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# Effects of Fatty Acid Amide Hydrolase (FAAH) Inhibitors in Non-Human Primate Models of Nicotine Reward and Relapse

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Inhibition of the enzyme fatty acid amide hydrolase (FAAH) counteracts reward-related effects of nicotine in rats, but it has not been tested for this purpose in non-human primates. Therefore, we studied the effects of the first- and second-generation *O*-arylcarbamate-based FAAH inhibitors, URB597 (cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester) and URB694 (6-hydroxy-[1,1'-biphenyl]-3-yl-cyclohexylcarbamate), in squirrel monkeys. Both FAAH inhibitors: (1) blocked FAAH activity in brain and liver, increasing levels of endogenous ligands for cannabinoid and α-type peroxisome proliferator-activated (PPAR-α) receptors; (2) shifted nicotine self-administration dose–response functions in a manner consistent with reduced nicotine reward; (3) blocked reinstatement of nicotine seeking induced by reexposure to either nicotine priming or nicotine-associated cues; and (4) had no effect on cocaine or food self-administration. The effects of FAAH inhibition on nicotine self-administration and nicotine priming-induced reinstatement were reversed by the PPAR-α antagonist, MK886. Unlike URB597, which was not self-administered by monkeys in an earlier study, URB694 was self-administered at a moderate rate. URB694 self-administration was blocked by pretreatment with an antagonist for either PPAR-α (MK886) or cannabinoid $CB_1$ receptors (rimonabant). In additional experiments in rats, URB694 was devoid of THC-like or nicotine-like interoceptive effects under drug-discrimination procedures, and neither of the FAAH inhibitors induced dopamine release in the nucleus accumbens shell—consistent with their lack of robust reinforcing effects in monkeys. Overall, both URB597 and URB694 show promise for the initialization and maintenance of smoking cessation because of their ability to block the rewarding effects of nicotine and prevent nicotine priming-induced and cue-induced reinstatement.

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## INTRODUCTION

Modulating the activity of the endogenous cannabinoid system has the potential to produce therapeutic effects for a wide range of medical and psychiatric disorders (Moreira and Lutz, 2008; Panlilio *et al*, 2013; Zanettini *et al*, 2011). One strategy for achieving such effects is to administer drugs that bind to cannabinoid $CB_1$ receptors. However, both agonists and antagonists at this receptor tend to have side effects that limit their usefulness as medications. For example, direct-acting $CB_1$ agonists such as $\Delta^9$-tetrahydrocannabinol (THC) exert antiemetic (Parker *et al*, 2011) and antispasmodic effects (Oreja-Guevara, 2012), but are also liable to produce dependence (Hall and Degenhardt, 2009) and amnesia (Ranganathan and D'Souza, 2006). On the other hand, $CB_1$ antagonists are effective in the treatment of obesity and tobacco addiction (Cahill and Ussher, 2011), but can also induce depressive states (Johansson *et al*, 2009).

A potentially safer and more targeted approach is to enhance levels of endogenously released cannabinoid $CB_1$ receptor ligands by inhibiting their degradation (O'Sullivan, 2007; Piomelli *et al*, 2006; Pistis and Melis, 2010). Fatty acid amide hydrolase (FAAH) provides the primary intracellular breakdown mechanism for the endocannabinoid anandamide (a partial agonist at the $CB_1$ receptor) and for several noncannabinoid members of its extended lipid family, including oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (agonists at the $\alpha$-subtype peroxisome proliferator-activated receptor (PPAR-$\alpha$)). Drugs that inhibit FAAH activity prolong and enhance the effects of these endogenous $CB_1$ and PPAR-$\alpha$ ligands when and where they are produced, and might therefore be better tolerated than

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exogenous ligands such as THC (Piomelli *et al*, 2006). This general strategy has been applied successfully, for example, with medications that increase endogenous levels of other neurotransmitters such as serotonin (Taylor *et al*, 2011) and dopamine (Godfrey, 2009).

In preclinical studies, FAAH-inhibiting drugs show promise for the treatment of tobacco addiction. Specifically, the FAAH inhibitor URB597 blocks reward-related effects of nicotine in behavioral, neurochemical, and electrophysiological procedures in rats, attenuating or preventing: (1) acquisition and reinstatement of nicotine self-administration behavior and nicotine-induced conditioned place preference (Forget *et al*, 2009; Scherma *et al*, 2008); (2) nicotine-induced increases in extracellular dopamine in the nucleus accumbens shell (Scherma *et al*, 2008); (3) nicotine-induced excitation of dopamine cells in the ventral tegmental area (Melis *et al*, 2008); and (4) nicotine-induced inhibition of medium spiny neurons in the nucleus accumbens shell (Luchicchi *et al*, 2010). However, despite its ability to block acquisition of nicotine self-administration in experimentally naive rats or to block reinstatement of nicotine seeking in rats that were currently abstinent, URB597 did not affect nicotine intake in rats that had an ongoing baseline of nicotine self-administration (Forget *et al*, 2009).

As mentioned above, FAAH inhibition indirectly activates both $CB_1$ and PPAR-$\alpha$ receptors by increasing endogenous levels of anandamide and OEA/PEA, respectively. Many of the positive effects exhibited by the FAAH inhibitor URB597 in animal models of nicotine reward and reward-related neurochemical and electrophysiological effects have also been demonstrated with selective PPAR-$\alpha$ agonists that have no activity at $CB_1$ receptors (Mascia *et al*, 2011; Melis *et al*, 2013; Panlilio *et al*, 2012). However, selective enhancement of endogenous levels of the $CB_1$ agonist anandamide can block the reinstatement of nicotine seeking in rats, and both PPAR-$\alpha$ and $CB_1$ receptors contribute to the ability of URB597 to block the effects of nicotine on neuronal signaling (Luchicchi *et al*, 2010; Melis *et al*, 2008). These findings suggest that FAAH inhibitors might be more effective than drugs that target only PPAR-$\alpha$ or only $CB_1$ receptors and should therefore continue to be developed as potential treatments for tobacco addiction.

Previous studies showing that FAAH inhibition modulates reward-related effects of nicotine have only been conducted in rodents, with rats and mice showing opposite effects. In mice, genetic deletion or pharmacological inhibition of FAAH was shown to enhance nicotine-induced conditioned place preference (CPP) (Merritt *et al*, 2008). Conversely, in rats, FAAH inhibition prevents the development and reinstatement of nicotine-induced CPP, the acquisition of nicotine self-administration, and nicotine-induced reinstatement of nicotine seeking (Scherma *et al*, 2008; Forget *et al*, 2009). The reasons for these species differences are not known, and it is not clear what species of laboratory animal will provide the best predictor of effects in humans. Nevertheless, given that substantial species-related differences exist (see also Muldoon *et al*, 2013), it could be valuable to determine the effects of FAAH inhibitors using behavioral models of nicotine reward in non-human primates that are phylogenetically closer to humans. Furthermore, as most previous research has involved a single chemical entity, URB597, it would also be valuable to investigate newer FAAH inhibitors with improved properties. An interesting compound in this regard is URB694 that displays greater plasma stability, higher water solubility, lower propensity to inhibit liver esterases, and higher potency as a FAAH inhibitor than its parent compound URB597 (Clapper *et al*, 2009; Vacondio *et al*, 2011).

In the present study, we evaluated the effects of URB597 and URB694 as potential treatments for tobacco addiction using behavioral models in squirrel monkeys, including nicotine self-administration and two models of relapse: nicotine priming-induced reinstatement and cue-induced reinstatement of nicotine seeking in abstinent animals. Relapse is particularly interesting because it is a significant obstacle to smoking cessation, occurring in most smokers who try to quit (Fiore *et al*, 2008). The potential of URB694 to produce rewarding effects was assessed by allowing monkeys access to URB694 for self-administration; earlier work had already shown that URB597, unlike THC and anandamide, had no rewarding effects in squirrel monkeys (Justinova *et al*, 2008). The potential for nonspecific disruption of operant behavior was studied by determining the effects of the FAAH inhibitors on food self-administration and cocaine self-administration in monkeys. Two additional studies were conducted in rats. First, drug-discrimination procedures were used to determine whether URB694 might produce interoceptive effects similar to either THC or nicotine that might indicate a liability to be abused or to trigger relapse. Second, *in vivo* microdialysis was used to determine whether URB694 might elevate levels of extracellular dopamine in the nucleus accumbens shell (as was tested earlier with URB597) (Scherma *et al*, 2008; Solinas *et al*, 2006), an effect that might convey abuse liability.

## MATERIALS AND METHODS

### Subjects

Male squirrel monkeys (*Saimiri sciureus*) weighing 0.9–1.1 kg and Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 300–350 g were housed in individual cages in temperature- and humidity-controlled rooms. Experiments were conducted at the Intramural Research Program of the National Institute on Drug Abuse in accordance with the guidelines of the National Research Council in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

In the primate studies, there were five separate groups of monkeys used in the behavioral experiments. Each animal had a unique numeric or alphanumeric identifier (nicotine self-administering group: 441, 431, 577, 572; anandamide self-administering group: 6754, 70F4, 42A, 67F4; THC self-administering group: 25B, 434, 66B2, 37B; cocaine self-administering group: 70F7, 2F19, 39B, 5045; food self-administering group: 1549, 30A, 34A, 27B). Each monkey was trained to self-administer either nicotine, cocaine, anandamide, THC, or food before the study and had experience with this reinforcer for over 5 years. The monkeys had no self-administration history with other reinforcers, except that the anandamide group had experience self-administering other cannabinoid class compounds. The monkeys in the nicotine group had not received any pharmacological treatment before testing with URB597, but

they were tested with cannabinoid CB1 antagonists and PPAR-$\alpha$ ligands before testing with URB694. A separate group of squirrel monkeys ($n=14$) was used for quantification of FAAH activity and levels of fatty acid ethanolamides (FAEs) in the brain and liver. In the rodent studies, 20 rats were used for drug discrimination, 15 rats for microdialysis, and 6 rats for pharmacokinetics.

### Drugs

Nicotine ((−)-nicotine hydrogen tartrate) (Sigma) was dissolved in saline solution (pH adjusted to 7.0). FAAH inhibitors URB597 and URB694 were synthesized at the Istituto Italiano di Tecnologia. URB597 (Carbamic acid, N-cyclohexyl-, 3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl ester) was dissolved in 5% Tween-80 and saline. URB694 (Carbamic acid, N-cyclohexyl-, 6-hydroxy[1,1'-biphenyl]-3-yl ester) was dissolved in vehicle containing 5% dimethylsulfoxide (DMSO), 5% Tween-80, and saline. THC (NIDA Drug Supply Program, Bethesda, MD) and anandamide (Tocris) were dissolved in a vehicle containing 1% ethanol and 1% Tween-80 and saline. (−)-Cocaine HCl (Sigma) was dissolved in saline. The PPAR-$\alpha$ antagonist MK886 (1-[(4-Chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-$\alpha$,$\alpha$-dimethyl-5-(1-methylethyl)-1H-Indole-2-propanoic acid; Tocris) was dissolved in 2–4% Tween-80, 4% DMSO, and sterile water. The cannabinoid $CB_1$ receptor antagonist/inverse agonist rimonabant (SR141716; N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide)) (NIDA Drug Supply Program) was dissolved in 2% Tween-80, 2% ethanol, and saline. Monkeys were given intravenous (IV) treatment injections in a volume of 1 ml/kg and intramuscular (IM) treatment injections in a volume of 0.3–0.5 ml/kg. Self-administered IV injections were always 0.2 ml. Rats were given intraperitoneal (IP) and subcutaneous (SC) injections in a volume of 1 ml/kg.

### Drug and Food Self-Administration Procedures in Monkeys

The IV catheterization, general procedure, and apparatus have been described in detail previously (Goldberg, 1973; Justinova *et al*, 2003). At the start of each 1-h self-administration session, the houselight was extinguished and a green light (discriminative stimulus) was presented, signaling the availability of nicotine. In the presence of the green light, 10 lever responses (10-response fixed ratio) produced a 0.2-s IV drug injection or a food pellet, turned off the green light, and illuminated an amber cue-light (injection-paired or conditioned stimulus) for 2 s. Each injection was followed by a 60-s timeout, during which the chamber was dark and lever presses had no programmed consequences. Separate groups of monkeys self-administered nicotine (30 μg/kg/injection; $n=4$), anandamide (30 μg/kg/injection; $n=4$), THC (4 μg/kg/injection; $n=4$), cocaine (30 μg/kg/injection; $n=3$), or food (190 mg/pellet; $n=3$).

### Effects of FAAH Inhibitors on Drug and Food Self-Administration in Monkeys

To determine whether they altered nicotine self-administration, the FAAH inhibitors URB597 or URB694 were administered at a dose of 1 mg/kg IV 30 min before each of five consecutive nicotine self-administration sessions, preceded by at least five consecutive baseline sessions of self-administration training in which vehicle was administered before each session. These sets of baseline and test sessions were conducted at nicotine doses of 10, 30, 56, and 100 μg/kg/injection to detect changes in the nicotine dose–effect curve. Various doses of the FAAH inhibitors were compared with each other by giving URB597 (0.1, 0.3, or 1 mg/kg IV) or URB694 (0.1 or 1 mg/kg IV) before self-administration of the 30 μg/kg/injection dose of nicotine. To determine whether the effects of the FAAH inhibitors were specific to nicotine self-administration, they were also tested with food and cocaine under self-administration conditions that paralleled the nicotine self-administration procedure.

### Self-Administration of the FAAH Inhibitor URB694 by Monkeys

To assess the abuse liability of the FAAH inhibitor URB694, the drug was made available for IV self-administration by monkeys that were originally trained to self-administer anandamide, THC, or cocaine. This was achieved by conducting at least five consecutive baseline sessions with the original training drug, followed by five consecutive sessions of vehicle self-administration (ie, extinction, during which responding decreased to low levels), and then five consecutive sessions of testing with URB694 (1 μg/kg/injection) available. In anandamide-trained monkeys, a range of URB694 doses (0.3, 1, 3, 10, and 30 μg/kg/injection) was made available for five consecutive sessions each to obtain dose–effect functions. Testing of each dose was always preceded and followed by five sessions of extinction. To assess the effects of $CB_1$ receptor blockade, rimonabant (1 mg/kg IM) was given 60 min before URB694 (1 μg/kg/injection) self-administration sessions. To assess the effects of PPAR-$\alpha$ blockade, MK886 (1 mg/kg IM) was given 45 min before URB694 (1 μg/kg/injection) self-administration sessions.

### Reinstatement of Drug Seeking by Noncontingent Exposure to Drugs in Monkeys

Priming-induced reinstatement testing consisted of baseline, extinction, and reinstatement phases. Monkeys were trained with a baseline of either nicotine or THC self-administration (as described above). During extinction sessions, vehicle was substituted for the training drug, but all other features of the self-administration schedule (ie, response requirement, visual stimulus presentations) were maintained. When responding reached a low, stable level in extinction ($<10$ injections/session, with no obvious increasing or decreasing trend), a reinstatement test was conducted by giving an IV priming injection of the training drug (0.1 mg/kg nicotine or 0.04 mg/kg THC) or vehicle immediately before the next session. The reinforcement schedule during the reinstatement test was the same as during extinction. The effects of URB694 and URB597 (each at a dose of 1 mg/kg IV, 30 min before the session) on reinstatement were assessed alone and in combination with nicotine priming in monkeys that had been trained with nicotine. URB694 (0.3 or 1 mg/kg IV, 30 min before the session) was also tested alone in monkeys

that had been trained with THC. In an additional test to determine whether the effects of URB694 on nicotine priming-induced reinstatement were mediated by PPAR-$\alpha$, the same procedure was used but with the PPAR-$\alpha$ antagonist MK886 (1 mg/kg IV) given in the home cage 45 min before the session and URB694 immediately before the session. To determine whether they might reinstate nicotine seeking when given alone (without nicotine or THC), URB597, URB694, and MK886 were also given during extinction sessions before vehicle-priming injections.

### Reinstatement of Drug Seeking by Response-Contingent Exposure to Nicotine-Associated Cues in Monkeys

Cue-induced reinstatement was used to model relapse induced by stimuli that were previously associated with nicotine delivery. The extinction phase for this procedure was similar to the extinction phase for the drug priming-induced relapse procedure, except that intravenous injections and injection-paired visual cues were discontinued during extinction (ie, there were no injections and no drug-paired visual cues presented upon the completion of an FR10). After response rates in extinction decreased to $<100$ lever presses per session, a test of cue-induced reinstatement was conducted by giving response-contingent IV saline and reinstituting presentation of injection-paired visual cues (ie, the green light went off and saline injection was paired with the amber cue light, followed by 60 s of timeout with all lights off) on the 10-response fixed ratio schedule. To determine whether FAAH inhibition would alter cue-induced reinstatement of nicotine seeking, URB597 (1 mg/kg, IV) and URB694 (1 mg/kg, IV) treatments were given 30 min before the test session. To determine whether the effects of the FAAH inhibitors on cue-induced reinstatement were mediated by PPAR-$\alpha$, MK886 (1 mg/kg IV) was given in the home cage 45 min before the test, and URB597 or URB694 treatment was administered 30 min before cue-induced reinstatement test. All cue-induced reinstatement tests were preceded by a baseline extinction session in which IM vehicle was given 45 min before the session. To determine their effects when given alone (without reinstituting cue presentation), URB597, URB694, and MK886 were also given before extinction sessions.

### Quantification of FAAH Activity and Levels of FAEs and 2-AG in Monkey Tissues

Monkeys received IV injection of URB694 (1 mg/kg; $n=4$), URB597 (1 mg/kg; $n=4$), or vehicle ($n=6$) 1 hour before euthanasia by administration of Euthasol (Virbac, Fort Worth, TX; 0.1 ml/kg i.v.). Tissue sampling and storage procedures have been described previously (Justinova *et al*, 2008). To asses FAAH activity, tissue samples from brain (cerebellum, ~100 mg) and liver (~100 mg) were homogenized in 10 volumes of ice-cold Tris-HCl buffer (50 mM, 5–9 vol, pH 7.5) containing 0.32 M sucrose and centrifuged at 1000 *g* for 10 min at 4 °C. Supernatants were collected and protein concentrations were determined using a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL). FAAH activity was measured at 37 °C for 30 min in 0.5 ml of Tris-HCl buffer (50 mM, pH 7.5) containing fatty acid-free bovine serum albumin (BSA) (0.05%, w/v), tissue homogenates (50 µg protein), 10 µM anandamide, and anandamide-[ethanolamine-$^3$H] (10 000 c.p.m., specific activity 60 Ci/mmol). Reactions were stopped with chloroform/methanol (1:1, 1 ml) and radioactivity was measured in the aqueous layers by liquid scintillation counting.

Levels of anandamide, PEA, OEA, and 2-arachidonoyl-*sn*-glycerol (2-AG) were measured by liquid chromatography/mass spectrometry (LC/MS). Frozen brain samples (midbrain, ~50 mg) were homogenized in methanol (1 ml) containing [$^2H_4$]-anandamide, [$^2H_4$]-PEA, [$^2H_4$]-OEA, and [2H8]-2-AG as internal standards. Analytes were extracted with chloroform (2 vol) and washed with water (1 vol). Organic phases were collected and dried under nitrogen. The organic extracts were fractionated by open-bed silica gel column chromatography (Cadas *et al*, 1997). Briefly, the extracts were dissolved in chloroform and loaded onto small glass columns packed with Silica Gel G (60-Å 230–400 Mesh ASTM; Whatman, Clifton, NJ). Lipids were eluted with chloroform/methanol (9:1, vol/vol). Organic phases were evaporated under nitrogen and reconstituted in 60 µl of methanol. Lipid levels were measured using an 1100-LC system coupled to a 1946A-MS detector (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization interface, as previously described (Astarita *et al*, 2008).

### Drug Discrimination in Rats

The general procedure and apparatus have been described previously (Le Foll and Goldberg, 2004). Drug-discrimination procedures were used to determine: (1) whether the FAAH inhibitor URB694, when given alone, produces interoceptive effects similar to THC or nicotine; and (2) whether URB694 can alter the interoceptive effects of THC or nicotine. Rats were trained under a discrete-trials schedule of food reinforcement (10 responses/pellet, 45 s timeout) in which responses on one lever produced a 45-mg food pellet when an injection of the training drug had been given before the session, and responses on the other lever produced food when a saline injection had been given. The training drug was nicotine (0.4 mg/kg SC, 10 min before the session) in one group and THC (3 mg/kg IP, 30 min before the session) in a separate group. Sessions lasted for 20 pellets or 30 min. URB694 (0.1–10 mg/kg IP) was given alone (40 min before the session), or URB694 (1 mg/kg IP, 30 min before the training drug) was given in combination with different doses of nicotine (0.01–0.4 mg/kg) or THC (0.1–3 mg/kg) in test sessions up to two times per week. During test sessions, food was delivered whenever there were 10 consecutive non-timeout responses on either lever.

### *In Vivo* Microdialysis in Freely Moving Rats

The general procedure and apparatus have been described previously (Solinas *et al*, 2007). Rats were surgically implanted with a concentric dialysis probe aimed at the shell of the nucleus accumbens (2 mm anterior and 1.1 mm lateral from bregma, 8.0 mm below the dura) (Paxinos and Watson, 2005) under anesthesia. Experiments were performed on freely moving rats 20–24 h after implantation. Ringer's solution (147.0 mM NaCl, 2.2 mM $CaCl_2$, and 4.0 mM KCl) was delivered at a constant flow rate of 1.0 µl/min. Collection of dialysate samples (20 µl/sample)

started after 90 min, with samples collected every 20 min and immediately analyzed by a high-performance liquid chromatography system coupled to electrochemical detection in order to quantify dopamine. Once stable dopamine values (<15% variability) were obtained for at least three consecutive samples, URB694 (3 mg/kg), URB597 (3 mg/kg), or vehicle (5% DMSO, 5% Tween-80, and saline) was administered IP. Probe location in the nucleus accumbens shell was determined histologically after each experiment, and only data from rats with correct probe placement were analyzed.

### Pharmacokinetic Studies with URB694 in Rats

URB694 was administered IP to catheterized Sprague-Dawley rats at 3 and 10 mg/kg dose in PEG400/Tween-80/Saline solution at 10:10:80 (vol/vol), respectively. Three animals were treated for each dose level. Blood samples were collected at 0, 15, 30, 60, 120, 240, and 480 min after drug administration. Control animals treated with vehicle only were also included. Plasma was separated from blood by centrifugation for 15 min at 3500 r.p.m. at 4 °C, collected in Eppendorf tubes, and frozen (−80 °C) until analysis.

Details of sample analysis are described in Supplementary Materials and Methods.

### Data Analysis

Counterbalanced assignment of treatment order for within-subject design was used in behavioral experiments. Experimenters were not blind to the treatment assignment. Calculations of response rates do not include responses or time elapsed during timeout periods. Self-administration responding after treatment with FAAH inhibitors was compared with the previous 1–3 consecutive sessions of vehicle treatment; for dose–effect curves, the last three sessions under each condition were averaged. Reinstatement data (extinction and reinstatement tests) represent the mean of 1–3 sessions under each condition. Rats were randomly assigned to groups in the microdialysis experiments. Microdialysis data were expressed as a percentage of basal dopamine values; basal values were the mean of three consecutive samples (differing from each other by <15%) taken immediately before the first injection of test compound or vehicle. Microdialysis and behavioral data were analyzed (SigmaStat, Systat Software) using one-way or two-way repeated measures analysis of variance (ANOVA). The *post hoc* analysis was performed either by Tukey's pairwise multiple comparisons or Bonferroni *t*-test (multiple comparisons *vs* control group). Differences between effects of vehicle and URB597 or URB694 on lipid levels and FAAH activity were analyzed using one-way ANOVA. Differences were considered statistically significant when $p<0.05$.

## RESULTS

### Effects of URB597 and URB694 on FAAH Activity and Levels of FAEs and 2-AG in Monkey Brain

Systemic administration of either URB597 or URB694 at an IV dose of 1 mg/kg fully inhibited FAAH activity in the brain (cerebellum shown) and liver of squirrel monkeys, measured 1 h after treatment (Figure 1a and b; effect of treatment; brain, $F_{2,11}=195.5$, $p<0.0001$; liver, $F_{2,11}=92.13$, $p<0.0001$). These findings are in agreement with those previously reported for mice (Clapper *et al*, 2009). Furthermore, concentrations of anandamide, PEA, and OEA, all of which are FAAH substrates (Cravatt *et al*, 1996), were significantly elevated in the midbrain of monkeys receiving URB597 or URB694, whereas levels of 2-AG, which is degraded by monoacylglycerol lipase (MGL), remained unaffected (Figure 1c–f; effect of treatment; anandamide: $F_{2,11}=61.99$, $p<0.0001$, PEA: $F_{2,11}=47.04$, $p<0.0001$, OEA: $F_{2,11}=72.56$, $p<0.0001$, 2-AG: $F_{2,10}=0.20$, $p=0.82$).

### FAAH Inhibition Decreases the Rewarding Effects of Nicotine

Dose–effect functions for nicotine self-administration had an inverted-U shape (Figure 2). Treatment with 1 mg/kg of either URB597 or URB694 shifted these functions to the right, consistent with a decrease in nicotine reward (interaction effect of nicotine dose and URB597 treatment on injections, Figure 2a: $F_{3,6}=12.41$, $p=0.006$; and response rate, Figure 2b: $F_{3,6}=39.02$, $p<0.001$) (interaction effect of nicotine dose and URB694 treatment on injections, Figure 2d: $F_{2,6}=148.47$, $p<0.001$; and response rate, Figure 2e: $F_{2,6}=190.74$, $p<0.001$). Under baseline conditions (vehicle pretreatment), the highest rates of nicotine injection (Figure 2a and d) and lever pressing (Figure 2b and e) were maintained at the 30 μg/kg dose of nicotine; when FAAH inhibitors were administered, the rates at this dose of nicotine were reduced to near the levels observed during extinction (marked 'V' on the abscissae in Figure 2). At doses of nicotine >30 μg/kg (ie, on the descending limb of the dose–effect function under baseline conditions), rates of injection, lever pressing, and nicotine intake increased after treatment with the FAAH inhibitors (Figure 2c, interaction effect of nicotine dose and URB597 treatment on intake, $F_{3,6}=9.54$, $p=0.011$; Figure 2f, interaction effect of nicotine dose and URB694 treatment on intake, $F_{2,6}=232.06$, $p<0.001$).

When the dose of nicotine was held constant (30 μg/kg per self-administered injection) and the doses of URB597 and URB694 treatments were changed, both FAAH inhibitors had dose-dependent effects (Figure 3a, URB597 dose × session interaction, $F_{10,20}=4.80$, $p<0.001$; Figure 3b, URB694 dose × session interaction, $F_{10,30}=3.03$, $p=0.009$), with the highest dose of each inhibitor (1 mg/kg) producing the most robust decrease. The effect of the highest dose of each FAAH inhibitor was consistent, with nicotine self-administration decreasing significantly in the first session of treatment and remaining low during the remaining treatment sessions, but returning to the baseline level when treatment was discontinued. The decreases in nicotine self-administration produced by the FAAH inhibitors were comparable to those produced by the $CB_1$ receptor antagonist/inverse agonist rimonabant (1 mg/kg; not shown; effect of rimonabant treatment on nicotine injections/session, $F_{5,10}=17.47$, $p<0.001$). The two FAAH inhibitors differed somewhat in potency, with URB694 being more potent. Pretreatment with URB597 at 0.1 mg/kg did not significantly alter responding for nicotine during any of the five sessions (all *p*'s >0.9), but doses of 0.3 and

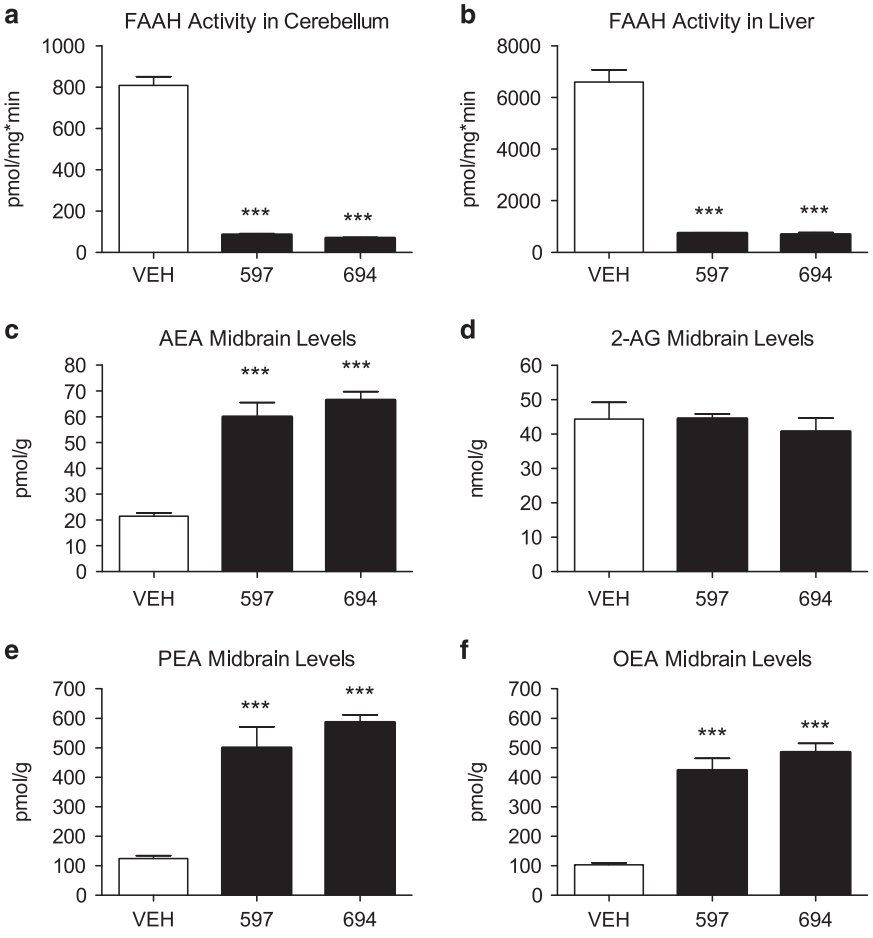


**Figure 1** Effect of URB597 or URB694 administration on FAAH activity and levels of FAEs in the brain of monkeys. (a, b) Inhibition of brain (a, cerebellum) and liver (b) FAAH activity 1 h after URB597, URB694 (both 1 mg/kg), or vehicle IV administration. (c–f) Midbrain concentrations of anandamide (c; AEA), 2-arachidonoylglycerol (d; 2-AG), PEA (e), and OEA (f) 1 h after vehicle, URB597, or URB694 (both 1 mg/kg) IV administration. Results are expressed as means ± SEM ($n = 4$–6). ***$P < 0.001$ *post hoc* comparisons with vehicle (VEH) conditions, Tukey's test.

1.0 mg/kg URB597 significantly decreased the number of self-administered nicotine injections (Figure 3a); paired comparisons showed that the effect of 1 mg/kg of URB597 was significantly greater than the effect of 0.1 mg/kg ($p = 0.02$) (0.1 *vs* 0.3 mg/kg: $p = 0.095$). URB694 had significant effects at lower doses than URB597, significantly decreasing responding for nicotine at all doses tested (0.03, 0.1, and 1 mg/kg) (Figure 3b, effect of URB694 dose, $F_{2, 30} = 8.91$, $p = 0.016$); paired comparisons showed that the 1 mg/kg dose of URB694 was significantly greater than those of both 0.03 and 0.1 mg/kg ($p = 0.018$ and $p = 0.04$, respectively) (0.03 *vs* 0.1, $p = 0.78$).

The PPAR-$\alpha$ antagonist MK886 was administered as a pretreatment to determine whether the FAAH inhibitors' indirect effects on PPAR-$\alpha$ might contribute to their ability to alter nicotine self-administration. The effects of both URB597 (Figure 3c; MK886 dose × session interaction, $F_{10, 20} = 2.56$, $p = 0.035$) and URB694 (Figure 3d; MK886 dose × session interaction, $F_{5, 15} = 3.99$, $p = 0.017$) on nicotine self-administration were reduced by MK886 pretreatment. The 1 mg/kg dose of MK886 was more effective than the 0.3 mg/kg dose at reducing the effects of URB597 (with the higher MK886 dose significantly different from vehicle pretreatment on all 5 days, $p$'s $< 0.05$). The effect of the lower dose of MK886 became progressively more apparent over the course of the 5 days of testing (differing significantly from vehicle pretreatment on days 3–5; $p$'s $< 0.05$). The effects of URB694 were also partially reversed by 1 mg/kg of MK886 (significantly different from vehicle treatment on days 2–5; $p$'s $< 0.01$), but the reversal was not as pronounced as with URB597.

The behavioral effects of FAAH inhibition in monkeys were specific to nicotine self-administration. URB597 did not alter the self-administration of food (pellets: $p = 0.08$; rate: $p = 0.11$) or cocaine (injections: $p = 0.24$; rate: $p = 0.50$) and neither did URB694 (food, pellets: $p = 0.91$, rate: $p = 0.53$; cocaine, injections: $p = 0.58$, rate $p = 0.41$) (Supplementary Figure S1).

## FAAH Inhibition Blocks Nicotine Priming-Induced Reinstatement of Nicotine Seeking

When vehicle was substituted for nicotine in the self-administration procedure (extinction), response and injection rates decreased to low rates (Figure 4a and b). When a noncontingent priming injection of nicotine (0.1 mg/kg, IV)

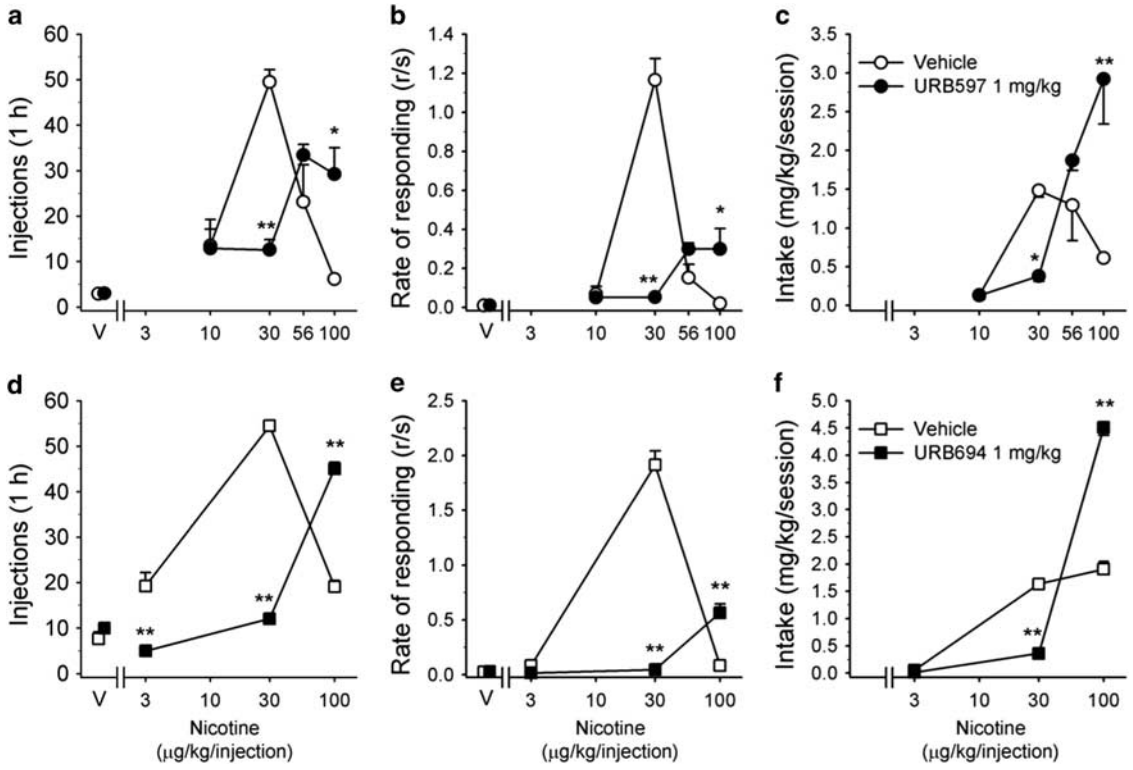


**Figure 2** Effect of URB597 or URB694 on nicotine self-administration in squirrel monkeys under a fixed-ratio 10 (FR10) schedule. (a–f) Pretreatment with URB597 or URB694 (both 1 mg/kg IV, 30 min before the session) caused significant (all $p$'s $<0.01$) rightward shifts of the nicotine dose–response curves compared with vehicle (V) pretreatment. Number of nicotine injections per 1-h session (a, d), overall response rates in the presence of the green light signaling nicotine availability (b, e), and total nicotine intake per session (c, f) are shown as a function of the nicotine dose (abscissae log scale). Each data point represents the mean ± SEM of the last three sessions under each nicotine condition and under vehicle conditions ($n=4$). *$P<0.05$, **$p<0.01$, *post hoc* comparisons of the effects of pretreatment with URBs *vs* vehicle treatment within each nicotine dose, Tukey's test.

was given (and responding continued to produce only visual cues and IV saline), lever pressing was reinstated (one-way RM ANOVA, Figure 4a, $F_{2,14}=35.72$, $p<0.001$; Figure 4b, $F_{2,14}=16.56$, $p<0.001$) to about the same levels that had been obtained at the peak of the nicotine dose–effect function when nicotine was still being delivered (as seen in Figure 2a and d). However, treatment with either URB597 or URB694 prevented this priming effect of nicotine (Figure 4a and b; both $p$'s $<0.001$ *vs* 'nicotine priming' conditions). Pretreatment with the PPAR-$\alpha$ antagonist MK886 (1 mg/kg) partially reversed the anti-reinstatement effects of FAAH inhibitors (both $p$'s$<0.01$ *vs* 'URB 1+nicotine priming' conditions). In contrast to the robust priming effect induced by reexposure to nicotine, the FAAH inhibitors did not induce reinstatement of nicotine seeking; when either URB597 or URB694 were given alone, there was only a slight, nonsignificant increase in lever-pressing (both $p$'s $>0.05$ *vs* 'vehicle priming" conditions). MK886 alone also did not reinstate extinguished nicotine seeking (both $p$'s $>0.99$ *vs* 'vehicle priming' conditions).

## FAAH Inhibition Blocks Cue-Induced Reinstatement of Nicotine Seeking

When response-contingent delivery of nicotine and its associated visual cues were both discontinued, response rates decreased to very low levels (Figure 4c and d, 'Vehicle +No cues' conditions). In tests of cue-induced reinstatement, presentation of response-contingent visual cues and IV injection cues (ie, saline injection) significantly reinstated lever pressing ('Vehicles+Cues' conditions; effect of treatment, Figure 4c, $F_{3,9}=154.73$, $p<0.001$; Figure 4d, $F_{3,9}=156.86$, $p<0.001$), producing approximately the same level of responding that had been obtained at the peak of the nicotine dose–effect function when nicotine and cues were still being delivered (as seen in Figure 2a and d). However, when either URB597 or URB694 was given before the session, cue-induced reinstatement was significantly reduced (Figure 4c and d; both $p$'s $<0.001$ *vs* 'Vehicle+Cues' conditions). Pretreatment with the PPAR-$\alpha$ antagonist MK886 (1 mg/kg) produced a modest but statistically significant reversal of this effect with URB694 (Figure 4d; $p=0.004$ *vs* 'URB 1+Cues' conditions), but not with URB597 (Figure 4c; $p=0.84$ *vs* 'URB 1+Cues' conditions).

## The FAAH Inhibitor URB694 Is Self-Administered at a Moderate Rate

Although its parent compound URB597 was not self-administered by squirrel monkeys in our previous study (Justinova *et al*, 2008), URB694 was self-administered at a moderate rate in the present study (effect of dose; Figure 5a, $F_{5,15}=9.05$, $p<0.001$; Figure 5b, $F_{5,15}=6.05$, $p=0.003$). An inverted U-shaped dose–effect function was obtained with

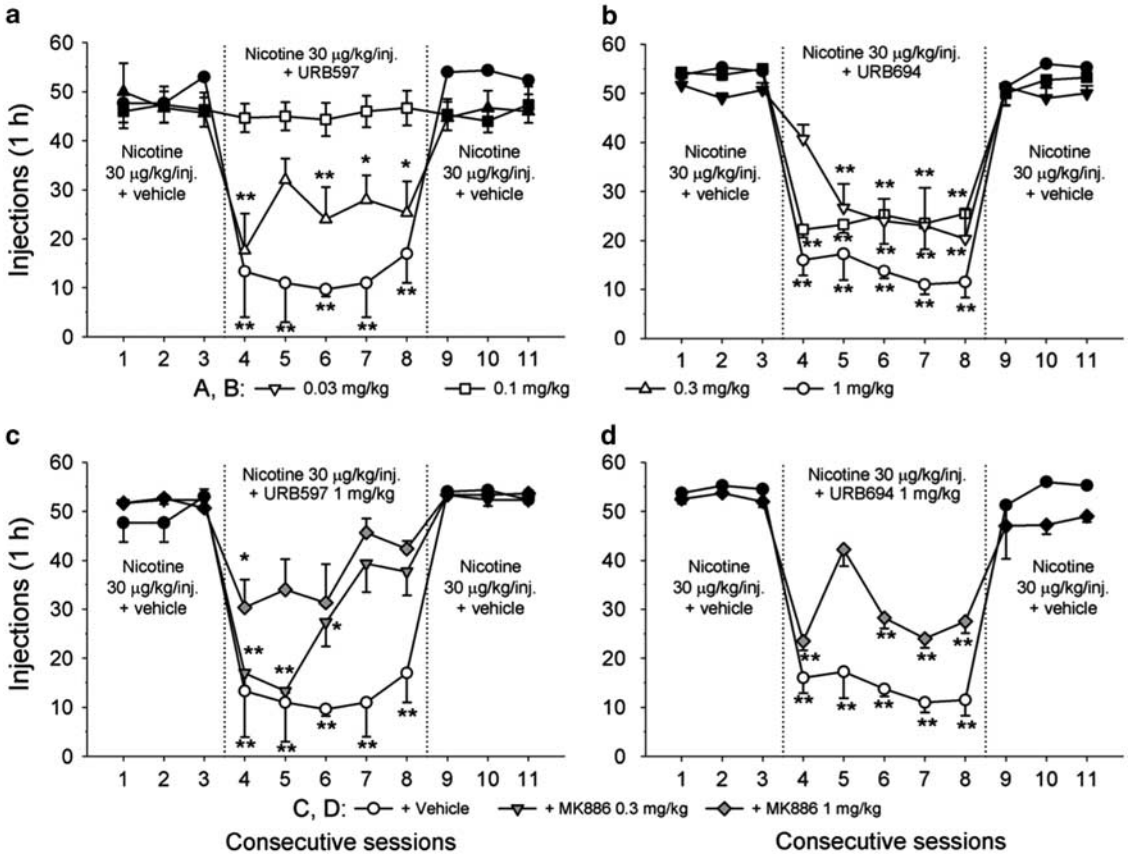


**Figure 3** Effect of different doses of URB597 or URB694 on self-administration of peak nicotine dose and reversal of these effects by PPAR-α antagonist MK886. (a, b) The 5-day treatment with URB597 (a; 0.1–1.0 mg/kg IV) or URB694 (b; 0.03–1.0 mg/kg IV) significantly decreased the number of 30 μg/kg injections of nicotine self-administered during 1 h sessions by squirrel monkeys under a fixed-ratio 10 (FR10) schedule (sessions 4–8) compared with vehicle treatment (sessions 1–3 and 9–11). (c, d) The blockade of nicotine self-administration by URB597 (c, 1 mg/kg) or URB694 (d; 1 mg/kg) was significantly reversed by MK866 (0.3 or 1 mg/kg IM, 45 min before the session). Number of nicotine injections per 1 h session is shown over consecutive sessions. Each data point represents the mean ± SEM ($n = 3$–4). $^{*}P < 0.05$, $^{**}p < 0.01$, *post hoc vs* the mean of the last three sessions with vehicle pretreatment (sessions 1–3), Bonferroni test.

URB694 in monkeys that had prior experience self-administering anandamide. However, the peak number of self-administered injections per session (at 1 μg/kg; Figure 5a) was only about half as high as that obtained with the training dose of anandamide (52.83 ± 1.45 *vs* 28.50 ± 1.17 injections per session), and the peak rate of lever pressing (Figure 5b; 1.81 ± 0.35 *vs* 0.19 ± 0.02 responses per second) and the total intake per session (Figure 5c; 1.58 ± 0.04 *vs* 0.03 ± 0.01 mg/kg/session) were substantially lower than those obtained with anandamide. As seen in Figure 5d and e, when URB694 (1 μg/kg) was offered to anandamide-trained monkeys after a series of extinction sessions (effect of extinction; Figure 5d, $F_{5,15} = 73.65$, $p < 0.001$; Figure 5e, $F_{5,15} = 27.75$, $p < 0.001$), self-administration rose to a moderate level on the first day and was maintained over 5 days of testing (effect of URB694 treatment; Figure 5d, $F_{5,15} = 4.34$, $p = 0.012$; Figure 5e, $F_{5,15} = 2.48$, $p = 0.079$). Treatment with the $CB_1$ receptor antagonist/inverse agonist rimonabant (1 mg/kg) before the session decreased URB694 self-administration by ~43% (effect of treatment; Figure 5f, $F_{5,15} = 3.51$, $p = 0.027$; Figure 5g, $F_{5,15} = 5.24$, $p = 0.006$). Pretreatment with the PPAR-α antagonist MK886 (1 mg/kg) had a similar effect to rimonabant, consistently decreasing URB694 administration by ~53% (effect of treatment; Figure 5f, $F_{5,15} = 8.36$, $p < 0.001$; Figure 5g, $F_{5,15} = 14.06$, $p < 0.001$).

URB694 (1 μg/kg) was also self-administered at moderate rates when it was offered after extinction to monkeys that had prior experience self-administering THC (effect of treatment; Supplementary Figure S2A, $F_{5,15} = 3.52$, $p = 0.026$; Supplementary Figure S2B, $F_{5,15} = 3.02$, $p = 0.044$) or cocaine (effect of treatment; Supplementary Figure S2C, $F_{5,10} = 9.46$, $p = 0.002$; Supplementary Figure S2D, $F_{5,10} = 8.54$, $p = 0.002$). The level of URB694 self-administration at the 1 μg/kg dose was about half as high as the rates maintained by the training doses of cocaine, THC, anandamide, and nicotine (Figures 2a and 5a, d, Supplementary Figure S2A and C).

## FAAH Inhibition by URB694 Does Not Reinstate the Seeking of THC

In contrast with the robust priming effect induced by reexposure to THC, the FAAH inhibitor URB694 did not induce reinstatement of THC seeking (Supplementary Figure S3; lever presses: $F_{3,6} = 73.4$, $p < 0.001$; rate:

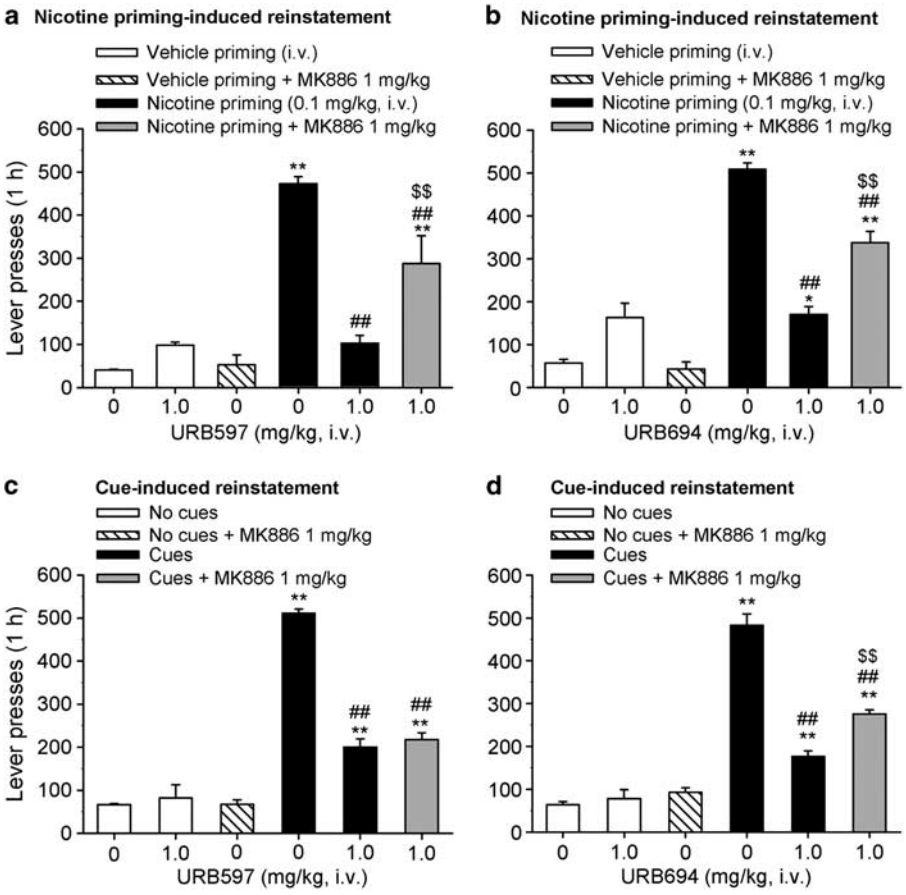


**Figure 4** Effect of URB597 or URB694 on relapse to nicotine seeking in abstinent squirrel monkeys. (a, b) During extinction, vehicle was substituted for nicotine. Treatment with URB597 (a) or URB694 (b; both 1 mg/kg IV, 30 min before the session) blocked the reinstatement of extinguished nicotine-seeking responses produced by a nicotine-priming injection (0.1 mg/kg IV, immediately before the session), and this effect was prevented by pretreatment with MK886 (1 mg/kg IM, 45 min before the session). $^{**}P<0.01$, *post hoc vs* 'vehicles+vehicle priming'; $^{\#\#}p<0.01$, *post hoc vs* 'vehicles+nicotine priming', $^{\$\$}p<0.01$, *post hoc vs* 'URB 1+nicotine priming', Tukey's test. (c, d) During extinction, injection-paired visual cues and response-contingent IV nicotine injections were removed. Treatment (the same as in a and b) with URB597 (c) or URB694 (d) blocked the reinstatement of extinguished nicotine-seeking responses induced by reintroduction of visual cues and IV saline injections. The effect of URB694, but not URB597, was partially reversed by pretreatment with MK886. $^{*}P<0.05$, $^{**}p<0.01$, *post hoc vs* 'vehicles+no cues'; $^{\#\#}p<0.01$, *post hoc vs* 'vehicles+cues', $^{\$\$}p<0.01$, *post hoc vs* 'URB 1+cues', Tukey's test. Total numbers of lever presses produced per 1 h session are shown (a–d). Each bar represents mean ± SEM ($n=4$). '0 mg/kg' represents vehicle in all panels.

$F_{3,6}=5.33$, $p=0.04$) in monkeys that had been trained to self-administer the cannabinoid agonist. That is, when URB694 (0.3 or 1 mg/kg) was given alone, there was no significant increase in lever pressing compared with extinction levels.

### URB694 Does Not Produce THC- or Nicotine-Like Interoceptive Effects in Rats

In the drug-discrimination procedure with rats trained to detect the effects of IP THC injections, THC itself produced a sigmoidal dose–effect function, with 100% of responding occurring on the THC-appropriate lever when rats received 3 mg/kg of the drug (the training dose). However, close to 0% of responses occurred on the THC-appropriate lever when rats received URB694 across a wide range of doses (0.1–10 mg/kg), indicating that URB694 did not exert THC-like interoceptive effects in these rats (Supplementary Figure S4A; effect of dose $F_{5,37}=0.34$, $p=0.89$). Moreover, when URB694 (1 mg/kg) was administered along with THC, the THC discrimination function was not significantly shifted (Supplementary Figure S4A; effect of URB694 × THC dose interaction, $F_{4,20}=1.31$, $p=0.30$). Similar results were obtained in a separate group of rats trained to discriminate the effects of nicotine. That is, URB694 alone did not produce a nicotine-like effect at any dose (Supplementary Figure S4B; effect of dose $F_{4,31}=1.54$, $p=0.21$), and combining URB694 with nicotine did not significantly shift the nicotine discrimination function (Supplementary Figure S4B; effect of URB694 × nicotine dose interaction, $F_{2,16}=3.22$, $p=0.067$). URB694 did not produce nonspecific depression of operant behavior; it had no significant effect on food-maintained response rates in the drug discrimination procedure when given alone (effect of dose; Supplementary Figure S4C, $F_{5,37}=0.88$, $p=0.50$, Supplementary Figure S4D, $F_{4,31}=1.22$, $p=0.32$) or in combination with THC (Supplementary Figure S4C, effect of URB694 × THC dose interaction, $F_{4,20}=1.97$, $p=0.14$) or nicotine (Supplementary Figure S4D, effect of URB694 × nicotine dose interaction, $F_{2,16}=0.59$, $p=0.57$).

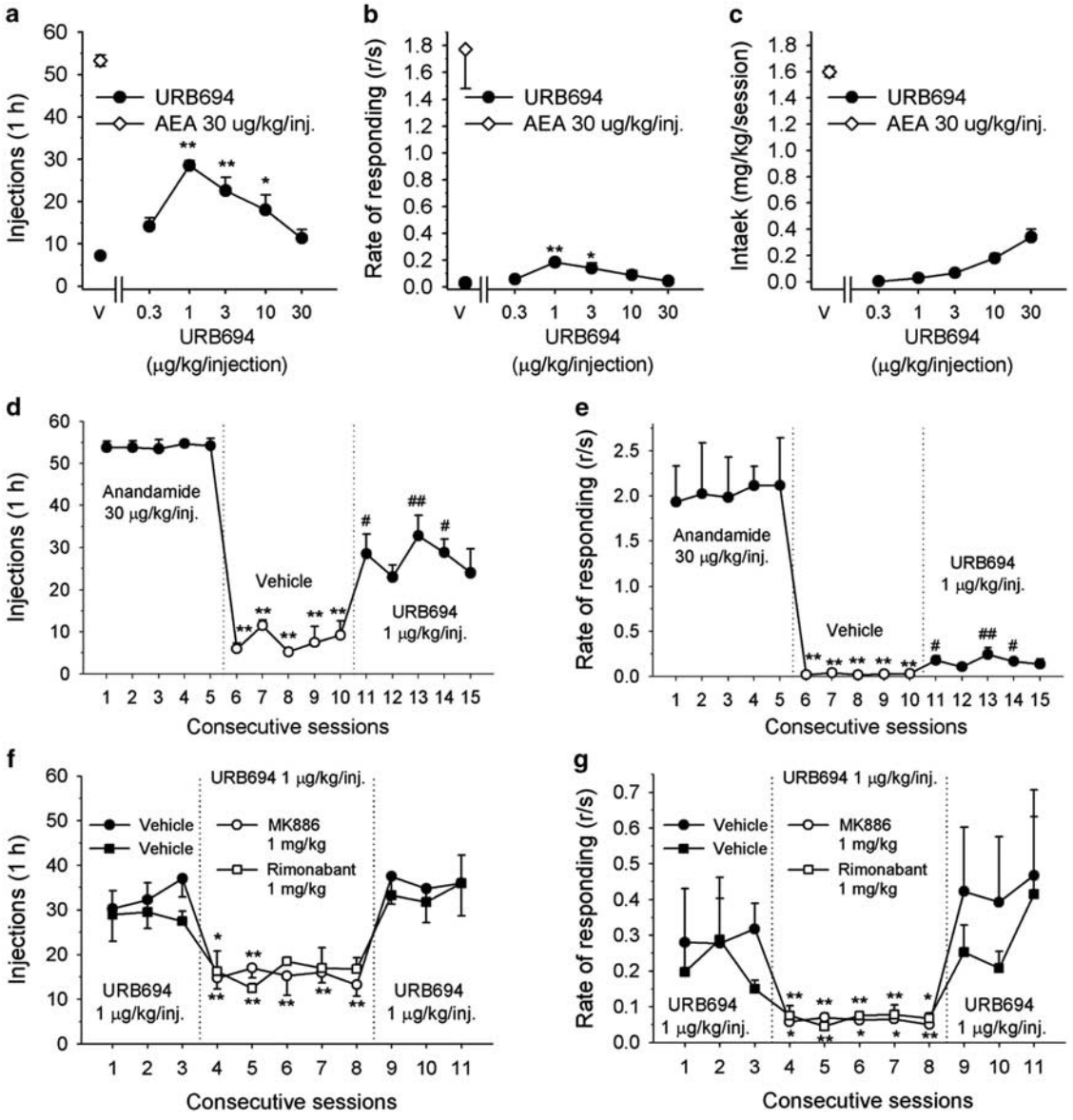


**Figure 5** Self-administration of URB694 under FR10 schedule in anandamide-experienced squirrel monkeys. (a–c) Effects of varying injection doses on self-administration of URB694. Number of URB694 injections per 1 h session (a), overall rates of responding in the presence of the green light signaling drug availability (b), and total URB694 intake per session (c) are shown as a function of injection dose (abscissae log scale). The baseline responding for 30 μg/kg injections of anandamide is also shown. Each point represents the mean ± SEM ($n = 4$) of the last three sessions under each dose condition and under a vehicle (V) condition. $^{*}P < 0.05$; $^{**}p < 0.01$, *post hoc vs* the vehicle condition, Bonferroni test. (d, e) Acquisition of URB694 self-administration. Number of injections per session (d) and overall rates of responding (e) during anandamide (30 μg/kg/injection) self-administration (sessions 1–5), vehicle extinction (sessions 6–10), and URB694 (1 μg/kg/injection) self-administration (sessions 11–15) are shown. Points represent means ± SEM ($n = 4$). $^{*}P < 0.05$, $^{**}p < 0.01$, *post hoc vs* the mean of the last three sessions of anandamide self-administration (sessions 3–5); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, *post hoc vs* the mean of the last three sessions of vehicle extinction (sessions 8–10), Bonferroni test. (f, g) Blockade of URB694 self-administration by pretreatment with rimonabant or MK886. Number of injections per session (f) and overall rates of responding (g) during URB694 (1 μg/kg/injection) self-administration are shown after IM pretreatment with vehicles (sessions 1–3 and 9–11), 1 mg/kg of rimonabant, or 1 mg/kg of MK886 (both sessions 4–8). Points represent means ± SEM ($n = 4$). $^{*}P < 0.05$, $^{**}p < 0.01$, *post hoc vs* the mean of the last three sessions of vehicle pretreatment (sessions 1–3), Bonferroni test.

### URB694 Does Not Increase Dopamine Outflow in the Rat Nucleus Accumbens Shell

In the microdialysis procedure (Supplementary Figure S5), levels of extracellular dopamine in the nucleus accumbens shell of rats were not significantly altered by administration of URB694 (3 mg/kg IP; $F_{9, 36} = 0.29$, $p = 0.97$), URB597 (3 mg/kg IP; $F_{9, 27} = 1.17$, $p = 0.35$), or their vehicle ($F_{9, 36} = 0.99$, $p = 0.47$). The effects of these treatments on dopamine levels did not differ from each other (Supplementary Figure S5; effect of time × treatment interaction, $F_{22, 132} = 0.33$, $p = 0.99$).

**Table 1** Pharmacokinetic Data for URB694 after IP Administration in Rats

| URB694 dose | 3 mg/kg | 10 mg/kg |
|---|---|---|
| AUC (ng × min/ml) | 6231 | 32 157 |
| Tmax (min) | 15 | 15 |
| Cmax (ng/ml) | 84 | 209 |
| Distribution volume (ml) | 33 857 | 56 917 |
| Clearance (ml/min/kg) | 481 | 286 |
| Half-life (elimination phase, min) | 48 | 137 |

### Pharmacokinetic Profile of URB694 in Rats

Prior work has shown that URB694 inhibits FAAH activity in the rat brain (Clapper *et al*, 2009), but the pharmacokinetic properties of this compound have not been reported. Because understanding these properties is relevant to interpretation of the effects of drugs on reward-related behaviors, which are strongly dependent on the kinetics of penetration into the brain, we administered URB694 (3 and 10 mg/kg, IP) to rats and measured circulating levels of the compound by LC/MS at various times (0–480 min) after injection. The results are shown in Supplementary Figure S6, and the calculated pharmacokinetic parameters for URB694 are listed in Table 1. As previously shown for URB597, URB694 displayed a relatively fast rate of elimination ($t_{1/2}$), consistent with the rapid first-pass metabolism demonstrated by this class of compounds (Piomelli *et al*, 2006).

## DISCUSSION

The two FAAH inhibitors tested in the present study (URB597 and URB694) attenuated the rewarding effects of nicotine in squirrel monkeys, as evidenced by downward and rightward shifts of the dose–response curves for nicotine self-administration. These results extend earlier findings indicating that URB597 blocks the acquisition of nicotine self-administration in rats (Scherma *et al*, 2008), and represents the first demonstration that FAAH inhibitors can also decrease nicotine self-administration that has already been acquired and maintained at a stable rate in animals. In addition, both FAAH inhibitors had robust effects in the reinstatement model of relapse to nicotine seeking in abstinent monkeys, blocking reinstatement triggered by reexposure to either nicotine itself or to nicotine-associated environmental cues; these findings suggest that FAAH blockade might prevent craving and relapse to smoking, the latter of which is widely considered the main obstacle to smoking cessation (Herd *et al*, 2009; Hughes *et al*, 2008). These findings in non-human primates provide the best preclinical evidence available that FAAH inhibitors could be beneficial as smoking-cessation medications in humans.

It was noted that when nicotine was offered at high doses, total nicotine intake increased after pretreatment with the FAAH inhibitors. This effect is consistent with a decreased rewarding effect of nicotine, but suggests that the effects of FAAH inhibition could be surmounted when taking nicotine at unit doses substantially higher than those that maintain maximum response rates under baseline conditions (ie, at doses on the descending limb of the dose–response curve that are not typically self-administered more than saline). It remains to be seen whether such attempts to surmount the effects of FAAH inhibition would occur in people who voluntarily initiate the treatment, and whether this would limit the effectiveness of the treatment. Nonetheless, it seems likely that in people motivated to quit, FAAH inhibition could be valuable for its ability to decrease the rewarding effects of nicotine and the relapse-inducing effects of nicotine and its associated cues.

At the same doses that blocked the rewarding effects of nicotine, URB597 and URB694 almost completely blocked central and peripheral FAAH activity in monkeys, increasing brain levels of anandamide, PEA, and OEA, all of which are substrates for FAAH. Brain levels of 2-AG, which are primarily controlled by MGL activity, were not affected by treatment with either URB694 or URB597. This result contrasts with those of our previous study (Justinova *et al*, 2008), in which brain levels of 2-AG were significantly decreased after pretreatment with a lower dose of URB597 in monkeys. There is no obvious explanation for this discrepancy, and this can only be resolved by additional experiments.

PEA and OEA are endogenous PPAR-$\alpha$ agonists, and it is notable that the PPAR-$\alpha$ antagonist MK886 reduced the effects of FAAH inhibition on nicotine self-administration and nicotine priming-induced reinstatement. These findings indicate that the indirect actions of FAAH inhibitors at PPAR-$\alpha$ contribute to their ability to attenuate nicotine's reinforcing effects, although the fact that the effects of FAAH inhibition were not completely reversed by MK886 suggest that PPAR-$\alpha$ is not the only mechanism. MK886 did not block the effects of URB597 on cue-induced nicotine seeking, and it produced only a slight attenuation of the effect of URB694 on cue-induced nicotine seeking. Thus, the involvement of PPAR-$\alpha$ in the ability of FAAH to block cue-induced reinstatement of nicotine seeking appears to be minimal. Some other possible mechanisms that might be involved are other PPAR subtypes, vanilloid receptors, and cannabinoid $CB_1$ receptors, or an interactive entourage effect of more than one of these mechanisms. For example, even though systemic administration of a selective $CB_1$ receptor agonist has been shown to increase nicotine self-administration in rats (Gamaleddin *et al*, 2012), indirectly enhancing endocannabinoid signaling by FAAH inhibition dampens nicotine-induced firing of dopamine cells in the nucleus accumbens through both PPAR-$\alpha$ and $CB_1$ receptors (Luchicchi *et al*, 2010; Melis *et al*, 2010). Moreover, it has been shown that potentiation of FAE signaling at PPAR-$\alpha$ by FAAH inhibition can have various consequences, including functional interactions with signaling at $CB_1$ receptors (Russo *et al*, 2007).

Although it was shown earlier that URB597 is not self-administered by squirrel monkeys (Justinova *et al*, 2008), URB694 was self-administered at a moderate rate in the present study. At most, monkeys self-administered URB694 at only about half of the rate of self-administration of the training drug, irrespective of whether the training drug used was anandamide, THC, or cocaine. Although monkeys trained in nicotine self-administration were not tested to determine whether they would self-administer URB597 or URB694, it seems likely that they would do so at rates similar to those seen in the anandamide-, THC-, and cocaine-trained

monkeys. Thus, the results suggest that the rewarding effects of URB694 are weaker than cocaine or THC, and that URB694 would not have high abuse potential. In fact, mild rewarding effects imply the absence of aversive effects and might be beneficial to a FAAH-inhibiting medication as far as compliance, especially in light of the aversive side effects that have prevented the use of drugs that antagonize endocannabinoid signaling, such as rimonabant (Le Foll *et al*, 2009). Importantly, neither of the FAAH inhibitors reinstated extinguished nicotine seeking when administered alone, suggesting they are not liable to induce relapse to nicotine use. Furthermore, the FAAH inhibitors did not affect cocaine- or food-reinforced behavior in monkeys under the same schedule of reinforcement used with nicotine, indicating that their effects on nicotine self-administration and reinstatement were selective for nicotine and not caused by nonspecific suppression of behavior.

Self-administration of URB694 was blocked by either rimonabant or MK886, pointing to an involvement of both cannabinoid $CB_1$ and PPAR-$\alpha$ receptors in this behavior. Despite this indication that the moderate rewarding effects of URB694 are at least partially caused by increases in brain levels of the $CB_1$ agonist anandamide, URB694 (as was shown with URB597 earlier in Justinova *et al*, 2008) did not induce relapse to drug seeking in monkeys trained to self-administer THC. Furthermore, URB694 and URB597 did not produce THC-like or nicotine-like discriminative-stimulus effects in rats, nor did they alter the ability of rats to discriminate the interoceptive effects of different THC or nicotine doses (see also Piomelli *et al*, 2006; Solinas *et al*, 2008). Finally, *in vivo* microdialysis studies in rats showed that —unlike drugs that are readily self-administered, including THC and anandamide (Justinova *et al*, 2011; Solinas *et al*, 2006)— both FAAH inhibitors failed to increase dopamine release in the nucleus accumbens shell.

In sum, the present results show that the FAAH inhibitors URB597 and URB694 selectively block nicotine reward and attenuate the ability of nicotine and nicotine-related cues to trigger relapse to nicotine seeking. Although URB694 has moderate reinforcing effects in drug-experienced monkeys, it does not provoke relapse to nicotine seeking after a period of abstinence in monkeys, and its discriminative-stimulus effects do not resemble those of either THC or nicotine in rats. Collectively, these findings suggest that URB597 and URB694 can safely and effectively decrease the abuse-related effects of nicotine in non-human primates, making them promising candidates for development as medications to promote smoking cessation.

## FUNDING AND DISCLOSURE

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## ACKNOWLEDGMENTS

We dedicate this manuscript to Dr Steven R Goldberg who passed away suddenly on 25 November 2014. He was a fantastic scientist, collaborator, mentor, and a friend, and he will be sorely missed. We thank Dr Ira Baum and Philip White for their excellent veterinary assistance during this study.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)