBREVIATIONS: CB, cannabinoid; THC, δ-9-tetrahydrocannabinol; FAAH, fatty acid amide hydrolase; AM-356, methanandamide, *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide; AM-404, N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide; UCM-707, N-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide; VDM-11, N-(4-hydroxy-2-methylphenyl) arachidonoyl amide; LY2318912, (5-[(4-azido-3-iodo-benzoylamino)-methyl]-tetrazole-1-carboxylic acid dimethylamide); OL-135, 7-phenyl-1-(5-pyridin-2-yl-oxazol-2-yl)-heptan-1-one; NAc, nucleus accumbens; SR141716, N-piperidino-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; VR, vanilloid receptor; Rim, rimonabant; ANOVA, analysis of variance; DA, dopamine.

(Freund et al., 2003; Piomelli, 2003; Di Marzo et al., 2004). A way to investigate whether exogenous and endogenous cannabinoids produce qualitatively similar effects is to use drug-discrimination procedures (Solinas et al., 2006b), with THC as the baseline training drug (Browne and Weissman, 1981; Wiley et al., 1995; Burkey and Nation, 1997; Jarbe et al., 2001; Solinas et al., 2003; Alici and Appel, 2004).

Among the endogenous ligands for cannabinoid CB_1 receptors, anandamide is the best-characterized (Freund et al., 2003; Piomelli, 2003; Di Marzo et al., 2004). Anandamide is synthesized on demand, binds with high affinity to extracellular CB_1 receptors, and is rapidly inactivated, presumably by a two-step process thought to involve active transport into neurons by a yet-to-be identified membrane transport mechanism followed by FAAH hydrolysis (Freund et al., 2003; Piomelli, 2003; Di Marzo et al., 2004; Moore et al., 2005), although the existence of a transport process has been a matter of debate (Glaser et al., 2005; Dickason-Chesterfield et al., 2006). It is probably because of this rapid inactivation that anandamide has failed to produce THC-like effects in previous drug-discrimination studies, because methanandamide, a longer-lasting synthetic analog of anandamide that is not metabolized by FAAH, produces complete generalization to THC training stimuli under similar conditions (Burkey and Nation, 1997; Jarbe et al., 2001). Pharmacological tools have been developed that inhibit these two mechanisms of anandamide inactivation (Piomelli, 2003). For example, compounds, such as AM-404 [N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide], VDM-11, UCM-707 [N-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide], and LY2318912, inhibit the transport of anandamide into neurons (Piomelli et al., 1999; De Petrocellis et al., 2000; Lopez-Rodriguez et al., 2003; Glaser et al., 2005; Moore et al., 2005), and compounds, such as cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB-597) and OL-135, inhibit the activity of FAAH (Kathuria et al., 2003; Lichtman et al., 2004). Administration of these compounds or genetic ablation of FAAH results in increases in anandamide concentrations in plasma and some brain areas (Giuffrida et al., 2000; Kathuria et al., 2003; Fegley et al., 2004; Lichtman et al., 2004; Bortolato et al., 2006) and in the potentiation and prolongation of many of the effects of anandamide (Calignano et al., 1997; Cravatt et al., 2001; Kathuria et al., 2003).

We recently found that both anandamide and methanandamide increase extracellular dopamine levels in the nucleus accumbens shell when injected intravenously (Solinas et al., 2006a), a characteristic effect of most drugs that have reinforcing or rewarding effects in experimental animals and that are abused by humans (Di Chiara, 2002; Wise, 2002). Anandamide and methanandamide produced similar peak effects, but the effects of methanandamide were more prolonged. Inhibition of FAAH by administration of URB-597 dramatically potentiated both the magnitude of peak elevations in dopamine levels in the nucleus accumbens shell produced by anandamide and the duration of these elevations (Solinas et al., 2006a). These results were obtained after i.v. injections of anandamide, a route of administration that might have reduced its hepatic first-passage metabolism, thus increasing concentrations of anandamide in the brain.

Because of previous failures to demonstrate significant THC-like discriminative effects of anandamide when given intraperitoneally or intramuscularly, we tested the hypothesis that anandamide would produce THC-like discriminative effects when administered intravenously. We first trained rats to discriminate i.p. injections of 3 mg/kg THC from saline with a single daily session procedure (Solinas et al., 2003) followed by training with a multiple sessions per day procedure until a baseline was reestablished and THC dose-response curves were determined by testing different i.p. and i.v. doses of THC. We determined whether i.v. or i.p. injections of anandamide alone or in combination with FAAH inhibition by URB-597 or transport inhibition by AM-404 or UCM-707 produced THC-like discriminative effects and compared these effects with those obtained with i.p. anandamide. We also compared effects of i.v. anandamide and methanandamide and assessed the receptors involved in the effects of anandamide by treating rats with specific cannabinoid CB_1 and vanilloid VR_1 receptor antagonists before administering anandamide. Finally, we performed parallel in vivo microdialysis experiments to compare these behavioral effects with the effects of i.p. and i.v. anandamide, given alone or in combination with FAAH inhibition by URB597 or transport inhibition by AM-404, on dopamine levels in the shell of the nucleus accumbens.

Materials and Methods

Subjects. For drug-discrimination studies, male Sprague-Dawley rats initially weighing 350 to 380 g (Charles River, Wilmington, MA) were housed individually. Weights of rats were gradually reduced to approximately 85% of free feeding by limiting daily access to food before the start of drug-discrimination training sessions. Once drug-discrimination sessions were started, weight was maintained at approximately 85% of free feeding by giving 15 g of food pellets shortly after the end of each daily session. Water was available ad libitum. For microdialysis studies, male Sprague-Dawley rats weighing 300 to 350 g (Charles River) were used. They were housed two per group with food and water available ad libitum. All rats were housed in temperature- and humidity-controlled rooms and were maintained on 12-h light/dark cycles. The lights were on from 6:45 AM to 6:45 PM, and experiments were conducted during the light phase. Animals used in these studies were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and 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), National Institutes of Health and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003).

Drugs. THC and rimonabant (SR141716, N-piperidino-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methylpyrazole-3-carboxamide) were obtained from the National Institute on Drug Abuse, NIH (Rockville, MD). Anandamide and capsazepine were purchased from Sigma/RBI (St. Louis, MO). UCM-707 was purchased from Tocris Cookson (Ellisville, MO). Methanandamide [AM-356, R-(+)-arachidonyl-1′-hydroxy-2′-propylamide] and AM-404 were provided by Dr. Alex Makriyannis (Center for Drug Discovery, Northeastern University, Boston, MA) and University of Connecticut, Center for Drug Discovery and Departments of Pharmaceutical Sciences and Molecular Cell Biology (Storrs, CT). URB-597 was provided by Dr. Daniele Piomelli (Department of Pharmacology, University of California, Irvine, CA). THC, 50 mg/ml in ethanol, was dissolved in a solution, 40% w/v of β-hydroxy-cyclodextrine (Sigma/RBI) for i.p. administration and in vehicle containing 2% Tween 80, 2% ethanol, and 96% saline for i.v. administration. All the other drugs were dissolved in

vehicle containing 2% Tween 80, 2% ethanol, and 96% saline and were injected in a volume of 1.0 ml/kg i.p. or i.v., with the exception of URB-597, which was dissolved in a vehicle containing 20% dimethyl sulfoxide in saline and injected in a volume of 2.0 ml/kg i.p.

Drug-Discrimination Apparatus. Standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber contained two levers separated by a recessed tray into which a pellet dispenser could deliver 45 mg of food pellets (F0021; Bioserv, Frenchtown, NJ). Each press of a lever with a force of 0.4 N through 1 mm was recorded as a response and was accompanied by an audible click. The operant-conditioning chambers were controlled by computers using a MED-PC software package (MED Associates, East Fairfield, VT).

Single Session per Day Training Procedure. Rats were trained under a discrete-trial schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 3 mg/kg THC and on the other lever after an injection of 1 ml/kg THC vehicle. Injections of THC or vehicle were given i.p. 30 min before the start of the session. At the start of the session, a white house light was turned on and, in its presence, the rats were required to make 10 consecutive responses (fixed-ratio 10 schedule of food delivery) on the lever appropriate to the presession treatment. The completion of 10 consecutive responses on the injection-appropriate lever produced delivery of a 45-mg food pellet and initiated a 45-s time-out during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the injection-inappropriate lever reset the fixed-ratio requirement on the injection-appropriate lever. After each time-out, the white house light was again turned on and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min had elapsed, whichever occurred first.

Discrimination-training sessions were conducted 5 days per week under a double alternation schedule (i.e., DDVVDDVV etc., where D = drug THC and V = vehicle). Training continued until there were eight consecutive sessions during which rats completed at least 90% of their responses during the session on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. A second phase of training was then initiated with a modified procedure.

Multiple sessions per Day Training Procedure. To maximize the number of tests with i.v. administrations, in relation to the duration of catheter patency, we conducted three consecutive discrimination sessions each test day and multiple sessions on some training days. These multiple sessions were run at 60-min intervals (from the start of one session to the start of the next session). To retain food intake similar to that with a single session per day procedure, we reduced the number of pellets that could be obtained during each of the three sessions to 10, for a maximum of 30 pellets per day. To habituate rats to multiple sessions per day and minimize stress that could disrupt behavior, we semirandomly alternated the number of sessions during training days, with one, two, or three discrimination sessions per day. During days with training sessions, rats were injected i.p. 30 min before each session with either vehicle or the 3-mg/kg dose of THC. THC was administered every second training day as with the normal single session per day training procedure (see above). During drug training days, when multiple sessions were conducted, THC was only injected before the last session of the day. The change to the multiple sessions per day training procedure initially resulted in some behavioral disruption, although no change in the ability of rats to discriminate THC from vehicle was found. Responding stabilized within 1 to 3 weeks.

After responding stabilized under the multiple sessions per day procedure, days with test sessions were initiated under a single alternation schedule (DTDVTVTVDT etc., where D = drug training day; V = vehicle training day; and T = test day). In this way, days with test sessions occurred with equal probability after training days with only saline vehicle sessions and training days with drug (THC) sessions. Test sessions were identical to training sessions, with the

exception that 10 consecutive responses on either one of the two levers ended the fixed-ratio trial and switching responding from one lever to the other lever reset the fixed-ratio requirement. Test sessions were conducted only if the criterion of 90% accuracy and not more than four incorrect responses during the first trial of each session was maintained during the two preceding training days. If rats failed to meet this criterion, additional days with training sessions were run according to the single alternation schedule until the criterion was met for at least two consecutive days.

Initially, under the multiple sessions per day procedure, test days with three different i.p. doses of THC, given in escalating order 30 min before each session over three consecutive sessions, were conducted to demonstrate that rats continued to correctly discriminate THC from vehicle in a dose-dependent manner. Rats then were catheterized, as described below, and test sessions with i.v. injections of THC and other drugs and drug combinations were started. During days with test sessions, rats were administered vehicle, THC, anandamide, or methanandamide i.v. 3 min before each of the three sessions, with or without pretreatment with the CB_1-receptor antagonist rimonabant, the vanilloid VR_1-receptor antagonist capsazepine, the anandamide-transport inhibitor AM-404, or the FAAH inhibitor URB-597. Doses of THC, anandamide, or methanandamide were given in an escalating order to reduce interference with the effects of the next dose due to accumulation. A third and final phase of the study was conducted with the one session per day procedure after most rats had developed blocked catheters and consisted of i.p. injections of anandamide with or without different anandamide-transport or FAAH inhibitors.

Two behavioral measures were used for analysis: 1) percentage of total lever-presses made on the THC lever, which gives a quantitative indication of how much the drug or the combination of drugs tested produce discriminative effects similar to those of the 3 mg/kg training dose of THC; 2) overall rate of lever-press responding, which gives an indication of any disruption of motor responses produced by the drug or the combination of drugs tested. When rates of responding were significantly reduced compared with basal levels, administrations of higher doses of that specific drug or combination of drugs were normally avoided.

Catheterization. Catheters were implanted in the right jugular vein under aseptic conditions using i.p. ketamine (60 mg/kg) and xylazine (10 mg/kg) anesthesia. Catheters consisted of approximately 4 cm of Silastic tubing (0.044 mm i.d., 0.814 mm o.d.) connected to vinyl tubing (Dural Plastics, 0.5 mm i.d., 1.0 mm o.d.) with a 23-g stainless steel tube and bonded with polyethylene shrink tubing. A 10-mm long section of Tygon tubing (5 mm o.d.) was glued to the catheter at the midpoint of the vinyl tubing and used as a subcutaneous anchor when the catheter exited the skin in the neck. The right external jugular vein was exposed by blunt dissection, and the silastic portion of the catheter was inserted into the vein and sutured into place. The vinyl portion of the catheter was passed subcutaneously to the back of the neck, where the tip exited and was obturated with a modified 23-g needle. The incision was closed with stainless wound clips. Catheters were flushed before and after each session with 0.1 ml of saline solution.

In Vivo Microdialysis. Concentric dialysis probes were prepared with AN69 fibers (Hospal Dasco, Bologna, Italy). In brief, two 4-cm pieces of silica-fused capillary tubes (the inlet and outlet tubing of the probes) were inserted into a 6-mm capillary dialyzing fiber (closed by a drop of glue on the other side), with the inlet tubing set at approximately 0.1 mm from the closed end of the fiber and the outlet set at 2.0 mm from the inlet tip. The open end of the dialysis membrane was then glued, and the protruding two silica-fused tubes were inserted and glued into a 22-G stainless steel needle (2.4 mm length). The needle was then secured to a CMA/10 clip (CMA/Microdialysis AB, Solna, Sweden) and mounted in a stereotaxic holder. The exposed dialyzing surface of the fibers, i.e., not covered by glue, was limited to the lowest 2.0-mm portion of the probes. During the same surgery session, rats were implanted with intravenous cathe-
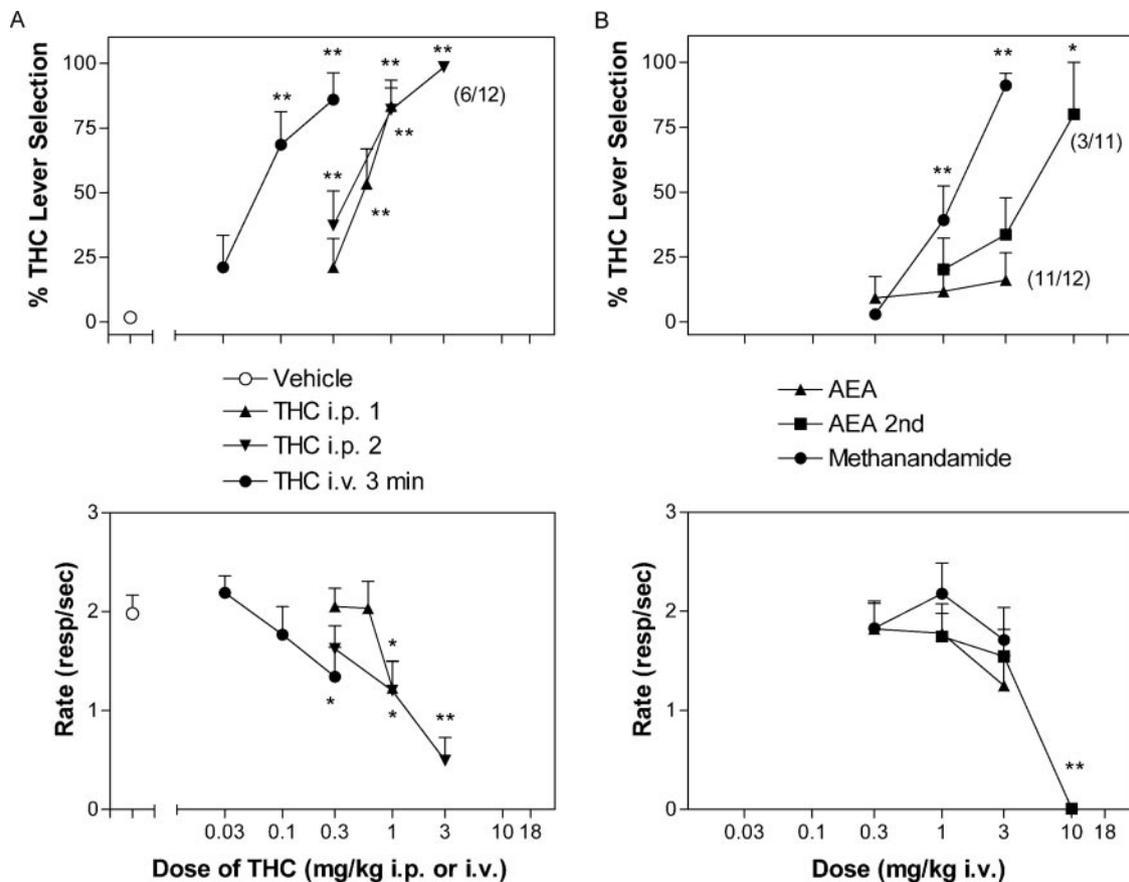
Fig. 1. Dose-response curves for discriminative effects of THC (A) and THC-like discriminative effects of anandamide and methanandamide (B) using a multiple sessions per day procedure. Ordinates: overall percentage of responses on the lever associated with THC administration (top) and overall rate of lever pressing expressed as responses per seconds (bottom) averaged over the entire session. Abscissae: dose in milligram/kilogram (log scale). Different i.p. doses of THC were administered 30 min before each of three consecutive sessions run at 60-min intervals (left). Different i.v. doses of THC, anandamide (AEA) or methanandamide were administered 3 min before each of three consecutive sessions ran at 60-min intervals (right). Repeated measures ANOVA followed by post hoc Dunnet's test: $*$ and $**$, $p < 0.05$ and $p < 0.01$ compared with vehicle. Numbers in parentheses at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested.

ters (as described above) and microdialysis probes. Rats were then placed in a stereotaxic apparatus where the skull was exposed and a small hole was drilled to expose the dura. A concentric dialysis probe aimed at the nucleus accumbens shell was then lowered into the brain (Fig. 1), in accordance with coordinates in the rat brain atlas by Paxinos and Watson (1998) (uncorrected coordinates, in mm: anterior, $+2.0$, and lateral, 1.1, from bregma; vertical, -7.9, from dura). After surgery, rats were placed in hemispherical CMA-120 cages (CMA/Microdialysis AB) equipped with overhead fluid swivels (Instech Laboratories Inc., Plymouth Meeting, PA) for connections to the dialysis probes and allowed to recover overnight.

Approximately 24 h after probe implant, experiments were performed on awake, freely moving rats in the same hemispherical home cages in which they recovered overnight from surgery. Ringer's solution (147.0 mM NaCl, 2.2 mM $CaCl_2$, and 4.0 mM KCl) was delivered by a 1.0-ml syringe operated by a BAS Bee Syringe Pump Controller (BAS West Lafayette, IN), through the dialysis probes at a constant flow rate of 1 μl/min. Collection of dialysate samples (10 μl) started after 30 min, and samples were taken every 10 min and immediately analyzed, as detailed below. After stable dopamine level values (less than 10% variability) were obtained for at least three consecutive samples (typically after approximately 1 h), rats were treated with drug, drug vehicle, or saline.

Analytical Procedure. Dialysate samples (10 μl) were injected without purification into a high-performance liquid chromatography apparatus equipped with a MD 150 $\times$ 3.2 mm column, particle size 3.0 μm (ESA, Chelmsford, MA) and a coulometric detector (5200a

Coulochem II; ESA) to quantify DA. The oxidation and reduction electrodes of the analytical cell (5014B; ESA) were set at $+125$ and -125 mV, respectively. The mobile phase, containing 100 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.5 mM n-octyl sulfate, and 18% (v/v) methanol (pH adjusted to 5.5 with Na_2HPO_4), was pumped by an ESA 582 (ESA) solvent delivery module at 0.60 ml/min. Assay sensitivity for DA was 2 fmol per sample.

Histology. At the end of the microdialysis experiments, rats were euthanized by pentobarbital overdose, and brains were removed and left to fix in 4% formaldehyde in saline solution. Brains were then cut on a Vibratome 1000 Plus (The Vibratome Company, St. Louis, MO) in serial coronal slices oriented according to the atlas by Paxinos and Watson (1998) to identify the location of the probes.

Data Analysis. Discriminative-stimulus data were expressed as the percentage of the total responses (on both levers) that were made on the THC-appropriate lever during the entire test session. Response-rate data were expressed as responses per second averaged over the session, with responding during time-out periods not included in calculations. The data from sessions during which rats did not complete at least one fixed ratio were excluded from analysis of drug-lever selection. All results are presented as group means ($\pm$S.E.M.). Statistical analysis of the ability of compounds to produce generalization to the discriminative effects of the training dose of THC was done using one-way ANOVA for repeated measures in comparison with vehicle treatments, followed when appropriate by the Dunnet's post hoc test. Statistical differences between the dose-response curves were evaluated by using two-way ANOVA for re-

peated measures followed by the Student-Newman-Keuls post hoc test. By nonlinear regression analysis using a sigmoidal dose-response (variable slope) equation, ED_{50} values were obtained for each compound; dose-response curves were considered significantly different when 95% confidence intervals of ED_{50} values did not overlap. Statistical analysis of the effect of any treatment on rates of responding was done by using one-way ANOVA for repeated measures in comparison with vehicle treatment, followed when appropriate by the Dunnet's post hoc test.

For microdialysis experiments, statistical analysis of differences in basal DA values (femtomole/10-μl sample $\pm$ S.E.M.) between all experimental groups was carried out with one-way ANOVA. Results shown in the figures are expressed as a percentage of basal dopamine values. Basal dopamine values were calculated as the mean of three consecutive samples (differing no more than 10%) immediately preceding the first drug or vehicle injection. All results are presented as group means ($\pm$S.E.M.). Statistical analysis was carried out using one- or two-way ANOVA for repeated measures over time applied to the data obtained from serial assays of dialysate dopamine normalized as percentage of basal dopamine values for each group, with results from treatments showing overall changes subjected to post hoc Tukey's test. A probability value of $P < 0.05$ was considered significant.

Results

Discrimination of THC. When the training procedure was changed from a single session per day to multiple sessions per day, operant behavior was often disrupted (rats tended to respond in the first session but not in subsequent sessions), and 5 to 15 training sessions were needed to reestablish consistent and stable behavior through all three consecutive sessions in a day. However, rats always consistently discriminated injections of saline from injections of THC (data not shown).

When test sessions started, discrimination of THC was clearly dose-dependent both when THC was injected i.p. and when THC was injected i.v. (Fig. 1A, top) [$F(3,33) = 18.727$; $p < 0.0001$ for low doses; $F(3,15) = 18.345$; $p < 0.001$ for higher doses; and $F(3,27) = 20.196$, $p < 0.0001$ for i.v. administration]; however, THC was approximately 10-fold more potent as a discriminative stimulus when injected i.v. than when injected i.p. (see ED_{50} values in Table 1, 0.57 and 0.48 versus 0.07). Using this multiple-session procedure, THC also produced response-rate depressant effects at the highest doses (0.3 mg/kg THC i.v. and 3 mg/kg i.p.) (Fig. 1A, bottom) [$F(3,33) = 5.010$; $p < 0.01$ for low doses; $F(3,33) = 10.302$; $p < 0.001$ for higher doses; $F(3,27) = 4.711$, $p < 0.01$ for i.v. administration]. The dose of 0.3 mg/kg THC produced response-rate depressant effects when injected i.v. but not

when injected i.p., suggesting that response-rate depressant effects are more pronounced with i.v. administration. It should be noted that, in these i.p. tests, to directly compare with i.v. injections, THC was injected 3 min and not 30 min before the session as it was injected during normal training sessions. This time difference explains the pronounced rate depression found in this study and not in our previous studies (Solinas et al., 2003, 2004).

THC-Like Discriminative Effects of Anandamide and Methanandamide. Lower i.v. doses of anandamide (0.3–3 mg/kg) did not produce any significant THC-like discriminative effects and had no significant effects on rates on responding (Fig. 1B, top) [$F(3,33) = 5.434$, $p < 0.01$]. When a high 10 mg/kg i.v. dose of anandamide was administered, however, significant THC-like discriminative effects were found (Fig. 1B, top) [$F(3,6) = 7.097$, $p < 0.05$], although this dose profoundly depressed rates of responding (Fig. 1B, bottom) [$F(3,30) = 34.172$, $p < 0.0001$] and most rats (8 of 11) ceased responding.

The metabolically stable analog of anandamide, methanandamide, produced dose-dependent THC-like discriminative effects at lower i.v. doses (0.3–3 mg/kg) (Fig. 1B, top) [$F(3,30) = 40.727$, $p < 0.0001$] without significantly affecting rates of responding (Fig. 1B, bottom), and rats responded almost exclusively on the THC-associated lever after injection of 3 mg/kg methanandamide.

Inhibition of FAAH Potentiates the Behavioral Effects of Anandamide. The main mechanism of neuronal inactivation of anandamide is cleavage to arachidonic acid and ethanolamine, which is catalyzed by FAAH (Freund et al., 2003; Piomelli, 2003; Di Marzo et al., 2004). Consistent with our previous results (Gobbi et al., 2005), the FAAH enzyme inhibitor URB-597 (0.03 or 0.3 mg/kg) did not produce THC-like discriminative effects and did not affect rates of responding (Fig. 2A, left). However, when a 0.3-mg/kg dose of URB-597, but not a lower dose of 0.03 mg/kg, was administered 40 min before the first of three consecutive drug-discrimination sessions, a low 1-mg/kg i.v. dose of anandamide that did not significantly alter rates of responding (Fig. 2A, bottom) produced significant THC-like discriminative effects (Fig. 2A, top) [$F(3,18) = 14.184$, $p < 0.0001$]. A higher 3-mg/kg dose of anandamide produced complete generalization to the THC training stimulus when it was given after 0.3 mg/kg URB-597 (Fig. 2A, top), but it also produced a significant depression of rates of responding when given in combination with either low [$F(3,15) = 5.008$, $p < 0.05$] or high

TABLE 1
ED_{50} values of selected treatments for drug discrimination experiments

Drug	Route	Dose Range Tested	ED_{50} (95% Confidence Interval)	n Responding/ n Tested[a]
		mg/kg		
THC	i.p.	0.3–1.0	0.59 (0.42–0.75)	12/12
THC	i.p.	0.3–3.0	0.48 (0.22–0.73)	6/12
THC	i.v.	0.03–0.3	0.074(0.04–0.10)	10/10
AEA	i.v.	1–10	N/A	3/11
Methanandamide	i.v.	0.3–3.0	1.10 (0.84–1.37)	11/11
URB 0.3 + AEA	i.p.+ i.v.	0.3–3.0	0.49 (0.09–0.72)	7/12
Rim 3 + URB 0.3 + AEA	i.p.+ i.v.	0.3–3.0	2.55 (0.89–4.38)	9/12
Rim 3 + methanandamide	i.p.+ i.v.	0.3–3.0	3.23 (2.42–4.04)	12/12
URB 0.3 + AEA	i.p.+ i.p.	3–10	3.26 (2.71–3.81)	6/6

N/A, not applicable; URB, URB-597.
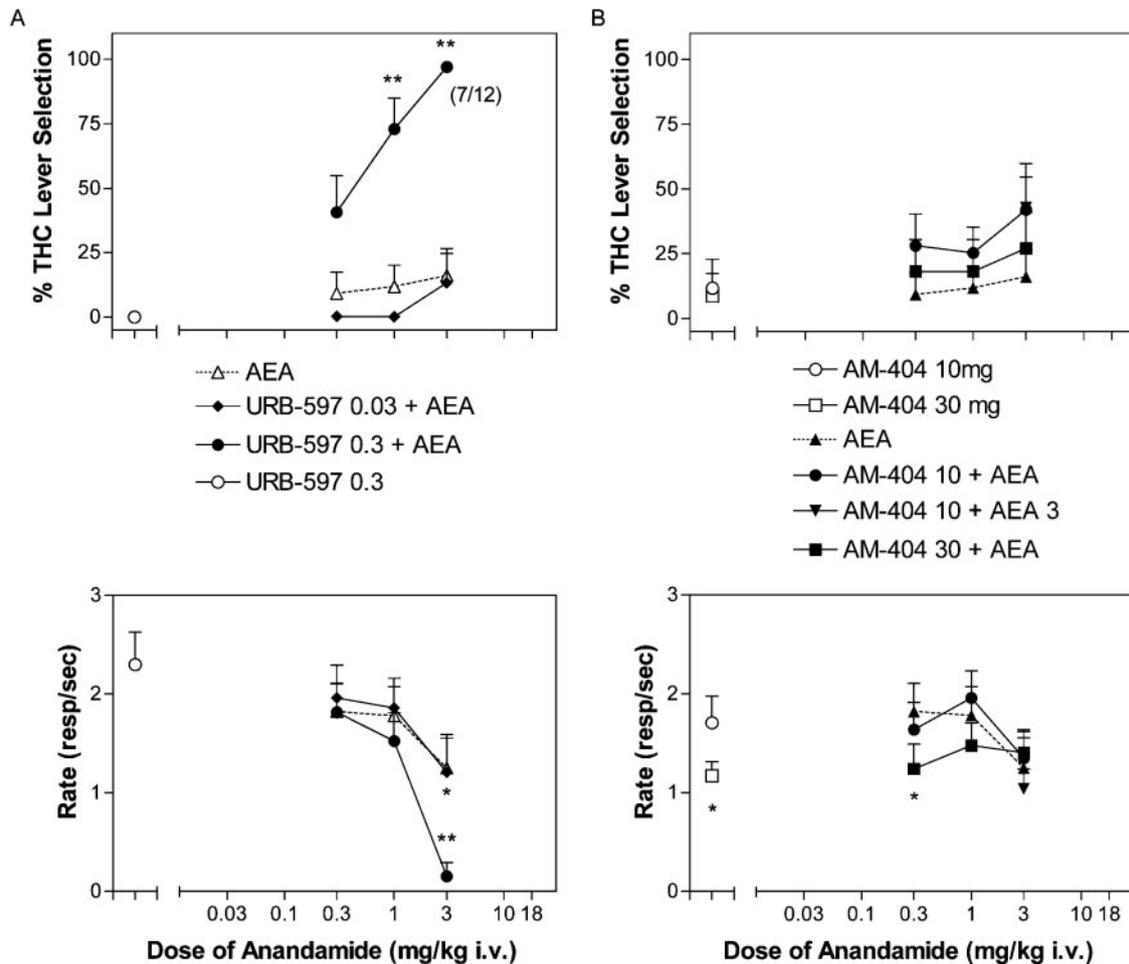[a] Refers to the highest dose tested.

Fig. 2. Inhibition of FAAH (A), but not of anandamide transport (B), dramatically potentiates the effects of i.v. anandamide. Ordinates: overall percentage of responses on the lever associated with THC administration (top) and overall rate of lever pressing expressed as responses per second (bottom) averaged over the entire session. Abscissae: dose in milligram/kilogram (log scale). Different doses of anandamide (AEA) were administered i.v. 3 min before each of three consecutive sessions ran at 60-min intervals. URB-597 was administered i.p. 40 min before the first session of the three consecutive sessions. AM-404 was administered i.p. 30 min before the first of the three consecutive sessions. To verify that the small effects of combinations of AM-404 and anandamide were not due to elimination of AM-404 during the duration of the experiment (approximately 2.5 h), in one test, AM-404 was given 60 min before a single 3-mg/kg dose of anandamide (administered i.v. 3 min before the session). Repeated measures ANOVA followed by post hoc Dunnet's test: $*$ and $**$, $p < 0.05$ and $p < 0.01$ compared with vehicle. Numbers in parentheses at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested.

doses of URB-597 $[F(3,24) = 18.162, p < 0.0001]$ (Fig. 2A, bottom).

Inhibition of Anandamide Transport Does Not Potentiate the Discriminative or Neurochemical Effects of Anandamide. The first step in rapid inactivation of anandamide is its transport from extracellular to intracellular space, which is believed to be mediated by a yet to be identified facilitated transport protein (Freund et al., 2003; Piomelli, 2003; Di Marzo et al., 2004; Glaser et al., 2005; Moore et al., 2005). This transport process seems to be selectively blocked by a number of ligands, including the most studied endocannabinoid transport inhibitor AM-404 (Beltramo et al., 1997). Administration of high doses of AM-404 (10 and 30 mg/kg 30 min before the session) did not produce any significant THC-like discriminative effects (Fig. 2B, top left), although 30 mg/kg AM-404 significantly reduced rates of responding $[F(2,16) = 3.787, p < 0.05]$ (Fig. 2B, bottom left). Surprisingly, neither 10 nor 30 mg/kg AM-404 significantly potentiated the ability of anandamide to produce THC-like discriminative effects (Fig. 2B, top right). Although 30 mg/kg AM-404 in combi-

nation with anandamide produced significant decreases in rates of responding compared with vehicle levels $[F(3,30) = 4.001, p < 0.05]$ (Fig. 2B, bottom right), these decreases were no greater in magnitude than those produced by AM-404 alone, suggesting that they were not due to potentiation of the rate-depressant effects of anandamide.

Because this lack of potentiation of the behavioral effects of anandamide by AM-404 was unexpected, we decided to test whether AM-404 would potentiate anandamide-induced elevations of extracellular dopamine levels in the nucleus accumbens shell, an effect of anandamide we previously characterized (Solinas et al., 2006a) that shows a high level of correlation with the behavioral results described here (see *Discussion*). As shown previously (Solinas et al., 2006a), 3 mg/kg i.v. anandamide significantly increased dopamine levels in the shell of the nucleus accumbens $[F(7,18) = 5.134, p < 0.0001]$ (Fig. 3). This effect was characterized by a fast short-lasting increase in dopamine levels followed by more modest but long-lasting elevations in dopamine levels. After i.p. administration of 10
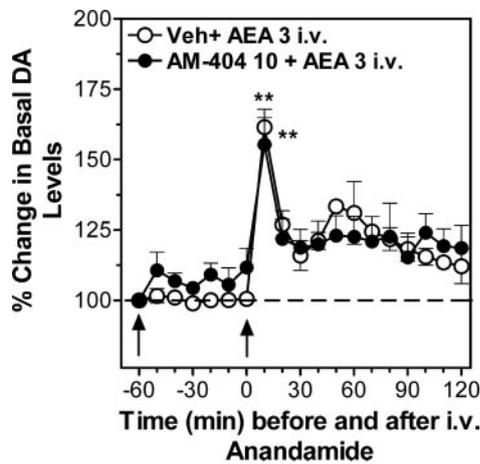
Fig. 3. AM-404 does not potentiate anandamide-induced elevations of DA levels in the shell of the NAc. Ordinates: amount of dopamine in the NAc in 10-min dialysate samples, expressed as percentage of basal values, uncorrected for probe recovery. Abscissae: time during the experiment. AM-404 or its vehicle was administered i.p. 60 min before i.v. administration of 3 mg/kg anandamide (AEA). Arrows represent the time of injection of AM-404 or vehicle pretreatment at time = −60 min, and the time of injection of anandamide at time = 0 min. Repeated measures ANOVA followed by post hoc Tukey's test: ∗ and ∗∗, $p < 0.05$ and $p < 0.01$ compared with corresponding basal DA levels.

mg/kg AM-404, anandamide still produced significant increase in dopamine levels [$F(5,18) = 5.405$, $p < 0.0001$], there was no change in the magnitude or duration of these

increases compared with anandamide treatment alone (Fig. 3).

Effects of Anandamide and Methanandamide Are Mediated by CB_1 Receptors. Consistent with our previous results (Solinas et al., 2003), a high 3-mg/kg dose of the cannabinoid CB_1 receptor antagonist rimonabant (SR141716) did not produce any THC-like discriminative effects, although rates of responding were significantly depressed compared with vehicle (Student's t test, $p < 0.001$) (Fig. 4, A and B, left). However, administration of 3 mg/kg rimonabant 30 min before the first of three consecutive sessions significantly reduced the ability of anandamide to produce THC-like discriminative effects [$F(1,6) = 19.466$, $p < 0.01$] (see Table 1 for ED_{50} values) (Fig. 4A, top right). Administration of 3 mg/kg rimonabant also produced modest alterations in the effects of anandamide on rates of responding [treatment × dose effects, $F(2,16) = 4.723$, $p < 0.05$]. At the two low doses of anandamide that did not significantly alter rates of responding, a slight depression of response rates was found after rimonabant treatment, whereas at the highest 3-mg/kg dose of anandamide, a slight reduction of the depressant effects of anandamide was found. The effects of rimonabant on response rates at the two lowest doses of THC were probably due to depressant effects of rimonabant on responding, in agreement with our previous finding that 3 mg/kg rimonabant significantly decreases rates of responding by itself when it is injected at short pretreatment times
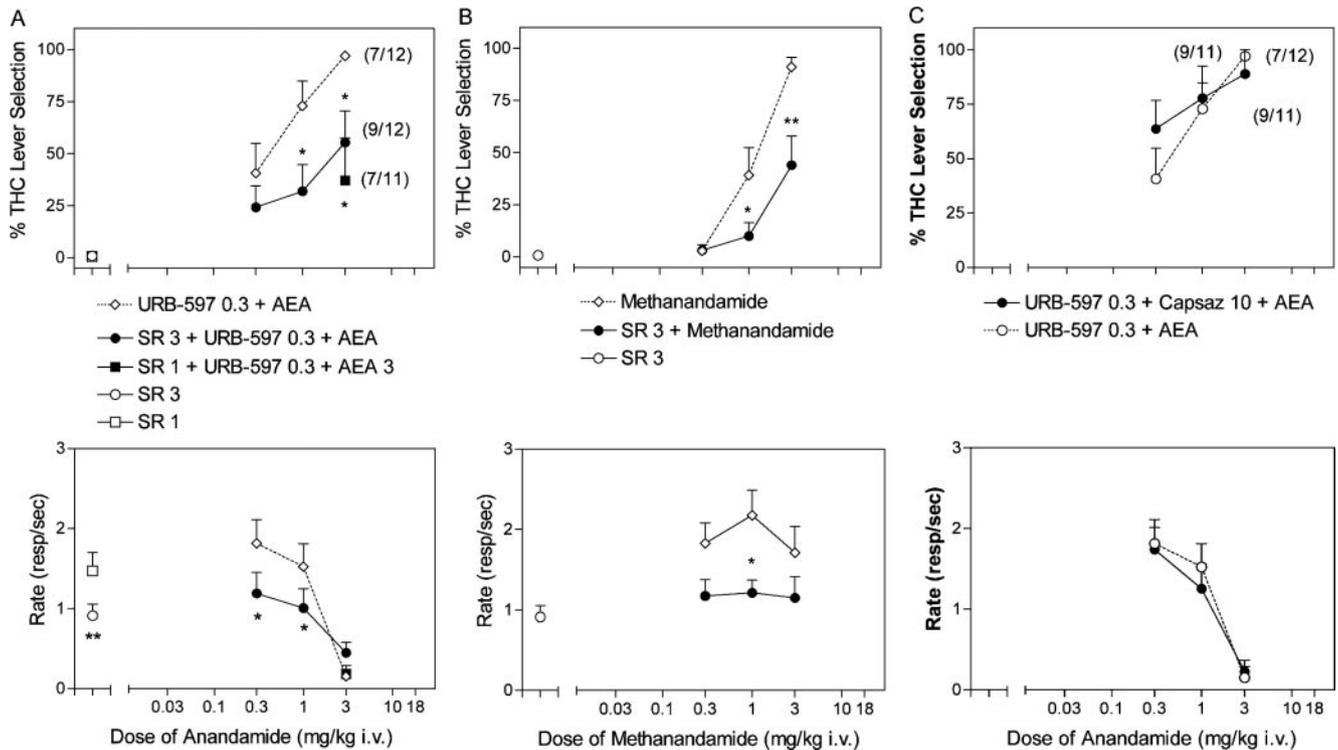

Fig. 4. The effects of anandamide (A) and methanandamide (B) depend on activation of cannabinoid CB_1 but not vanilloid VR_1 (C) receptors. Ordinates: overall percentage of responses on the lever associated with THC administration (top) and overall rate of lever pressing expressed as responses per second (bottom) averaged over the entire session. Abscissae: dose in milligram/kilogram (log scale). Rimonabant (SR) was administered i.p. 40 min before the first session. Capsazepine (Capsaz) was administered i.p. 15 min before the first session. Different doses of anandamide (AEA) and methanandamide were administered i.v. 3 min before each of three consecutive sessions ran at 60-min intervals. In one test, 1 mg of rimonabant was given i.p. 60 min before the session in combination with 0.3 mg/kg URB-597 and a single 3-mg/kg dose of anandamide (administered i.v. 3 min before the session), because this dose and pretreatment time was previously found to be optimal for antagonism of the discriminative effects of THC (Solinas et al., 2003). Repeated measures ANOVA followed by post hoc Student-Newman-Keuls test: ∗ and ∗∗, $p < 0.05$ and $p < 0.01$ compared with the respective dose of anandamide or methanandamide alone. Numbers in parentheses at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested.
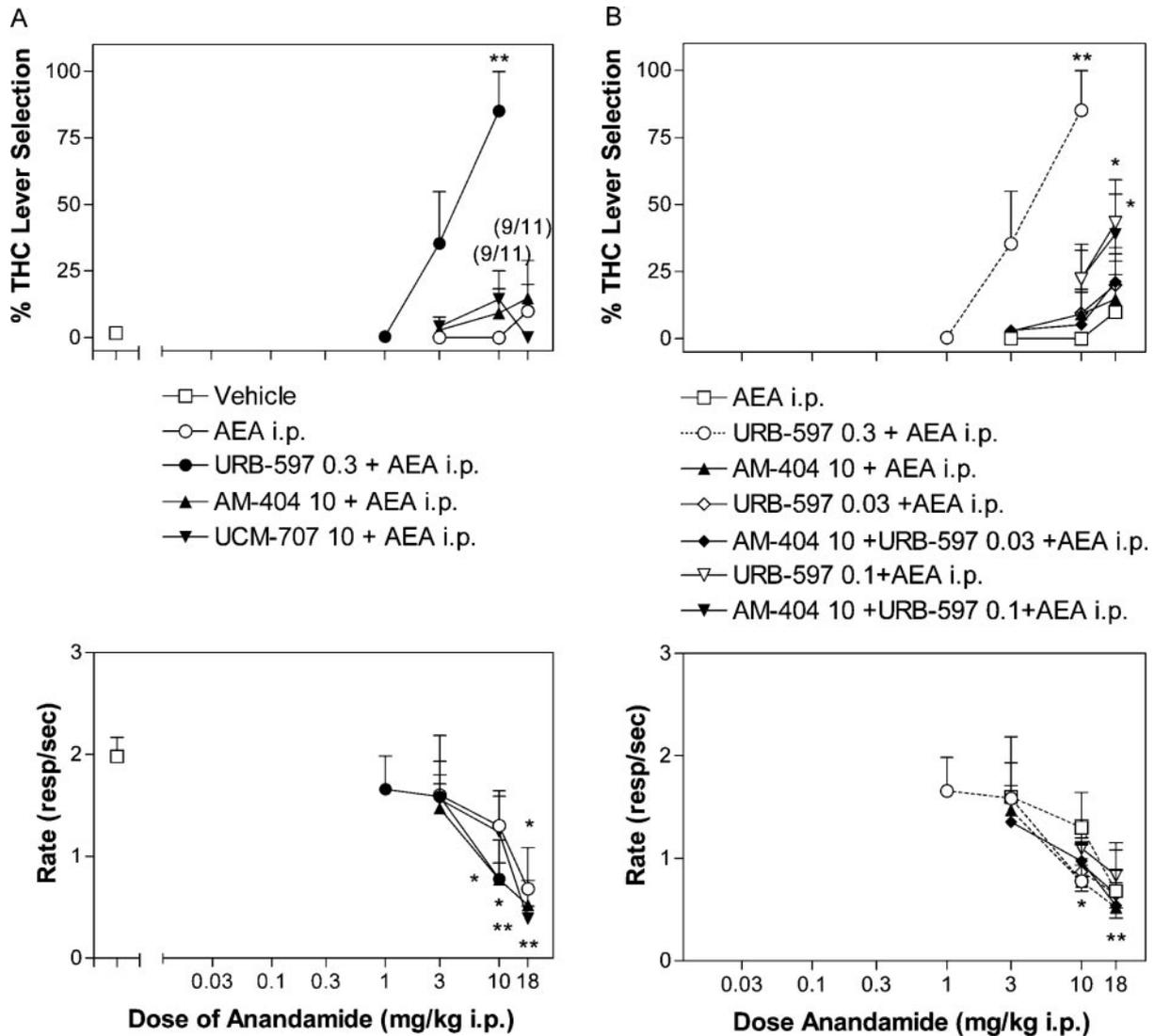
Fig. 5. Potentiation of the discriminative effects of i.p. anandamide by FAAH inhibition with URB-597 but not by inhibition of anandamide uptake with AM-404 or UCM-707 (A) and lack of synergistic effects of URB-597 and AM-404 (B). Ordinates: overall percentage of responses on the lever associated with THC administration (top) and overall rate of lever pressing expressed as responses per second (bottom) averaged over the entire session. Abscissae: dose in milligram/kilogram (log scale). AM-404 (10 mg/kg), URB-597 (0.3 mg/kg), and UCM-707 (10 mg/kg) were administered i.p. 60, 40, and 30 min, respectively, before single test sessions. Different doses of anandamide (AEA) were administered i.p. 3 min before sessions. Repeated measures ANOVA followed by post hoc Dunnet's test: ∗ and ∗∗, $p < 0.05$ and $p < 0.01$ compared with vehicle. Numbers in parentheses at higher doses indicate the number of rats that completed at least one fixed ratio during the session over the total number of rats in which the dose was tested.

(Solinas et al., 2003). In addition, rimonabant did not reverse the marked response rate depressant effects of the highest 10 mg/kg i.v. dose of anandamide, suggesting that these rate depressant effects of anandamide are mediated by mechanisms independent from CB$_1$ receptor activation. When we used a rimonabant dose and pretreatment time that was able to completely block the discriminative effects of THC in a previous study (1 mg/kg rimonabant 60 min before the session) (Solinas et al., 2003), rimonabant significantly reduced the ability of anandamide to produce THC-like discriminative effects (Student's t test, $p < 0.001$); however, again it was not able to reverse the depressant effects of anandamide on rates of responding.

Administration of 3 mg/kg CB$_1$-receptor antagonist rimonabant 30 min before the first of three consecutive sessions significantly reduced the ability of methanandamide to produce THC-like discriminative effects, as it did with anand-

amide [treatment effect: $F(1,10) = 7.521$, $p < 0.01$ and treatment × dose effect $[F(1,10) = 10.187$, $p < 0.05]$ (see Table 1 for ED$_{50}$ values) (Fig. 4B, top). This dose of rimonabant also significantly reduced the slight (nonsignificant compared with baseline values) increase in rates of responding produced by methanandamide $[F(2,20) = 3.938$, $p < 0.05]$ (Fig. 4B, bottom).

Discriminative Effects of Anandamide Are Not Mediated by Vanilloid VR$_1$ Receptors. It is known that anandamide at very high doses activates vanilloid VR$_1$ receptors (Zygmunt et al., 1999). To exclude involvement of VR$_1$ receptors in the THC-like discriminative effects of anandamide, we administered the VR$_1$ antagonist capsazepine at a dose of 10 mg/kg i.p. 30 min before the first of three consecutive sessions. Capsazepine alone did not produce THC-like effects and did not affect rates of responding (Fig. 4C, left). In addition, capsazepine did not block the ability of

anandamide to produce THC-like discriminative effects and did not alter the depressant effects of anandamide on rates of responding (Fig. 4C, left).

Inhibition of FAAH, but Not Inhibition of Transport, Potentiates the Behavioral and Neurochemical Effects of Intraperitoneal Anandamide. In previous studies using drug-discrimination techniques, anandamide was always administered i.p. (Wiley et al., 1995; Burkey and Nation, 1997; Jarbe et al., 2001). Thus, we tested whether in our setting i.p. injections of anandamide would produce generalization to the discriminative effects of the THC training stimulus and whether inhibition of FAAH would potentiate the effects of i.p. injections of anandamide. Anandamide, when given alone by the i.p. route, did not produce THC-like discriminative effects (Fig. 5A), even at high doses that depressed rates of responding [$F(3,15) = 4.161$, $p < 0.05$]. However, when 0.3 mg/kg URB-597 was administered 40 min before the session, an i.p. dose of 10 mg/kg anandamide produced complete generalization to the THC training stimulus (Fig. 5A, top) [$F(1,10) = 10.187$, $p < 0.05$] with an ED_{50} of 3.261 (see Table 1). URB-597 also potentiated the depressant effects of anandamide on rates of responding, as demonstrated by the finding that the 10 mg/kg i.p. dose of anandamide that was ineffective by itself produced a significant decrease in rates of responding after administration of URB-597 (Fig. 5A, bottom) [$F(3,15) = 4.034$, $p < 0.05$]. In contrast, neither AM-404 (10 mg/kg i.p.) nor UCM-707 (10 mg/kg i.p.; a structurally different and more selective inhibitor of anandamide transport) potentiated the discriminative or rate depressant effects of anandamide.

In further experiments, we tested whether AM-404 could potentiate the effects of anandamide when given in combination with threshold doses of URB-597. A dose of 0.03 mg/kg URB-597 did not potentiate the effects of anandamide, but a higher dose of 0.1 mg/kg URB-597 produced a small potentiation of the effects of anandamide [$F(1,6) = 7.674$, $p < 0.05$]. However, 10 mg/kg AM-404 did not alter the effects of anandamide in combination with either the 0.03 or 0.1 mg/kg dose of URB-597.

Finally, we tested the effects of i.p. anandamide on dopamine levels in the shell of the nucleus accumbens. An i.p. dose of 10 mg/kg anandamide (Fig. 6) did not increase dopamine levels when given alone. However, when FAAH was blocked by 0.3 mg/kg URB-597 i.p., anandamide produced a small but sustained increase in dopamine levels [treatment effect: $F(1,8) = 13.756$, $p < 0.01$; time effect: $F(16, 128) = 1.920$, $p < 0.05$; treatment $\times$ time effect: $F(16, 128) = 3.160$, $p < 0.0001$] (Fig. 6).

Discussion

In these experiments, anandamide produced discriminative effects qualitatively similar to those of THC, but this was clearly revealed only after URB-597 inhibition of the metabolic cleavage of anandamide by FAAH. Intracellular degradation by FAAH appeared to be the main mechanism for anandamide inactivation in brain areas mediating THC-like discriminative effects of anandamide, because the transport inhibitors AM-404 and UCM-707 did not potentiate the THC-like discriminative effects of anandamide. We also replicated our previous finding (Solinas et al., 2006a) that anandamide, like THC, increases dopamine levels in the nucleus accum-

Fig. 6. Inhibition of FAAH potentiates anandamide-induced elevations of DA levels in the shell of the NAc. Ordinates: amount of dopamine in 10-min dialysate samples from the NAc, expressed as percentage of basal values, uncorrected for probe recovery. Abscissae: time during the experiment. URB-597 or its vehicle was administered i.p. 60 min before i.p. administration of 10 mg/kg anandamide (AEA). Arrows represent the time of injection of URB-597 or vehicle pretreatment at time = −40 min, and the time of injection of anandamide at time = 0 min. Repeated measures ANOVA followed by post hoc Tukey's test: *, $p < 0.05$ compared with corresponding basal DA levels. * and $$, $p < 0.05$ and $p < 0.01$ compared with the corresponding time point of vehicle + anandamide.

bens shell in rats when given intravenously, an effect potentiated by URB-597 but not by AM-404. These results support the idea that anandamide, an endogenous brain constituent, can produce psychotropic and neurochemical effects similar to THC and provide new insights about the relative importance of different mechanisms of inactivation for the central effects of anandamide.
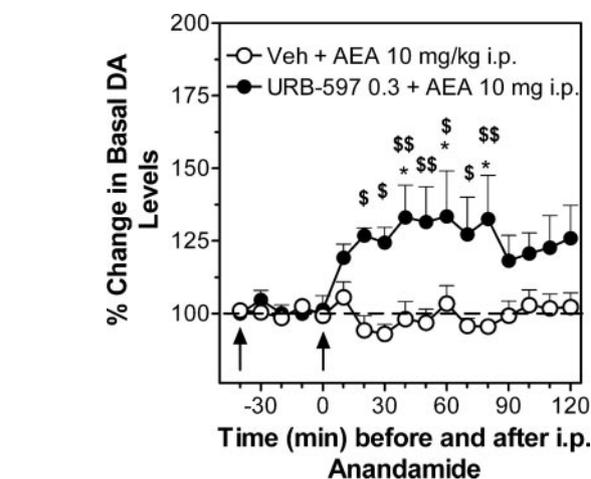
Previous studies in which anandamide was injected i.p. found no generalization to THC training stimuli (Burkey and Nation, 1997; Jarbe et al., 2001), partial generalization (Alici and Appel, 2004), or generalization only at very high doses of anandamide that depressed rates of responding (Wiley et al., 1995). Our results support the idea that high doses of anandamide are necessary to produce THC-like discriminative effects and that these doses produce secondary depression of responding, which may interfere with measurement of THC-like discriminative effects. It is likely that these high doses are necessary to overcome the rapid and massive inactivation of anandamide by FAAH so that brain anandamide concentrations can reach levels sufficient to activate brain CB_1 receptors. Administration of these high doses of anandamide may be accompanied by rapid and massive formation of anandamide metabolites that may be responsible for depression of responding through non-CB_1 receptor mechanisms (Wiley et al., 2006).

We could not obtain ED_{50} values for the discriminative effects of anandamide alone because of its pronounced depressant effects on responding, but observation of dose-response curves suggests that FAAH inhibition by URB-597 produces at least a 3- to 10-fold increase in the THC-like discriminative effects of anandamide. This is consistent with previous studies showing that genetic ablation (Cravatt et al., 2001) or pharmacological blockade (Kathuria et al., 2003; Fegley et al., 2005) of FAAH greatly potentiates other behavioral effects of anandamide. Although potentiation of THC-like discriminative effects of anandamide by FAAH inhibi-

tion was dramatic, potentiation of depressant effects of anandamide on rates of lever-press responding was less pronounced. In fact, after URB-597 treatment, complete generalization to the THC training stimulus was obtained in the absence of rate depression at the 1-mg/kg i.v. dose of anandamide (see Fig. 2A). Thus, anandamide metabolites, and not anandamide itself, may be primarily responsible for depressant effects of anandamide on rates of responding, because both inhibition of FAAH by URB-597 and resistance to FAAH with methanandamide resulted in reduced rate-depressant effects.

Compounds such as AM-404 and UCM-707 are thought to inhibit the transport of anandamide into neurons (Piomelli et al., 1999; De Petrocellis et al., 2000; Lopez-Rodriguez et al., 2003; Glaser et al., 2005; Moore et al., 2005), and administration of AM-404 at doses (5–10 mg/kg) similar to those used in the present experiments increases anandamide concentrations in plasma and some brain areas (Giuffrida et al., 2000; Fegley et al., 2004) and potentiates and prolongs many of physiological and behavioral effects of anandamide (Beltramo et al., 1997; Calignano et al., 1997; Bortolato et al., 2006). Thus, inhibitors of endocannabinoid transport, such as AM-404, were expected to produce a potentiation of behavioral and neurochemical effects of anandamide similar to the potentiation produced by the FAAH inhibitor URB-597. Instead, AM-404 did not potentiate either the discriminative effects of anandamide or its depressant effects on rates of responding and also did not potentiate dopamine-releasing effects of anandamide in the accumbens shell. Although AM-404 binds to different targets, including vanilloid VR_1 receptors (Zygmunt et al., 2000), such actions are not likely to explain this lack of effect because a more selective inhibitor UCM-707 (Lopez-Rodriguez et al., 2003) was also ineffective in potentiating discriminative effects of anandamide. It is possible to speculate that, at least in brain areas involved in the discriminative effects of THC, passive diffusion into cells driven by FAAH-dependent concentration gradients, rather than transport processes (Glaser et al., 2005), determines the magnitude and duration of action of systemically administered anandamide. It is also possible that brain concentrations reached after the systemic administration of exogenous anandamide were higher than those found under physiological conditions and that effective blockade of transport would not produce a measurable effect.

The ability of both anandamide and methanandamide to produce THC-like discriminative effects were mediated by cannabinoid CB_1 receptors, as demonstrated by significant reductions of these discriminative effects after treatment with the CB_1 receptor antagonist rimonabant. This is consistent with previous drug-discrimination studies with methanandamide (Jarbe et al., 2006) and in agreement with a prominent role of cannabinoid CB_1 receptors in the in vivo effects of anandamide (Howlett et al., 2002; Pertwee and Ross, 2002; Freund et al., 2003). Importantly, depressant effects of anandamide on rates of responding did not seem to depend on cannabinoid CB_1 receptors, as has been reported previously (Di Marzo et al., 2001), because rimonabant was not able to significantly reduce them.

It is known that anandamide at high doses can also activate VR_1 vanilloid receptors (Zygmunt et al., 1999). However, the ability of anandamide to produce THC-like discriminative or rate-depressant effects did not appear to be mediated by VR_1 receptors, because there was a complete lack of effect of the VR_1 antagonist capsazepine. Although these findings suggest that the THC-like discriminative effects of anandamide are independent of VR_1 receptors, this cannot be taken as evidence that all of the behavioral effects of anandamide are independent of VR_1 receptors.

In this study, we used mostly i.v. injections of anandamide. We chose this route of administration for two reasons. First, in previous studies, i.p. injections of anandamide generally failed to produce THC-like discriminative effects, and second, our recent results using in vivo microdialysis (Solinas et al., 2006a) suggest that anandamide is very effective when given i.v. because of rapid brain penetration and lower first-passage hepatic metabolism. When anandamide was given i.p., it produced THC-like discriminative effects and elevated dopamine levels in the shell of the nucleus accumbens only when FAAH was inhibited, again suggesting that fast metabolism represents a major cause of lack of effects of anandamide when given alone. When given i.v., anandamide only produced THC-like discriminative effects at a high dose that almost completely suppressed lever-press responding in most rats. However, rats that continued lever-press responding at the high 10-mg/kg i.v. dose of anandamide all consistently selected the THC-lever. This indicates that, even with i.v. administration, systemically administered anandamide is rapidly metabolized, and only at very high doses does it reach sufficient concentrations in the brain (or in specific brain areas) to activate cannabinoid CB_1 receptors in a way that results in significant behavioral and neurochemical effects.

There were some differences in the effects of anandamide and methanandamide on THC discrimination compared with their effects on accumbal dopamine levels. For example, 3 mg/kg i.v. anandamide increased dopamine levels but did not produce THC-like discriminative effects; anandamide and methanandamide were similarly potent in elevating dopamine levels in the nucleus accumbens (Solinas et al., 2006a), but methanandamide was clearly more potent in producing THC-like discriminative effects; the combination of URB-597 and 10 mg/kg i.p. anandamide produced THC-like discriminative effects similar to the combination of URB-597 and 3 mg/kg i.v. anandamide, but dopamine elevations were much lower after i.p. (present study) compared with i.v. injections (Solinas et al., 2006a). Thus, although dopamine neurotransmission in the shell of the nucleus accumbens could be one of the neurochemical mechanisms involved in the discriminative effects of THC, it is clear that it is not the only interoceptive cue for THC discrimination. This is consistent with previous findings (Browne and Weissman, 1981) and our unpublished findings that strong indirect-acting dopaminergic agonists, such as cocaine and amphetamine, do not produce THC-like discriminative effects.

In conclusion, when given i.v. or i.p., anandamide can produce discriminable THC-like effects that are revealed or markedly enhanced by FAAH inhibition. In addition, anandamide elevates dopamine levels in the nucleus accumbens shell in a manner similar to that of THC and other abused drugs (Di Chiara, 2002; Wise, 2002). The present findings, together with our previous findings that i.v. anandamide produces marked reinforcing effects in nonhuman primates (Justinova et al., 2005) that are similar to the reinforcing effects of THC (Tanda et al., 2000), support the hypothesis that anandamide serves as an endogenous THC-like neuro-

modulator that participates in the signaling of rewarding/reinforcing events. This suggests that events that trigger the synthesis and release of anandamide may induce a THC-like state that includes various aspects of effects of THC, such as reward or reinforcement, increased appetite, and anxiolytic or anxiogenic effects. However, inhibitors of FAAH, such as URB-597, do not trigger formation and release of anandamide but instead elevate endogenous anandamide levels in brain areas where anandamide is synthesized and released, and FAAH inhibitors appear to have selective behavioral effects, such as anxiolytic and antidepressant effects, without the undesirable effects that would suggest potential for abuse (Gobbi et al., 2005; Solinas et al., 2005, 2006a; present findings).

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