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RAPID COMMUNICATION

Anandamide, an Endogenous Ligand of the Cannabinoid Receptor, Induces Hypomotility and Hypothermia In Vivo in Rodents

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CRAWLEY, J. N., R. L. CORWIN, J. K. ROBINSON, C. C. FELDER, W. A. DEVANE AND J. AXELROD. *Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents.* PHARMACOL BIOCHEM BEHAV **46**(4) 967–972, 1993.—Anandamide (arachidonylethanolamide), an arachidonic acid derivative isolated from the porcine brain, displays binding characteristics indicative of an endogenous ligand for the cannabinoid receptor. The functional activity of anandamide was tested in vivo using behavioral and physiological paradigms in laboratory rodents. At IP doses from 2 to 20 mg/kg in mice, anandamide significantly decreased spontaneous motor activity in a Digiscan open field. Rectal body temperature significantly decreased at doses of 10 and 20 mg/kg in rats. At doses from 0.03 to 30 mg/kg, anandamide had no significant effect on chow consumption in ad lib fed rats. Over the dose range of 2–20 mg/kg, anandamide did not show anxiolytic properties in the mouse light ⇄ dark exploration model of anxiety. Over the dose range of 0.3–3 mg/kg, anandamide had no effect on choice accuracy or session duration in the delayed nonmatching to sample memory task (DNMTS) in rats. These results demonstrate that anandamide has biological and behavioral effects in awake rodents, some of which are similar to the reported actions of THC.

Marijuana Cannabis Receptor Rodent Behavior Sedation Anxiety Feeding Body temperature Memory

A RECEPTOR which specifically binds delta-9-tetrahydrocannabinol (THC), the principal psychoactive compound in marijuana, and related compounds was recently discovered, and has been cloned in rat and human brain (11,19,34,35). The cannabinoid receptor is coupled to a G-protein (11) and inhibits cAMP accumulation (17,26). The cannabinoid receptor is localized in rat, guinea pig, dog, monkey, and human brains, with high concentrations in the olfactory bulb, striatum, globus pallidus, hippocampus, amygdala, substantia nigra, and cerebellum (22,23,31,34), sites involved in a variety of behaviors.

Anandamide, arachidonylethanolamide, was recently discovered as the first natural ligand for the cannabinoid receptor (12). Anandamide competitively inhibits binding of [^{3}H]HU-243, a specific ligand for the cannabinoid receptor, with an inhibition constant of 52 nM in rat synaptosomal membranes (12). Anandamide inhibits calcium currents in neuroblastoma cells (30), inhibits calcium channel antagonist binding (29), stimulates arachidonic acid and intracellular calcium release in Chinese hamster ovary (CHO)m5 and human cannabinoid receptor (CHO-HCR) cells (16), and inhibits forskolin-stimulated adenylate cyclase activity in COS (monkey kidney) cells (47). Nanomolar concentrations of anandamide inhibit the twitch response of the isolated murine vas deferens (12). Further characterization of the biological and behavioral effects of anandamide are now necessary to elucidate its functional activity at the cannabinoid receptor. Several standard behavioral and physiological paradigms were chosen to characterize the actions of anandamide in vivo. These included quantitation of exploratory locomotion, anxiety-related behavior, body temperature, food consumption, and performance on a spatial memory task, behaviors which have been

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shown to be affected by THC, its analogs, or marijuana in animals or humans (1,2,4–6,13,20,21,24,25,32,36–40,44). During the course of these experiments, another laboratory independently reported decreased motor activity and decreased body temperatures after anandamide treatment (18), and a related abstract was presented at a conference meeting (33). The present findings, therefore, serve to replicate and extend the results of Fride and Mechoulam (18), demonstrating behavioral and physiological effects of anandamide in vivo.

METHODS

Anandamide was synthesized as previously described (12) and prepared in a vehicle containing 1% ethanol, 1% Emulphor EL-620 (GAF Corporation, New York), and 98% physiological saline. Vehicle or anandamide was administered by IP injection in a volume of 5 ml/kg for mice and 1 ml/kg for rats. All animal procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and approved by the National Institute of Mental Health Animal Care and Use Committee. Due to the Schedule 1 designation of delta-9-tetrahydrocannabinol by the U.S. Drug Enforcement Agency, regulations prevented our laboratory from obtaining Schedule 1 authorization at the present time. The experiments described below were therefore unable to incorporate the positive control of THC into the experimental design. However, paradigms were chosen that were shown to be positive for THC in other publications and/or are standard in our laboratory and others.

Open Field Locomotor Activity

Male C57/BL6J mice (Jackson Laboratories, Bar Harbor, ME) were tested for spontaneous exploratory activity immediately after injection of anandamide or vehicle. Ambulatory activity, the parameter most representative of spontaneous exploratory locomotion (8,9), was quantitated by an automated Digiscan Animal Activity Monitor (Omnitech Instruments, Columbus, OH) at 5-min intervals.

Light–Dark Transitions Test for Anxiolytics

The two-chambered light ⇌ dark mouse exploration model of anxiety, previously described as sensitive and specific to anxiolytic drugs, including diazepam, chlordiazepoxide, and clonazepam (7) as well as cholecystokinin type B (CCK-B) antagonists (27) and neurosteroids (9), was used to quantitate anxiety-related behavior. The number of transitions between the large, open, lit compartment and the small, enclosed, dark compartment were quantitated automatically for 10 min, beginning 1 min after IP injection with anandamide or vehicle.

Body Temperature

Rectal temperature was measured in male Sprague–Dawley rats (250–400 g, Taconic Farms, Germantown, NY) using a CMA/150 temperature controller (Bioanalytical Systems, West Lafayette, IN) before, 15 min after, and 30 min after IP injection of saline, anandamide, or vehicle.

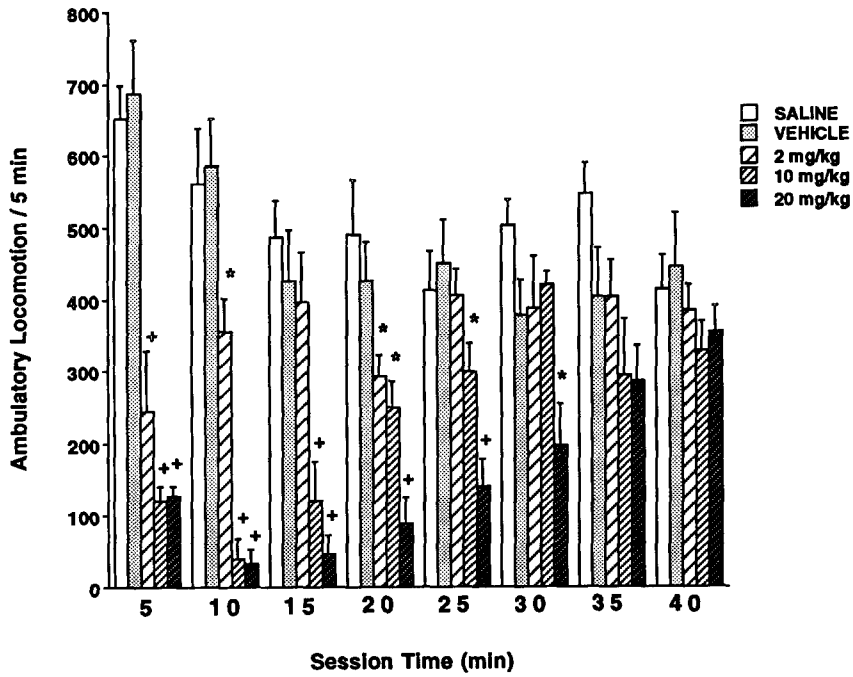

FIG. 1. Anandamide reduced spontaneous motor activity in the automated photocell-equipped Digiscan open field, at IP doses of 2, 10, and 20 mg/kg (*$p < .05$, +$p < .01$ as compared to the vehicle control group). No significant differences were detected between saline (SAL) and vehicle (VEH) control groups. Data are expressed as cumulative ambulatory activity units at 5-min session intervals, beginning immediately after IP treatment, shown as mean ± SE, $N = 5$ mice per treatment group.

Feeding

Consumption of standard Purina Rat Chow was assessed in male Sprague-Dawley rats in the home cage during the first 3 h of the dark cycle. Chow was provided in containers hung on the front of the cage, and was weighed immediately prior to, 30 min after, and 60 min after drug administration, with spillage collected on paper placed under the cages.

Delayed Nonmatching to Sample (DNMTS)

Male Sprague-Dawley rats, maintained on a restricted water schedule, were on the DNMTS task as previously described (14,41,45). Briefly, this operant task requires the subject to press the lever opposite to the lever signaled on the previous sample phase, where a randomized delay of 1, 5, or 15 s separates the sample from the trial. Sixty trials were presented daily, using 0.1 ml water as the reinforcer. Randomized anandamide or vehicle injections were administered IP twice weekly, on Tuesdays and Thursdays, with intervening baseline days to assess stability of performance.

Statistical Analysis

Statistical analyses were conducted on Superanova™ (version 1.0) statistical software. Open field data were analyzed by repeated-measures analysis of variance (ANOVA), followed by a one-way analysis of variance at each time point, and a Student Newman-Keuls post hoc test for significance of comparisons between treatment groups at each time point significant in the one-way ANOVA. Light ⇄ dark transitions data were analyzed by a one-way ANOVA, followed by a Student Newman-Keuls post hoc test. Rectal temperature data were analyzed by two-way ANOVA (Time × Treatment with time as a repeating variable) followed by one-way ANOVA at each time point and Student Newman-Keuls post hoc test for significant differences between treatment groups. Food intake data were analyzed by one-way ANOVA and by Student Newman-Keuls post hoc test. DNMTS performance was analyzed by repeated-measures ANOVA. Secondary measures were analyzed by one-way ANOVA. Post hoc comparisons were made by Dunnett's test.

RESULTS

A dose-related decrease in spontaneous ambulatory activity is shown in Fig. 1, beginning within the first 5-min interval after anandamide administration. Repeated-measures ANOVA showed an effect of dose, $F(4, 16) = 18.36$, $p < .0001$); an effect of time, $F(7, 112) = 394.1$, $p < .001$; and a Dose × Time interaction, $F(7, 28) = 12.3$, $p < .001$. One-way ANOVA was significant for doses at time points of 5 min, $F(4, 20) = 26.2$, $p < .0001$; 10 min, $F(4, 20) = 26.3$, $p < .0001$; 15 min, $F(4, 20) = 12.2$, $p < .0001$; 20 min, $F(4, 20) = 11.9$, $p < .0001$; 25 min, $F(4, 20) = 10.1$, $p < .0001$; and 30 min, $F(4, 20) = 5.35$, $p < .005$. Student Newman-Keuls post hoc analysis of treatment groups found significant decreases in ambulatory activity in comparison to vehicle control for 20 mg/kg anandamide up to 30 min after treatment, for 10 mg/kg anandamide up to 25 min after treatment, and for 2 mg/kg anandamide up to 20 min after treatment. Vehicle treatment was not significantly different from saline treatment at any time point.

The light ⇄ dark exploration model of anxiety detected no anxiolytic effect of anandamide. One-way ANOVA was

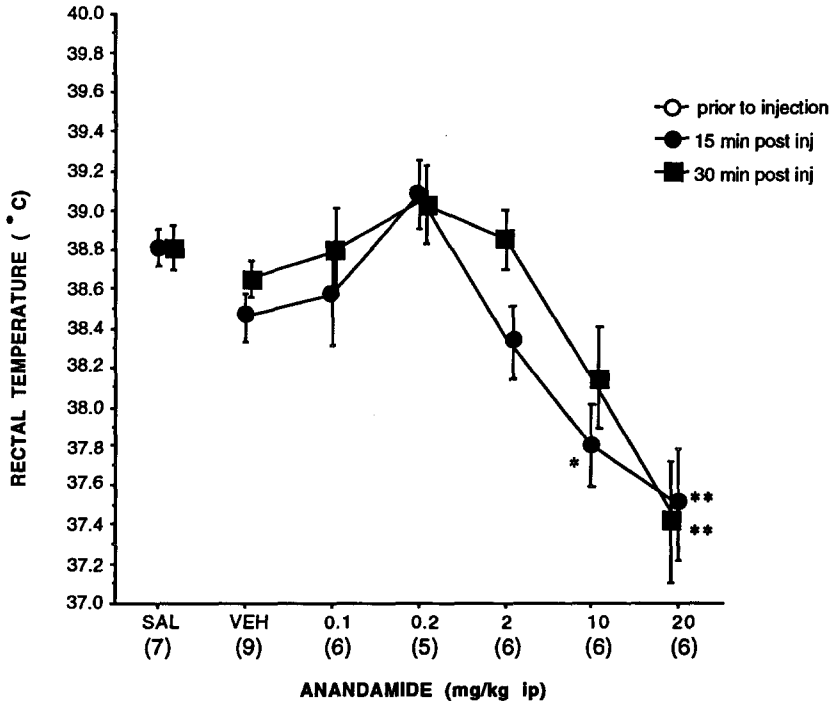

FIG. 2. Anandamide decreased rectal temperature of rats at doses of 10 and 20 mg/kg IP, as compared to vehicle controls. Numbers in parentheses indicate the number of rats per treatment group. Data are expressed as mean ± SE. $*p < .05$, $**p < .01$ as compared to the vehicle control group at the same time point.

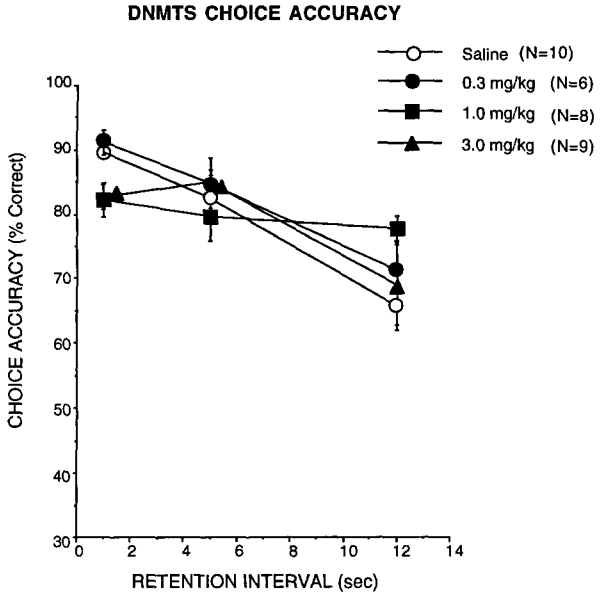

FIG. 3. Anandamide produced no significant effects at any dose on choice accuracy in the delayed nonmatching to sample memory paradigm. Data are expressed as mean ± SE.

significant for dose, $F(5, 29) = 9.1$, $p < .01$. No increase in number of light ⇌ dark transitions was seen at any dose, as compared to vehicle controls ($N = 5$ mice per treatment group; data as treatment group mean ± SE: saline = 56 ± 4, vehicle = 45 ± 10, 0.02 mg/kg = 58 ± 5, 0.1 mg/kg = 51 ± 4, 0.5 mg/kg = 54 ± 4, 2.0 mg/kg = 21 ± 5). A significant decrease in number of transitions ($p < .01$) and an increase in total seconds spent in the dark compartment ($p < .01$) were found at the highest dose tested, 2.0 mg/kg, which is representative of behavioral sedation in this paradigm (7) and consistent with the decrease in ambulatory locomotion at the dose of 2 mg/kg shown in Fig. 1.

Anandamide decreased rectal temperature in rats, as shown in Fig. 2: ANOVA, Time × Dose interaction, $F(12, 74) = 7.275$, $p < .0001$. Prior to injection, there were no differences between groups, ANOVA, $F(6, 37) = 1.471$, NS). At 15 min postinjection, body temperature was decreased at 10 and 20 mg/kg anandamide, ANOVA, $F(6, 37) = 7.587$, $p < .0001$; $p < .05$ at 10 mg/kg, $p < .01$ at 20 mg/kg by Student Newman-Keuls. At 30 min postinjection, temperature was decreased at 20 mg/kg anandamide, ANOVA, $F(6, 37) = 7.837$, $p < .0001$; $p < .01$ by Student Newman-Keuls.

Anandamide had no effect on 30-min or 60-min rat chow consumption, as compared to vehicle controls. Mean grams consumed ± SE for each 60-min treatment group ($N = 5$–12 per treatment group) were saline = 1.32 ± 0.2, vehicle = 0.83 ± 0.3, anandamide 0.03 mg/kg = 0.52 ± 0.2, 0.06 mg/kg = 0.73 ± 0.5, 0.1 mg/kg = 1.53 ± 0.4, 0.2 mg/kg = 0.64 ± 0.4, 0.3 mg/kg = 1.67 ± 0.5, 1.0 mg/kg = 0.24 ± 0.1, 3.0 mg/kg = 1.14 ± 0.4, 10 mg/kg = 0.08 ± 0.03, 30 mg/kg = 0.06 ± 0.3; ANOVA, $F(10, 104) = 3.6$, $p < .0004$. Student Newman-Keuls analysis revealed no significant difference between any group and either the vehicle or saline control groups. Intake for all groups at 30 min was less than one gram, $F(10, 104) = 1.63$, NS. Chow intake did not differ between groups treated with saline (1.32 ± 0.2) and vehicle (0.83 ± 0.3), $F(1, 40) = 2.44$, NS. Several of the rats treated with 30 mg/kg anandamide were observed lying down on their sides or flat on the cage floor 10 min after being injected, indicating an ataxic or nonspecific effect at the highest dose.

As shown in Fig. 3, no effect of anandamide was found on correct DNMTS responses at any dose, at any delay interval, as compared to saline controls, $F(3, 29) = 0.25$, NS. Data from the 10.0-mg/kg dose were not plotted because three of seven subjects did not complete at least 20 trials. All subjects showed notable slowing in responding at the 10-mg/kg dose. The 10.0-mg/kg dose of anandamide produced an increase in a secondary measure, session duration, $F(4, 32) = 4.3$, $p < .007$. This increase in time to complete the session may indicate a sedative or nonspecific action of the 10.0-mg/kg dose of anandamide, consistent with the mouse data shown in Fig. 1. Other secondary measures of DNMTS performance, including frequency of discrimination errors, latency to make choice responses, frequency of error trials followed by other errors, and distribution of errors within a session, were not significantly different after anandamide treatment (0.3, 1.0, 3.0, and 10.0 mg/kg) as compared to vehicle controls.

DISCUSSION

Anandamide produced significant behavioral and physiological effects when administered IP to mice and rats. Dose-dependent sedation was seen in an open field paradigm, over the range of 2–20 mg/kg, similar to doses of THC which produced reductions in spontaneous motor activity (5,6,38). The time course of the sedative effect of anandamide may be shorter than the time course of the sedative effect of THC, in that spontaneous motor activity returned to levels not significantly different than vehicle 20–30 min after IP anandamide injection. Anandamide decreased rectal temperature in rats at IP doses of 10 and 20 mg/kg, qualitatively similar to the ability of THC to reduce body temperature (3,42,46). No significant effects of anandamide were detected in standard paradigms for anxiety-related behaviors, feeding, or working memory. Anxiolytic activity of cannabidiol, a nonpsychoactive analog which does not bind to the cannabinoid receptor, has been reported in the elevated plus-maze task (21). THC has been reported to decrease food intake, though at doses of THC that also induce conditioned taste aversions (2–32 mg/kg), suggesting that food intake reductions after these doses of cannabinoids may be due to nonspecific behavioral effects (13,15,28,43). THC has been shown to produce decreased choice accuracy as retention interval increases in DNMTS performance in rats (24). The present results suggest that anandamide does not mimic all of the actions of THC and other cannabinoids. Several interpretations are possible. Anandamide may have a short half-life in vivo, poor penetration into the brain when administered IP, or other unexplored biochemical properties of this endogenous compound. Alternatively, different behavioral paradigms may be more likely to detect additional biological actions of anandamide, and/or anandamide may decrease motor activity and body temperature by a mechanism unrelated to its binding to the cannabinoid receptor.

The present findings are consistent with the first reports of ataxia and hypothermia after anandamide administration in mice (18,33). In addition, while some cannabinoids have significant effects on anxiety, feeding, and memory (2,3,21,24, 36,38,42,46), the present results show no effect of anandamide on standard animal models of anxiety, feeding, and memory. The five behavioral and physiological paradigms employed to

characterize the effects of anandamide, an endogenous ligand of the THC receptor, demonstrate that anandamide has biological actions in vivo which are similar to some of the biological actions of cannabinoids in the mammalian central nervous system.

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