

Pharmacological Evaluation of Cannabinoid Receptor Ligands in a Mouse Model of Anxiety: Further Evidence for an Anxiolytic Role for Endogenous Cannabinoid Signaling

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ABSTRACT

Extracts of *Cannabis sativa* have been used for their calming and sedative effects for centuries. Recent developments in drug discovery have suggested that modulation of neuronal endogenous cannabinoid signaling systems could represent a novel approach to the treatment of anxiety-related disorders while minimizing the adverse effects of direct acting cannabinoid receptor agonists. In this study, we evaluated the effects of direct cannabinoid receptor agonists and antagonists and endocannabinoid-modulating drugs on anxiety-like behavior in mice using the elevated-plus maze. We found that the direct CB₁ receptor agonists (1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol (CP 55,940) (0.001–0.3 mg/kg) and 2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo [1,2,3-*de*]-1,4-benzoxazinyl-(1-naphthalenyl)methanone mesylate (WIN 55212-2) (0.3–10 mg/kg) increased time spent on the open arms (T_o) at low doses only. At the highest doses tested, both compounds altered overall locomotor activity. In contrast, Δ^9 -tetrahydrocannabinol (0.25–10 mg/kg) produced a dose-dependent reduction in T_o .

The endocannabinoid uptake/catabolism inhibitor 4-hydroxyphenylarachidonylamide (AM404) (0.3–10 mg/kg) produced an increase in T_o at low doses and had no effect at the highest dose tested. The fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597) (0.03–0.3 mg/kg) produced a monophasic, dose-dependent increase in T_o . The CB₁ receptor antagonists *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR141716) (1–10 mg/kg) and *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251) (1–10 mg/kg) produced dose-related decreases in T_o . These data indicate that activation of CB₁ cannabinoid receptors reduces anxiety-like behaviors in mice and further support an anxiolytic role for endogenous cannabinoid signaling. These results suggest that pharmacological modulation of this system could represent a new approach to the treatment of anxiety-related psychiatric disorders.

Marijuana is widely used throughout the world for recreational and therapeutic purposes (Adams and Martin, 1996). A common reason given for continued marijuana use in certain populations is reduction in anxiety and relaxation; however, adverse reactions, including heightened anxiety and panic, are common and widely cited reasons for discontinuation of marijuana use (Szuster et al., 1988; Thomas, 1996; Reilly et al., 1998). The adverse effects of marijuana are more

pronounced during novel or stressful environmental conditions, after consumption of large doses of cannabis, and in naive users (Abel, 1971; Gregg et al., 1976; Naliboff et al., 1976).

Animal data parallel these clinical observations. Direct agonists of the cannabinoid receptor type-1 (CB₁), the molecular target of the principal psychoactive component of marijuana, Δ^9 -tetrahydrocannabinol (THC), produce biphasic effects on anxiety. Relatively low doses produce anxiolytic-like effects in animals, whereas higher doses produce an anxiogenic profile (for review see Viveros et al., 2005). In addition, prior exposure of animals to stress sensitizes them to the anxiogenic effects of cannabinoid agonists, and stress inter-

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ABBREVIATIONS: CB₁, cannabinoid receptor type 1; 2-AG, 2-arachidonylglycerol; AEA, *N*-arachidonylethanolamine; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; WIN 55212-2, 2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo [1,2,3-*de*]-1,4-benzoxazinyl-(1-naphthalenyl)methanone mesylate; SR141716, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; URB597, cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester; CP 55,940, (1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; AM404, 4-hydroxyphenylarachidonylamide; THC, Δ^9 -tetrahydrocannabinol; T_o , time spent on the open arms of the elevated plus maze.

acts synergistically with cannabinoid agonists to activate stress-related brain regions, including the central amygdala (Hill and Gorzalka, 2004; Patel et al., 2005b). Although these data suggest that appropriate modulation of cannabinoid systems could have therapeutic potential in the treatment of anxiety-related neuropsychiatric disorders, the biphasic and context-dependent effects of direct CB₁ receptor agonists limit their therapeutic potential.

Recent data suggest that pharmacological agents that enhance endogenous cannabinoid (eCB) signaling provide a novel approach to the treatment of anxiety-related disorders. The identification and characterization of the molecular substrates of eCB signaling have led to the development of pharmacological agents that augment eCB signaling. eCBs, which include *N*-arachidonyl ethanolamine (AEA) and 2-arachidonylglycerol (2-AG), are synthesized by neurons via independent enzymatic cascades. After release, AEA and 2-AG and are thought to be recaptured by neurons and degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase, respectively (for review see Piomelli, 2003). Activation of presynaptic CB₁ receptors leads to a reduction in neurotransmitter release, and eCB signaling probably plays a role in retrograde inhibition of neurotransmitter release in many brain regions. The localization of eCBs, CB₁ receptors, FAAH, and monoacylglycerol lipase within brain regions subserving emotion and motivation [i.e., the prefrontal cortex, nucleus accumbens, amygdala, and hypothalamus (Tsou et al., 1998a,b; Marsicano and Lutz, 1999; Katona et al., 2001; McDonald and Mascagni, 2001; Gulyas et al., 2004)] is also consistent with a role for this system in the regulation of emotional behavior.

Specifically, brain AEA content is significantly increased by the FAAH inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597) (Kathuria et al., 2003). URB597 reduces anxiety-like behaviors in the elevated zero maze and isolation-induced ultrasonic vocalizations in pups (Kathuria et al., 2003) and reduces restraint stress-induced corticosterone release (Patel et al., 2004), all with linear dose-response relationships. In contrast to direct CB₁ receptor agonists, URB597 does not induce place preference in rodents, suggesting that it could be devoid of the abuse potential inherent in direct CB₁ agonists (Gobbi et al., 2005). Another target for the development of indirect agonists of the CB₁ receptor is the eCB transport/uptake process, because inhibition of neuronal accumulation of the eCBs increases availability of eCBs AEA and 2-AG at CB₁ receptors (Hajos et al., 2004). AM404 (4-hydroxyphenylarachidonylamide) is an inhibitor of eCB uptake and an inhibitor of FAAH (Jarrahian et al., 2000). AM404 reduces restraint-induced corticosterone release in a biphasic manner, with only low doses producing a reduction in corticosterone release (Patel et al., 2004). Thus, the dose-response properties of AM404 are more similar to those of the direct CB₁ agonist (1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol (CP 55,940) than the FAAH inhibitor URB597. In summary, URB597 seems to be devoid the biphasic properties of direct CB₁ agonists and AM404 (Kathuria et al., 2003; Patel et al., 2004; Gobbi et al., 2005) and does not interact with environmental stress to potentiate activation of the central amygdala (Patel et al., 2005b). For these reasons, selective inhibitors of FAAH could be viable alternatives to

direct CB₁ agonists for the development of cannabinoid-based antianxiety treatments.

Although numerous studies exploring the effects of exogenous cannabinoids and eCB signaling in the modulation of anxiety have been published, comparative data using a variety of ligands and dose ranges within the same laboratory have not been compiled. Thus, the purpose of these studies was to evaluate the effects of direct and indirect CB₁ receptor agonists and CB₁ antagonists on anxiety behaviors using the elevated-plus maze paradigm in mice. We used wide dose ranges and multiple drugs from each class to clarify the role of eCB signaling in the regulation of anxiety-related behaviors.

Materials and Methods

Animals and Drugs. All experiments were carried out in accordance with the National Institutes of Health *Guide for the Use and Care of Laboratory Animals*. ICR male mice (21–24 g) were used in all experiments (Harlan, Madison WI). All animals were housed on a 12:12 light/dark cycle with lights on at 6:00 AM. Animals had ad libitum access to food and water. All experiments were conducted between 9:00 AM and 12:00 PM. Each animal was used only once; approximately 300 mice were used in this study.

AM404 and AM251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] were purchased from Tocris Cookson (Ellisville, MO). URB597 (cyclohexylcarbamic acid 3'-carbamoyl-biphenyl-3-yl ester) was purchased from Cayman Chemical (Ann Arbor, MI). SR141716 [rimonabant; *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl] was a gift from the NIDA Drug-Supply Program (Research Triangle Park, NC). CP 55,940 [5-(1,1-dimethylheptyl)-2-[5-hydroxypropyl]cyclohexyl]phenol] was a gift from Pfizer Central Research (Groton, CT). (*R*)-(+)-WIN 55212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo [1,2,3-*de*]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate)] was purchased from Sigma Chemical (St. Louis, MO).

All drugs, with the exception of URB597, were dissolved in ethanol-Emulphor vehicle (18:1:1, saline/Emulphor/ethanol); URB597 was dissolved in dimethyl sulfoxide-Emulphor vehicle (18:1:1 saline/Emulphor/dimethyl sulfoxide). All drugs were delivered by i.p. injection 30 min before experimentation in a volume of 10 ml/kg. Each experimental group (i.e., the mice included in a particular session) included vehicle-treated mice.

Elevated-Plus Maze. For each treatment group, eight to 10 animals were used. Animals were habituated to the testing room for at least 2 h before experimentation. The elevated-plus maze (San Diego Instruments, San Diego, CA) was constructed from beige plastic and consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) that extended from a central platform (5 × 5 cm). The maze was elevated 40 cm above the floor. Experiments began by placing a single mouse on the central platform facing an open arm. During the first 5 min of free exploration, the number of entries into (defined as an animal placing all four paws onto an arm) and time spent in open and closed arms were recorded by a trained observer blind to treatment condition. The maze was cleaned thoroughly between animals using a 50% ethanol solution.

Statistical Analysis. Percentage time spent in open arm exploration [(time spent in open arms/(time spent in open arms + time spent in closed arms)] × 100, absolute time spent in open arm exploration, and percentage open arm entries [(number of open arm entries/(number of open arm entries + number of closed arm entries)] × 100) were considered measures of anxiety; total arm entries provided a measure of overall locomotor activity. Between group comparisons were conducted by one-way ANOVA followed by Dunnett's test; *p* < 0.05 was considered significant throughout.

Results

Effects of CB₁ Receptor Agonists on Anxiety-Like Behaviors. The high-efficacy CB₁ receptor agonist CP 55,940 significantly increased the percentage time spent in open arm exploration at 0.01, 0.03, and 0.3 mg/kg compared with vehicle treatment, whereas 0.1 mg/kg was not significantly different from control (Fig. 1). CP 55,940 significantly increased time spent in open arm exploration, percentage open arm entries, and number of total arm entries at 0.3 mg/kg only. At the 0.3 mg/kg dose of CP 55,940, mice exhibited a stereotypic behavior characterized by repetitive and rapid entries into open arms. Given the large increase in overall activity at this dose, the validity of this test as a measure of anxiety is likely compromised.

A second high-efficacy CB₁ receptor agonist, WIN 55212-2, significantly increased the percentage time spent in open arm exploration at 1 and 3 mg/kg compared with vehicle treatment, whereas 10 mg/kg was not significantly different from control (Fig. 2). WIN 55212-2 also significantly increased time spent in open arm exploration and percentage open arm entries at 1 and 3 mg/kg compared with vehicle treatment, whereas 10 mg/kg was not significantly different from control on either measure. WIN 55212-2 significantly increased the total number of arm entries at 3 mg/kg and significantly decreased the total number of arm entries at 10 mg/kg, thus compromising the validity of this test as a measure of anxiety at these doses.

THC, at doses of 1, 2.5, and 10 mg/kg, significantly decreased percentage time spent in open arm exploration (Fig. 3). THC significantly reduced time spent in open arm exploration and percentage open arm entries at 2.5 and 10 mg/kg. THC did not affect the total number of arm entries at any of the doses tested.

Effects of CB₁ Receptor Antagonists on Anxiety-Like Behaviors. To determine whether tonic eCB signaling affects anxiety-like behaviors, we assessed the effects of the CB₁ receptor antagonists AM251 and SR141716 on elevated-plus maze performance in male mice. AM251 significantly decreased the percentage time spent in open arm exploration at 3 and 10 mg/kg compared with vehicle treatment (Fig. 4). AM251 also decreased the time spent in open arm exploration at 3 and 10 mg/kg and decreased the percentage open arm entries at 10 mg/kg. AM251 did not significantly affect the total number of arm entries at any of the doses examined.

SR141716 significantly reduced the percentage time spent in open arm exploration at doses of 3 and 10 mg/kg (Fig. 5). At 10 mg/kg, SR141716 produced a significant reduction in the time spent in open arm exploration. SR141716 did not significantly effect the percentage open arm entries or the total number of arm entries at any of the doses tested.

Effects of eCB Modulators on Anxiety-Like Behaviors. To determine whether pharmacological augmentation of eCB signaling has effects similar to direct CB₁ receptor activation, we assessed the effects of eCB modulators URB597 and AM404 on anxiety-like behaviors using the elevated-plus maze. URB597 significantly increased the percentage time spent in open arm exploration at 0.1 and 0.3 mg/kg (Fig. 6). URB597 also significantly increased the time spent in open arm exploration and the percentage of open arm entries at 0.1 mg/kg. URB597 did not affect the total number of arm entries at any of the doses tested.

AM404 significantly increased the percentage time spent in open arm exploration at 1 and 3 mg/kg, whereas a dose of 10 mg/kg was not different from control (Fig. 7). AM404 also significantly increased the time spent in open arm exploration at 3 mg/kg with a dose of 10 mg/kg producing no effect

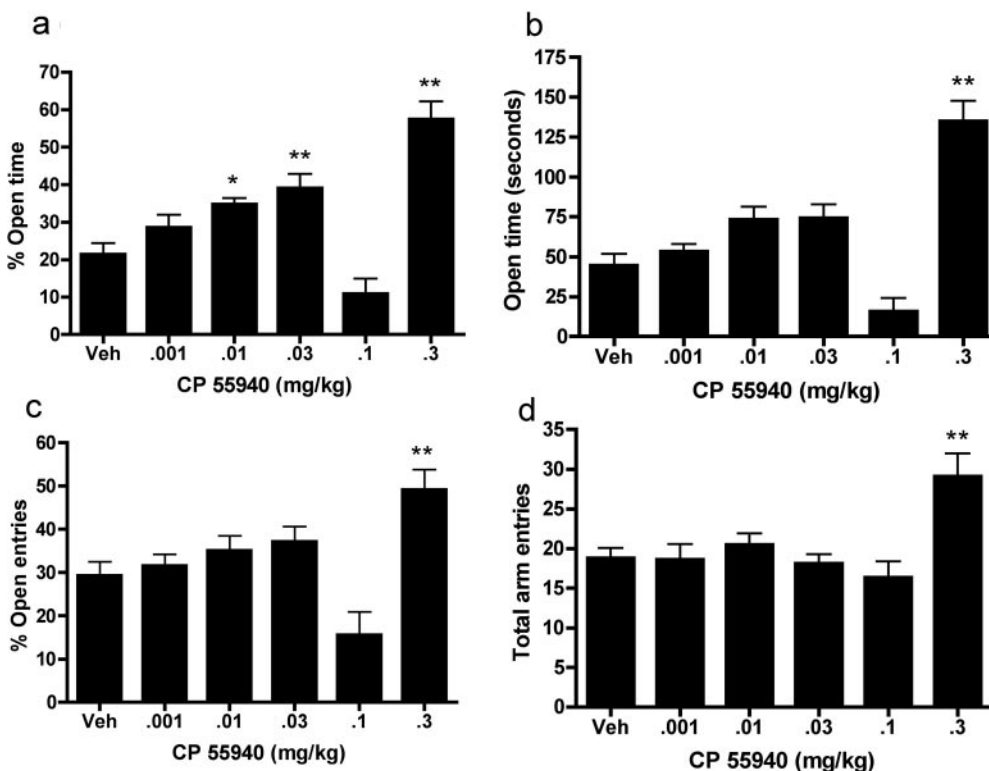


Fig. 1. Effects of CP 55,940 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$; **, $p < 0.01$, significantly different from vehicle control.

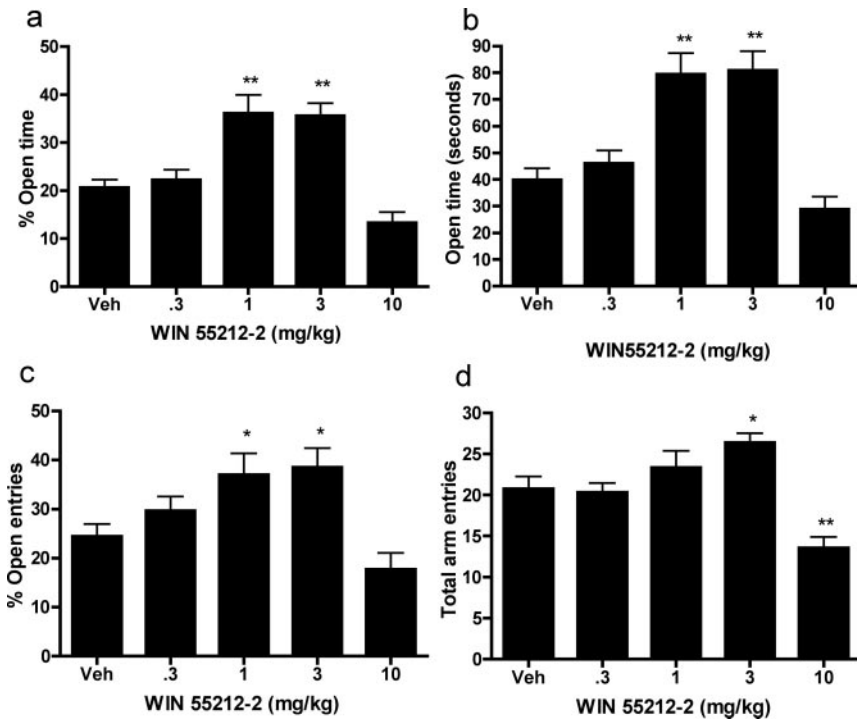


Fig. 2. Effects of WIN 55212-2 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$; **, $p < 0.01$, significantly different from vehicle control.

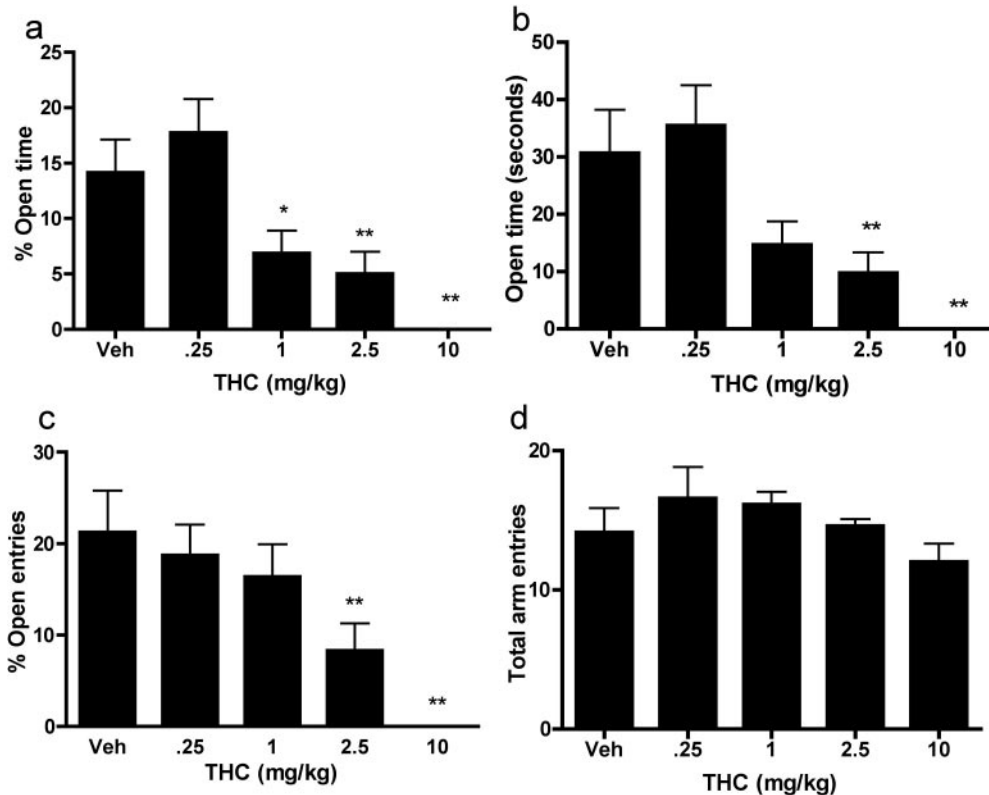


Fig. 3. Effects of THC on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$; **, $p < 0.01$, significantly different from vehicle control.

compared with control. AM404 did not significantly increase the percentage of open arm entries or affect the total number of arm entries at any of the doses tested.

Discussion

The laboratory study of the effects of cannabinoids on anxiety-like behaviors in rodents has been hampered by

some unique properties of the cannabinoids compared with classic anxiolytics, such as benzodiazepines. Specifically, the effects of cannabinoids on anxiety-like behaviors are dependent on the pharmacological properties and selectivity of the available ligands, dose, species and strain, basal and previous stress exposure, and possibly other variables (see Viveros et al., 2005 for discussion of these issues). Although many previous studies have explored the role of various cannabi-

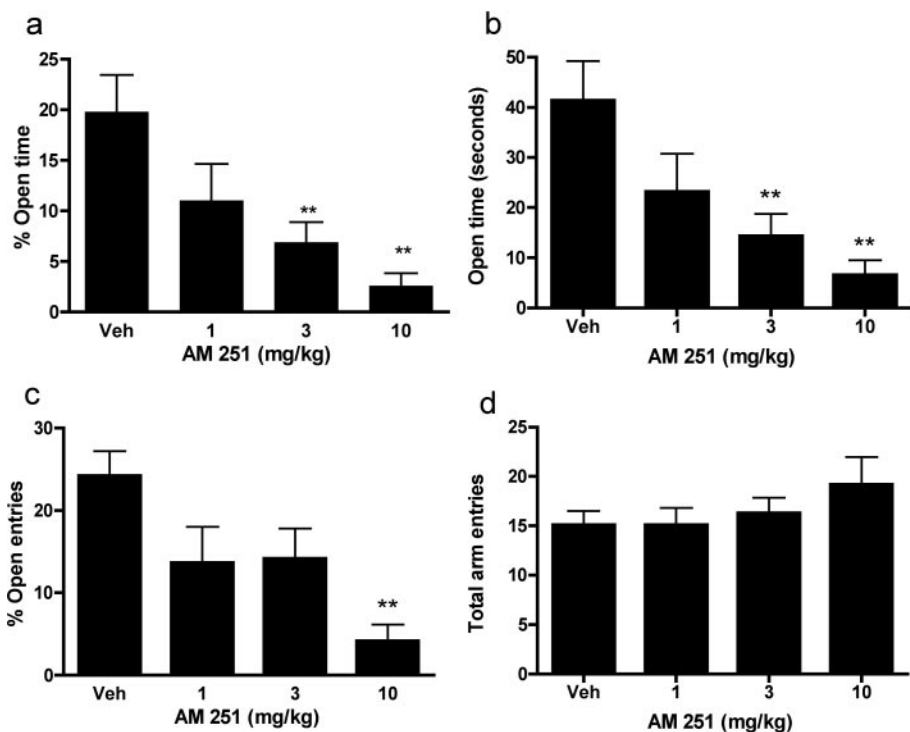


Fig. 4. Effects of AM251 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. **, $p < 0.01$, significantly different from vehicle control.

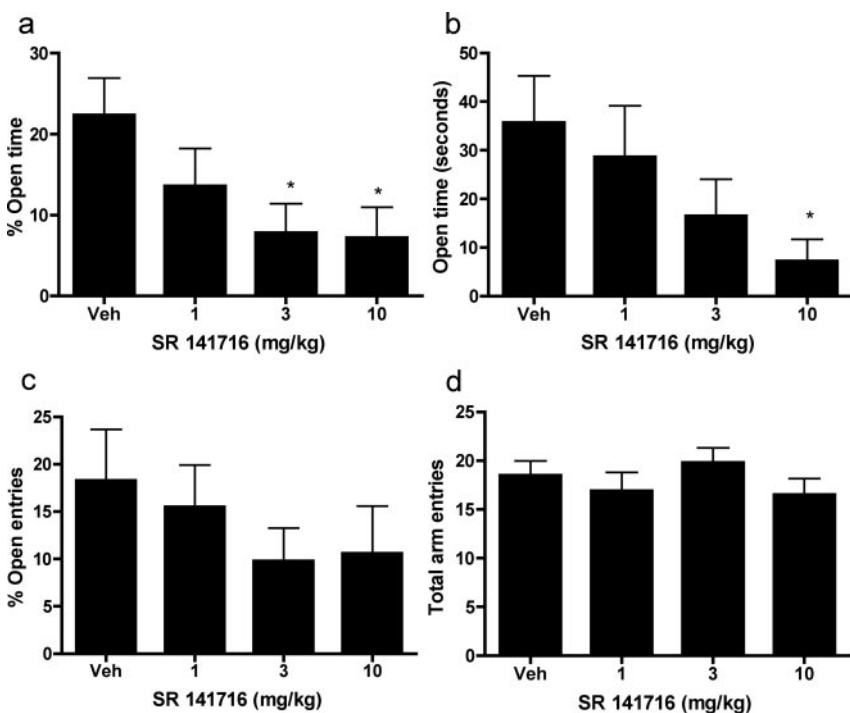


Fig. 5. Effects of SR141716 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$, significantly different from vehicle control.

noid ligands on anxiety-like behavior using the elevated-plus maze, the lack of multiple ligand and dose comparisons combined with the lack of comparability between studies (based on the reasons cited above) have hampered the development of a clear picture of the effects of cannabinoids on anxiety. The purpose of this study was to compare broad dose ranges of several structurally diverse direct CB₁ cannabinoid receptor agonists, two CB₁ antagonists, and eCB-modulating compounds to gain insight into the role of CB₁ receptor signaling in the physiological modulation of anxiety.

We evaluated three direct acting CB₁ receptor agonists, CP

55,940, WIN 55212-2, and THC, on the behavior of mice on the elevated-plus maze. The dose-response relationship for CP 55,940 is complex. Between 1 and 30 $\mu\text{g}/\text{kg}$ CP 55,940 produced a dose-related increase in behavior that is consistent with a reduction in the fearfulness or anxiety of the mice. However, at a dose of 100 $\mu\text{g}/\text{kg}$, this trend was reversed completely and no significant differences from vehicle were seen. When the dose was increased further, stereotypic behaviors were observed and a significant increase in the total number of arm entries was seen. This change in behavior, which probably reflects a large increase in overall loco-

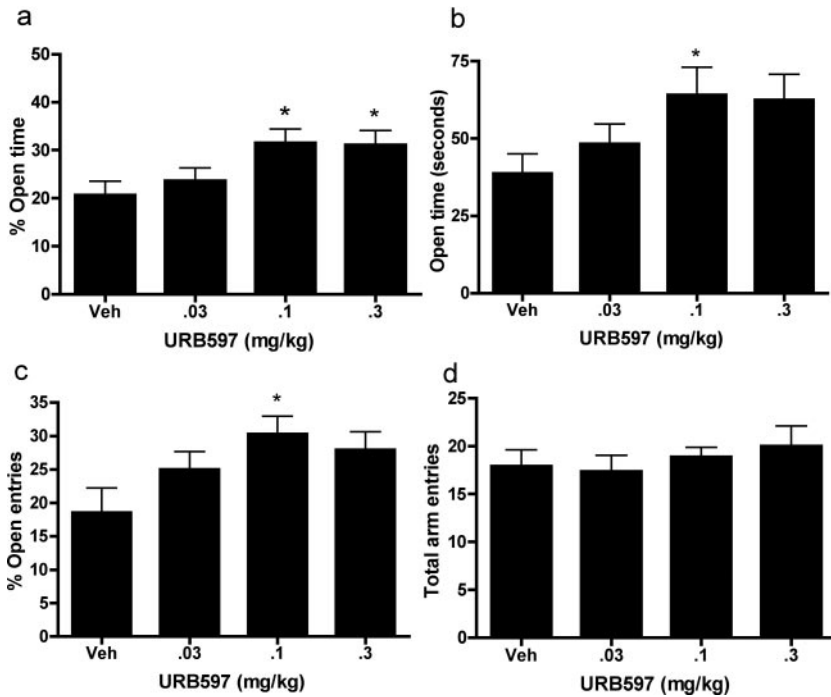


Fig. 6. Effects of URB 597 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$; **, $p < 0.01$, significantly different from vehicle control.

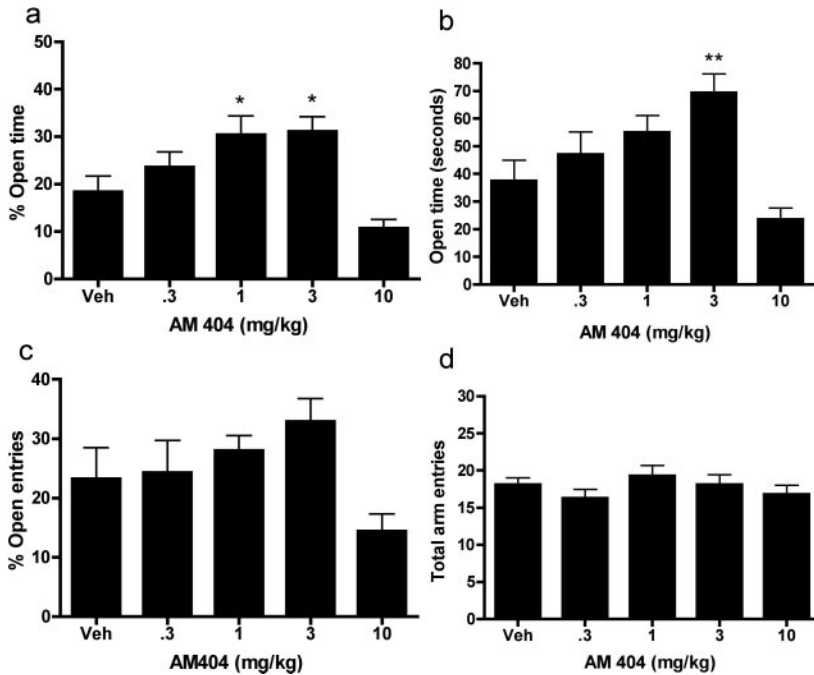


Fig. 7. Effects of AM404 on % open time (a), absolute open time (b), % open arm entries (c), and number of total arm entries (d) during a 5-min exposure to the elevated-plus maze. *, $p < 0.05$; **, $p < 0.01$, significantly different from vehicle control.

motor activity, compromises the validity of the behavioral assay as a measure of anxiety at this dose. Therefore, these data indicate that, at doses of less than 30 $\mu\text{g}/\text{kg}$, CP 55,940 produces anxiolytic-like effects in mice; however, doses of 100 $\mu\text{g}/\text{kg}$ or higher are either ineffective or produce data that cannot be interpreted because of locomotor effects. A somewhat similar pattern of effects was observed with WIN 55212-2. A relatively low dose (1 mg/kg) produced increased open arm entries and time on the open arms compared with vehicle, whereas the effects of doses of 3 mg/kg and higher were confounded by changes in total arm entries. These data are generally consistent with previous findings in mice and rats that low doses of high-efficacy CB_1 agonists produce

anxiolytic effects, whereas higher doses have no effect or are possibly anxiogenic (Haller et al., 2004; Marco et al., 2004).

In contrast, the low-efficacy CB_1 agonist THC displayed a linear, dose-dependent anxiogenic effect similar to the findings of Onaivi et al. (1990). Although the reasons for the differences between CP 55,940/WIN 55212-2 and THC are not clear, one probable contribution is the difference in efficacy among these agonists. Both CP 55,940 and WIN 55212-2 exhibit high efficacy for CB_1 receptor activation, whereas THC is quite low in comparison [approximately 10% WIN 55212-2 (Kearn et al., 1999)]. In fact, when GDP/GTP exchange is used as an assay for efficacy, THC has lower efficacy than the endocannabinoids AEA and 2-AG (Kearn et al.,

1999; Hillard, 2000). As a result, THC will act as an antagonist at CB₁ receptors that have high occupancy by endogenous ligands. This possibility is supported by our data that the CB₁ receptor antagonists SR141716 and AM251 also produce anxiogenic effects. Other studies have also concluded that THC can act as a physiological antagonist under certain conditions (Kelley and Thayer, 2004; Straiker and Mackie, 2005). In light of recent data that CB₁ receptor agonists can differentially direct signaling to specific subtypes of G-proteins within specific cells, it is also possible that the three agonists studied induce different signaling pathways, even though they activate the same receptor (Mukhopadhyay and Howlett, 2005).

Our finding that THC does not produce anxiolytic-like effects in this model is interesting in light of the anxiolytic and calming subjective effects of cannabis intoxication in humans. One explanation is that *Cannabis sativa* contains over 100 distinct compounds (Brenneisen and el Sohly, 1988), some of which could be anxiolytic by CB₁- or non-CB₁ receptor-dependent mechanisms. In support of this possibility, we and others have shown that cannabidiol, a compound found in *C. sativa* preparations that does not interact with CB₁ receptors, exerts robust anxiolytic effects in mice via an unknown mechanism (unpublished data; Guimaraes et al., 1994). It is possible that other compounds found in plant preparations could act together with THC in producing subjective experiences that occur following cannabis use. However, this notion is not supported by data indicating only minor differences between the subjective effects of pure THC and marijuana in humans (Hart et al., 2002; Wachtel et al., 2002).

We also evaluated the effects of two CB₁ receptor antagonists, AM251 and SR141716, on the behavior of mice in the elevated-plus maze. Both compounds reduce the time spent on the open arms and therefore exhibit anxiogenic-like effects in mice. These findings are consistent with some previous reports (Navarro et al., 1997; Arevalo et al., 2001; Haller et al., 2004). However, in another study, SR141716 was found to produce an anxiolytic effect that was also observed in CB₁^{-/-} mice, indicating that this effect was not mediated by CB₁ receptors (Haller et al., 2002). However, AM251 had no effect on the activity of CB₁^{-/-} mice in the elevated-plus maze, suggesting that the alternative mechanism is selective for SR141716 (Haller et al., 2004). In addition, acute SR141716 administration in rats also produces anxiolytic effects (Griebel et al., 2005). These authors suggest that the basal level of anxiety determines the net effect of SR141716 on anxiety-like behaviors. This is supported by data demonstrating that an anxiolytic effect of SR141716 is revealed in mice previously exposed to the maze apparatus (Rodgers et al., 2003). Because previous exposure to the maze decreased the time spent in open arm exploration (i.e., produced an anxiogenic profile), the anxiolytic effects of SR141716 are more pronounced when animals have increased basal levels of anxiety. However, in contrast to these previous studies, we failed to reveal any anxiolytic effects of SR141716, although it does seem that AM251 is more potent than SR141716 in producing anxiogenic effects in male ICR mice. Taken together, our present results, as well as those of others (Kathuria et al., 2003; Haller et al., 2004; Patel et al., 2004), indicate that global activation of CB₁ receptor signaling shifts the internal state of mice toward a reduction in fearfulness and

increased exploration. However, it is also clear that this effect is dose-sensitive, suggesting that CB₁ receptor activation is not purely "antianxiety."

To further evaluate the role of eCB signaling in the modulation of anxiety, we determined the effects of eCB-modulating compounds URB597 and AM404 on elevated-plus maze performance in mice. URB597, a compound that increases brain AEA content (as well as other *N*-acylethanolamines, including *N*-palmitoylethanolamine and *N*-oleoylethanolamine) via inhibition of FAAH activity (Kathuria et al., 2003; Patel et al., 2005a), produced a linear, dose-dependent anxiolytic effect. This finding replicates data by Piomelli and coworkers (Kathuria et al., 2003), demonstrating a linear, dose-dependent anxiolytic effect of this compound in the elevated-zero maze in rats. Although it is possible that higher doses of URB597 could convert the dose-response curve from monophasic to biphasic, the highest dose used (0.3 mg/kg) has been shown previously to produce maximal FAAH inhibition and increased AEA content beginning 15 min after administration (Kathuria et al., 2003). Furthermore, higher doses could reduce the selectivity of this compound for FAAH relative to other serine hydrolase enzymes. Interestingly, AM404, like direct-acting CB₁ agonists, produces a biphasic effect on anxiety, with low doses producing anxiolytic effects and the highest dose having no effect. AM404 is thought to exert its actions by increasing the availability of both AEA and 2-AG for CB₁ receptors by inhibiting their reuptake and/or degradation (Freund et al., 2003). These findings suggest that selective augmentation of AEA signaling may be preferred over the nonselective enhancement of AEA and 2-AG signaling as a novel approach to developing antianxiety pharmacotherapeutics. Similar profiles for these compounds were demonstrated with regard to inhibition of stress-induced corticosterone release. URB597 produced linear, dose-dependent inhibition of stress-induced corticosterone release, whereas AM404 produced a biphasic effect (Patel et al., 2004). We have previously shown that acute restraint stress decreases AEA content within the amygdala and forebrain; thus, normalization of brain AEA signaling by FAAH inhibitors or eCB transport inhibitors during stress could underlie the anxiolytic properties of these compounds (Patel et al., 2005c).

Lastly, although many of the effects of URB597 and AM404 on anxiety-related behaviors are blocked by CB₁ receptor antagonists (Kathuria et al., 2003; Patel et al., 2004; Hill and Gorzalka, 2005), these compounds could exert effects on behavior independent of CB₁ receptor activation. For example, URB597 increases brain content of *N*-oleoylethanolamine, which can activate peroxisome proliferator-activated receptor- α (Fu et al., 2003). We are currently testing the hypothesis that the effects of URB597 and AM404 are mediated via CB₁ receptors by determining their effects on anxiety behaviors in CB₁^{-/-} mice.

In summary, we have evaluated the effects of broad dose ranges of multiple direct cannabinoid receptor agonists, antagonists, and eCB-modulating compounds on anxiety-like behaviors in mice using the elevated-plus maze. We conclude that eCB signaling is part of an endogenous anxiolytic neuromodulatory system and that inhibition of FAAH activity represents a viable and promising approach to the development of treatments for anxiety-related psychiatric disorders. Preclinical data suggest that these compounds will avoid the

adverse effects of direct CB₁ agonists, including abuse potential and biphasic dose-response relationships.

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