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Behavioral effects of the novel potent cannabinoid CB1 agonist AM 4054

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Abstract

Due to the ubiquity of the CB1 cannabinoid receptor throughout the nervous system, as well as the many potential therapeutic uses of CB1 agonist-based interventions, it is desirable to synthesize novel probes of the CB1 receptor. Here, the acute behavioral effects of systemic (i.p.) administration of the putative novel CB1 full agonist AM 4054 were tested in rats. In Experiment 1, a dose range (0.15625 – 1.25 mg/kg) of AM 4054 produced effects consistent with CB1 agonism in the cannabinoid tetrad of tasks in rats, including induction of analgesia, catalepsy, hypothermia, and locomotor suppression. These effects were reversed with the CB1-selective inverse agonist AM 251 in Experiment 2, indicating that AM 4054 produced CB1 receptor-mediated effects. Analysis of open-field activity indicated that the reduction in locomotion is more consistent with general motor slowing than anxiogenesis. AM 4054 (0.0625 – 0.5 mg/kg) also dose-dependently reduced fixed-ratio 5 (FR5) operant responding for food in Experiment 3, and microanalysis of the timing and rate of lever pressing indicated a pattern of suppression similar to other CB1 agonists. Minimum doses of AM 4054 (0.125 – 0.3125 mg/kg) required to produce significant effects in these behavioral assays were lower than those of many CB1 agonists. It is likely that AM 4054 is a potent pharmacological tool for assessment of cannabinoid receptor function.

Keywords

cannabinoid; motor control operant; microanalysis

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1. Introduction

1.1 Rationale

The cannabinoid CB1 receptor is densely expressed in brain tissue (Herkenham et al., 1991; Egertová and Elphick, 2000), as well as peripheral tissue (Wenger et al., 2001; Howlett et al., 2002). CB1 agonists therefore hold therapeutic promise for treating a variety of conditions, including pain, inflammation, gastrointestinal disorders, and hyperkinetic symptoms (Müller-Vahl, 2003; Izzo and Coutts, 2005; Adam et al., 2012; Arevalo-Martin et al., 2012). The CB1 receptor is also believed to regulate cognitive processes (McLaughlin et al., 2005a; de Oliveira Alvares et al., 2008; Pedroza-Llinás et al., 2013). It is therefore crucial to develop novel pharmacological probes that target the CB1 receptor. In particular, potent CB1 agonists (such as CP 55,940 [Johnson and Melvin, 1986] and HU 210 [Devane et al., 1992; Ottani et al., 2002]) are desirable because they produce greater effects per unit cost, and because they can be delivered via microinjection in lower quantity.

1.2 Behavioral assays of cannabinoid CB1 agonists

The current paper represents early behavioral characterization of AM 4054, a novel adamantyl cannabinoid; biochemical results indicate that it is a cannabinoid full agonist with nanomolar affinity for CB1 receptors ($K_i = 4.9$ nM) and threefold preference for CB1 binding, compared with CB2 (Thakur et al. submitted). In comparison, CP 55,940 and HU 210 have similar affinity for CB1 and CB2 (Pertwee et al., 2005a). In a recent investigation, AM 4054 induced diuresis and hypothermia in rats with ED_{50} values of 0.06 and 0.1 mg/kg, respectively (Paronis et al., 2013). These effects were reversed by the CB1 inverse agonist rimonabant, suggesting that they were mediated by CB1 agonism; similarly, both rimonabant and AM 4113, a putative CB1 neutral antagonist, antagonized the discriminative stimulus effects of AM 4054 in primates (Kangas et al., 2013).

In rodents, CB1 agonist-like behavioral activity has been defined by similar potency on a tetrad of tasks developed by Martin and colleagues (Martin et al., 1991), of which the hypothermia assay is one part. The remaining assays include the induction of catalepsy, and of analgesia, and the suppression of spontaneous locomotion. Wiley and Martin (2003) have also suggested that, because other drug classes (especially dopamine D2 antagonists) produce cannabinoid-like effects on some of the tetrad tasks, CB1 agonist activity should be verified by reversing the behavioral effects with a CB1-selective antagonist/inverse agonist such as rimonabant (a.k.a. SR 141716A; Rinaldi-Carmona et al., 1994), AM 251 (Gatley et al., 1996) or AM 1387 (McLaughlin et al., 2006). Although these compounds exhibit inverse agonist activity (Landsman et al., 1997; Pertwee, 2005b; McLaughlin et al., 2006), they have been shown to antagonize the effects of CB1 agonists, even at doses that do not produce behavioral effects when administered alone (Järbe et al., 2002, 2004; McLaughlin et al., 2005b). Indeed, the hypothermic and discriminative stimulus effects of AM 4054 were reversed by the CB1 inverse agonist rimonabant, and also the CB1 neutral antagonist AM 4113 (Bergman et al., 2008; Kangas et al., 2013; Paronis et al., 2013). As CB1 agonists have sometimes been shown to be anxiogenic (Rey et al., 2012), it is possible that a reduction in spontaneous locomotion could result from thigmotaxis, in which movement is restricted to

motion near walls, which are relatively enclosed (Prut and Belzung, 2003). Another novel adamantyl cannabinoid agonist, AM 411, was shown to reduce both outside (thigmotaxic movements) and inside line crossings (within the relatively open middle of the field) in the open field task (McLaughlin et al., 2005b). This indicated that anxiety, if present, was not sufficient to suppress locomotion as seen in that study.

CB1 agonists are also known to reduce food-reinforced operant responding on various schedules, including the fixed-ratio 5 (FR5; in which every fifth response is reinforced; Carriero et al., 1998; McLaughlin et al., 2005b), and FR10 (Järbe et al., 2003; De Vry and Jentzsch, 2004). As CB1 receptor activation is believed to increase food motivation (Giuliani et al., 2000; Williams and Kirkham, 2002), it is likely that reductions in responding for food and other reinforcers is nonmotivational in nature. Indeed, AM 4054 was recently reported to inhibit FR30 responding for shock termination (Desai et al., 2013). In a previous study using an FR5 schedule, which generates high rates of responding, AM 411 was shown to shift the distribution of interresponse times (IRTs; measured as time between onset of consecutive responses) away from fast responses (IRTs < 250 ms), and enhance the proportion of slower responses (McLaughlin et al., 2005b). Similar effects were shown with CB1 agonists AM 356, CP 55,940, ⁹-THC, and WIN 55,212-2 (Carriero et al., 1998). Thus, the overall profile of the suppressive effects of CB1 agonists can be used to help characterize these drugs.

In the present studies, AM 4054 was assessed using a variety of behavioral tasks. In Experiment 1 a modified version of the cannabinoid tetrad (Martin et al., 1991) was employed; analgesia, catalepsy, and hypothermia were tested in one group of animals, while locomotion in the open field was examined in a separate group, with inside and outside crossings counted separately in order to try to eliminate anxiety as a confound. To attempt to reverse these effects, in Experiment 2, the CB1 inverse agonist AM 251 was administered to a group of rats prior to administration of AM 4054. It was hypothesized that AM 251 would block the effects of AM 4054 without producing any effects when given alone. In Experiment 3, animals trained on an FR5 schedule of lever-pressing for food reinforcement were administered AM 4054 in a within-subjects design.

2. Material and Methods

2.1 Animals

The three experiments used a total of 128 adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 350–450 g at the outset of testing. Rats were doubly housed in a colony room with a 12 h light-dark cycle (lights on 0800–2000). Water was available ad libitum throughout the study except during testing; food was available ad libitum except in Experiment 3 (a food-maintained operant design), in which rats were food-restricted to no less than 85% of free-feeding body weight. As the operant schedule used was reinforcement-dense, modest weight gain was noted during food restriction. Animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Connecticut, and all methods were in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011).

2.2 Drugs

AM 4054 and AM 251 were synthesized in the Center for Drug Discovery, Northeastern University. All injections were administered i.p.; drug was prepared in DMSO, Tween-80 (both from Sigma, St. Louis, MO), and physiological saline in a concentration of 1:2:7, respectively. In Experiment 1 (Tetrad Task), AM 4054 was administered in doses of 0.15625, 0.3125, 0.625, 1.25 mg/kg, as well as vehicle. A 0.3 mg/kg dose of AM 4054 (or its vehicle) was administered in Experiment 2 (Reversal of Tetrad), because this dose was close to the ED₅₀ in Experiment 1. Rats in Experiment 2 were also pretreated with vehicle, 2.0, or 4.0 mg/kg AM 251. Based on the results of Experiments 1 and 3, Experiment 3 (Suppression of Operant Responding) utilized lower doses of 0.0625, 0.125, 0.25, and 0.5 mg/kg AM 4054, in addition to vehicle. Doses of AM 4054 were selected based on pilot testing. Doses of AM 251 were based on previous research on reversal of tetrad effects with other CB1 agonists (McLaughlin et al., 2005b).

2.3 Behavioral Procedures

The tetrad employed in Experiments 1 and 2 consists of measures of analgesia, catalepsy, hypothermia, and locomotion, while Experiment 3 involved operant lever pressing behavior. All tetrad tasks were carried out by experimenters blind to treatment.

2.3.1 Analgesia—Analgesic effects were assessed using a tail flick device (Ugo Basile, Comerio, Italy). Each animal was loosely wrapped in a towel to limit spontaneous movement. Its tail was exposed and placed in contact with a combination heat source/photo sensor which, along with a built-in stopwatch, was activated by the experimenter depressing a pedal. The heat source and stopwatch were automatically stopped following the first tail flick by the animal, and tail flick latency (in s) was taken as the measure of analgesia. To prevent tissue damage, trials were discontinued after 10 s if no tail flick had been recorded. In these cases, a value of 10 s was entered.

2.3.2 Catalepsy—Catalepsy was measured using a bar apparatus fixed 13 cm above a wooden stand. Rats were placed with forepaws draped over the bar and hindpaws on the base of the device. Stepdown latency was measured by a separate observer holding a timer. Data presented are the sum of two trials.

2.3.3 Hypothermia—Animals were held loosely by an experimenter, while a thin, flexible thermistor probe (Fisher Scientific, Pittsburgh, PA) was then inserted 6 cm into the animal's rectum. Temperature was recorded when it ceased to fluctuate for at least 5 s. Ambient temperature of the examination room was 22° C.

2.3.4 Locomotion—In Experiment 1, locomotion was measured in an open field. The custom-built wooden open field consisted of a square (115 cm X 115 cm) floor covered with clear Plexiglas. The floor was painted black with white lines across the length and width spaced 23 cm apart, forming a 5 X 5 grid. Walls of the device were 44 cm high, and subjects were tested in the dark, except for two red lights clipped to the top of the walls in opposite corners of the apparatus. Trials were begun when an experimenter placed the rat in one of the four corners (varied between trials) with its head oriented toward the center. A different

experimenter counted all line crossings during the 18-min session, divided between inside and outside crossings to confirm that any motor effects were not solely due to anxiety. If the reduction in crossings were due to an overall motor impairment, both inner and outer crossings would be expected to decrease at similar doses (McLaughlin et al. 2005b). An outside crossing was any movement of all four paws into, or between, any of the 16 squares along the wall of the open field. Inside crossings were defined as placement of all four paws into, or between, the nine innermost squares of the grid. The results of Experiment 1 suggested that AM 4054 affected both inside and outside crossings similarly; therefore, in Experiment 2 locomotion was assessed using custom-built stabilimeter cages that assayed locomotion automatically. These Plexiglas cages (28 X 28 X 28 cm) were each housed in sound-attenuating cubicles. The floor consisted of two rectangular wire mesh panels (27 X 13 cm each) mounted on a Plexiglas frame. Both panels were connected by a single metal rod that served as a fulcrum, permitting enough rotation to depress one of four microswitches mounted in each corner. This movement was induced by locomotion of the animals within and between the four quadrants of the chamber. The number of microswitch closures was counted by a Pentium II computer running a custom-written program in MedState Notation. As with the open field task, test sessions were 18 min in length.

2.3.5 Operant conditioning—Eight Med Associates (Georgia, VT) operant boxes were used for daily sessions that were 30 min in length. Each box contained a flat operant lever approximately 2 cm from a food tray into which reinforcers (45 mg Noyes pellets, Research Diets, New Brunswick, NJ) were delivered by a food hopper outside of the chamber. The experimental protocol was controlled by a Pentium III computer running custom-written programming in MedState Notation, via a Med Associates interface. All sessions were 30 min in length. Following one day of magazine training, during which all responses were reinforced and pellets were delivered noncontingently every 30 s, a CRF task was begun in which every response was reinforced. Once animals reached a criterion of at least 300 responses in three consecutive daily sessions, they were transferred to an FR5 schedule, in which one reinforcer is delivered following every fifth response. Drug testing was begun once the overall number of responses stabilized between sessions, and all animals were emitting at least 1200 responses per day. Training took approximately 8 weeks total.

2.4 Experiments

2.4.1 Experiment 1 (Tetrad Task)—40 animals were tested for catalepsy, followed by analgesia, and then hypothermia. Doses listed above ($n = 8$ per dose) were also administered in random order 30 min prior to the start of the catalepsy assay. AM 4054 was administered i.p. 30 min prior to open field testing of an additional 40 animals.

2.4.2 Experiment 2 (Reversal of Tetrad)—Subjects ($n = 40$) were tested in random order following assignment to one of five groups, each involving 2 injections: a 60 min pretreatment with 2.0 or 4.0 mg/kg AM 251 (or its vehicle), followed by 30 min pretreatment with AM 4054 (or its vehicle). A dose of 0.3 mg/kg AM 4054 was selected as it approximated the ED_{50} in Experiment 1. Doses of AM 251, as well as the injection regimen and drug conditions, were assigned as previously reported (McLaughlin et al. 2005b). The five groups were thus: 2.0 mg/kg AM 251 followed by 0.3 mg/kg AM 4054;

4.0 mg/kg AM 251 followed by 0.3 mg/kg AM 4054; AM 4054-alone (vehicle of AM 251 followed by 0.3 mg/kg AM 4054); AM 251-alone (4.0 mg/kg followed by vehicle of AM 4054); and lastly a Vehicle condition in which the vehicle of AM 251 was administered 30 min prior to injection of the vehicle of AM 4054. Each animal was tested for spontaneous locomotion in the stabilimeter cages, as well as tests of catalepsy, analgesia, and hypothermia.

2.4.3 Experiment 3 (Suppression of Operant Responding)—Rats ($n = 8$) were trained to respond reliably on an FR5 schedule prior to beginning the drug regimen. A counterbalanced, within-subjects design was used, such that each subject was exposed to one dose condition every week in a pseudorandom order. Animals were injected i.p. 30 min prior to operant training once per week. Data (not shown) from the other sessions during the week were analyzed to ensure that drug effects did not carry over from one test session to the following week's test session.

2.5 Data Analysis

Data were analyzed using SPSS 21 (Chicago, IL). For the measures of tail flick latency and hypothermia, one-way analysis of variance (ANOVA) was performed for the main effect of dose. Nonorthogonal planned comparisons (Keppel and Wickens, 2004) were used as post hoc tests, in which four (i.e., $k-1$, where k = number of groups) analyses are performed. Some measures contained at least one condition with a mean at or near zero, which violated assumptions of normality and necessitated use of nonparametric statistics. For the locomotion and catalepsy measures, the Kruskal-Wallis test was used. Where significant effects were found, post hoc comparisons with the Mann-Whitney U test were performed. As the FR5 task was a within-subjects design, the Friedman test was used to assess the overall main effect, and Wilcoxon signed-rank test was used as a post hoc test. For all parametric or nonparametric post hoc analyses, the number of tests was limited to four. For Experiments 1 and 3, each dose of AM 4054 was compared with the vehicle condition. In Experiment 2, which tested for reversal of the effects of AM 4054, data from the AM 4054-alone group, as well as the AM 251-alone group, were compared to the Vehicle group. A significant difference between the AM 4054-alone group and vehicle was anticipated for each measure. No differences were expected between the AM 251-alone group and the Vehicle condition. Reversal with AM 251 was investigated by comparing animals pretreated with 2.0 or 4.0 mg/kg AM 251, followed by 0.3 mg/kg AM 4054, with the AM 4054-alone group (which received vehicle, followed by 0.3 mg/kg AM 4054). In Experiment 3, the ED_{50} was ascertained using curve-fitting in GraphPad Prism (version 3.0; La Jolla, CA) in which the span was entered as the mean of responding in the vehicle condition, and the plateau was entered as zero.

2.6 Microanalysis of Operant Responding

Further analysis of the operant response record was performed by measuring the length of all IRTs and creating a distribution of 21 IRT bins. To account for differences in overall responding between sessions, IRTs are expressed as a percentage of that session's total IRTs (i.e., responses minus 1). The first 20 bins each measured 250 ms in width; hence, all IRTs which were less than or equal to 5 s ($250 \text{ ms IRT bins} \times 20 \text{ bins} = 5 \text{ s}$) were sorted into one

of the first 20 bins. Bin 21 does not span 250 ms, rather, it contains all pauses, defined as IRTs > 5.0 s. During baseline conditions of FR5 responding, Bin 1 (containing IRTs > 250 ms) and Bin 2 (containing IRTs > 250 ms, and < 500 ms) together contain the majority of IRTs, while the other bins contain a much smaller proportion. As such, Bin 1 and Bin 2, as well as Bin 21 were singled out for further dose analysis using one-way ANOVA. The IRT distribution was therefore submitted to two-way ANOVA with dose and bin as factors. As IRTs summed to 100%, a significant main effect of dose was not possible; however, a significant dose X bin interaction was taken to indicate that AM 4054 altered the distribution of operant responses.

3. Results

3.1 Experiment 1 (Tetrad Task)

A significant dose effect was found for tail flick latency (Fig. 1a, $F(4,35) = 21.8$, $p < .001$). As shown in Figure 1b, AM 4054 also increased step-down latency in the bar test, a measure of catalepsy ($\chi^2(4) = 18.2$, $p < .001$). Post hoc analyses revealed significant differences between each dose of AM 4054 and vehicle. For the hypothermia measure, one data point was > 4 standard deviations from the mean and was removed from the vehicle group. It was found that AM 4054 dose-dependently reduced body temperature (Fig. 1c; $F(4,34) = 2.92$, $p = .035$). According to planned comparisons, the 0.3125, 0.625, and 1.25 mg/kg doses significantly induced analgesia (Fig. 1a) and lowered rectal temperature (Fig. 1c) relative to vehicle.

The Kruskal-Wallis test revealed that AM 4054 significantly suppressed open field crossings ($\chi^2(4) = 29.25$, $p < .001$). Separate analyses of outside ($\chi^2(4) = 29.25$, $p < .001$) and inside ($\chi^2(4) = 35.36$, $p < .001$) crossings yielded similar results and are shown in Figure 2a and 2b, respectively. The Mann-Whitney U test was used as a post hoc analysis, and all doses of AM 4054 were significantly different from vehicle (p 's < .001) for all open field measures.

3.2 Experiment 2 (Reversal of Tetrad)

Regardless of whether parametric (for the tail flick and hypothermia assays) or nonparametric analyses (for locomotion and catalepsy) were used, four (k-1) comparisons were planned following each significant dose effect: both the AM 251-alone and the AM 4054-alone conditions were compared with the vehicle condition. Both of the conditions in which rats were pretreated with AM 251 and then administered 0.3 mg/kg AM 4054 were analyzed for differences from the AM 4054-alone condition. As seen in Figure 3, a significant main effect was found for tail flick latency (Fig. 3a; $F(4,35) = 9.48$, $p < .001$), catalepsy (Fig. 3b, $\chi^2(4) = 19.0$, $p < .001$), rectal temperature (Fig. 3c; $F(4,35) = 28.7$, $p < .001$), and locomotion (Fig. 3d; $\chi^2(4) = 16.9$, $p = .002$). In all four tetrad tasks, planned post hoc comparisons revealed a significant difference in the expected direction between the group treated with AM 4054 alone, and the vehicle-only condition. In every case, this effect was attenuated by pretreatment with AM 251, as indicated by significant differences between AM 4054-treated rats pretreated with vehicle, and AM 4054-treated rats pretreated with either 2.0 or 4.0 mg/kg AM 251. As expected, there were no differences between rats administered 4.0 mg/kg AM 251 alone and rats treated with vehicle.

3.3 Experiment 3 (Suppression of Operant Responding)

AM 4054 significantly impaired FR5 operant responding ($\chi^2(4) = 20.5$, $p < .001$), according to the Friedman test. Post hoc analysis with the Wilcoxon signed ranks test revealed significant differences (p 's $< .05$) from vehicle at the 0.125, 0.25, and 0.5 mg/kg doses, as shown in Figure 4. The ED_{50} of this effect was determined to be 0.0998 mg/kg. Analysis of the IRT bin distribution was restricted to the vehicle, 0.0625, and 0.125 mg/kg doses, as the majority of rats emitted no responses at higher doses. As expected, 3 X 21 ANOVA of the distribution of the IRT bins yielded no main effect of dose for the percent-transformed data, but a main effect of bin ($F(20,140) = 8.86$, $p < .001$) and a significant dose X bin interaction ($F(40,280) = 1.89$, $p = .002$). These effects are shown in Figure 5. AM 4054 dose-dependently decreased responding in Bin 1 (IRTs < 250 ms; $F(2,14) = 4.70$, $p = .027$) and in Bin 2 (251 ms $<$ IRTs < 500 ms; $F(2,14) = 5.84$, $p = .014$), but not in any other bin, including Bin 21, containing IRTs > 5 s, which were defined as pauses in responding.

4. Discussion

Consistent with its other known behavioral effects (Bergman et al., 2008; Desai et al., 2013; Paronis et al., 2013), AM 4054 exhibited characteristic features of a CB1 agonist. As has been suggested (Wiley and Martin, 2003), CB1 agonists can be distinguished from the effects of other drugs on the tetrad by comparing potency across the tasks. Whereas the dopamine antagonist haloperidol was 43-fold more potent at reducing locomotion versus other tetrad tasks in mice, 9 -THC did not exhibit a potency difference greater than 2.4 between any two tasks (Wiley and Martin, 2003). In the current study, AM 4054 produced significant differences from vehicle at 0.3125 mg/kg in the tail flick and hypothermia measures, and 0.15625 mg/kg in the open field and catalepsy assays. While it is likely that AM 4054 would decrease movement at lower doses than those tested, the dose-response function for operant responding (Fig. 4) indicates nonsignificant suppression at 0.0625 mg/kg.

There is agreement between the current paper and previous reports (Bergman et al., 2008; Paronis et al., 2013), at least regarding hypothermia, with a calculated ED_{50} of 0.1 mg/kg (Paronis et al., 2013). However, the latter report revealed significant hypothermia (at 0.1 mg/kg and higher) only after 240 min, whereas in the current paper hypothermia was found approximately 60 min after injection, but only at doses of at least 0.3125 mg/kg. Further assessment will be needed on a variety of different tasks to completely characterize the potency and profile of AM 4054.

Effects on the tetrad were reversed with AM 251, as evidenced by significant differences between animals pretreated with 2.0 or 4.0 mg/kg AM 251 and 0.3 mg/kg AM 4054, and those pretreated with vehicle and 0.3 mg/kg AM 4054. In an identical dosing regimen, 2.0 mg/kg AM 251 was found to reverse the effects of 5.0 mg/kg AM 411 on the tetrad (McLaughlin et al., 2005b). These findings corroborate reports of reversal of the hypothermic effect of AM 4054 with the CB1 inverse agonist rimonabant (Paronis et al., 2013), and the CB1 neutral antagonist AM 4113 (Bergman et al., 2008).

In the open field (Fig. 2), AM 4054 inhibited outside and inside crossings. As subjects were drug-naïve at the outset of testing, and were only tested once, an anxiogenic reaction to the novel drug state and open field environment is plausible. On the other hand, the significant reduction of both kinds of movement suggests that heightened anxiety alone cannot explain the pattern of locomotor suppression. Instead, as CB1 receptors are densely expressed in brain areas related to locomotion, such as nucleus accumbens, basal ganglia, and cerebellum (Egertová and Elphick, 2000), it is likely to be a direct motor effect of CB1 receptor stimulation.

Similarly, the suppression of operant responding corresponded to a large reduction in the fastest responses, even at relatively low doses (up to 0.125 mg/kg) that permitted several hundred responses in each session. Unlike other putative CB1 agonists such as AM 411 (McLaughlin et al., 2005b), CP 55,940, AM 356, ⁸-THC, or WIN 55,212-2 (Carriero et al., 1998), AM 4054 did not significantly increase the proportion of pause IRTs (Bin 21); however, as seen in Figure 5, a rightward shift is seen in the peak of the IRT distribution: 0.125 mg/kg produces a peak in the fourth bin (comprising IRTs between 750 and 1000 ms) and significantly fewer of the fastest IRTs, seen in Bins 1 and 2, indicating a substantial slowing of responses. The pattern of lever-pressing suppression seen with AM 4054 is therefore similar to that produced by other putative CB1 agonists in that fast responding is slowed dramatically (Carriero et al., 1998; McLaughlin et al., 2005b).

The most notable difference between AM 4054 and several other CB1 agonists is its potency. An ED₅₀ of 0.0998 mg/kg was calculated for the overall suppression of operant responding, and significant differences from vehicle were found at 0.125 mg/kg for lever pressing, 0.15625 mg/kg for catalepsy and open field locomotion, and 0.3125 mg/kg for the tail flick and hypothermia assays. These values are lower than the minimal significant dose of AM 356, ⁸-THC, and WIN 55,212-2 (Carriero et al., 1998) or AM 411 (McLaughlin et al., 2005b) on the FR5 task. AM 4054 therefore exhibits similar potency to CP 55,940, which has shown an ED₅₀ of approximately 0.07 mg/kg on suppression of FR5 responding, and in striking similarity, also produced significant differences from vehicle at 0.125 mg/kg and also abolished responding at doses of 0.25 and 0.5 mg/kg (Carriero et al., 1998). In contrast, a dose of at least 1.8 mg/kg ⁹-THC was found to be necessary to significantly reduce open field locomotion, and a dose of 5.6 mg/kg (Järbe et al., 2002) was needed to reduce activity to approximately the same extent as 0.15625 mg/kg of AM 4054 in the present paper. Therefore, AM 4054 likely exhibits a minimum 30-fold higher behavioral potency relative to ⁹-THC on suppression of locomotion. On the other hand, the potency of AM 4054 is slightly less than that of HU 210 (Ferrari et al., 1999) or AM 2389 (Paronis et al., 2013), although AM 4054 may be slightly more selective for the CB1 receptor relative to the CB2 receptor compared to HU 210 or CP 55,940, which may have similar potency for both cannabinoid receptor subtypes (Pertwee, 2005a). The potency of AM 4054 has recently been confirmed in other species; in the squirrel monkey, it suppressed FR30 responding with an ED₅₀ that was 37 times lower than that of ⁹-THC (Desai et al., 2013).

5. Conclusion

It is concluded that AM 4054 produces effects similar to putative CB1 agonists such as AM 411 (McLaughlin et al., 2005b), and has a behavioral potency similar to CP 55,940. Future studies should provide an additional behavioral characterization of AM 4054, and also should further elucidate its onset and duration of action. Operant lever-pressing tasks, such as that used in Experiment 3, provide a sensitive and reliable assay for such investigations (e.g. McLaughlin et al., 2010).

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Highlights

- AM 4054 is a novel, potent cannabinoid CB1 receptor agonist.
- We sought to characterize its behavioral effects in rats.
- AM 4054 induced analgesia, catalepsy, and hypothermia, and reduced locomotion.
- Effects on this tetrad of tasks were reversed by the CB1 inverse agonist AM 251.
- ED₅₀ for response inhibition was < 0.1 mg/kg, similar to most potent cannabinoids.

Fig. 1.

AM 4054 produces cannabinoid-like effects at relatively low doses. (a) AM 4054 reduced response to pain in the tail-flick test; (b) catalepsy is induced, as measured by increased latency to move forepaws from an elevated bar; (c) reduction in rectal temperature by AM 4054. * $p < .05$, ** $p < .01$ difference from vehicle-treated animals via planned comparisons.

Fig. 2.

Effects of AM 4054 on spontaneous locomotion in an open field are shown. Outside crossings (a; i.e., movement into or between areas along the walls) and inside crossings (b; i.e., movement into or between areas that are not adjacent to the walls) were counted separately to ascertain whether AM 4054 induced thigmotaxis. ** $p < .01$ reduction versus vehicle-treated controls.

Fig. 3.

Reversal of CB1 agonist-like effects on the cannabinoid tetrad using the CB1 inverse agonist AM 251. Based on the results of Experiment 1 (Figs. 1–2), a dose of 0.3 mg/kg AM 4054 was selected and produced significant differences from vehicle (** $p < .01$). Pretreatment with 2.0 and 4.0 mg/kg AM 251 prior to administration of AM 4054 significantly reversed (a) analgesia in the tail-flick test, (b) catalepsy, (c) hypothermia, and (d) suppression of locomotion. † $p < .05$, †† $p < .01$ difference between subjects treated with 2.0 or 4.0 mg/kg AM 251 prior to AM 4054, and those treated with vehicle prior to AM 4054.

Fig. 4. AM 4054 dose-dependently reduced lever-pressing on an FR5 schedule. ED_{50} of the effect was determined to be 0.0998 mg/kg. * $p < .05$ decrease from the vehicle condition.

Fig. 5.

Significant alteration of the interresponse time (IRT) distribution. The 0.25 and 0.5 mg/kg doses were excluded due to the near-total abolition of responding they produced. The remaining doses yielded a significant dose X IRT interaction, and a dose-dependent reduction in Bin 1 and Bin 2 (* $p < .05$ via one-way ANOVA of each bin). These results suggest that the suppression in responding seen at these doses may be due to a slowing of the fastest responses.