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Behavioral effects of the novel cannabinoid full agonist AM 411

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Abstract

AM 411 ((-)-1-adamantyl-Δ8-tetrahydrocannabinol) is a novel full agonist at cannabinoid CB1 receptors. The present studies were conducted to provide behavioral characterization of this compound in rats. It was hypothesized that AM 411 should produce behavioral effects similar to known cannabinoid agonists, and that these effects should be inhibited by co-treatment with a CB1 antagonist. In Experiments 1 and 2, AM 411 dose-dependently produced behaviors consistent with CB1 agonism, including analgesia, hypothermia, catalepsy and reductions in locomotion, which were blocked by a CB1-selective antagonist. In Experiment 3, AM 411 produced a dose-dependent suppression of lever-pressing on a fixed-ratio 5 (FR5) schedule, a task known to be sensitive to administration of CB1 agonists. Detailed analysis of the temporal patterns of operant responding showed that AM 411 altered the distribution of interresponse times. Experiment 4 showed that AM 411 decreased relative interior activity in the open field, which is suggestive of an anxiogenic effect. It is concluded that AM 411 produces CB1 agonist-like behavior with potency between that of WIN 55,212-2 and AM 356. AM 411 could be a useful tool for understanding the behavioral and neural effects of CB1 receptor stimulation.

Keywords: Tetrahydrocannabinol; Motor; Analgesia; Hypothermia; Anandamide; Anxiety

1. Introduction

Stimulation of cannabinoid CB1 receptors can produce a variety of behavioral actions. These include locomotor suppression, catalepsy, ataxia, memory and attentional impairments, hyperphagia, and reduced reactivity to painful stimuli (Childers and Breivogel, 1998). In addition to delta-9-tetrahydrocannabinol (Δ9-THC), the main psychoactive constituent of marijuana, several other compounds have been developed with CB1 agonist activity, including CP 55,940 (Johnson and Melvin, 1986), WIN 55,212-2 (Ward et al., 1989), and HU-210 (Devane et al., 1992a). These compounds, as well as the endogenous cannabinoid receptor ligands arachidonoylethanolamide (anandamide; Devane et al., 1992b) and 2-arachidonyl glycerol (2-AG; Mechoulam et al., 1995), have become useful tools for elucidating the functions of the CB1 receptor in normal and pathological conditions, and for exploring the potential ameliorative benefits of CB1 ligands in varied diseases such as Tourette's Syndrome (Muller-Vahl et al., 2002), multiple sclerosis (Consroe et al., 1997; Baker et al., 2001; but see Killestein et al., 2002), anorexia due to AIDS (Beal et al., 1995), cancer chemotherapy (Nelson et al., 1994), and Alzheimer's disease (Volicer et al., 1997).

It is critical to develop novel CB1 agonists in order to provide research tools for studying CB1 receptor function, and also because of the potential therapeutic benefit of these compounds. For this reason, the present paper provides behavioral characterization of AM 411, which is a novel full agonist at CB1 receptors. AM 411 ((-)-1-adamantyl-Δ8-tetrahydrocannabinol) is a classical cannabinoid with an adamantyl side chain (Fig. 1; Khanolkar et al., 2000). In an

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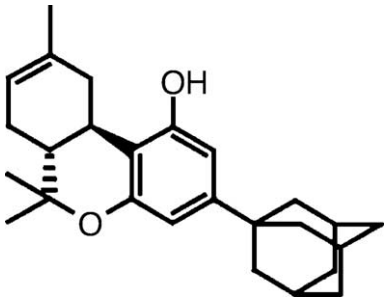

Fig. 1. Structure of (-)-1-adamantyl-Δ8-tetrahydrocannabinol (AM 411; from Khanolkar et al., 2000).

initial study, it was reported that AM 411 is a partially selective cannabinoid receptor ligand, with an approximately tenfold binding preference for the CB1 receptor (Ki=5.9 nM) relative to the CB2 (Ki=52 nM) receptor (Tartal et al., 2002). More recently, some of the biochemical characteristics of AM 411 have been described in detail (Luk et al., 2004). AM 411 was identified as a full agonist of CB1 receptors in terms of the modification of inwardly rectifying potassium channels on Xenopus oocytes. The EC50 for this effect was reported to be 29.5 nM (Luk et al., 2004), which was slightly more potent than WIN 55,212-2, and also was more potent than AM 356, anandamide, and Δ9-THC. Based upon these biochemical results, it was hypothesized that AM 411 should produce behavioral actions consistent with the known effects of CB1 stimulation. In an initial behavioral report, AM 411 was found to reduce spontaneous locomotion in the open field, an effect reversed with the CB1 antagonist SR 141716A (Järbe et al., 2004). In the present studies, adult male Sprague–Dawley rats were used to assess the effects of two doses of AM 411 on a variety of behavioral tasks. In Experiment 1, a modified version of the tetrad of tasks associated with CB1 receptor activation (Martin et al., 1991) was used. These tasks included suppression of spontaneous locomotion as measured in small automated locomotor cages, induction of catalepsy (as measured by the bar test), hypothermia, and analgesia using the tail-flick test. In order to test the hypothesis that these behavioral effects resulted from CB1 receptor stimulation, Experiment 2 examined the ability of the selective CB1 antagonist AM 251 (Gatley et al., 1996; McLaughlin et al., 2003) to block the effects of AM 411. Previous reports from our laboratory have shown that CB1 agonists produce a reliable and dose-dependent suppression of operant responding on a fixed-ratio 5 (FR5) operant schedule (Carriero et al., 1998; Arizzi et al., 2004). Therefore, in Experiment 3 we studied the effect of AM 411 on FR5 lever pressing. Detailed analyses of the temporal characteristics of lever pressing were conducted in order to determine the specific pattern of effects produced by AM 411, because previous research has shown that these effects can be used to characterize the effects of drugs and brain lesions that impair motor function (Salamone et al., 1993; Carriero et al., 1998; Correa et al., 2003a; Arizzi et al., 2004). Because compounds with CB1 activity are often found to produce anxiogenic effects, particularly at high doses (Arévalo et al., 2001; Genn et al., 2004), reductions in spontaneous locomotion may reflect effects related to anxiety. Treatments that produce anxiety are known to reduce the relative amount of motor activity that is conducted in the more exposed inner portion of an open field (Prut and Belzung, 2003). Therefore, Experiment 4 addressed the question of whether AM 411, at doses that reduced locomotion in the first experiment, would produce differential patterns of ambulation in the inner and outer portions of the open field.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats housed in a colony room with a 12 h light–dark cycle (lights on 0800–2000 hours) were used for these experiments. All experiments were conducted during the light part of the cycle. For the studies of locomotion, catalepsy, hypothermia and analgesia, food and water were available ad libitum throughout the experiments. For the operant behavior studies, rats were food deprived to 85% of their free-feeding body weight, although water was available ad libitum in the home cage throughout the experiments. Animal protocols were approved by the institutional animal care and use committee, and the methods were in accord with the guidelines for the care and use of laboratory animals set forth by the National Research Council.

2.2. Drugs

AM 411 and AM 251 were synthesized in the laboratory of Alex Makriyannis, in the School of Pharmacy at the University of Connecticut. All injections were administered i.p. Vehicle for both compounds consisted of DMSO, Tween-80 (both from Sigma, St. Louis, MO), and 0.9% saline in a 1:2:7 ratio. Across all experiments, AM 411 was administered in a dose range of 0.3125–5.0 mg/kg i.p. These doses were determined by extensive pilot studies.

2.3. Assessment of locomotion, catalepsy, hypothermia and analgesia

For assessment of locomotor activity, rats were placed in small locomotor chambers (28×28×28 cm). The floor of each chamber consisted of two wire mesh panels (27×13 cm) connected by a metal rod though the center which served as a fulcrum for the floor panels. Locomotion by the subjects produced a slight deflection of one or more floor panels, which closed one or more of four microswitches mounted on the exterior of the chamber. Closure of the microswitches was detected and counted by an external computer running a custom program written in

QBasic, by means of an interface (Med Associates). Animals were tested for an 18-min session. The chambers were novel to the subjects at the time of testing to ensure a high baseline of locomotor counts. Immediately following the locomotion session, subjects were moved to an adjoining room and allowed to habituate for 5 min. Subjects were then tested for catalepsy by placement of both forelimbs over a thin metal bar fixed 13 cm above a wooden stand. Subjects were timed for latency to remove one or both forelimbs, or to jump onto the bar. Two trials were taken for each subject, and latencies were summed. Subjects were then tested for latency on a tail-flick apparatus (Ugo Basile, Italy) as a measure of analgesia. Animals were wrapped lightly in a cloth towel or shirt to prevent spontaneous movement. Each subject's tail was exposed and placed in contact with a combination heat source and photosensor which was turned on using an experimenter-operated foot pedal. Any movement of the tail was detected by the photosensor, which then turned off the heat source and stopped a built-in timer. A cutoff of 10 s was set to prevent tissue damage. Following a single trial on the tail-flick apparatus, each subject's temperature was taken with a pliable, water-resistant thermistor inserted 6 cm into the animal's rectum. The thermistor was attached to a digital thermometer (Fisher Scientific); temperatures were allowed to stabilize for at least 5 s before being recorded.

2.4. Operant lever pressing

Experiment 3 tested the effects of AM 411 on suppression of lever pressing on an FR5 task, in which every fifth response on a lever is rewarded with a food pellet. Subjects ($n=11$) were food-restricted to 85% of free-feeding body weight. Each was placed in an operant chamber for a 30 min daily session. Following a training session in which a single 45 mg food pellet was delivered every 30 s or following each lever press, subjects were placed on a fixed-ratio 1 (FR1) schedule for two weeks. Rats were then transferred to an FR5 schedule. When performance on the FR5 schedule stabilized, drug sessions were conducted once per week as described below. Pellets were nutritionally complete and subjects typically received all of their food in the form of pellets received during the session (approximately 13–15 g per day). Testing was conducted in Med Associates (St. Albans, VT) operant chambers controlled by custom software written in QBASIC via Med Associates interface equipment with temporal resolution of 1.0 ms.

2.5. Assessment of open field locomotion and relative interior activity

The open field chamber had a square (115 cm × 115 cm) wooden floor painted black with white lines every 23 cm, forming a five-by-five grid. The floor was covered with a clear Plexiglas sheet and had walls 44 cm in height. Rats were tested for 18 min sessions in a darkened room with dim red lamps above two corners. The open field apparatus and experimental room were both novel to the subject, and each subject was tested only once. Rats were initially placed in one of the corners and faced toward the center of the apparatus. An experimenter blind to treatment separately counted inner and outer horizontal crossings, defined as movement of all four paws from one black square to another, using a hand counter. Outer crossings were defined as movements into one of the 16 squares adjacent to the walls, either from another outer square or from an inner square. Inner crossings were defined as movements into one of the nine squares within the open field which did not touch a wall, from either an outer square or another inner square (Correa et al., 2003b).

2.6. Experiments

2.6.1. Experiment 1

Locomotion, Catalepsy, Analgesia, and Hypothermia. In Experiment 1, 30 rats were randomly assigned one of three treatments: vehicle, 2.5, or 5.0 mg/kg AM 411. Following a 30-min pretreatment, rats were tested on a battery of tasks as described above (i.e., locomotion, catalepsy, tail flick, and hypothermia). Each rat received only one drug treatment, but was assessed on all four behavioral tasks.

2.6.2. Experiment 2

Effects of AM 251 on the behavioral actions of AM 411. In Experiment 2 a new batch of animals ($n=41$) was randomly assigned to one of five groups in order to examine reversal of the behavioral effect of AM 411, using the CB1 inverse agonist/ antagonist, AM 251. Three of the groups received either vehicle ($n=8$) or 2.0 ($n=8$) or 4.0 ($n=9$) mg/kg of AM 251 60 min prior to behavioral testing, followed by 5.0 mg/kg of AM 411 30 min later. Another group ($n=8$) received 4.0 mg/kg of the antagonist, AM 251, followed by a vehicle injection. The last group ($n=8$) received one vehicle injection 60 min prior to testing, followed by another vehicle injection 30 min later. The dose of AM 411 was selected based on the results of Experiment 1. The dose range of AM 251 was selected based on previous findings of suppression of food-reinforced behavior at these doses (McLaughlin et al., 2003). Subjects were tested in identical behavioral conditions to those in Experiment 1.

2.6.3. Experiment 3

Effects of AM 411 on FR5 lever pressing. In Experiment 3, rats were trained on the FR5 task as described above until stable baseline performance was reached (i.e., two weeks with >1000 responses per 30 min). After training, the drug testing phase began. Each rat ($n=11$) received baseline testing four days each week, and on the 5th day received a drug treatment. Drug test days were conducted once per week, and over the course of the experiment each rat received all drug treatments in counterbalanced order. Drug

treatments consisted of i.p. injections of either vehicle or AM 411 at doses of 0.3125, 0.625, 1.25, 2.5, and 5.0 mg/kg i.p. (30 min prior to testing).

2.6.4. Experiment 4

Effects of AM 411 on open field locomotion and relative interior activity. Rats ($n=43$) were handled for five days prior to experimentation. Subjects were randomly assigned to one of three treatment conditions: vehicle injection ($n=14$), 2.5 mg/kg AM 411 ($n=14$), 5.0 mg/kg ($n=15$) AM 411, all administered i.p. 30 min prior to testing.

2.7. Data analyses

Effects for Experiment 1 and Experiment 2 were analyzed using one-way analysis of variance (ANOVA). Individual dose effects were analyzed using planned comparisons (Keppel, 1982) in which each group was compared to vehicle. Data points identified by SYSTAT 7.0 as outliers (data+/−3 S.E.M. from group mean) were removed from the analysis. In Experiment 2 an effect of AM 411 was considered to be reversed by AM 251 if planned comparisons revealed that the group that received AM 251 plus AM 411 significantly differed from the group receiving AM 411 only. One subject in the operant responding study produced an aberrant number of responses (>3 S.E.M. lower than group mean) in the vehicle condition and was removed from the analysis. Total response data in Experiment 3 were analyzed using a repeated-measures ANOVA, and individual dose effects were analyzed using planned comparisons. Additionally, interresponse time (IRT), defined as time between onset of consecutive responses, was recorded. Fast IRTs (<5.0 s) were sorted based on length into one of twenty 250 ms bins. Pauses (IRT>5 s) were also recorded. A two-way ANOVA was used in which dose and IRT bin were within-subjects factors. Because there was a significant dose × IRT bin interaction (see below), individual bins were assessed for significant effects using ANOVA (linear regression of dose-related effects using analysis of residual variances). For Experiment 4, total activity counts were analyzed using one-way ANOVA, as were inside and outside crossings. Furthermore, inside counts were expressed as a percentage of total activity counts (i.e., relative interior activity) and analyzed with a one-way ANOVA.

3. Results

3.1. Experiment 1. Locomotion, catalepsy, analgesia, and hypothermia induced by AM 411

As seen in Fig. 2, AM 411 produced significant effects on all four behavioral components of Experiment 1. It significantly suppressed spontaneous locomotion (2a; $F(2,27)=10.80$, $p<.001$), increased the duration of the

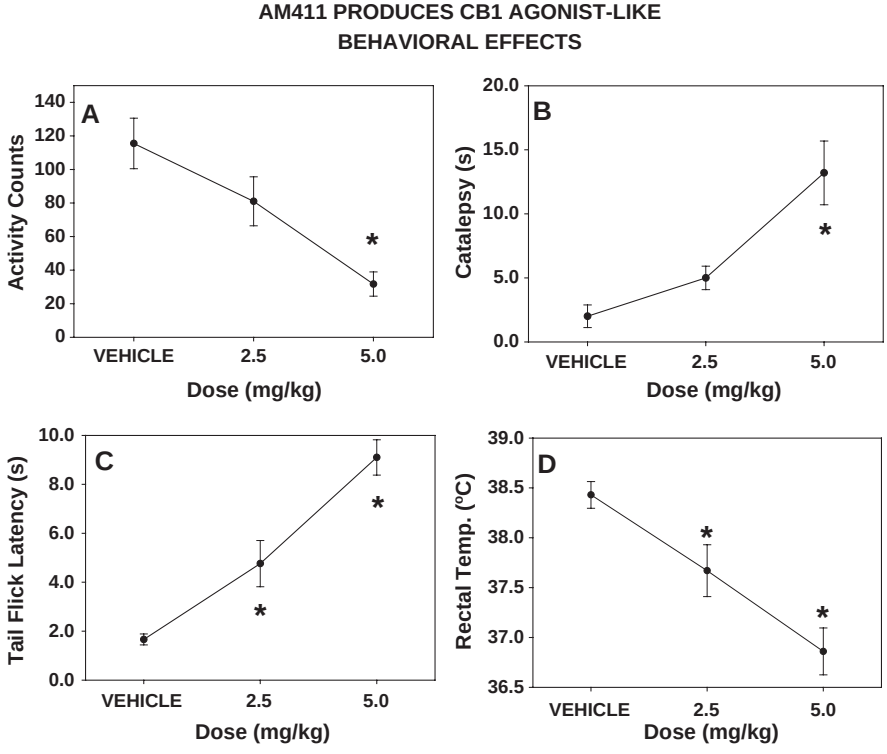

Fig. 2. AM 411 dose— dependently produces behaviors consistent with CB1 receptor activation, including (A) decreased spontaneous locomotion, (B) induction of catalepsy, (C) analgesia on the tail-flick test, and (D) induction of hypothermia. Data points represent group means (+/−S.E.M.). *$p<0.05$; **$p<0.01$ difference between dose of AM 411 and vehicle using planned comparisons.

catalepsy response (2b, $F(2,27)=7.20$, $p<.005$), increased tail-flick latency (2c; $F(2,27)=28.63$, $p<.001$), and lowered body temperature (2d; $F(2,27)=13.14$, $p<.001$). Planned comparisons revealed that both doses were different from vehicle ($p<.05$) on the tail-flick and hypothermia measures but only the high dose (5.0 mg/kg) was different from vehicle in the locomotion and catalepsy tests.

3.2. Experiment 2: Effects of AM 251 on behavioral actions of AM 411

The behavioral effects of AM 411 were reversed by co-administration of the CB1-selective antagonist/inverse agonist AM 251. With locomotion, there was an overall significant group effect (Fig. 3a; $F(4,36)=4.74$, $p<.004$). Planned comparisons revealed that the groups that received AM 411 alone ($p<0.01$) and AM 251 alone ($p<0.01$) significantly differed from vehicle. Also, the suppression of locomotion induced by AM 411 was significantly reversed by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<0.01$). There was a significant overall group difference with the catalepsy measure (Fig. 3b; $F(4,21)=4.72$, $p<.01$). AM 251 alone had no effect, while AM 411 alone showed a significant induction of catalepsy compared to vehicle ($p<0.01$). AM 411-induced catalepsy was significantly reduced by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<0.01$). In the tail-flick test (Fig. 3c), ANOVA revealed a significant overall effect ($F(4,36)=7.83$, $p<.001$). AM 251 alone had no significant effect, while AM 411 did produce increased tail flick latency ($p<0.01$). AM 411-induced tail-flick analgesia was significantly reduced by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<0.01$). Core body temperature (Fig. 3d; $F(4,34)=3.00$, $p<.05$) was also signifi-

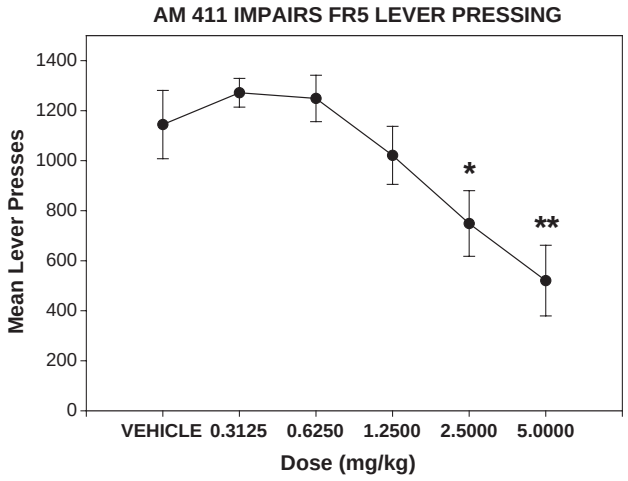

Fig. 4. AM 411 dose—dependently impairs operant responding on an FR5 task (ED50=4.93 mg/kg). Data points represent group means (+/−S.E.M.) for each operant session. *$p<0.05$; **$p<0.01$ difference between dose of AM 411 and vehicle using planned comparisons.

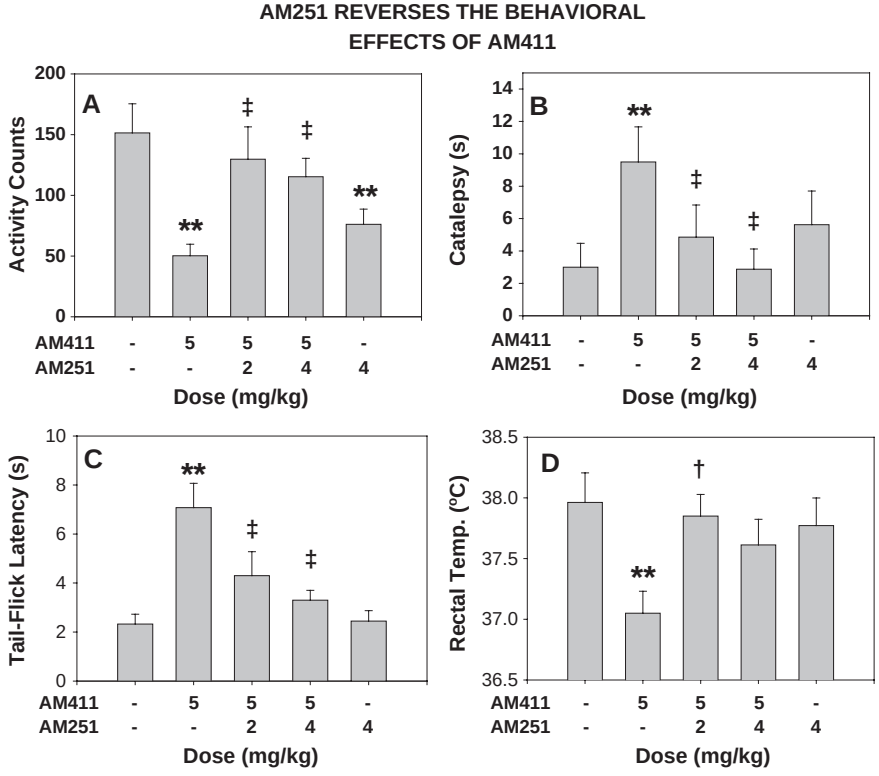

Fig. 3. AM 251 antagonizes the actions of AM 411 on tasks in the tetrad. Bars represent group means (+/−S.E.M.).*$p<0.05$; **$p<0.01$ difference between AM 411 alone and vehicle or between AM 251 alone and vehicle. †$p<0.05$; ‡$p<0.01$ difference between AM 251-pretreated animals and AM 411 alone using planned comparisons.

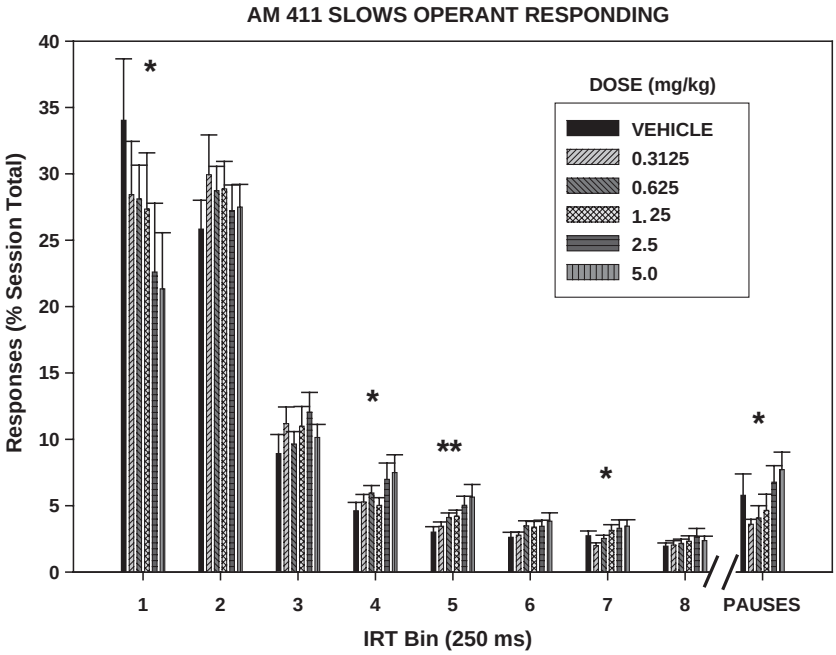

Fig. 5. AM 411 slows fast responding and increases pausing in operant responding on an FR5 task. Bins 1-8, representing all interresponse times under 2.0 s, are shown (each Bin was 250 ms; see text for further details), as well as pauses, defined as all interresponse times over 5.0 s. Note that Bins 9–20 are omitted because responses sorted into these bins were minimal and produced no significant effects. Bars represent mean (+/−S.E.M.) proportion of session responses falling into each IRT bin for each dose. *$p<0.05$; **$p<0.01$.

cantly different across groups. AM 251 alone had no significant effect, while AM 411 produced significant hypothermia compared to vehicle ($p<0.01$). AM 411-induced hypothermia was significantly reduced by co-administration of AM 251 at the 2.0 mg/kg dose ($p<0.05$), but not the 4.0 mg/kg dose.

3.3. Experiment 3. Effects of AM 411 on FR5 lever pressing

Fig. 4 shows that administration of AM 411 led to a significant dose-related suppression of FR5 responding ($F(5,45)=8.85$, $p<.001$). Planned comparisons revealed that both the 2.5 and 5.0 mg/kg doses were different from vehicle. To determine the ED50 of this effect, the dose-response data were subjected to one-phase exponential decay using Graphpad Prism software in which the plateau was entered as 0 and the span was fixed to the mean performance of the vehicle group ($M=1144.273$). The ED50 of the curve was determined to be 4.93 mg/kg. Interresponse times (IRTs) were sorted into 21 time bins. Fast responses (IRT < 5.0 s) were sorted into 20 bins, 250 ms each. Pauses (IRTs greater than 5.0 s) were sorted into one time bin. To account for differences in overall responding between doses, bin data were expressed as a percentage of total responses per session; thus, Bin 1 represented the proportion of responses in a session which occurred between 0 and 250 ms following the previous response onset, Bin 2 data are the proportion of IRTs between 251 and 500 ms, and so on. A two-way, repeated measures ANOVA revealed a significant interaction between dose and bin ($F(95,855)=1.77$, $p<.001$), suggesting that AM 411 produced changes in the overall distribution of IRTs. Dose-related trends within individual bins were analyzed using ANOVA. A significant ($p<.05$) dose-related decrease in Bin 1 (percent IRTs < 251 ms) was found, as was a significant dose-related increase in Bins 4, 5, and 7 (Fig. 5). No other bins of fast IRTs produced significant trends. A significant dose-related effect also was found for pauses (IRTs > 5.0 s). Furthermore, time spent not responding (i.e., total session time within pauses) was significantly increased

Table 1
Effects of AM 411 on temporal factors of operant responding

Dose (mg/kg)	Vehicle	0.3125	0.625	1.25	2.5	5.0
Time not responding (s)†	600.6 (100.9)	579.7 (66.9)	618.5 (102.4)	887.4 (101.4)**	1097.6 (118.6)**	1245.1 (144.7)**
Average pause length (s)†	12.2 (2.1)	15.1 (3.0)	19.4 (4.4)	33.5 (9.3)	67.7 (37.2)	159.6 (64.5)**
Local response rate (responses per s responding)	1.086 (0.051)	1.064 (0.045)	1.054 (0.053)	1.082 (0.059)	0.961 (0.052)	0.975 (0.098)

Values represent session means (S.E.M.). See text for detailed definitions of variables. †Overall F-ratio for dose effect reached significance, $p<.05$. *$p<.05$; **$p<.01$ difference from vehicle using planned comparisons where appropriate.

(Table 1; $F(5,45)=8.57$, $p<.001$), as was average pause length (time not responding divided by number of pauses; $F(5,45)=3.41$, $p<.05$). In addition, a measure of the local response rate was created to examine overall changes in fast responses (IRTs < 5.0 s) only, and was defined as number of fast IRTs divided by time spent responding (i.e., session length—time spent in pauses). Unlike individual analyses of bin data, no significant overall effect was found for average local response rate ($F(5,45)=0.99$ ns).

3.4. Experiment 4. Effects of AM 411 on locomotion and relative interior activity in the open field

AM 411 significantly suppressed overall locomotion in the open field (Fig. 6A; $F(2,40)=31.21$, $p<.001$), as well as locomotion in the outside ($F(2,40)=29.48$, $p<.001$) and inside ($F(2,40)=12.81$, $p<.001$) portions of the open field. Relative interior activity (i.e., activity within the inner portion of the open field as a percentage of total activity) also was analyzed. There was a dose-related decrease in relative interior activity induced by AM 411 (Fig. 6B; $F(2,35)=9.89$, $p<.001$). Planned comparisons revealed significant differences between both doses of AM 411 and vehicle in all analyses.

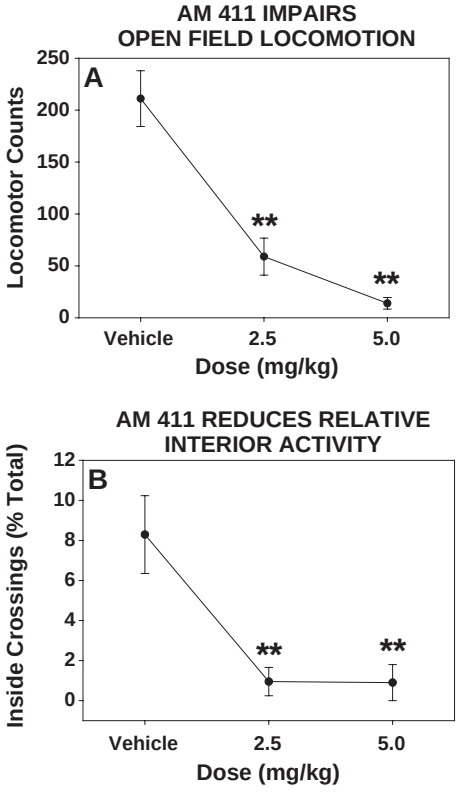

Fig. 6. (A). AM 411 reduced locomotion in the open field. Data points represent mean (+/−S.E.M.) number of total locomotor counts in the open field. (B) AM 411 reduced relative interior activity (i.e., percent of total activity that was within the interior portion of the open field; see text for details). $^{**}p<0.01$ difference between dose of AM 411 and vehicle using planned comparisons.

4. Discussion

In Xenopus oocytes, AM 411 produced 100% of the maximal response of WIN 55,212-2, but partial CB1 agonists such as Δ9-THC did not, indicating that AM 411 is a full agonist at the CB1 receptor (Luk et al., 2004). In the same paper, AM 411 was characterized by higher potency (EC50 = 29.5 nM) and affinity (Ki = 7 nM) than WIN 55,212-2 (105.5 and 10 nM, respectively). Moreover, a 10-fold preference for CB1 (Ki = 5.9 nM) over CB2 (Ki = 52.0 nM) receptors was reported for this compound (Tartal et al., 2002). In the current study, AM 411 produced effects in vivo similar to other CB1 agonists in the tetrad test, including locomotor suppression, catalepsy, analgesia, and hypothermia (Martin et al., 1991). Furthermore, these effects were reversed by the CB1-selective antagonist AM 251 at doses that are known to be behaviorally active in rats (Hildebrandt et al., 2003; McLaughlin et al., 2003). AM 411 dose—dependently suppressed operant lever—pressing in a manner similar to the effects previously shown by other CB1 agonists (Carriero et al., 1997; Arizzi et al., 2004). In addition, AM 411 reduced locomotion and relative interior activity in the open field, which suggests that an anxiogenic effect was induced at doses that also suppressed motor activity. Together with the biochemical data, the results of these behavioral studies indicate that AM 411 is an agonist of cannabinoid receptors that induces centrally mediated CB1-related behavioral effects.

It has been suggested recently (Wiley and Martin, 2003) that the tetrad test is sensitive to the effects of several classes of non-cannabinoid drugs. However, CB1-agonists appear to be characterized by similar potency in all four tests, and by reversal of these effects by an antagonist/inverse agonist such as SR 141716A (Rinaldi-Carmona et al., 1994). In the current study, AM 411 produced significant effects on all four behavioral measures (i.e., locomotion, catalepsy, analgesia, hypothermia) in the dose range of 2.5–5.0 mg/kg, with the 5.0 mg/kg dose being significant with all four tests. In the second experiment, effects of 5.0 mg/kg of AM 411 were reversed by co-administration of the CB1 antagonist AM 251. The 2.0 mg/kg dose of AM 251 produced significant reversal of the effects of AM 411 on all four tasks, while the 4.0 mg/kg dose of AM 251 produced a significant reversal of the effects of AM 411 on three of the four tasks. AM 251 alone also was found to produce a significant suppression of spontaneous locomotion. High doses of CB1 antagonists, such as SR 141716A, have been found to inhibit open field ambulation (Järbe et al., 2002). The mechanism of action of this effect is most likely different from that produced by AM 411, as subjects treated with both compounds exhibited locomotor counts similar to vehicle, indicating that the effects of both drugs tend to counteract each other. Taken together, these results indicate that AM 411 produces behavioral effects that are characteristic of CB1 receptor stimulation.

Although the present studies used behavioral tests associated with CB1 receptor activity, we cannot conclude that AM 411 lacks CB2 activity in the dose range tested. AM 411 exerts a moderate preference for CB1 receptors relative to CB2 receptors compared to other classical cannabinoids (Howlett et al., 2002), but it is less CB1 selective than several other compounds, including methanandamide, ACPA, ACEA and O-1812 (Khanolkar et al., 1996; Hillard et al., 1999; Di Marzo et al., 2001). As CB2 receptors are not found in brain of rodents or primates (Howlett et al., 2002), few behavioral assays exist. However, the CB2 receptor may modulate pain in inflammatory (Hanus et al., 1999) and noninflammatory states (Ledent et al., 1999; Zimmer et al., 1999; Malan et al., 2002), via local peripheral mechanisms. Future studies of the potential CB2-related effects of AM 411 should therefore study the ability of a CB2 antagonist such as AM 630 (e.g., Malan et al., 2001; Ibrahim et al., 2003; Yoon and Choi, 2003; Johanek and Simone, 2004) to block analgesic and anti-inflammatory effects of AM 411. In terms of central cannabinoid effects, it also is important to recognize that there may be additional cannabinoid receptors present in the brain that are not of the CB1 subtype. For example, the cannabinoid agonist WIN 55,212-2 may activate a receptor on hippocampal glutamatergic terminals, which do not express CB1 receptors (Katona et al., 1999). WIN 55,212-2 binds to brain membrane of CB1 knockout mice, albeit with lower affinity than in wildtype animals (Breivogel et al., 2001). However, as the modulation of hippocampal physiological responses by WIN 55,212-2 was not antagonized by AM 251 (Hajos and Freund, 2002), this putative receptor is not likely to be responsible for the effects of AM 411 described in Experiment 2 above.

The role of cannabinoid systems in modulating anxiety is complex, and remains poorly understood (Genn et al., 2004). In some studies, cannabinoid agonists have been shown to be anxiogenic (Arévalo et al., 2001; Genn et al., 2004), while in other papers anxiolytic effects have been reported (Haller et al., 2002). In addition, there are conflicting reports about the anxiety-related effects of CB1 receptor knockouts (Martin et al., 2002; Degroot and Nomikos, 2004; Haller et al., 2002). It has been suggested that cannabinoid agonists tend to produce anxiolytic effects at low doses, but anxiogenic effects at higher doses (Genn et al., 2004). In the present study, the doses of AM 411 that reduced spontaneous locomotion in automated locomotor cages also reduced overall line crossings in the open field. Relative interior activity in the open field also was suppressed, suggesting that rats selectively avoided the more exposed inner region, and ambulated relatively more near the high walls of the apparatus. This effect has also been shown using anxiogenic drugs, such as benzodiazepine inverse agonists, and it has been suggested that changes in relative interior activity can be used as a behavioral marker of anxiolytic or anxiogenic effects of drugs (Prut and Belzung, 2003). In the case of AM 411, when interior and exterior activity counts were analyzed separately, exterior activity was significantly suppressed at both doses, suggesting that there also was an overall motor effect. Taken together with the reduction of locomotion and induction of catalepsy in Experiment 1, and the impairment in lever-pressing Experiment 3, the most likely explanation is that AM 411 inhibits locomotion by impairing motor control, but that this drug also produces anxiogenic effects in the same dose range. These anxiogenic effects may contribute to the overall suppression of open field locomotion that is seen under novel conditions, by producing both absolute and relative decreases in interior activity.

In addition to having effects on locomotion, catalepsy, analgesia and hypothermia, AM 411 also suppressed operant lever pressing on an FR5 schedule within the same dose range. Several other cannabinoid agonists also have been shown to have this effect (Carriero et al., 1998; Arizzi et al., 2004). The ED50 for AM 411 in the present study was 4.9 mg/kg, which means that AM 411 was less potent than CP 55,940 and WIN 55,212-2, approximately equipotent with delta-8-tetrahydrocannabinol and anandamide, and more potent than AM 356, for the production of this effect (Carriero et al., 1998; Arizzi et al., 2004). It is recognized that suppression of operant responding is not a selective test for CB1 agonists, and in fact many other drugs, including CB1 antagonists (Freedland et al., 2000; McLaughlin et al., 2003), also have this effect, although it is noteworthy that CB1 agonists and antagonists most likely reduce operant responding via separate mechanisms. Pretreatment with a low dose of a CB1 agonist will reverse, rather than augment, a CB1 antagonist-induced reduction in responding (Freedland et al., 2000). Likewise, pretreatment with a CB1 antagonist will reverse an impairment in responding produced by a CB1 agonist (Baskfield et al., 2004). CB1 antagonists are thought to reduce operant responding because of appetite suppression or food aversion (McLaughlin et al., 2003; in press), while CB1 agonists are thought to reduce lever pressing because of motor suppressant effects (Carriero et al., 1998). Although the suppression of operant responding is not unique to any particular drug category, studies involving operant behavior can be useful for characterizing the potency and time course of drug effects, and for studying tolerance on a task characterized by consistent behavioral baselines. In addition, considerable information can be learned by examining the effects of drugs, including CB1 agonists, on the microstructure of operant behavior, and by comparing the results with those obtained using other behavioral tasks (Liao and Fowler, 1990; Salamone et al., 1993; Cousins and Salamone, 1996; Carriero et al., 1997, 1998; Correa et al., 2003a). In the present study, the effects of AM 411 were characterized by alterations in the overall distribution of IRTs, and by dose-related reductions in the relative number of fast IRTs (i.e., Bin 1). Although there was no overall effect on the average local rate of responding, there were substantial increases in the relative number of pauses, average pause length, and

total time spent not responding. These analyses indicate that there was a slight effect on response speed during periods of responding (i.e., relatively fewer fast responses), but that the major effect of AM 411 was to fragment periods of responding and dramatically reduce time spent responding. Behavioral observations indicated that AM 411-treated rats were akinetic, and at higher doses they showed signs of ataxia. These observations, coupled with the parallel studies showing reductions in locomotion and induction of catalepsy produced by AM 411, indicate that AM 411 suppressed operant responding largely because of motor impairments that led to long periods of time without responding. This conclusion is similar to that reached in previous studies (Romero et al., 1995, 1996; Martin et al., 1991), including those involving detailed behavioral analyses of the suppression of FR5 responding with CB1 agonists (Carriero et al., 1998). Although the precise anatomical basis of the effects of CB1 agonists on operant responding is not known, it is likely that this effect is due to actions on CB1 receptors in brain regions involved in motor function, such as basal ganglia (Marsicano and Lutz, 1999; Sanudo-Peña et al., 1999; Julian et al., 2003; El-Banoua et al., 2004) or cerebellum (Dar, 2000).

We conclude that AM 411 produces behavioral effects consistent with those actions that are typically produced by cannabinoid agonists that have effects on CB1 receptors. AM 411 may be a useful tool for exploring the effects CB1-receptor mediated processes including analgesia and motor control, and also memory (Heyser et al., 1993; Hampson et al., 2003), attention (Presburger and Robinson, 1999), and feeding (Williams and Kirkham, 2002). Given the in vitro data showing that AM 411 is resistant to desensitization in comparison to other CB1 full agonists (Luk et al., 2004), future studies should assess the development of behavioral tolerance of with repeated administration of this compound. Initial behavioral studies indicate that the suppression of locomotion and operant responding produced by AM411 does show tolerance over the first 12 days of administration (Winston et al., unpublished observations), and additional research must be conducted to compare these effects with those of other CB1 agonists.

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