Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis

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Although anecdotal reports suggest that cannabis may be used to alleviate symptoms of depression, the psychotropic effects and abuse liability of this drug prevent its therapeutic application. The active constituent of cannabis, Δ^9-tetrahydrocannabinol, acts by binding to brain CB_1 cannabinoid receptors, but an alternative approach might be to develop agents that amplify the actions of endogenous cannabinoids by blocking their deactivation. Here, we show that URB597, a selective inhibitor of the enzyme fatty-acid amide hydrolase, which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide, exerts potent antidepressant-like effects in the mouse tail-suspension test and the rat forced-swim test. Moreover, URB597 increases firing activity of serotonergic neurons in the dorsal raphe nucleus and noradrenergic neurons in the nucleus locus ceruleus. These actions are prevented by the CB_1 antagonist rimonabant, are accompanied by increased brain anandamide levels, and are maintained upon repeated URB597 administration. Unlike direct CB_1 agonists, URB597 does not exert rewarding effects in the conditioned place preference test or produce generalization to the discriminative effects of Δ^9-tetrahydrocannabinol in rats. The findings support a role for anandamide in mood regulation and point to fatty-acid amide hydrolase as a previously uncharacterized target for antidepressant drugs.

depression | endocannabinoid | fatty-acid amide hydrolase | serotonin | URB597

Cannabis elicits in humans a complex subjective experience, a combination of mood elevation, heightened sensitivity to external stimuli, and relaxation (1), which results from the interaction of its main psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), with CB_1 cannabinoid receptors in the brain (2). Functional imaging studies have shown that this drug-induced state is associated with changes in cerebral blood flow and glucose metabolism in limbic and paralimbic areas of the cortex (3, 4) that are involved both in the control of normal emotional behavior and the pathogenesis of depression (5).

The idea that the mood-elevating properties of cannabis might be harnessed to treat depression was proposed first in the mid-19th century, but soon was disputed on account of the multiple side effects and inconsistent efficacy of the drug (6). Surprisingly, this controversy is still unsettled. Indeed, although clinical trials of cannabis in affective disorders have yielded mixed results (7, 8), many patients continue to report benefits from its use in primary or secondary depressive syndromes (9–13). One likely explanation for these contrasting data is suggested by the diversity of functions served by CB_1 receptors in the brain (14), which makes it difficult to separate the mood-elevating actions of Δ^9-THC from its unwanted psychotropic effects. Synthetic cannabinoid agonists target the same receptors engaged by Δ^9-THC and are limited, therefore, by equally narrow therapeutic indexes.

An alternative way of enhancing cannabinoid function might be to use drugs that interfere with the deactivation of the endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) (15). We have recently described a class of such drugs, which act by blocking the intracellular hydrolysis of anandamide by fatty-acid amide hydrolase (FAAH) (16, 17). The index compound of this class, URB597, inhibits FAAH activity with nanomolar potency and has no affinity for CB_1 receptors or other cannabinoid-related targets (16, 18, 19). This high degree of selectivity is paralleled by a lack of overt cannabinoid-like actions: For example, even when administered at doses that completely inhibit brain FAAH activity, URB597 does not cause catalepsy, hypothermia, or hyperphagia, three key signs of cannabinoid intoxication in the rodent (16). Notably, however, URB597 elicits profound anxiolytic-like effects in rats, which are prevented by the CB_1 antagonist rimonabant (16). These findings suggest that FAAH inhibitors such as URB597 may selectively modulate mood states by enhancing anandamide's interaction with a subset of brain CB_1 receptors that are normally engaged in the processing of emotional information. Here, we further tested this hypothesis, first, by examining the impact of URB597 on emotional and hedonic behavior and, second, by determining whether URB597 influences brain monoamine pathways that participate in the control of mood and reward.

Materials and Methods

Animals. We used male C57BL/6 mice (25–30 g; Charles River Laboratories, Raleigh, NC), Wistar rats (200–350 g; Charles River Laboratories; Harlan Labs, Milan) or Sprague–Dawley rats (225–275 g; Charles River Laboratories). The animals were housed at constant room temperature and humidity under a 12-h light/dark cycle. Food and water were available *ad libitum* in all but drug discrimination experiments, in which rats were slightly food-deprived (20). All procedures were approved by local institutional care and use committees and followed the Guidelines for the Care and Use of Mammals in Neuroscience and

Conflict of interest statement: No conflicts declared.

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; DRN, dorsal raphe; FAAH, fatty-acid amide hydrolase; 5-HT, serotonin; FST, forced swim test; NE, norepinephrine; TST, tail suspension test; 2-AG, 2-arachidonoylglycerol.

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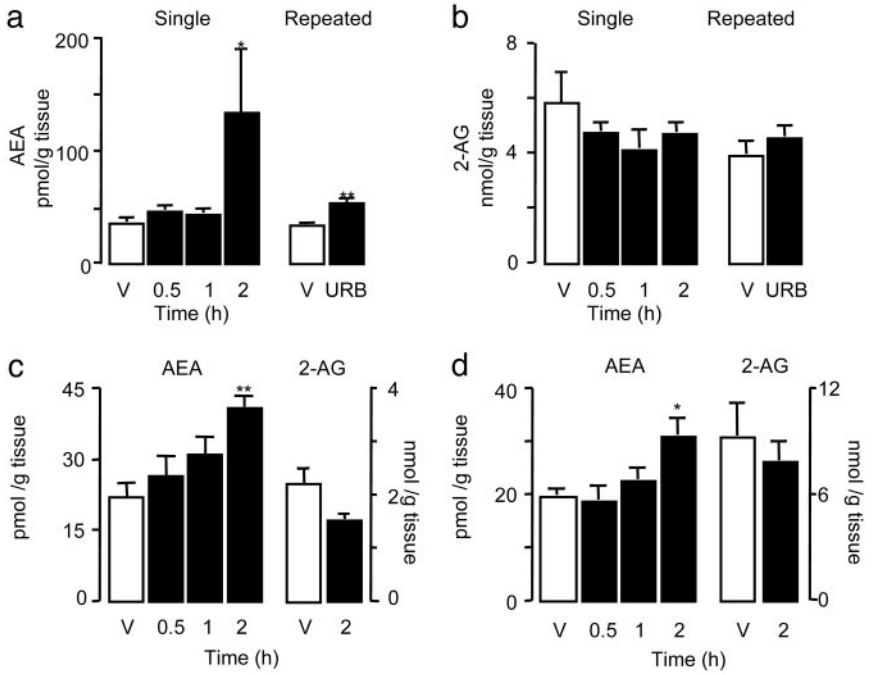

Fig. 1. Time-dependent effects of URB597 on endocannabinoid levels in rat brain. (*a* and *b*) Effects of URB597 on anandamide (AEA) (*a*) and 2-AG (*b*) in hippocampus after single (0.1 mg·kg^{-1}, i.p.) or repeated injections (0.1 mg·kg^{-1}, i.p., once daily for 4 days). (*c* and *d*) Effects of a single URB597 administration on anandamide and 2-AG in cortex (*c*) and midbrain (*d*). Vehicle, open bars; URB597, filled bars. *, $P < 0.05$ vs. vehicle; **, $P < 0.01$ vs. vehicle.

Behavioral Research (National Research Council 2004), and guidelines released by the Italian Ministry of Health (D.L. 116/92) and the Canadian Institutes of Health Research.

Drugs. URB597 was synthesized as described in ref. 18, rimonabant and Δ^9-THC were from the National Institute on Drug Abuse, and all other drugs were from RBI-Sigma (St. Louis).

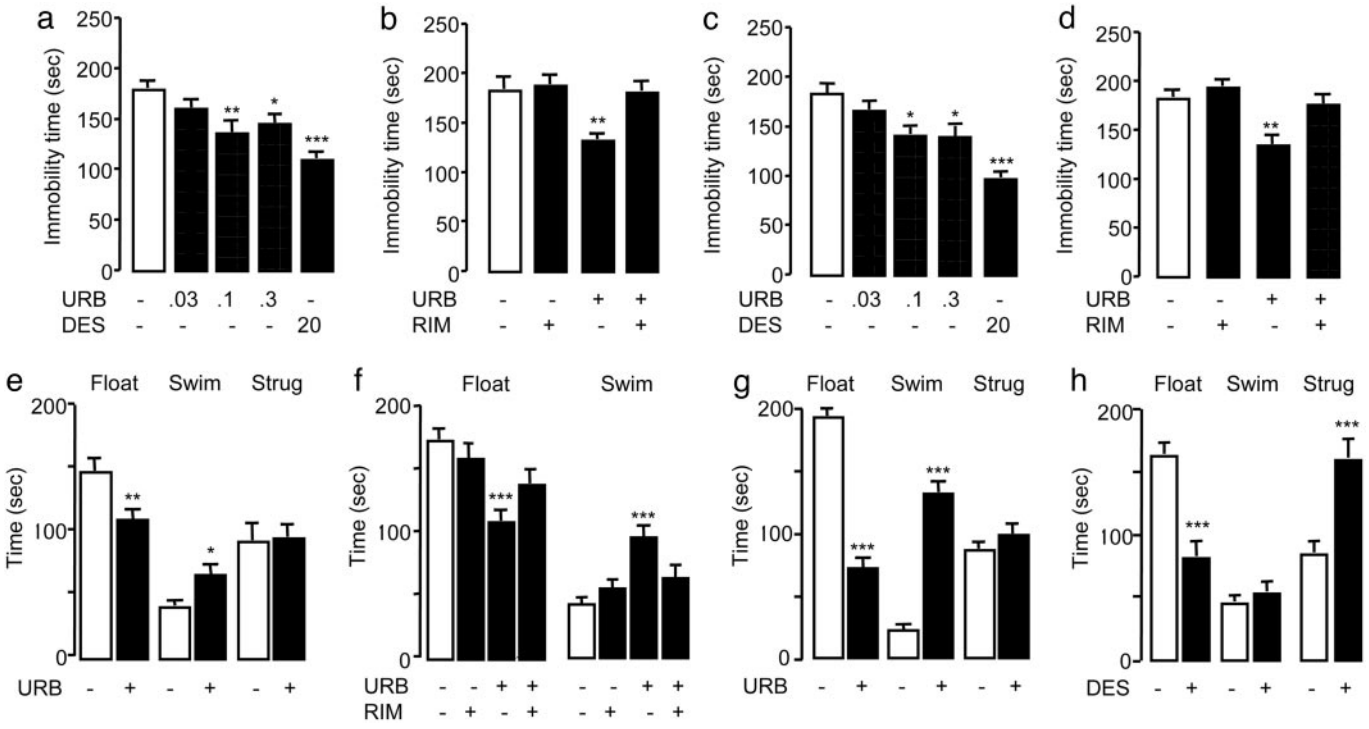

Fig. 2. Antidepressant-like effects of URB597 in (*a–d*) mouse TST and (*e–h*) rat FST. (*a* and *c*) Effects of URB597 (mg·kg^{-1}, i.p.) and desipramine (DES, 20 mg·kg^{-1}, i.p.) in the TST after single (*a*) or repeated (*c*) administration (once daily for 4 days). (*b* and *d*) Single injection of rimonabant (RIM) (1 mg·kg^{-1}, i.p., 30 min before URB597, 0.1 mg·kg^{-1}) prevents the effects of single (*b*) or repeated (*d*) URB597 administration. (*e–g*) Effects of URB597 in the FST: (*e*) effects of single URB597 injection; (*f*) single injection of rimonabant prevents the effects of URB597; (*g*) effects of multiple URB597 injections. (*h*) Effects of repeated desipramine injections (15 mg·kg^{-1}, i.p., once daily for 4 days). *, $P < 0.05$ vs. vehicle; **, $P < 0.01$ vs. vehicle; ***, $P < 0.001$ vs. vehicle.

Drug preparation and vehicles are described in *Supporting Methods*, which is published as supporting information on the PNAS web site. We administered all drugs by i.p. or i.v. injection in 1–2 ml·kg^{-1} of vehicle.

Receptor Binding. Radioligand-binding assays were conducted at the National Institute of Mental Health Psychoactive Drug Discovery Program of Case Western Reserve University (available upon request) by using 10 μM URB597.

Behavioral Tests. We conducted the tail suspension test (TST) in C57BL/6 mice (21), the forced swim test (FST) (22) and the conditioned place preference test in Wistar rats (23), and the drug discrimination test in Sprague–Dawley rats (20) (see *Supporting Methods*).

***In Vivo* Electrophysiological Recordings.** We performed dorsal raphe (DRN) and locus ceruleus recordings in Sprague–Dawley rats as described in ref. 24 (see *Supporting Methods*).

***In Vivo* Microdialysis.** *In vivo* microdialysis was performed in awake, freely moving Wistar rats (25) (see *Supporting Methods*).

Neurochemical Analyses. We dissected brain regions of Wistar rats and quantified endocannabinoids by HPLC/mass spectrometry (19).

Statistical Analyses. Results are expressed as mean ± SEM. Statistical significance was evaluated by using the Student *t* test or, when appropriate, one-way analysis of variance (ANOVA) followed by a Dunnett's or Tukey's post hoc test.

Results

Effects on Rat Brain Anandamide Levels. We first examined whether URB597 prevents anandamide deactivation in three brain regions that are involved in the control of emotions: hippocampus, prefrontal cortex and DRN (5). As expected from studies in refs. 16 and 19, URB597 (0.1 mg·kg^{-1}, i.p.) produced a slow accumulation of anandamide in the hippocampus, which was significant 2 h after drug administration and was maintained upon repeated dosing (0.1 mg·kg^{-1}, i.p., once daily for 4 days, measured 2 h after final injection) (Fig. 1*a*). URB597 also increased hippocampal levels of the noncannabinoid fatty-acid ethanolamide palmitoylethanolamide (PEA) (26, 27) while causing no acute or persistent increase in 2-AG content (Fig. 1*b*; see also Table 1, which is published as supporting information on the PNAS web site). Similar increases in anandamide and PEA, but not 2-AG levels, were observed in the rat prefrontal cortex and midbrain (Fig. 1 *c* and *d* and Table 1).

Antidepressant-Like Properties. We next tested the effects of URB597 in two models of stress-coping behavior, the mouse TST and the rat FST, which are widely used to assess antidepressant-like properties of drugs. In the TST, single injections of URB597 (0.03–0.3 mg·kg^{-1}, 2 h before testing) elicited a dose-dependent decrease in time spent in immobility (Fig. 2*a*). This effect was maximal at a dose of 0.1 mg·kg^{-1}, was comparable to that produced by two clinically used antidepressants, desipramine (20 mg·kg^{-1}, i.p.) (Fig. 2*a*) and paroxetine (10 mg·kg^{-1}, i.p.; Table 2, which is published as supporting information on the PNAS web site), and was prevented by the CB_1 antagonist rimonabant (1 mg·kg^{-1}, i.p.; 30 min before URB597) (Fig. 2*b*). Repeated injections of URB597 (0.03–0.3 mg·kg^{-1}, i.p., once daily for 4 days) elicited a similar response (Fig. 2*c*), which was also blocked by a single rimonabant injection (Fig. 2*d*) (1 mg·kg^{-1}, i.p.; 30 min before last URB597 injection).

In the FST, URB597 (0.1 mg·kg^{-1}, i.p., 2 h before testing) significantly shortened floating time and prolonged swimming time but did not affect struggling time (Fig. 2*e*). These effects were attenuated by rimonabant (Fig. 2*f*) and were maintained upon repeated URB597 injections (0.1 mg·kg^{-1}, i.p., once daily for 4 days) (Fig. 2*g*). The response pattern evoked by URB597 resembled that reported for fluoxetine (28) but differed from that produced by desipramine (15 mg·kg^{-1}, i.p., once daily for 4 days), which prolonged struggling time without affecting swimming time (Fig. 2*h*). The significance of this difference is unclear.

Effects on Conditioned Place Preference and Stimulus Discrimination. URB597 might influence stress-coping behaviors by altering endocannabinoid-mediated reward mechanisms (29). To test this possibility, we investigated the rewarding properties of URB597 in the conditioned place preference test (23). The effects of cannabinoid agonists in this model are highly variable, and contrasting data have been reported (29). In our laboratory, rats housed in an enriched environment develop preference for the cannabinoid agonist WIN 55,212-2 (1 mg·kg^{-1}, i.p.), whereas rats housed in a normal environment do not (data not shown). Irrespective of housing conditions, however, rats treated with URB597 (0.03–0.3 mg·kg^{-1}, i.p., 90-min or 2-h treatment) during the training phase exhibited no shift in preference toward the URB597-associated environment (Fig. 3*a* and data not shown). We next asked whether URB597 produces in rats an interoceptive state similar to that elicited by Δ^9-THC. We trained rats to discriminate Δ^9-THC (3 mg·kg^{-1}, i.p.) from vehicle in a two-lever operant drug-discrimination procedure (Fig. 3*b*) and then tested them either with WIN 55,212-2 (0.1–3 mg·kg^{-1}, i.p., 40 min before 30-min sessions) or URB597 (0.1–3 mg·kg^{-1}, i.p., 40 min or 2 h before 30-min sessions). WIN 55,212-2 produced a complete generalization to the discriminative effects of Δ^9-THC (Fig. 3*b*) and, as observed with a high 5.6 mg·kg^{-1} dose of Δ^9-THC, decreased motor responding at its highest dose (Fig. 3*c*). By contrast, URB597 had no such effect at any of the doses or pretreatment times tested (Fig. 3 *b* and *c*). The results indicate that URB597 does not produce rewarding effects or mimic the interoceptive state elicited by Δ^9-THC.

Regulation of Serotonergic Transmission. To assess whether URB597 influences brain monoaminergic transmission (30), we first measured spontaneous activity of serotonin (5-HT) neurons in the DRN of anesthetized rats. Single injections of URB597 (0.03–0.3 mg·kg^{-1} i.v.) evoked a slow increase in 5-HT neuron firing activity (Fig. 4*a*), which was half-maximal at a dose of ≈0.06 mg·kg^{-1} (Fig. 4*b*) and was blocked by rimonabant (1 mg·kg^{-1}, i.v.) (Fig. 4*c*). Repeated injections of URB597 (0.1 mg·kg^{-1}, i.p., once daily for 4 days) evoked an even stronger response, which was also reversed by rimonabant (1 mg·kg^{-1}, i.p.) (Fig. 4*d*) (single injections: vehicle, 1.42 ± 0.05 Hz; URB597, 2.57 ± 0.2 Hz; $P < 0.001$, $n = 176$; repeated injections: vehicle, 1.36 ± 0.2 Hz; URB597, 3.24 ± 0.5 Hz; $P < 0.001$, $n = 137$). Three additional aspects of repeated URB597 treatment are worth noting. First, the treatment increased the number of bursting neurons in the DRN, a pattern of activity that is associated with enhanced 5-HT release in DRN terminal fields (percent bursting cells; single injection: vehicle, 8.1%; URB597, 11.5%; repeated injections: vehicle, 12.7%; URB597, 76.3%; $P < 0.001$; $n = 161$) (31). Second, repeated URB597 did not affect the responsiveness of 5-HT neurons to local iontophoretic administration of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (Fig. 4*e*), suggesting that URB597, unlike classical antidepressants (32), did not produce desensitization of 5-HT_{1A} autoreceptors. Finally, repeated URB597 enhanced 5-HT outflow in the hippocampus, as assessed by *in vivo* microdialysis in awake rats (Fig. 4*f*), whereas a single injection of URB597 had no such effect (Fig. 4*f*) even when the 5-HT reuptake inhibitor citalopram (1 mM) was added to the microdialysis perfusate (data not shown). Irrespective of dosing reg-

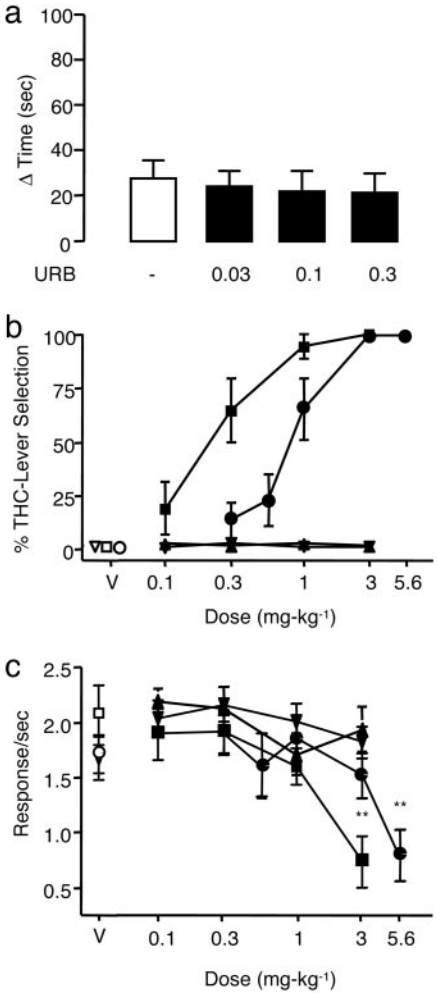

Fig. 3. Motivational profile of URB597. (*a*) Effects of URB597 (0.03–0.3 mg·kg^{-1}, i.p., 2-h treatment) in the rat conditioned place preference. Δ Time, difference in time spent in the nonpreferred compartment between post- and preconditioning sessions. (*b* and *c*) Effects of WIN 55,212-2 (■), Δ^9-THC (●), URB597 2 h before test session (▼), and URB597 40 min before test session (▲) in rats trained to discriminate 3 mg·kg^{-1} Δ^9-THC from vehicle. Shown are the percent of responses on the Δ^9-THC associated lever (*b*) and rate of lever pressing over the entire 30-min session (*c*). Open symbols represent respective vehicles. **, $P < 0.01$ vs. vehicle.

imen, URB597 did not increase 5-HT outflow in the prefrontal cortex (Fig. 4*g*).

Regulation of Noradrenergic Transmission. Next, we measured spontaneous activity of norepinephrine (NE)-releasing neurons in the locus ceruleus of anesthetized rats. Single URB597 injections (0.1 mg·kg^{-1}, i.v.) evoked a slow increase in NE neuron activity (Fig. 5*a*), which was blocked by rimonabant (1 mg·kg^{-1}, i.v.) (Fig. 5*b*). Repeated URB597 injections (0.1 mg·kg^{-1}, i.p., once daily for 4 days) evoked a similar response, which was also sensitive to rimonabant (1 mg·kg^{-1}, i.p.) (Fig. 5*b*). Microdialysis studies showed, however, that neither single nor repeated URB597 treatment had any effect on NE outflow in the prefrontal cortex (Fig. 6, which is published as supporting information on the PNAS web site) (single, $t = 0.55$, nonsignificant; repeated, $t = 1.93$, nonsignificant).

Target Selectivity. URB597 (10 μM) did not significantly displace the binding of radioactively labeled ligands from a panel of 47 receptors, transporters, and ion channels, which included 5-HT$_{1a}$, 5-HT$_{1b}$, 5-HT$_{1d}$, 5-HT$_{1e}$, 5-HT$_{2a}$, 5-HT$_{2c}$, 5-HT$_3$, 5-HT$_6$, and 5-HT$_7$; adrenergic α_{1a}, α_{1b}, α_{2a}, α_{2b}, α_{2c}, β_1, and β_2; dopamine D_1–D_5; muscarinic m_{1-5}; nicotinic $\alpha_2\beta_2$, $\alpha_2\beta_4$, $\alpha_3\beta_2$, $\alpha_3\beta_4$, $\alpha_4\beta_2$, and $\alpha_4\beta_4$; CB_1 and CB_2; histamine H_1 and H_2; μ and κ opiate; σ_1 and σ_2; 5-HT transporter (SERT); NE transporter (NET); dopamine transporter; multidrug resistance protein-1; and HERG channel.

Discussion

We have used the selective FAAH inhibitor URB597 to examine whether anandamide signaling modulates brain circuits involved in the control of mood and emotion. Our results show that administration of URB597, at doses that inhibit FAAH activity and elevate brain anandamide levels, enhances stress-coping behaviors and increases spontaneous firing of serotonergic and noradrenergic neurons in the midbrain. These actions are blocked by the CB_1 antagonist rimonabant and are not accompanied by overt rewarding effects. We interpret these findings to indicate that endogenous anandamide interacts with a subset of brain CB_1 receptors that concertedly regulate monoaminergic neurotransmission and stress responses. This interaction can be magnified, and consequently unmasked, by blocking intracellular anandamide degradation with URB597.

Three lines of evidence suggest that anandamide modulates the emotional response to stress. First, stressful stimuli affect anandamide mobilization in brain regions that are involved in the control of emotions. In rats, for example, an electric shock to the paw elevates anandamide levels in the midbrain (33), whereas in mice, physical restraint decreases anandamide levels in the amygdala (34). Second, pharmacological blockade or genetic ablation of CB_1 receptors exacerbates normal reactions to acute stress, presumably by disabling an endocannabinoid modulation of these reactions (35–38). Third, URB597 prolongs the time spent by rats in the open quadrants of an elevated maze (16), reduces the number of ultrasonic vocalizations emitted by rat pups after parental separation (16), lowers restraint stress-induced corticosterone release in mice (39), and prolongs nonopioid stress-induced analgesia in rats (33). All these effects are prevented by CB_1 receptor blockade. The present results expand the pharmacological profile of URB597 to include the potentiation of stress-coping behaviors in the TST and FST, two widely used screens for antidepressant drugs, and point to the dual regulation of 5-HT and NE neurotransmission as a possible neural substrate for these actions.

The 5-HT and NE systems of the midbrain serve important adaptive functions in the response to acute stress, and long-term alterations in their activity may contribute to the development of depression (5). Indeed, the ability to enhance monoaminergic transmission is a distinguishing feature shared by all antidepressant drugs, irrespective of their specific mechanism of action (30). Importantly, however, a dual 5-HT and NE activation reminiscent of that produced by URB597 is seen only with a restricted group of antidepressants, which include venlafaxine (dual 5-HT/NE reuptake inhibitor), nefazodone (5-HT$_2$ antagonist), and mirtazapine (α_2 adrenergic antagonist). Clinical evidence suggests that these "atypical" antidepressants display greater efficacy and faster onset of action compared with 5-HT reuptake inhibitors and improved side-effect profile compared with tricyclics and monoaminoxidase inhibitors (30). Our results indicate that URB597 may offer similar advantages, which might be further enhanced by the acute anxiolytic-like properties of this drug (16).

The addictive properties of Δ^9-THC are a major obstacle to the development of cannabinoid-based therapeutics. Thus, it is particularly important that URB597 does not mimic the hedonic and interoceptives states evoked by direct-acting cannabinoid

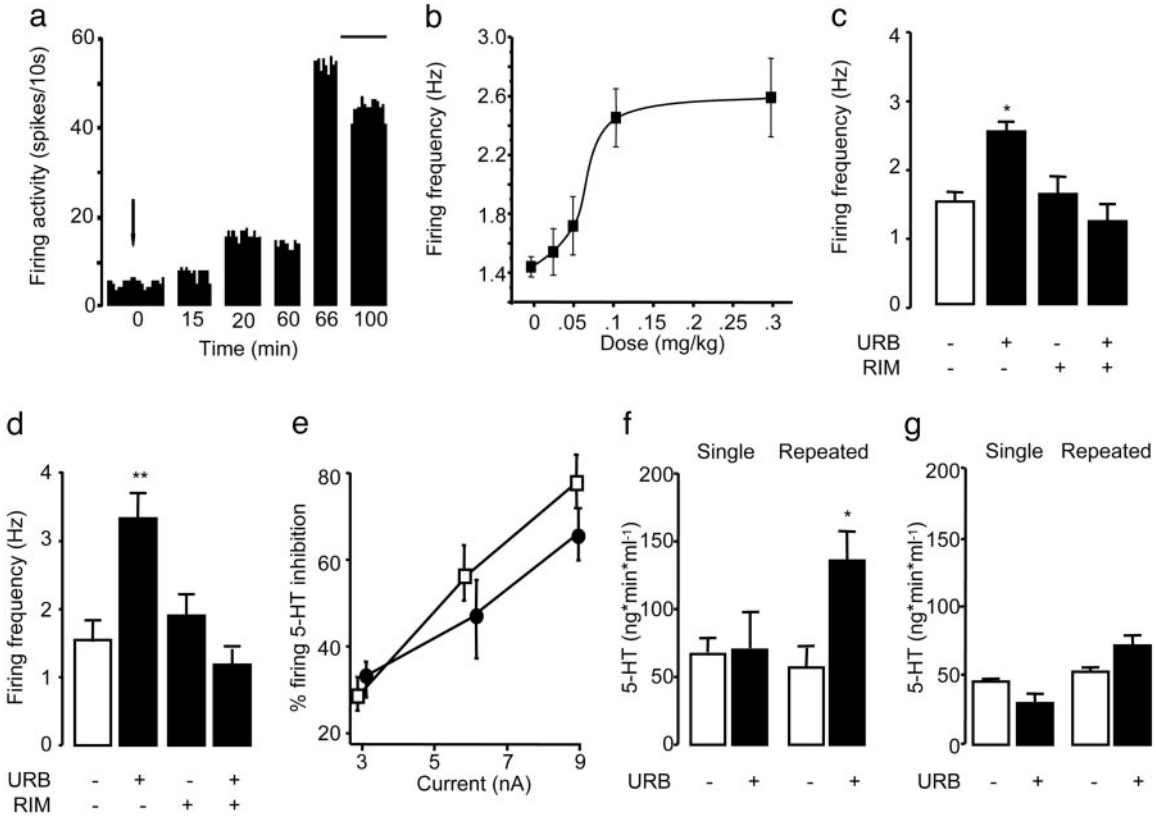

Fig. 4. Effects of URB597 on 5-HT neuron firing in the rat DRN. (*a*) Integrated firing rate histogram of DRN neurons, illustrating the time-dependent effects of URB597; arrow indicates time of URB597 injection (0.1 mg·kg^{-1}, i.v.; calibration bar: 1 min). (*b*) Dose-dependent effects of URB597 on spontaneous firing rate. (*c* and *d*) Single administration of rimonabant (RIM) (1 mg·kg^{-1}, i.v.) prevents the effects of single (0.1 mg·kg^{-1}) (*c*) and repeated (*d*) URB597 injections (0.1 mg·kg^{-1}, i.p., once daily for 4 days) on 5-HT neuron firing. (*e*) Repeated URB597 administration does not affect the response of 5-HT neurons to 8-hydroxy-2-(di-*n*-propylamino)tetralin, expressed as percent inhibition of 5-HT-neuron firing rate. Open symbols represent vehicle. (*f* and *g*) Effects of single or repeated URB597 injections on 5-HT outflow over 3 h in hippocampus (*f*) and prefrontal cortex (*g*) of awake rats. *, $P < 0.05$ vs. vehicle; **, $P < 0.01$, vs. vehicle.

agonists. This lack of cannabimimetic activity is consistent with the fact that URB597 does not elicit catalepsy, hypothermia, or other classical signs of CB_1 activation (16).

Our experiments do not elucidate the neural substrates underlying the antidepressant-like properties of URB597. Indeed, although the results highlight a possible role of midbrain monoaminergic nuclei, the contribution of such nuclei and the sequence of events leading to their activation remain unknown. Despite these open questions, our findings provide a preclinical validation for URB597 as an antidepressant agent with dual 5-HT- and NE-enhancing activity.

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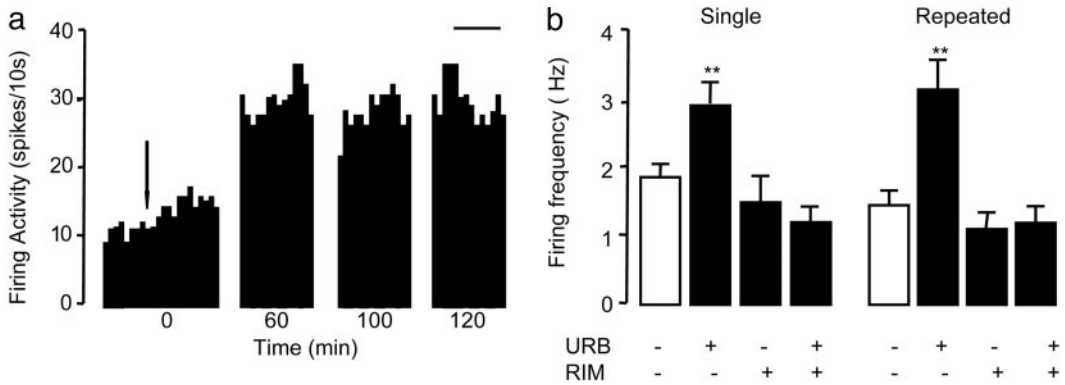

Fig. 5. Effects of URB597 on NE neuron firing in the rat locus ceruleus. (*a*) Integrated firing rate histogram of locus ceruleus neurons, illustrating the time-dependent effects of URB597 (0.1 mg·kg^{-1}, i.v.). Arrow, time of URB597 injection; calibration bar, 1 min. (*b*) Effects of single injection (0.1 mg·kg^{-1}, i.v.) (*Left*) or repeated injections (0.1 mg·kg^{-1}, i.p., once daily for 4 days) (*Right*) of URB597 on NE firing activity, and blockade of these effects by single injection of rimonabant (RIM) (1 mg·kg^{-1}, i.v.). Bars represent mean ± SEM firing activity (Hz) of neurons recorded 20–120 s after injection. **, $P < 0.01$ vs. vehicle.

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