Cannabimimetic Properties of Ajulemic Acid

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ABSTRACT

Side effects of marijuana-based drugs and synthetic analogs of Δ⁹-tetrahydrocannabinol (Δ⁹-THC), including sedation and dysphoria, have limited their therapeutic application. Ajulemic acid (AJA), a side-chain synthetic analog of Δ⁹-THC-11-oic acid, has been reported to have anti-inflammatory properties without producing undesired psychoactive effects. Moreover, it has been suggested that AJA does not interact with cannabinoid receptors to produce its pharmacological effects. The aim of the present study was to conduct a thorough evaluation of the pharmacological effects of AJA then to determine whether actions at cannabimimetic receptor (CB), mediated these effects. This study evaluated the psychoactive and analgesic effects of AJA by examining its cannabimimetic properties in ICR mice (i.e., antinociception, catalepsy, hypothermia, and hypomobility), its discriminative stimulus effects in Long Evans rats trained in a two-lever Δ⁹-THC (3.0 mg/kg) versus vehicle drug discrimination procedure, and its antihyperalgesia effects in a rat model of inflammatory pain [complete Freund’s adjuvant (CFA)-induced mechanical hyperalgesia]. Lastly, antagonism tests with SR 141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride], CB, receptor antagonist, were conducted. These studies demonstrated that AJA shares a number of CB₁-medi- 
ed pharmacological properties with Δ⁹-THC, including cannabimimetic, discriminative stimulus, and antihyperalgesic effects. Furthermore, a separation between doses that produced antinociception and those that produced the other pharmacological effects in mice was not observed. Moreover, AJA showed nearly equipotency for therapeutic efficacy in the CFA model and for substitution in Δ⁹-THC discrimination. In summary, this study shows that AJA, like Δ⁹-THC, exhibits psychoactive and therapeutic effects at nearly equal doses in preclinical models, suggesting similar limitations in their putative therapeutic profiles.

Cannabis sativa (marijuana plant) has been used since antiquity for its presumed therapeutic, as well as for its euphoric effects. Although Δ⁹-tetrahydrocannabinol (Δ⁹-THC) has been identified as the major psychoactive ingredient in C. sativa (Gaoni and Mechoulam, 1971), difficulty in dissociating unwanted side effects, such as sedation and psychotropic effects, from therapeutic effects has limited clinical application of Δ⁹-THC-based drugs. For example, dronabinol, an orally administered synthetic version of Δ⁹-THC, has been developed as an appetite stimulant and antiemetic for use in chronic diseases such as AIDS and cancer (for review, see Ben Amar, 2006). In addition, recent evidence suggests oral Δ⁹-THC may be effective as an adjunct to opioid analgesics (Roberts et al., 2006). The therapeutic utility of Δ⁹-THC, however, has been limited due to patient complaints of dysphoria and unpleasant subjective effects (Ben Amar, 2006). Previous research has suggested that Δ⁹-THC carboxylic acid, one of the acid metabolites of Δ⁹-THC, lacks psychoactive properties of the parent compound and yet retains antinociceptive and other effects (Burstein et al., 1988; Doyle et al., 1990). Since this metabolite has a relatively low potency, structural changes that increased potency and stability of Δ⁹-THC analogs in previous structure-activity relationship studies (Loe et al., 1973; Mechoulam et al., 1988; Compton et al., 1993) were applied to the structure Δ⁹-THC carboxylic acid. The resulting compound, ajulemic acid (AJA), substitutes a 1’1-dimethylheptyl side chain for the pentyl group of Δ⁹-THC and changes the Δ⁹-THC core structure to a more stable confirmation, Δ⁹-THC (Fig. 1).

To date, the efficacy of AJA has been demonstrated in numerous pain and inflammation studies (for review, see Wiley, 2005). These findings are equivocal, however, in that effects have been found with a single dose or a restricted range of doses or have been reported in different strains or ABBREVIATIONS: Δ⁹-THC, Δ⁹-tetrahydrocannabinol; AJA, ajulemic acid (1’1’-dimethylheptyl-Δ⁹-tetrahydrocannabinol-11-oic acid); CFA, complete Freund’s adjuvant; MPE, percentage of maximal possible effect in tail-flick test; SR 141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride; SR144528, N-[1(S)-endo-1,3,3-trimethyl bicyclo [2,2,1] heptan-2-yl]-5-(4-chloro-3-methyl-phenyl)-1-(4methylbenzyl)-pyrazole-3-carboxamide; FR-10, fixed-ratio 10; VEH, vehicle; CB, cannabinoid receptor; ANOVA, analysis of variance; CL, confidence limit.
Effects of AJA were compared with same species and with similar dosing parameters. First, the more fully the analgesic and side-effect profile of AJA in the range. Therefore, the present study was designed to elucidate might be either present or absent in the therapeutic dose not included a careful evaluation of behavioral effects that used similar methods (Mitchell et al., 2005). Potencies for AJA reported in two of these studies (Burstein et al., 1998; Dyson et al., 2005), showed that AJA reduced experimentally induced pain or inflammation in rats (Burstein et al., 1998; Dyson et al., 2005; Mitchell et al., 2005). Potencies for AJA reported in two of these studies (Burstein et al., 1998; Dyson et al., 2005), however, were 10-fold greater in magnitude than the single dose reported to be effective in the only other published study that used similar methods (Mitchell et al., 2005).

Unfortunately, previous analgesic studies with AJA have not included a careful evaluation of behavioral effects that might be either present or absent in the therapeutic dose range. Therefore, the present study was designed to elucidate more fully the analgesic and side-effect profile of AJA in the same species and with similar dosing parameters. First, the effects of AJA were compared with Δ9-THC in mice in a well established tetrad of tests, in which cannabinoids produce antinociception (“therapeutic effect”) and locomotor suppression, hypothermia, and catalepsy (presumed indices of “side effects”) (Martin et al., 1991). Second, doses of AJA that produced antihyperalgesic effects in a rat model of chronic pain were compared with those that produced substitution for Δ9-THC in a rat drug discrimination procedure. Since the results of Δ9-THC drug discrimination in animals has been shown to predict marijuana-like intoxication in humans (Balster and Prescott, 1992), the combined use of these two procedures (rat model of chronic pain and Δ9-THC discrimination) were used to determine the degree of separation between a therapeutic effect (analgesia) of AJA and its unwanted psychotropic effects in rats. Taken together, these studies represent a thorough pharmacological evaluation of AJA, a synthetic analog of a Δ9-THC metabolite, that addresses the question of whether or not the analgesic effects of this compound can be accessed without inducing unwanted side effects typically associated with Δ9-THC.

Subjects

Male ICR mice (25–32 g), purchased from Harlan (Dublin, VA), were housed in groups of five. All mice were kept in a temperature-controlled (20–22°C) environment with a 14/10-h light/dark cycle and received food and water ad libitum. Separate mice were used for testing each drug dose in the in vivo procedures.

Naïve adult male Long Evans rats obtained from Harlan were individually housed in a temperature-controlled (20–22°C) vivarium with a 12-h light/dark cycle. All rats were allowed to acclimate to the vivarium for at least a week before the experiments commenced. During the drug discrimination studies, rats were maintained within a weight range of 400 to 450 g by restricted postsession feeding. They had ad libitum water in their home cages. Separate rats were used for the CFA studies. These rats had food and water ad libitum in their home cages, except during test sessions. Guidelines of the International Association for the Study of Pain (IASP, 1983), regarding the ethics of animal experimentation, were followed throughout the study. All animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Virginia Commonwealth University and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press, 1996).

Apparatus

Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH). A standard tail-flick apparatus and a digital thermometer (Fisher Scientific, Pittsburgh, PA) were used to measure antinociception and rectal temperature, respectively. The ring immobility device consisted of an elevated metal ring (diameter, 5.5 cm; height, 16 cm) attached to a wooden stand.

For the drug discrimination studies, rats were trained and tested in standard operant conditioning chambers (BR5/LVE Inc., Laurel, MD or Lafayette Instruments Co., Lafayette, IN) housed in sound-attenuated cubicles. Pellet dispensers delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a food cup on the front wall of the chamber between two response levers. Fan motors provided ventilation and masking noise for each chamber. House lights located above the food cup were illuminated during training and testing sessions. A microcomputer with Logic 1 Interface (MED Associates, Georgia, Vermont) and MED-PC software (MED Associates) were used to control schedule contingencies and to record data.

Noxious testing in CFA-treated rats occurred in response to mechanical stimulation of the hindpaw. A mechanical stimulus was applied to the right (affected) hindpaw with an analgesy meter (Ugo Basile, Varese, Italy), a device with a dome-shaped plastic tip (diameter, 1 mm) that applies a linearly increasing pressure (acceleration caused by gravity) to the dorsal surface of the hindpaw, with the tip applied to the region of the paw just proximal to the third digit. The amount of force (acceleration caused by gravity) required to elicit a withdrawal attempt was recorded.

Procedure

Mouse Tetrad. Before testing in the pharmacological procedures, mice were acclimated to the experimental setting (ambient temperature, 22–24°C) for at least 1 h. The effects of oral and i.v. administration of Δ9-THC and AJA on spontaneous locomotion, tail-flick, ring immobility, and rectal temperature (the “tetrad”) were assessed.

Preinjection control values were determined for rectal temperature and tail-flick latency (in seconds). Five minutes following i.v. or 15 min following p.o. administration, individual mice were placed into a wooden stand. Fan motors provided ventilation and masking noise for each chamber. House lights located above the food cup were illuminated during training and testing sessions. A microcomputer with Logic 1 Interface (MED Associates, Georgia, Vermont) and MED-PC software (MED Associates) were used to control schedule contingencies and to record data.

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inhibition of activity of the vehicle group. Then, tail-flick latencies were redetermined 35 min after drug administration. Antinociception was calculated as percentage of maximal possible effect [%MPE = ([test − control latency]/[10 − control]) × 100]. Control latencies typically ranged from 1.5 to 4.0 s. Rectal temperatures were reassessed 45 min after drug administration. Rectal temperature values were expressed as the difference between control temperature (before injection) and temperatures following drug administration (change in degrees Celsius). Next, at 55 min postdrug administration, mice were placed on the ring immobility apparatus for 5 min. The total amount of time (in seconds) that the mouse remained motionless was measured. This value was divided by 300 s and multiplied by 100 to obtain a percentage immobility rating. The criterion for ring immobility was the absence of all voluntary movement, including snout and whisker movement. Different mice were tested with each dose of each drug. For antagonist tests, SR 141716A (3 and 10 mg/kg), SR144528 (10 mg/kg), or vehicle were administered i.v. 10 min before an i.v. injection of Δ^2-THC (10 mg/kg) or AJA (10 mg/kg; a dose that rendered maximal effects when administered alone).

**Drug Discrimination.** All rats were trained to press one lever following administration of 3 mg/kg Δ^2-THC and to press another lever after injection with vehicle (1:1.18 ratio of Emulphor:ethanol: saline), each according to a fixed-ratio 10 (FR-10) schedule of food reinforcement. Completion of 10 consecutive responses on the injection-appropriate lever resulted in delivery of a food reinforcer. Each response on the incorrect lever reset the response requirement on the correct lever. The position of the drug lever was varied among the group of rats. The daily injections for each rat were administered in a double alternation sequence of training drug and vehicle. Rats were injected and returned to their home cages until the start of the experimental session. Training occurred during 15-min sessions conducted 5 days a week (Monday to Friday) until the rats had met three criteria during 8 of 10 consecutive sessions: first completed FR-10 on the correct lever, percentage of correct lever responding greater than 80% for the entire session, and response rate greater than 0.4 responses/s.

Following successful acquisition of the discrimination, stimulus substitution tests with test compounds were conducted on Tuesdays and Fridays during 15-min test sessions. Training continued on Mondays, Wednesdays, and Thursdays. During test sessions, responses on either lever delivered reinforcement according to an FR-10 schedule. To be tested, rats must have completed the first FR and made at least 80% of all responses on the injection-appropriate lever on the preceding day’s training session. In addition, the rat must have met these same criteria during at least one of the training sessions with the alternate training compound (Δ^2-THC or vehicle) earlier in the week. Dose-effect determinations with the training drug Δ^2-THC and, then, with AJA were conducted in each rat. Doses of each compound were administered in ascending order. Subsequently, antagonist tests were conducted with Δ^2-THC and AJA to evaluate the ability of SR 141716A or SR144528 to block the Δ^2-THC-like discriminative stimulus effects of AJA. Throughout the study, control tests with vehicle and the training drug were conducted during the week before the start of each dose-effect curve determination.

**Nociceptive Testing in Complete Freund’s Adjuvant-Treated Rats.** Before induction of the arthritic or nonarthritic state on day 0, 14 male rats went through a habituation procedure that consisted of being handled, weighed, and exposed to the paw pressure test on four separate occasions prior to the induction of the arthritic or nonarthritic state on day 0. Subsequently, eight rats were injected s.c. with 0.1 ml of 5.0 mg/ml CFA (heat-killed Mycobacterium; Difco Laboratories, Detroit, MI), and six rats were injected s.c. with 0.1 ml of vehicle (VEH) (mineral oil) in the dorsal surface of the right hindpaw under light isoflurane anesthesia. On day 0, the day of the CFA/VEH injection, the mean weight of the rats was 273 g (±4.3 S.E.M.).

Nociceptive testing occurred between days 12 and 28, inclusive. Before each test session, paw thickness measurements (millimeters) of the hindpaws were obtained with a digital caliper. Then, each rat was lightly restrained in a towel for testing. Preliminary tests indicated that in VEH- and CFA-treated rats, injection of the vehicle followed by paw pressure testing at 30-min intervals for up to six times resulted in no systematic change in paw pressure thresholds; hence, Δ^2-THC and AJA were tested using a cumulative dosing schedule. Δ^2-THC was tested on day 12, and AJA was tested on day 14. Immediately after the baseline, paw pressure threshold of the right hindpaw was recorded, rats were injected with the first dose of Δ^2-THC or AJA, and thresholds of the right hindpaw were redetermined 30 min later. Immediately after these tests, the next drug dose was administered such that each successive dose increased the total drug concentration by 0.5 log units. This cycle of drug administration followed by threshold determination continued every 30 min until maximal antinociception was achieved or until the largest dose to be tested was administered. Solubility issues with AJA prevented testing doses higher than 30 mg/kg.

Antagonism studies were conducted in CFA-treated rats using the CB1 receptor antagonist SR 141716A (10 mg/kg). On day 26, following the determination of paw thickness measurements, baseline paw pressure thresholds were determined for the right hindpaw. Next, SR 141716A was injected, and thresholds of the right hindpaw were redetermined 30 min later. Subsequently, the highest dose of AJA (30 mg/kg) was injected, and 30 min later, thresholds were redetermined. On day 28, a similar testing protocol was used with the exception that a dose of 10 mg/kg Δ^2-THC was administered instead of 30 mg/kg AJA.

**Drugs.** Δ^2-THC (National Institute on Drug Abuse, Rockville, MD) was suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ), and saline in a ratio of 1:1:18. SR 141716A (National Institute on Drug Abuse) and AJA (Organix, Inc., Woburn, MA) were also mixed in 1:1.18 vehicle. For the drug discrimination and the CFA studies, Δ^2-THC or AJA were injected i.p. 30 min pretest. For behavioral tests with mice, all drugs were administered i.v. in the tail vein or p.o. by oral gavage, as indicated, at a volume of 0.1 ml/10 g. Preession injection intervals for each drug were chosen based upon previous research with these drugs in our lab or on values obtained in the literature and were as follows. For i.v. and p.o. dose-effect determinations in the mouse tetrad, mice were injected 5 or 15 min, respectively, prior to the start of tests. For antagonist tests in the tetrad, mice were injected i.v. with SR 141716A, SR144528, or vehicle followed 10 min later by injection with Δ^2-THC, AJA, or vehicle. For antagonist tests in drug discrimination, rats were injected i.p. with SR 141716A, SR144528, or vehicle followed 10 min later by injection with Δ^2-THC or AJA or vehicle.

**Data Analysis**

**Mouse Tetrad.** Antinociception was calculated as percentage of maximal possible effect [%MPE = ([test − control latency]/[10 − control]) × 100]. Rectal temperature values were expressed as the difference between control temperature (before injection) and temperatures following drug administration (change in degrees Celsius). The percentage inhibition was calculated as the difference between baseline paw pressure thresholds; hence, Δ^2-THC and AJA were tested using a cumulative dosing schedule. Δ^2-THC was tested on day 12, and AJA was tested on day 14. Immediately after the baseline, paw pressure threshold of the right hindpaw was recorded, rats were injected with the first dose of Δ^2-THC or AJA, and thresholds of the right hindpaw were redetermined 30 min later. Immediately after these tests, the next drug dose was administered such that each successive dose increased the total drug concentration by 0.5 log units. This cycle of drug administration followed by threshold determination continued every 30 min until maximal antinociception was achieved or until the largest dose to be tested was administered. Solubility issues with AJA prevented testing doses higher than 30 mg/kg.

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change in rectal temperature was estimated at $-6^\circ\text{C}$, and maximal percentage ring immobility was 60%. ANOVAs were conducted separately for p.o. and i.v. tests. Dunnett’s test was used for post hoc comparison when appropriate. Antagonism tests also were evaluated with ANOVAs as a function of drug followed by Dunnett’s test when appropriate.

**Drug Discrimination.** For each test session, percentage of responses on the drug lever and response rate (responses per second) was calculated. Full substitution was defined as $\leq 80\%$ $\Delta^8$-THC lever responding. ED$_{50}$ values were calculated separately for each drug using least-squares linear regression analysis, followed by calculation of 95% confidence limits by the method of Bliss (1967). Potency ratio values with a 95% confidence interval were calculated by the method of Colquhoun (1971). Repeated measures ANOVAs with Dunnett’s post hoc tests ($\alpha = 0.05$) were used to determine differences in drug lever responding during antagonism tests and response rates both compared with vehicle control. Since rats that responded less than 10 times during a test session did not press either lever a sufficient number of times to earn a reinforcer, their drug lever selection data were excluded from analysis, but their response rate data were included in mean response rate.

**Nociceptive Testing Complete Freund’s Adjuvant-Treated Rats.** Right hindpaw thickness measurements and baseline paw pressure thresholds for the right hindpaw of VEH- and CFA-treated rats were averaged across the test days, and differences in the paw thickness and paw pressure between VEH- and CFA-treated rats were determined using a Student’s $t$ test. For dose-effect testing in VEH- and CFA-treated rats, right hindpaw pressure thresholds following administration of the $\Delta^8$-THC or AJA were converted to a percentage of a maximal possible effect based upon a maximal 500g of pressure using the following equation: $\%$ antinociception = [(observed – baseline)/500g – baseline)] × 100. Antinociception was operationally defined as drug-induced increases in paw pressure thresholds above nondrug baseline thresholds with 100% antinociception equal to 500g. The dose-effect data from CFA-treated rats were also analyzed to determine the antihyperalgesic effects of $\Delta^8$-THC and AJA.

Antihyperalgesia was operationally defined as drug-induced increases in paw pressure thresholds in CFA-treated rats above nondrug baseline thresholds with 100% antihyperalgesia equal to 500g. The dose-effect data from CFA-treated rats were also analyzed to determine the antihyperalgesic effects of $\Delta^8$-THC and AJA.

Differences between the baseline right hindpaw pressure thresholds and thresholds following SR141716A alone and SR141716A in combination with $\Delta^9$-THC or AJA in CFA-treated rats were determined using a repeated measures ANOVA followed by Bonferroni post hoc test. The significance level was set at $p < 0.05$.

**Results**

**Mouse Tetrad Dose-Effect Testing of $\Delta^9$-THC and AJA following p.o. and i.v. Administration.** $\Delta^9$-THC and $\Delta^8$-THC active in all four tests following p.o. (Fig. 2) and i.v. (Fig. 3) administration. With each route of administration, both drugs significantly ($p < 0.05$) and dose-dependently decreased spontaneous activity and rectal temperature and produced antinociception and catalepsy. ED$_{50}$ values for $\Delta^9$-THC and AJA were consistently higher following p.o. administration (Table 1) than after i.v. injection (Table 2). To assess the separation between analgesia and other

**Fig. 2.** Effects of oral gavage administration of $\Delta^9$-THC and AJA on spontaneous activity (A), antinociception (B), rectal temperature (C), and catalepsy (D). Mice were pretreated p.o. with $\Delta^9$-THC or AJA 15 min prior to the beginning of tests. Values represent the mean (±S.E.M.) of 5 to 12 mice per group. + and #, significant difference for $\Delta^9$-THC or AJA, respectively ($p < 0.05$, Dunnett post hoc test) relative to the vehicle condition.
pharmacological effects, potency ratios of antinociception to other effects were calculated. These potency ratios revealed that orally administered AJA was equally potent at producing antinociception and catalepsy but was significantly more potent at producing antinociception than at decreasing spontaneous activity (Table 1). With i.v. administration, AJA was equipotent at decreasing spontaneous activity and producing antinociception but was significantly more potent at producing antinociception than at producing catalepsy (Table 2). In comparison, oral Δ⁹-THC produced a similar pattern of potency ratios as oral AJA, although the exact values were different. Intravenously administered Δ⁹-THC produced antinociception and catalepsy at approximately equal potencies but was significantly less potent at producing antinociception than at decreasing spontaneous activity.

To determine whether the cannabimimetic effects induced by Δ⁹-THC and AJA were mediated via CB₁ or CB₂ receptors, a dose of 10 mg/kg Δ⁹-THC or AJA, shown to produce maximal effects in the mouse tetrad, was administered i.v. followed by injections of vehicle, 3 or 10 mg/kg SR141716A, or 10 mg/kg SR144528. As shown in Table 3, SR141716A dose-dependently blocked all four of the cannabimimetic effects of both Δ⁹-THC and AJA. In contrast, the CB₂ antagonist

### Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Δ⁹-THC Potency Ratio (Compared with Antinociception)</th>
<th>AJA Potency Ratio (Compared with Antinociception)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀ in mg/kg (95% CL)</td>
<td>ED₅₀ in mg/kg (95% CL)</td>
</tr>
<tr>
<td>SA</td>
<td>40 (22–74)</td>
<td>108 (66–175)</td>
</tr>
<tr>
<td>MPE</td>
<td>15 (10–23)</td>
<td>37 (29–46)</td>
</tr>
<tr>
<td>RI</td>
<td>&lt;10</td>
<td>1.6 (0.7–4.8, N.S.)</td>
</tr>
<tr>
<td>RT</td>
<td>33 (25–44)</td>
<td>59 (46–75)</td>
</tr>
</tbody>
</table>

N.S., not significant; N.D., not determined; SA, percent inhibition of spontaneous activity; RT, change in rectal temperature in degrees Celsius; RI, ring immobility. ED₅₀ for each drug are expressed in milligrams per kilogram with 95% confidence intervals in parentheses. Potency ratios (and 95% confidence limits) for each drug were calculated with respect to the ED₅₀ for producing antinociception.

* Potency for the measure is significantly different than potency for producing antinociception (p < 0.05).

### Table 2

<table>
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<th>AJA Potency Ratio (Compared with Antinociception)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀ in mg/kg (95% CL)</td>
<td>ED₅₀ in mg/kg (95% CL)</td>
</tr>
<tr>
<td>SA</td>
<td>0.5 (0.2–1.2)</td>
<td>2.5 (1.5–4.3)</td>
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<tr>
<td>MPE</td>
<td>1.3 (0.8–2.0)</td>
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<tr>
<td>RI</td>
<td>1.0 (0.7–1.9)</td>
<td>3.1 (2.2–4.5)</td>
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<tr>
<td>RT</td>
<td>1.0 (0.5–2.2)</td>
<td>3.5 (2.1–5.9)</td>
</tr>
</tbody>
</table>

N.S., not significant; N.D., not determined; SA, percent inhibition of spontaneous activity; RT, change in rectal temperature in degrees Celsius; RI, ring immobility. ED₅₀ for each drug are expressed in milligrams per kilogram with 95% confidence intervals in parentheses. Potency ratios (and 95% confidence limits) for each drug were calculated with respect to the ED₅₀ for producing antinociception.

* Potency for the measure is significantly different than potency for producing antinociception (p < 0.05).
SR144528 (10 mg/kg) failed to block any of these cannabimimetic effects.

**Drug Discrimination.** Both $\Delta^8$-THC and AJA fully and dose-dependently substituted for $\Delta^9$-THC, with $ED_{50} = 0.5$ (95% CL, 0.3–1) and 3.6 (95% CL, 2.1–6.3) mg/kg, respectively (Fig. 4, top). SR 141716A (10 mg/kg; $p < 0.05$) blocked the $\Delta^9$-THC-like discriminative stimulus effects exhibited by AJA (10 mg/kg), whereas SR144258 (10 mg/kg) did not ($p > 0.05$). Response rates were not significantly decreased by any dose of $\Delta^8$-THC whereas a significant response rate decrease was observed following administration of the 30 mg/kg dose of AJA ($p < 0.05$). Hence, for both AJA and $\Delta^8$-THC, complete substitution was observed at doses that did not produce significant response rate suppression.

**Paw Thickness and Baseline Paw Pressure Thresholds in VEH- and CFA-Treated Rats.** A significant difference in mean right hindpaw thickness between VEH- and CFA-treated rats was observed across the test days (3.9 ± 0.1 versus 7.5 ± 0.3 mm, respectively). Baseline paw pressure thresholds were significantly different between VEH- and CFA-treated rats across the test days (143 ± 10 g versus 73 ± 6 g, respectively; $p < 0.05$).

**Dose-Effect Testing of $\Delta^8$-THC and AJA in VEH- and CFA-Treated Rats.** In CFA-treated rats, both $\Delta^8$-THC and AJA produced dose-dependent increases in antinociception with $\Delta^8$-THC producing a 97% antinociceptive effect at a dose of 3.0 mg/kg ($ED_{50}$ value, 0.09 mg/kg (95% CL, 0.05–0.16)), whereas AJA produced a 67% antinociceptive effect at the highest dose tested (30 mg/kg) ($ED_{50}$ value, 5.6 mg/kg; 95% CL, 5.6–24) (Fig. 5).

Based upon a 500g maximal possible effect, $\Delta^8$-THC produced dose-dependent increases in antinociception in CFA-treated rats ($ED_{50}$ value, 4 mg/kg; 95% CL, 2.7–6.1), whereas in VEH-treated rats, $\Delta^8$-THC’s maximal effect was less than 45% (data not shown). AJA failed to produce marked antinociception in VEH- or CFA-treated rats (data not shown). Analysis of the maximal antinociceptive effect at the highest dose tested for $\Delta^8$-THC and AJA in VEH- and CFA-treated rats indicate that there was a main effect of drug treatment, such that the antinociceptive effect obtained with $\Delta^8$-THC in CFA-treated rats (75 ± 13%) was greater than the antinociceptive effects of AJA in VEH- and CFA-treated rats ($p < 0.05$).

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**Table 3**

<table>
<thead>
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<th></th>
<th>THC (10 mg/kg)</th>
<th>AJA (10 mg/kg)</th>
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<tr>
<td></td>
<td>Veh</td>
<td>SR1–3</td>
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<tr>
<td>SA</td>
<td>79 (8.9)</td>
<td>32 (15)</td>
</tr>
<tr>
<td>MPE</td>
<td>88 (13)</td>
<td>74 (12)</td>
</tr>
<tr>
<td>RI</td>
<td>46 (5.6)</td>
<td>11* (5.5)</td>
</tr>
<tr>
<td>RT</td>
<td>-4.1 (0.5)</td>
<td>-1.3* (0.3)</td>
</tr>
</tbody>
</table>

*Significant difference ($p < 0.05$, Dunnett post hoc test) relative to the vehicle-$\Delta^8$-THC condition.

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**Fig. 4.** Effects of AJA and $\Delta^8$-THC on percentage of $\Delta^8$-THC lever responding (top) and response rates (bottom) in rats trained to discriminate 3.0 mg/kg $\Delta^8$-THC from vehicle. Points above VEH and $\Delta^8$-THC represent the results of control tests with vehicle and 3 mg/kg $\Delta^8$-THC conducted before each dose-effect determination. Points above SR 141716A and SR144258 represent the antagonism tests with 10 mg/kg SR 141716A or 10 mg/kg SR144258, respectively, administered prior to an injection of 10 mg/kg AJA. For each dose-effect curve determination, values represent the mean (±S.E.M.) of seven to eight rats except for percentage of $\Delta^8$-THC lever responding at 30 mg/kg AJA, $n = 5$, and during the antagonism tests, $n = 5$.

**Fig. 5.** Antihyperalgesic effects of $\Delta^8$-THC and AJA in CFA-treated rats ($n = 10$). This graph represents the ability of each drug to return baseline thresholds of CFA-treated rats to the baseline thresholds of VEH-treated rats. For tests with $\Delta^8$-THC, baseline thresholds for VEH- and CFA-treated rats were 127 and 70 g, respectively. For tests with AJA, baseline thresholds for VEH- and CFA-treated rats were 141 and 65 g, respectively. Therefore, 100% antinociception for $\Delta^8$-THC and AJA represent 127 and 142 g, respectively.
ceptive effect obtained with $\Delta^9$-THC in VEH-treated rats (17 ± 5%) and was greater than the antinociceptive effect obtained with AJA in VEH-treated (12 ± 3%) and CFA-treated (30 ± 13%) rats.

**Antagonism of $\Delta^9$-THC and AJA with SR 141716A.** SR 141716A antagonized the $\Delta^9$-THC and AJA-induced increases in paw pressure thresholds in CFA-treated rats (Table 4). Thresholds following SR 141716A administration alone and in combination with $\Delta^9$-THC were not significantly different from baseline paw pressure thresholds. Thresholds following SR 141716A administration alone and in combination with AJA were not significantly different from baseline paw pressure thresholds.

**Discussion**

Similar to previous studies (Compton et al., 1992, 1993; Wiley et al., 1998; Martin et al., 1999, 2002), this study demonstrated that $\Delta^9$-THC produced characteristic cannabinoid pharmacological effects in mice, including antinociception, catalepsy, hypothermia, and hypomobility (Martin et al., 1991). This cannabinimimetic profile occurred with oral and i.v. routes of administration, with $\Delta^9$-THC being approximately 8- to 76-fold more potent following i.v. administration. AJA shared $\Delta^9$-THC’s profile of cannabinoid effects in mice after oral and i.v. administration and was also more potent after i.v. injection. These results are consistent with their moderate binding affinities for CB$_1$ receptor ($K_i$ = 32.3 nM for AJA and 80.3 nM for $\Delta^9$-THC) (Rhee et al., 1997).

Previous studies with AJA have shown that it produces antinociceptive effects in acute models of pain, including hot-plate, formalin, and writhing tests and tail clip in the mouse (Burstein et al., 1992, 1998; Sumarivallwa et al., 2004; Dyson et al., 2005) and in rats using the hot-plate and tail-clip tests (Dajani et al., 1999). In addition, previous reports have shown decreased activity or other indication of motor impairment; however, in most studies, but not all (Sumarivallwa et al., 2004; Mitchell et al., 2005), these deficits in motor behavior occurred at doses higher than the antinociceptive ED$_{50}$ dose (Dajani et al., 1999; Dyson et al., 2005). Although these results seem to indicate therapeutic selectivity or separation of effects, most previous studies did not include full dose-effect determinations. Our results are consistent with previous reports in that AJA produced antinociceptive and locomotor suppressive effects; however, unlike some of the other studies, our data indicate little separation of antinociceptive and other pharmacological effects including locomotor suppression, hypothermia, and catalepsy. Potency ratios for AJA showed that, across routes of administration, potency for either decreasing activity (i.v.) or inducing catalepsy (p.o.) did not differ significantly from potency to produce antinociception. These results suggest that AJA may be efficacious as an analgesic agent in acute pain (as is $\Delta^9$-THC), but that it is likely to produce side effects such as sedation. This pharmacological profile is similar to that observed with dronabinol, synthetic $\Delta^9$-THC (for review, see Ben Amar, 2006).

Several mechanisms of actions for the various pharmacological effects of AJA have been suggested, including activation of CB$_1$ or CB$_2$ receptors (Rhee et al., 1997), interaction with peroxisome proliferated-activated receptor $\gamma$ (Liu et al., 2003), and inhibition of cyclooxygenase-2 or 5-lipoxygenase (Zurier et al., 1998). The present study demonstrates that AJA acts at the CB$_1$ receptor to produce typical cannabinimimetic effects and that peripheral CB$_2$ receptors do not play a role in these effects. Specifically, the CB$_1$ receptor antagonist SR 141716A attenuated the ability of AJA to produce antinociception and catalepsy and to decrease spontaneous activity and rectal temperatures, whereas the CB$_2$ antagonist, SR144528, did not. The present study underscores the ability of the mouse tetrad to reveal CB$_1$-mediated cannabinimimetic profiles of novel drugs such as AJA. Similarity of tetrad effects, however, may be limited in its application as a tool for determining therapeutic indices of novel compounds. The tetrad has been shown to reveal false positives in classes of drugs pharmacologically distinct from cannabinoids (e.g., central nervous system depressants and antipsychotic drugs), although positive results across tests are less consistent for noncannabinoid drugs, and their effects are not blocked by the CB$_1$ antagonist SR 141716A (Wiley and Martin, 2003).

Although some promise has been demonstrated for AJA as an acute antinociceptive agent, much research has focused on AJA’s effects in chronic pain conditions. In rats, Mitchell et al. (2005) tested a single dose of AJA (10 mg/kg) and demonstrated that it reduced allodynia in neuropathic and inflammatory pain models. Interestingly, this dose was smaller than the 30 mg/kg dose of AJA that reversed CFA-induced hyperalgesia in the present study. Another important finding from the current study is that SR 141716A antagonized AJA-induced increases in paw pressure thresholds in CFA-treated rats. This finding is consistent with the report by Dyson et al. (2005) that demonstrated that SR 141716A reversed the effects of AJA on the mechanical hyperalgesia induced by partial sciatic ligation. As discussed above, the mechanism by which AJA produces its therapeutic effects remains unclear. However, the blockade of AJA-induced increases in paw pressure thresholds of pain threshold by SR 141716A in the present study supports the notion that AJA may be involved in the antihyperalgesic effects of AJA.

To date, preclinical research has shown that AJA is a potentially promising therapeutic agent for the treatment of chronic inflammation and pain. The present study supports the notion by demonstrating that AJA produced an antihyperalgesic effect at the highest dose tested, although this was not a complete blockade of pain. Given that AJA and $\Delta^9$-THC

### TABLE 4

<table>
<thead>
<tr>
<th>Cannabinoid Drug</th>
<th>Baseline (Predrug)</th>
<th>SR 141716A (10 mg/kg)</th>
<th>SR 141716A (10 mg/kg) and Drug</th>
<th>THC or AJA (Alone)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC (10 mg/kg)</td>
<td>71 (13)</td>
<td>79 (13)</td>
<td>77 (7.5)</td>
<td>391 (56)</td>
</tr>
<tr>
<td>AJA (30 mg/kg)</td>
<td>83 (6.1)</td>
<td>77 (6.4)</td>
<td>89 (9.6)</td>
<td>193 (57)</td>
</tr>
</tbody>
</table>

*Data represent the effects of THC of AJA alone as determined in the cumulative dosing procedure.
showed promising antinociceptive/antihyperalgesic effects in CFA and other models of chronic pain in the present study and others (Burstein et al., 1998; Zurier et al., 1998; Dajani et al., 1999; Mitchell et al. 1998, 2005), determination of the degree to which AJA also shares Δ9-THC’s intoxicating effects is crucial.

This crucial evaluation was determined using Δ9-THC versus vehicle two-lever drug discrimination, a well accepted animal model of subjective effects of marijuana in humans (Balster and Prescott, 1992). In contrast with the tetrad animal model of subjective effects of marijuana in humans, the vehicle two-lever drug discrimination, a well accepted degree to which AJA also shares psychoactive properties at therapeutic doses (Burstein et al., 2004), the present study demonstrates that AJA shares Δ9-THC’s discriminative stimulus properties, suggesting that it may produce intoxicating side effects in humans. Previously, Martin et al. (1995) demonstrated that in a study of 11-nor-Δ9-THC dimethylheptyl carboxylic acid fully substituted for Δ9-THC at a dose of 10 mg/kg. This compound only differs from AJA in that the double bond within the aromatic ring is in the ninth position for 11-nor-Δ9-THC dimethylheptyl carboxylic acid, whereas it is in the eighth position in AJA. Unfortunately, in that study, only one dose was tested; thus, comparisons of ED50 values are impossible. In this study, AJA produced full and dose-dependent substitution, and nearly equal ED50 values were obtained for discriminative stimulus effects (substitution for Δ9-THC) and therapeutic effects (antihyperalgesia in the CFA model). Interestingly, AJA did not produce antinociception in nonarthritic rats, whereas Δ9-THC did. The results of the present study also do not support the hypothesis that the role of CB1 receptors is insignificant at therapeutically relevant doses of AJA (Burstein et al., 2004) since its discriminative stimulus effects and antihyperalgesic effects were blocked by the CB1 receptor antagonist, SR 141716A.

AJA has been tested in phase I and phase II clinical trials. Results from published clinical trials were inconclusive. Although the authors reported analgesia in neuropathic pain patients in the absence of psychotropic effects (Karst et al., 2003), this conclusion is somewhat misleading because it is likely that subthreshold doses were used (for further discussion, see Wiley, 2005). The absence of psychotropic effects in these patients is clear from presentation of the incidence of side-effect profile; however, AJA showed only minimal efficacy, if any, as an analgesic. AJA-induced analgesic effects were statistically significant only for one of the two daily assessments. Only a trend was evident for the other time point. In addition, the magnitude of the significant effect was notably low and approximated the magnitude of changes in analgesia that were observed with different sequences of presentation of drug and vehicle in this crossover design. Based upon these findings, it is likely that a higher dose would be required for adequate pain control. Since the psychoactivity of higher doses was not assessed in this study, it is currently impossible to determine whether or not this type of side effect would co-occur with clinical efficacy of AJA in neuropathic pain patients. Based upon the results of our study, we would predict that marijuana-like intoxication would accompany clinical analgesic efficacy for AJA.

Comprehensive evaluation of the therapeutic versus deleterious effects of AJA has been a main goal of the present study. Although it has been suggested that AJA is therapeutically active at doses lower than those doses that produce undesired effects, the present data provide little evidence of separation of analgesic activity and other effects. Specifically, this study has shown that AJA, like Δ9-THC, induces psychoactive properties including a decrease in spontaneous activity, catalepsy, and Δ9-THC-like discriminative stimulus effects (undesired effects) at doses similar to those that produce antinociception or antihyperalgesia, respectively (desired effects). Moreover, contrary to previous research that has suggested that AJA’s therapeutic effects are not mediated via action at CB1 receptors, this study demonstrated that AJA’s effects, both therapeutically for pain and psychoactively, are CB1-mediated. These findings also underscore the importance of thoroughly evaluating the pharmacological characteristics of novel Δ9-THC-like compounds, such that therapeutic and deleterious effects are considered simultaneously.

References


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