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

G. Gobbi*†, F. R. Bambico*‡, R. Mangieri§¶, M. Bortolato§¶, P. Campolongo§, M. Solinas†, T. Cassano**, M. G. Morgese**, G. Debonnel*, A. Duranti††, A. Tontini††, G. Tarzì††, M. Mor‡‡, V. Trezza‡, S. R. Goldberg§§, V. Cuomo*, and D. Piomelli*††

*Department of Psychiatry, McGill University, Montréal, QC, Canada H3A 3V2; †Department of Psychiatry, Centre de Recherche Fernand Seguin, Université de Montréal, Montréal, QC, Canada H3A 1A1; ‡Department of Pharmacology and Center for Drug Discovery, University of California, Irvine, CA 92697-4625; §Department of Human Physiology and Pharmacology, University of Rome “La Sapienza,” 00185 Rome, Italy; ¶Department National of the Recherche Scientifique Unité Mixte de Recherche 6187, University of Poitiers, 86000 Poitiers, France; **Department of Biomedical Sciences, University of Foggia Medical School, 71100 Foggia, Italy; ††Institute of Medicinal Chemistry, University of Urbino “Carlo Bo,” 61029 Urbino, Italy; ‡‡Pharmaceutical Department, University of Parma, 43100 Parma, Italy; and §§Preclinical Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224

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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, Δ²-tetrahydrocannabinol, acts by binding to brain CB₁ 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 locus coeruleus. These actions are prevented by the CB₁ 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 CB₁ receptors that are normally engaged by blockade of anandamide in mood regulation and point to fatty-acid amide hydrolase as a previously uncharacterized target for antidepressant drugs.

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, Δ²-tetrahydrocannabinol (Δ²-THC), with CB₁ 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).

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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 Δ⁹-THC were from the National Institute on Drug Abuse, and all other drugs were from RBI-Sigma (St. Louis).

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.

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. 1a). URB597 also increased hippocampal levels of the noncannabinoid fatty-acid ethanolamide palmitoylethanolamide (PEA) (26, 27) while causing no increase in anandamide levels in the hippocampus, which was significant 2 h after final injection (Fig. 1a). URB597 also increased hippocampal levels of the noncannabinoid fatty-acid ethanolamide palmitoylethanolamide (PEA) (26, 27) while causing no increase in anandamide levels in the hippocampus, which was significant 2 h after final injection (Fig. 1a).

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

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, i.v.) evoked a slow increase in NE neuron activity (Fig. 5b), which was blocked by rimonabant (1 mg/kg, i.p.) (Fig. 5b). Repeated URB597 injections (0.1 mg/kg, i.v., once daily for 4 days) evoked a similar response, which was also sensitive to rimonabant (1 mg/kg, i.p.) (Fig. 5b). 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 \( \mu \)M) 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\(_2a\), 5-HT\(_2c\), 5-HT\(_3\), and 5-HT\(_7\); adrenergic \( \alpha_{1a} \), \( \alpha_{1b} \), \( \alpha_{2a} \), \( \alpha_{2b} \), \( \beta_1 \), and \( \beta_2 \); dopamine D\(_1\)–D\(_5\); muscarinic m\(_1\)–m\(_5\); nicotinic \( \alpha_3\beta_2 \), \( \alpha_7\beta_4 \), \( \alpha_9\beta_2 \), \( \alpha_9\beta_4 \), \( \alpha_9\beta_5 \), and \( \alpha_9\beta_2 \); CB\(_1\) and CB\(_2\); histamine H\(_1\) and H\(_3\); \( \mu \) and \( \kappa \) opioid; \( \delta \) and \( \sigma_2 \); 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 consequently 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 parenteral 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 (\( \alpha_2 \) 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 monoaminooxidase 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 \( \Delta^2\)-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 interoceptors states evoked by direct-acting cannabinoid
agonists. This lack of cannabimimetic activity is consistent with the fact that URB597 does not elicit catalepsy, hypothermia, or other classical signs of CB1 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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