Title
Anxiolytic-like properties of the anandamide transport inhibitor AM404

Permalink
https://escholarship.org/uc/item/32r5x8rf

Journal
Neuropsychopharmacology, 31(12)

ISSN
0893-133X

Authors
Bortolato, M
Campolongo, P
Mangieri, RA
et al.

Publication Date
2006-12-04

DOI
10.1038/sj.npp.1301061

License
CC BY 4.0

Peer reviewed
Anxiolytic-Like Properties of the Anandamide Transport Inhibitor AM404

INTRODUCTION

The endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) may contribute to the regulation of mood and emotion. In this study, we investigated the impact of the endocannabinoid transport inhibitor AM404 on three rat models of anxiety: elevated plus maze, defensive withdrawal and separation-induced ultrasonic vocalizations. AM404 (1–5 mg kg⁻¹, intraperitoneal (i.p.)) exerted dose-dependent anxiolytic-like effects in the three models. These behavioral effects were associated with increased levels of anandamide, but not 2-AG, in the prefrontal cortex and were prevented by the CB₁ cannabinoid antagonist rimonabant (SR141716A), suggesting that they were dependent on anandamide-mediated activation of CB₁ cannabinoid receptors. We also evaluated whether AM404 might influence motivation (in the conditioned place preference (CPP) test), sensory reactivity (acoustic startle reflex) and sensorimotor gating (prepulse inhibition (PPI) of the startle reflex). In the CPP test, AM404 (1.25–10 mg kg⁻¹, i.p.) elicited rewarding effects in rats housed under enriched conditions, but not in rats kept in standard cages. Moreover, AM404 did not alter reactivity to sensory stimuli or cause overt perceptual distortion, as suggested by its lack of effect on startle or PPI of startle. These results support a role of anandamide in the regulation of emotion and point to the anandamide transport system as a potential target for anxiolytic drugs.

Neuropsychopharmacology (2006) 31, 2652–2659. doi:10.1038/sj.npp.1301061; published online 15 March 2006

Keywords: anandamide; anxiety; AM404; conditioned place preference; prepulse inhibition

The endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) may contribute to the regulation of mood and emotion. In this study, we investigated the impact of the endocannabinoid transport inhibitor AM404 on three rat models of anxiety: elevated plus maze, defensive withdrawal and separation-induced ultrasonic vocalizations. AM404 (1–5 mg kg⁻¹, intraperitoneal (i.p.)) exerted dose-dependent anxiolytic-like effects in the three models. These behavioral effects were associated with increased levels of anandamide, but not 2-AG, in the prefrontal cortex and were prevented by the CB₁ cannabinoid antagonist rimonabant (SR141716A), suggesting that they were dependent on anandamide-mediated activation of CB₁ cannabinoid receptors. We also evaluated whether AM404 might influence motivation (in the conditioned place preference (CPP) test), sensory reactivity (acoustic startle reflex) and sensorimotor gating (prepulse inhibition (PPI) of the startle reflex). In the CPP test, AM404 (1.25–10 mg kg⁻¹, i.p.) elicited rewarding effects in rats housed under enriched conditions, but not in rats kept in standard cages. Moreover, AM404 did not alter reactivity to sensory stimuli or cause overt perceptual distortion, as suggested by its lack of effect on startle or PPI of startle. These results support a role of anandamide in the regulation of emotion and point to the anandamide transport system as a potential target for anxiolytic drugs.

Neuropsychopharmacology (2006) 31, 2652–2659. doi:10.1038/sj.npp.1301061; published online 15 March 2006

Keywords: anandamide; anxiety; AM404; conditioned place preference; prepulse inhibition
other consequences of CB1 receptor activation, such as whether such effects, if present, may be associated with et al, 2003), may exert anxiolytic-like effects. The second is whether such effects, if present, may be associated with other consequences of CB1 receptor activation, such as perceptual distortion and liability to addiction (Hollister, 1998; D’Souza et al, 2005). To begin to address these issues, in the present study we have investigated the impact of a prototypical anandamide transport inhibitor, the compound AM404, on a series of behavioral models relevant to potential emotional and cognitive outcomes of endocannabinoid deactivation blockade.

MATERIALS AND METHODS

Animals

We used 10-day old Wistar pups for ultrasonic vocalization emission tests, adult male Sprague–Dawley rats (250–300 g) for startle and prepulse inhibition (PPI) tests, and adult male Wistar rats (200–350 g) for all other tests. Some of the rats tested for conditioned place preference (CPP) were housed under enriched conditions, consisting in exposing the rats for 6 weeks to a varied set of daily exchanged toys in a structured cage environment, as well as daily handling sessions. All procedures met the National Institutes of Health guidelines for the care and use of laboratory animals and those of the Italian Ministry of Health (D.L. 116/92).

Drugs

AM404 and WIN55212-2 were from Tocris Cookson (Avonmouth, UK); morphine, diazepam, apomorphine and dizocilpine (MK801) from Sigma Aldrich (St Louis, USA); and rimonabant (SR141716A) from the National Institute on Drug Abuse (NIDA).

Endocannabinoid Analyses

At 45 min after injection with AM404 (2.5–10 mg kg−1, intraperitoneal (i.p.)) or vehicle (polyethylene glycol, Tween 80, saline solution; 5:5:90 vol/vol), we anesthetized the rats with halothane and promptly decapitated them with a guillotine. Brains were removed within approx. 1 min after decapitation, frozen in 2-methylbutane and stored at −80°C until analysis. We placed frozen brains on a cutting block and sliced them into 1 mm sections. Prefrontal cortex was taken from approximately 2.20 to 4.20 mm rostral to bregma. Hippocampus was dissected from approximately 2.20 to 6.20 mm caudal to bregma. Thalamus was dissected from approximately 2.20 to 4.20 mm caudal to bregma. Tissues were immediately frozen on dry ice and stored at −80°C until analysis. We extracted endocannabinoids and related lipids with methanol–chloroform, fractionated them by open-bed silica gel chromatography and quantified them by isotope-dilution liquid chromatography/mass spectrometry (LC/MS), as described by Fegley et al (2004).

Elevated Plus Maze

Adult Wistar rats were placed in the central platform of the test apparatus (Pellow et al, 1985) and video recorded for 5 min in a dim light, sound-attenuated environment. The maze comprised two open arms (50 × 10 cm2) and two closed arms (50 × 10 × 40 cm3) that extended from a common central platform (10 × 10 cm2). The apparatus, made of Plexiglas (gray floor, clear walls), was elevated to a height of 60 cm above the floor level. AM404 and vehicle were injected 30 min before testing. Behavioral analyses were performed by blinded observers, using the Observer 3.0 software (Noldus, Wageningen, the Netherlands). Percent time spent in open arms, number of head dips and stretched attend postures were measured as described by Griebel et al (2002).

Defensive Withdrawal

At 45 min after treatment, adult Wistar rats were placed in a small cylindrical stainless-steel chamber (11 cm diameter × 21 cm length) opened at one end, alongside one of the four walls of an open field (90 × 90 cm2) (Takahashi et al, 2001), and video recorded for 15 min in a dim light, sound-attenuated environment. Behavioral analyses were performed by blinded observers, using the Observer 3.0 software (Noldus, Wageningen, the Netherlands). The latency to leave the chamber and the total amount of time spent in the open field were measured.

Isolation-Induced Ultrasonic Vocalizations

Vocalizations were recorded in 10-day-old Wistar rat pups, as described by Cagiano et al (1988). Briefly, a single male pup was randomly removed from each litter, weighed and placed in a shallow glass dish located 15 cm under a microphone, connected to a sound detector. Vocalizations were recorded 30 min after treatment, for 15 s and expressed as percent change from baseline.

Startle and PPI of Startle

The startle reflex was assessed as described by Bortolato et al (2005). At 45 min after treatment with either AM404 or vehicle, rats were placed in a startle reflex apparatus (Med Associates, St Albs, USA) for a 5 min acclimatization period with a 70 dB background noise, which continued for the remainder of the session. Each session consisted of three consecutive sequences of trials. During the first and the third sequence, the rats were presented with five pulse-alone trials of 115 dB. The second sequence consisted of 50 trials in pseudo-random order, including 12 pulse-alone trials, 30 trials of pulse preceded by 73, 76, or 82 dB prepulses (10 for each level of prepulse loudness), and eight no stimulus trials of 115 dB. The second sequence consisted of five pulse-alone trials. Intertrial intervals were selected randomly between 10 and 15 s. Acoustic devices and startle cages were connected to a computer, which detected and analyzed all chamber variables using customized software. Percent PPI was calculated with the following formula: 100 − [(mean startle amplitude for prepulse–pulse trials/mean startle amplitude for pulse-alone trials) × 100].
CPC

CPC was evaluated as described by Gobbi et al (2005). The experiment consisted of three consecutive phases. The CPC apparatus consisted of four rectangular plastic shuttle boxes (30 × 60 × 30 cm), each divided by a guillotine door into two distinct compartments of equal size, containing different visual and tactile cues. Visual cues were present on the walls, which were either brown or black and white striped; tactile cues were present in the floor, being either grid or chequered. All cues were present in the compartments in a counterbalanced order. The experimental room was sound attenuated and dimly lit. In phase I the animals were habituated to CPC boxes for 3 days and their initial side preferences were recorded. Phase II lasted 12 days and consisted of six alternated presentations of AM404 (1.25–10 mg kg\(^{-1}\), i.p.), WIN55,212-2 (1 mg kg\(^{-1}\), i.p.) or morphine (5 mg kg\(^{-1}\), s.c.) and vehicle. Specifically, on odd days rats received one of the drugs or vehicle and were immediately placed in the nonpreferred compartment (separated from the other by a guillotine door) for 60 min, while on even days rats received vehicle and were placed in the preferred compartment for 60 min. On the test day (phase III), the animals were given no treatment and were placed in the cage with free access to both sides for 15 min. Drug-induced differences were assessed from the time spent in the nonpreferred compartment between postconditioning and preconditioning tests. Since environmental enrichment has been shown to positively affect mood and cognitive abilities in rats (Larsson et al, 2002; Renner and Rosenei, 1987), we conducted CPC tests in animals housed under both standard and enriched conditions.

Statistical Analyses

Results are expressed as the mean ± SEM of \(n\) experiments. All analyses were conducted using Statistica (Statsoft, Tulsa, USA). The significance of differences between groups was determined by one- or two-way analysis of variance (ANOVA) followed by Tukey’s or Spjotvoll–Stoline’s tests for multiple comparisons, as appropriate.

RESULTS

Effects of AM404 on Anandamide Levels in the Brain

The inhibitory effects of AM404 on anandamide transport have been previously characterized both in vitro and in vivo (Beltramo et al, 1997, 2000; Piomelli et al, 1999; Fegley et al, 2004). To assess its impact on brain anandamide levels, we administered AM404 to adult Wistar rats and measured endocannabinoid content in the prefrontal cortex, hippocampus and thalamus by LC/MS 45 min after injection. AM404 caused a dose-dependent increase in anandamide levels in prefrontal cortex (\(F_{4,41} = 3.72, p < 0.05\); \(p < 0.05\) for post hoc comparisons between vehicle and AM404 5 mg kg\(^{-1}\)), hippocampus (\(F_{4,37} = 7.73, p < 0.001\); \(p < 0.001\) for post hoc comparisons between vehicle and AM404 10 mg kg\(^{-1}\)) and thalamus (\(F_{4,25} = 3.12, p < 0.05\); \(p < 0.05\) for post hoc comparisons between vehicle and AM404 10 mg kg\(^{-1}\)) (Figure 1a–c). In contrast, the drug had no effect on the levels of 2-arachidonoylglycerol (2-AG), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) in any of the brain regions examined (Figure 1d–f and data not shown). As OEA and PEA are two FAAH substrates, which are markedly elevated after administration of FAAH inhibitors (Fegley et al, 2005), the present results confirm that AM404 does not affect FAAH activity in vivo (Fegley et al, 2004). As expected, the direct-acting cannabinoid agonist WIN55,212-2 (1 mg kg\(^{-1}\), i.p.) had no effect on brain anandamide content (Figure 1a–c).

Effects of AM404 in the Elevated Plus Maze Test

Next, we examined whether the ability of AM404 to increase anandamide levels in the brain might be associated with a modulation of anxiety responses, as previously shown for the FAAH inhibitor URB597 (Kathuria et al, 2003). In a preliminary open field test, Wistar rats received different doses of AM404 (1–10 mg kg\(^{-1}\), i.p., 30 min before trials) to assess the effects of this drug on locomotor and exploratory activities. In agreement with previous results (Beltramo et al, 2000), only the dose of 10 mg kg\(^{-1}\) was found to elicit a significant reduction of locomotor activity (number of crossings: vehicle: 71.5 ± 7.9; AM404 2.5 mg kg\(^{-1}\): 64.1 ± 9.3; AM404 5 mg kg\(^{-1}\): 62.3 ± 11.2; AM404 10 mg kg\(^{-1}\): 46.2 ± 7.8, \(p < 0.05\) in comparison with vehicle; \(n = 11–16\) per group; session duration: 10 min). We then tested the effects of AM404 in the elevated plus maze (Pellow et al, 1985). Rats treated with doses of AM404 that did not affect locomotor activity (0.5–5 mg kg\(^{-1}\), i.p., 30 min before trials) spent a longer time in the open arm of the plus maze than did vehicle-injected rats (Figure 2a) (\(F_{5,53} = 6.21, p < 0.001\);
Effects of AM404 on Defensive Withdrawal

As an additional test of the anxiolytic-like properties of AM404, we evaluated the effects of this agent on defensive withdrawal in Wistar rats (Takahashi et al., 2001). AM404 (1–5 mg kg⁻¹, i.p.) elicited a dose-dependent increase in time spent outside the box (Figure 3a) (F₂,27 = 3.57, p < 0.05) and a dose-dependent decrease in latency to leave the box (Figure 3b) (F₂,27 = 4.18, p < 0.05). Both responses were significantly reduced by rimonabant (1 mg kg⁻¹, i.p.) (Figure 3a and b) (total time: F₃,36 = 3.69, p < 0.05; latency: F₃,36 = 2.93, p < 0.05).

Effects of AM404 on Ultrasonic Vocalization Test

Concurrent results were obtained in the ultrasonic vocalization test in Wistar rat pups (Cagiano et al., 1988). In this test, AM404 (0.5–5 mg kg⁻¹, i.p.) exerted anxiolytic-like effects (Figure 3c) (F₅,60 = 4.85, p < 0.05 for post hoc comparisons between vehicle and AM404 1 mg kg⁻¹; p < 0.01 for post-hoc comparisons between vehicle and AM404 2 mg kg⁻¹) at doses that did not alter axillary temperature or locomotor activity (data not shown). These responses were comparable to those produced by diazepam (0.25 mg kg⁻¹, i.p.) (p < 0.01; data not shown), and were abrogated by rimonabant (1 mg kg⁻¹, i.p.) (Figure 3c) (F₃,24 = 6.54, p < 0.001). The inverse U-shaped effect of

---

**Figure 2** Effects of AM404 (0.5–5 mg kg⁻¹, i.p.) or diazepam (DIA, 2.5 mg kg⁻¹, i.p.) in the rat elevated plus maze test, and reversal of these effects by rimonabant (RIM). (a and d) AM404 and diazepam were injected 30 and 60 min before testing, respectively. Percent time spent in the open arms (%)time open); (b and e) Number of head dips (HDIPS); (c and f) Number of stretched attend postures (SAP). *p < 0.05; **p < 0.01 compared to vehicle (VEH); ***p < 0.001 compared to vehicle (VEH); ###p < 0.001 compared to AM404 5 mg kg⁻¹; n = 8–11 per group.

**Figure 3** Effects of AM404 (1–5 mg kg⁻¹, i.p.) in the (a–b) rat defensive withdrawal and (c) ultrasonic vocalization tests, and reversal of these effects by rimonabant (RIM). (a) Time spent outside the box; (b) Latency to leave the box; (c) Ultrasonic vocalizations in 10-day old rat pups. *p < 0.05, **p < 0.01 compared to vehicle (VEH); ***p < 0.005; ****p < 0.001 compared to AM404 5 mg kg⁻¹ in defensive withdrawal and 1 mg kg⁻¹ in ultrasonic vocalizations; n = 10–12 per group.
AM404 observed in this test was likely due to a greater sensitivity of the pups to the effects of the drug.

Effects of AM404 on Startle and PPI of Startle

To determine whether the anxiolytic-like actions of AM404 might be associated with altered vigilance and reactivity to sensory stimuli, a common effect of both anxiolytic drugs and cannabinoid agonists (Koelaga, 1989; Bahri and Amir, 1994), we measured startle magnitude in Sprague–Dawley rats after administration of AM404 (2.5–10 mg kg\(^{-1}\), i.p.), WIN55,212-2 (5 mg kg\(^{-1}\), i.p.) or diazepam (2.5–5 mg kg\(^{-1}\), i.p.). Diazepam increased startle latency at the 2.5 and 5 mg kg\(^{-1}\) doses (data not shown), and decreased startle amplitude at the dose of 5 mg kg\(^{-1}\) (Figure 4a). WIN55,212-2 produced a significant reduction of startle amplitude, but did not affect startle latency (Figure 4a and data not shown) (\(F_{5,62} = 4.2\), \(p < 0.01\), \(p < 0.05\) for post hoc comparisons between diazepam and vehicle and WIN55,212-2 and vehicle) (Bortolato et al, 2005; Abduljawad et al, 1997). By contrast, AM404 did not affect any startle-related parameter at the doses tested (Figure 4a). We then examined the impact of AM404 (2.5–10 mg kg\(^{-1}\), i.p.) on PPI of the startle reflex, the disruption of which is widely utilized as a model of perceptual distortion (Swerdlow et al, 2000). We compared the effects of AM404 with those of WIN55,212-2 (1 mg kg\(^{-1}\), i.p.), apomorphine (0.25 mg kg\(^{-1}\), s.c.) and dizocilpine (0.1 mg kg\(^{-1}\), s.c.). AM404 was ineffective in this test at all doses examined (Figure 4b). As previously shown, WIN55,212-2 was also unable to disrupt PPI (Bortolato et al, 2005), while dizocilpine significantly decreased it (Figure 4b) (\(F_{2,38} = 5.26; p < 0.01\)); a similar decrease was observed with apomorphine (\(F_{2,38} = 3.77; p < 0.05\)) (data not shown).

Effects of AM404 on CPP

Finally, we examined the motivational effects of AM404 in the CPP test, and compared them to those produced by WIN55,212-2 (1 mg kg\(^{-1}\), i.p.) and morphine (5 mg kg\(^{-1}\), s.c.). As shown in Figure 5a, Wistar rats housed under standard nonenriched conditions exhibited a significant shift in preference towards the morphine-associated compartments (\(F_{6,48} = 6.89, p < 0.001\) for main treatment effect; \(p < 0.05\) for post hoc comparison between vehicle and morphine), but not to the compartments associated with either AM404 (1.25–10 mg kg\(^{-1}\), i.p.) or WIN55,212-2. In contrast, rats housed under enriched conditions displayed a significant shift in preference toward the environment associated with AM404, WIN55,212-2, or morphine (Figure 5b). In particular, enriched AM404-treated rats (1.25–10 mg kg\(^{-1}\), i.p.) exhibited a dose-dependent shift in preference, which was significantly higher than controls for the 2.5 mg kg\(^{-1}\) dose (Figure 5b).
DISCUSSION

The present study shows that the endocannabinoid transport inhibitor AM404 selectively increases levels of anandamide, but not 2-AG, in the rat prefrontal cortex, hippocampus and thalamus, three brain regions that are intimately involved in the regulation of stress and emotion (Nestler et al., 2002; Cahill and McGaugh, 1998). This biochemical response is accompanied by marked anxiolytic-like effects, which are prevented by the CB1 receptor antagonist rimonabant. In contrast, AM404 exerts only modest motivational effects in the CPP test and does not influence startle reflex or PPI of startle.

The anxiolytic-like effects elicited by AM404 and the sensitivity of these effects to rimonabant suggest that endogenously produced anandamide is involved in the regulation of anxiety, plausibly via activation of brain CB1 receptors. Such a mechanism might also account for the antidepressant-like effects of AM404, which were recently documented using the rat forced-swim test (Hill and Gorzalka, 2005). Our results complement other lines of evidence suggesting a role for anandamide in the modulation of emotional responses to stress. Stressful stimuli affect anandamide mobilization in brain regions that are involved in the control of emotion. In rats, for example, a single electric shock to the paw elevates anandamide levels in the midbrain (Hohmann et al., 2005), while in mice physical restraint decreases anandamide levels in the amygdala (Patel et al., 2004). Moreover, pharmacological blockade or genetic ablation of CB1 receptors exacerbates normal reactions to acute stress, presumably by disabling an endocannabinoid modulation of these reactions (Navarro et al., 1997; Haller et al., 2004; Uriguen et al., 2004). Finally, the FAAH inhibitor URB597 enhances stress-coping behaviors in a rimonabant-sensitive manner (Kathuria et al., 2003; Gobbi et al., 2005), suggesting that anandamide interacts with a subgroup of CB1 receptors in the brain that regulate stress responses.

Brain CB1 receptors are predominantly localized on axon terminals of γ-aminobutyric acid (GABA)ergic and glutamatergic neurons, and their activation inhibits the release of GABA and glutamate in the hippocampus, amygdala and other regions of the brain (for review, see Freund et al., 2003). Thus, the modulation of either inhibitory or excitatory transmitter systems may be involved in the regulation of emotional behavior by anandamide. In addition, anandamide might also influence the release of anxiogenic neuromodulators, such as corticotropin-releasing factor and cholecystokinin-octapeptide (Weidenfeld et al., 1994; Beinfeld and Connolly, 2001).

In contrast with previous experiments in mice (Fernandez-Espejo and Galan-Rodriguez, 2004), we found that AM404 does not affect startle reflex and PPI in Sprague-Dawley rats. Differences in animal species and experimental protocol are likely to account for this discrepancy. For example, those studies were performed in a local colony of Swiss mice, which did not show either loudness dependence in baseline PPI or dose dependence in PPI disruption induced by acute administration of AM404. Irrespective of possible explanations, our findings clearly indicate that blockade of anandamide transport has little impact on sensory reactivity in Sprague-Dawley rats. Moreover, since PPI disruption is considered to be a predictor of perceptual impairment and hallucinatory potential (Swerdlow et al., 2000), the results also suggest that AM404 may be devoid of acute psychotomimetic effects.

AM404 produced an inverse U-shaped response in the CPP paradigm, but only when administered to rats housed under enriched conditions. At the dose of 2.5 mg kg⁻¹, the effect of AM404 was similar to that elicited by the direct-acting cannabinoid agonist WIN55,212-2, albeit lower than that produced by morphine. Conversely, no response to either AM404 or WIN55,212-2 was observed in rats kept under nonenriched conditions. These results are consistent with those of other investigations, showing that environmental factors can greatly affect responses in the CPP test (Bowling and Bardo, 1994; Smith et al., 2003). As such, they might help interpret some of the discrepancies reported on the effects of cannabinoid agonists in this model (Gardner, 2005). In addition, our findings are consistent with recent findings indicating that both anandamide (Justinoa et al., 2005) and AM404 are intravenously self-administered by squirrel monkeys (Justinova and Goldberg, 2005).

Previous reports have shown that AM404 does not closely mimic the spectrum of pharmacological responses produced by direct cannabinoid agonists, since it does not elicit catalepsy, acute antinociception or hypothermia (Beltramo et al., 1997, 2000; Fegley et al., 2004). These differences have been attributed to the ability of AM404 to inhibit endocannabinoid transport without directly activating CB1 receptors (Beltramo et al., 1997, 2000). Recently, the existence of an endocannabinoid transport system has been questioned in favor of a simple diffusion mechanism, whereby anandamide accumulation may be solely driven by an inward concentration gradient maintained by FAAH-mediated hydrolysis (Glaser et al., 2003). In this context, the actions of AM404 have also been ascribed to its ability to serve as a FAAH substrate and to compete with anandamide for FAAH activity. However, the discovery that genetic deletion of FAAH does not affect anandamide internalization in neurons argues against this possibility and in favor of the transporter hypothesis (Fegley et al., 2004; Alger, 2004; Ortega-Gutierrez et al., 2004). Additional support to this hypothesis comes from the recent discovery of potent nonaliphatic inhibitors of endocannabinoid transport, which has led to the identification of a high-affinity binding site presumably involved in the transport process (Moore et al., 2005). Noteworthy, the effects produced by AM404 are markedly different from those exerted by the FAAH inhibitor URB597 in at least two respects. First, AM404 does not affect brain levels of OEA and PEA, which are enhanced by URB597 (Kathuria et al., 2003; Fegley et al., 2005). Second, although AM404 mirrors the anxiolytic-like effects of URB597 (Kathuria et al., 2003), the
latter does not exert any significant motivational effect in the CPP model, irrespective of housing conditions (Gobbi et al., 2005).

In addition to its inhibitory actions on anandamide transport, AM404 interacts in vitro with several unrelated pharmacological targets, such as vanilloid TRPV1 receptors (Zygmunt et al., 2000) and sodium channels (Nicholson et al., 2003). While the in vivo relevance of these effects is still unclear, we cannot rule out that they might contribute to the pharmacological properties of AM404. Nevertheless, the ability of selective doses of the CB1 receptor antagonist rimonabant to prevent the anxiolytic-like actions of AM404 suggests that such actions can be ascribed to anandamide-mediated activation of CB1 receptors. To date, AM404 remains the best characterized among a small series of endocannabinoid transport inhibitors that have been developed. Indeed, the compound AM1172 (Fegley et al., 2004) also acts as a direct partial agonist of CB1 receptors, while other agents, such as VDM11, have been shown to interact with multiple targets (Kelley and Thayer, 2004). UCM707 induces hypomotility and antinociception (de Lago et al., 2002), but its impact on behavior is still incompletely documented. Thus, the compounds mentioned above offer little or no advantage over AM404 for in vivo studies. The recent availability of new anandamide transport inhibitors, such as LY2183240 (Moore et al., 2005), will allow a more detailed characterization of this target in the future. In conclusion, while our results point to the anandamide transport system as a target for anxiolytic drugs, they also highlight the need to fully characterize this system at the molecular level, and to develop more advanced probes to validate its therapeutic potential.

ACKNOWLEDGEMENTS
The present study was supported by grants from National Institute on Drug Abuse (DA-12447 and DA-3412) and the National Alliance for Research on Schizophrenia and Depression (to DP), and Ministero dell’Istruzione, Università e Ricerca (to VC and GLG). MB was a NIDA INVEST Depression (to DP), and Ministero dell’Istruzione, Università e Ricerca (to VC and GLG). MB was a NIDA INVEST Depression (to DP), and Ministero dell’Istruzione, Università e Ricerca (to VC and GLG). We thank Oliver Arguello and Marco Orrù for experimental assistance.

REFERENCES
Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D (1999). Dopamine activation of...


