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Evaluation of Cannabimimetic Discriminative Stimulus Effects of Anandamide and Methylated Fluoroanandamide in Rhesus Monkeys

JENNY L. WILEY,* KEITH M. GOLDEN,* WILLIAM J. RYAN,† ROBERT L. BALSTER,* RAJ K. RAZDAN† AND BILLY R. MARTIN*

*Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613
†Organix, Inc., Woburn, MA 01801

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WILEY, J. L., K. M. GOLDEN, W. J. RYAN, R. L. BALSTER, R. K. RAZDAN AND B. R. MARTIN. *Evaluation of cannabimimetic discriminative stimulus effects of anandamide and methylated fluoroanandamide in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **58**(4) 1139–1143, 1997.—In previous research arachidonylethanolamide (anandamide) has been shown to produce behavioral effects in mice characteristic of psychoactive cannabinoids, including antinociception, catalepsy, hypothermia, and hypomotility. However, differences have also been found between anandamide and Δ^9-tetrahydrocannabinol (Δ^9-THC), with anandamide having lower potency, a more rapid onset, and shorter duration of action than Δ^9-THC. Although it can produce Δ^9-THC–like discriminative stimulus effects in rats, anandamide also produces concomitant response rate decreasing effects, whereas with Δ^9-THC there is a better separation of these two behavioral effects. The present study was designed to examine the discriminative stimulus effects of anandamide in rhesus monkeys trained to discriminate Δ^9-THC from vehicle. While anandamide failed to produce reliable substitution for Δ^9-THC and did not reduce response rates at doses up to 10 mg/kg, 2-methylarachidonyl-2′-fluoroethylamide (methylated fluoroanandamide), a putative stable analog of anandamide, produced full dose-dependent substitution for Δ^9-THC at doses that caused no significant changes in response rates. These results suggest that systemically administered anandamide may be metabolized in monkeys before behaviorally active concentrations could reach the brain and further suggest that the metabolically more stable analog of anandamide, methylated fluoroanandamide, may aid in the discovery of functional properties of the endogenous cannabinoid system. © 1997 Elsevier Science Inc.

Δ^9-tetrahydrocannabinol Anandamide Fluoroanandamide Drug discrimination Cannabinoids Rhesus monkey

ARACHIDONYLETHANOLAMIDE (anandamide), the first putative endogenous ligand for cannabinoid receptors, was originally discovered in porcine brain (11). It has since been found that anandamide binds to central cannabinoid (CB1) receptors in rat brain (7,11) and to cloned human cannabinoid receptors (13). It shares characteristic cannabinoid behavioral effects in mice with Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive ingredient of the marijuana plant, including antinociception, hypothermia, catalepsy, and suppression of locomotor activity (9,14,18); however, anandamide has lower potency, a more rapid onset, and shorter duration of action (18). In rats trained to discriminate either Δ^9-THC or the synthetic bicyclic cannabinoid CP 55,940 from vehicle, anandamide substituted for Δ^9-THC or CP 55,940, but only at doses that also decreased response rates (19). These results suggest that there are differences, as well as similarities, in the behavioral effects of Δ^9-THC and anandamide in rodents.

The purpose of the present study was to investigate the behavioral effects of anandamide in nonhuman primates in a two-lever drug discrimination procedure. Δ^9-THC discrimination represents an animal model of the subjective effects of cannabis intoxication in humans (3). The procedure has pharmacological specificity in both rats and rhesus monkeys (4,22). In rhesus monkeys, plant-derived cannabinoids, Δ^9-

Requests for reprints should be addressed to Dr. Jenny Wiley, Department of Pharmacology and Toxicology, Medical College of Virginia, P.O. Box 980613, Richmond, VA 23298-0613.

THC and Δ8-THC, and synthetic cannabinoids with novel structures, such as CP 55,940, WIN 55,212-2 and 1-butyl-2-methyl-3-(1-naphthoyl)indole, that bind to the CB1 receptor (8,16,17), substitute for Δ^9-THC (15,22). In contrast, the nonpsychoactive cannabinoid, 1-heptyl-2-methyl-3-(1-naphthoyl) indole (16), and drugs of other classes do not reliably substitute for Δ^9-THC in this model (22). For classical cannabinoids, the potency of substitution for Δ^9-THC in rats is highly correlated with binding affinity at the CB1 receptor (8), suggesting that the discriminative stimulus effects of cannabinoids are receptor mediated. This hypothesis is strengthened by the observation that substitution of classical, bicyclic, and aminoalkylindole cannabinoids for Δ^9-THC or CP 55,940 can be blocked by the CB1 receptor antagonist SR141716A in rats and rhesus monkeys (21,23). In Experiment 1 of the present study, we examined the discriminative stimulus effects of anandamide in rhesus monkeys trained to discriminate Δ^9-THC from vehicle. In Experiment 2, we tested 2-methylarachidonyl-2′-fluoroethylamide (methylated fluoroanandamide; Fig. 1), a putative stable analog of anandamide, in this procedure.

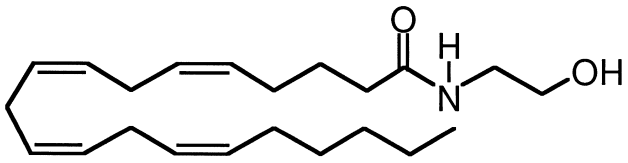
METHOD

Subjects

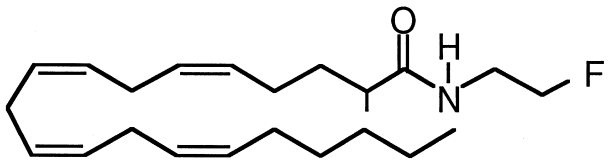
Four adult male rhesus monkeys (8–12 kg), individually housed in a temperature-controlled environment with 12 L:12 D (lights on at 0700 h), were food-deprived for 20 h prior to each daily session (Monday–Friday). Each monkey received a multivitamin and his daily food supplement (135–205 g of monkey chow) following daily sessions. Monkeys had free access to water at all times.

Apparatus

Monkeys were tested in their home cages. During experimental sessions, removable two-lever panels, equipped with two primate response levers and associated stimulus lights (Coulbourn Instruments, Lehigh Valley, PA) and 1-g pellet dispensers (BRS/LVE, Laurel, MD), were attached to the front of each cage. A food cup was located between the two response levers. Lights were located above the food cup and above each lever. A microcomputer with Logic '1' interface (MED Associates, East Fairfield, VT) and MED-PC software (MED Associates) was used to control the scheduling of reinforcement delivery and to record data.



Anandamide



2-Methylarachidonyl-2'-fluoroethylamide

FIG. 1. Chemical structures of anandamide and 2-methylarachidonyl-2′-fluoroethylamide (methylated fluoroanandamide).

Drugs

Δ^9-THC (National Institute on Drug Abuse, Rockville, MD) was dissolved in a 1:1 mixture of absolute ethanol and Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ) and diluted with saline to form a stock suspension of 10 mg/ml Δ^9-THC in a vehicle of emulphor:ethanol: saline (1:1:18) (6). Lower concentrations were obtained by further dilution with the vehicle solution. Doses of anandamide and methylated fluoroanandamide (synthesized in our laboratories) were mixed as needed in a vehicle of 1:1:18 emulphor:ethanol:saline. Injections of Δ^9-THC were given 30 min before the start of the session. Anandamide and methylated fluoroanandamide were administered 15 min presession, except where specifically noted. In the first study, individual training doses of Δ^9-THC were obtained for the monkeys by adjusting the volume of a 1.2 mg/ml suspension of Δ^9-THC. In the second study, Δ^9-THC was administered at a volume of 0.1 ml per kg of body weight. Injections of anandamide and methylated fluoroanandamide were also given at a volume of 0.1 ml per kg of body weight. All drugs were injected intramuscularly (IM) in the thigh.

General Procedure

Monkeys had been previously trained to discriminate Δ^9-THC from vehicle in a two-lever drug discrimination procedure (15) and had participated in several other studies (20,22,23). In the present study, discrimination training continued with a double alternation sequence of Δ^9-THC and vehicle injections. Training doses were determined individually for each monkey in experiment 1 and ranged from 0.08–0.16 mg/kg, IM (see Fig. 2 legend for specific training dose for each monkey). Food reinforcement was obtained under a fixed-ratio 50 (n = 1; Experiment 1 only) or a fixed-ratio 100 (n = 3) schedule. Responses on the incorrect lever reset the ratio requirement on the correct lever. Training sessions consisted of four trials of 5 min each, alternating with three 15-min time out periods for a total session duration of 65 min. During trials, the lights over both levers were illuminated. The light over the food cup was illuminated during pellet delivery. During time-out periods, lights over the levers were turned off and responses were recorded but had no programmed consequences. Test sessions consisted of a single 5-min trial, during which consecutive responses on either lever were reinforced according to the fixed-ratio schedule.

Experiment 1

In Experiment 1, anandamide was tested for substitution for Δ^9-THC. Replications were performed with doses of anandamide that produced greater than 80% Δ^9-THC–lever responding. Control tests with vehicle and Δ^9-THC were conducted before and after the anandamide dose–effect curve determination. To examine the effect of a shorter presession injection interval, a single injection of 10 mg/kg anandamide was given 5 min before the start of a test session. Anandamide

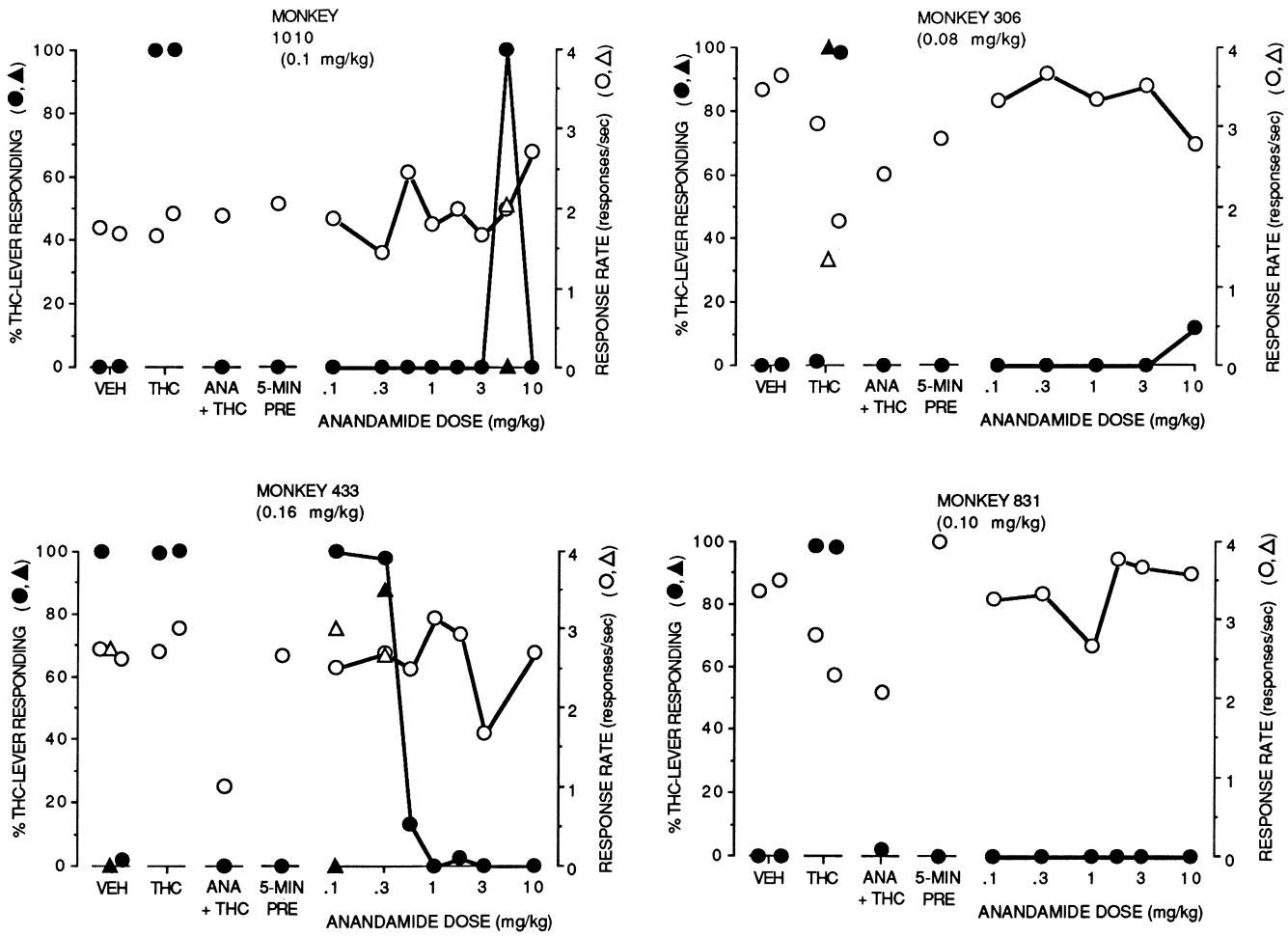


FIG. 2. Effects of anandamide (IM, 15-min presession) on percentage of Δ^9-THC–lever responding (filled symbols) and response rates (open symbols) in four rhesus monkeys trained to discriminate Δ^9-THC from vehicle. (Training doses for each monkey are given on each panel in parentheses below the monkey's number.) Points above VEH and THC represent the results of control tests with vehicle and the training dose of Δ^9-THC conducted before and after substitution tests with anandamide. Points above ANA + THC represent results of tests with a combination of anandamide (5.6 mg/kg) and Δ^9-THC (0.01 mg/kg in monkeys 433 and 1010 and 0.03 mg/kg in monkeys 306 and 831). Points above 5-MIN PRE represent results of tests with anandamide (10 mg/kg) conducted 5 min prior to the start of the test session. Second determinations of data points are indicated by filled (% THC-lever responding) and open (response rate) triangles.

(5.6 mg/kg) was also tested in combination with subthreshold doses of Δ^9-THC (0.01 or 0.03 mg/kg) that did not substitute for the training dose in the most recent Δ^9-THC dose–effect curve determination in these monkeys [see (22)].

Experiment 2

Dose–effect curves for Δ^9-THC and methylated fluoroanandamide were determined approximately 1 year after tests with anandamide in three of the original four monkeys. During the intervening period, the Δ^9-THC training dose had been raised to 0.3 mg/kg, IM, for all monkeys. All other experimental conditions remained the same, with each monkey being tested under a fixed ratio 100 schedule of food reinforcement in Experiment 2.

Data Analysis

For each test session, percentage of responses on the Δ^9-THC lever and response rate (responses/sec) were calculated individually for each monkey. Data on lever selection for monkeys that did not respond at all during the session are not presented on the figures. Substitution for Δ^9-THC was indicated by greater than 80% drug-lever responding.

RESULTS

The results of substitution tests with anandamide in Δ^9-THC–trained rhesus monkeys are presented as individual subject data (Fig. 2). Anandamide failed to produce consistent generalization from Δ^9-THC in any of the four monkeys tested; i.e., the few doses that produced full substitution during initial testing of the anandamide dose–effect curve failed to do so upon retest. Doses of anandamide up to 10 mg/kg did not decrease response rate in these monkeys. Testing 10 mg/kg of anandamide with a 5-min interval between injection and start of the session produced predominantly vehicle lever responding in all monkeys, as did the combination of 5.6 mg/kg anandamide and a subthreshold dose of Δ^9-THC. Responding

during control tests with vehicle and Δ^9-THC generally occurred on the correct lever, although monkey 433, who showed some Δ^9-THC–like effects at low doses of anandamide, also had a tendency to make errors during vehicle control test sessions.

In contrast to the predominantly negative results obtained with anandamide, Δ^9-THC and methylated fluoroanandamide fully substituted for Δ^9-THC in a dose-dependent manner in all three of the monkeys tested (Fig. 3, upper panels). In each monkey, methylated fluoroanandamide was more potent than Δ^9-THC. Response rates were decreased in only one monkey (Monkey 306) at the highest dose of Δ^9-THC, but were unaffected by any of the other doses of either drug in any of the monkeys (Fig. 3, lower panels).

CONCLUSIONS

In rhesus monkeys, anandamide did not produce consistent substitution for Δ^9-THC. When substitution did occur, it generally was not observed upon retest and could be explained by occasional failures in stimulus control. Further, anandamide (5.6 mg/kg) failed to augment responding on the drug lever following injection with a subthreshold dose of Δ^9-THC (either 0.01 or 0.03 mg/kg). Response rates were not decreased at any dose of anandamide, suggesting that behaviorally active doses were not attained. Unfortunately, the limits of solubility (100 mg/ml) were reached for anandamide prepared for IM injection at a reasonable volume. In rats, substitution of anandamide for Δ^9-THC or CP 55,940 was accompanied by reduction in response rates (19). If the cannabimimetic effects of anandamide occur only at response rate-decreasing doses in monkeys as well, it is possible that anandamide was not administered to the monkeys in doses sufficient to engender Δ^9-THC-like discriminative stimulus effects. In rats, anandamide metabolism to arachidonic acid, ethanolamine, and other catabolites occurs very quickly via hydrolytic degradation by amidases (10,12), a process that can be blocked by the amidase inhibitor, phenylmethylsulfonyl fluoride [PMSF; (7,10)]. Although anandamide metabolism in monkeys has not been studied, it is likely that anandamide is also metabolized rapidly following systemic administration in this species, as suggested by the fact that shortening the injection interval from 15 to 5 min had no effect on percentage of drug-lever responding or response rates. It is possible that differences in route of administration (IP for rats and IM for monkeys) may have contributed to the observed differences between the species. Alternatively, anandamide substitution for Δ^9-THC may be species specific.

Examination of the monkey anandamide data revealed the unusual finding that doses of anandamide administered to monkeys without behavioral activity were equivalent on a mg/kg basis to those producing effects in rats. Other psychoactive cannabinoids, such as Δ^9-THC, CP 55,940, and WIN 55,212-2, are approximately 10-fold more potent in monkeys than in rats (15,22). With rare exception, monkeys are more sensitive than rats to the effects of a variety of other classes of drugs and usually require much lower doses for pharmacological activity (5).

To determine whether a metabolically more stable anandamide analog might substitute for Δ^9-THC in rhesus monkeys, we synthesized methylated fluoroanandamide. Previous work with this compound found that it was fourfold more potent than anandamide in mice at producing suppression of spontaneous activity and approximately equipotent at producing antinociception (2). In addition, the binding affinity of methylated fluoroanandamide to the CB1 receptor was greater than that of anandamide [K_i = 89 + 10 nM; (2)] and, unlike anandamide and several of its other analogs (1,2), was

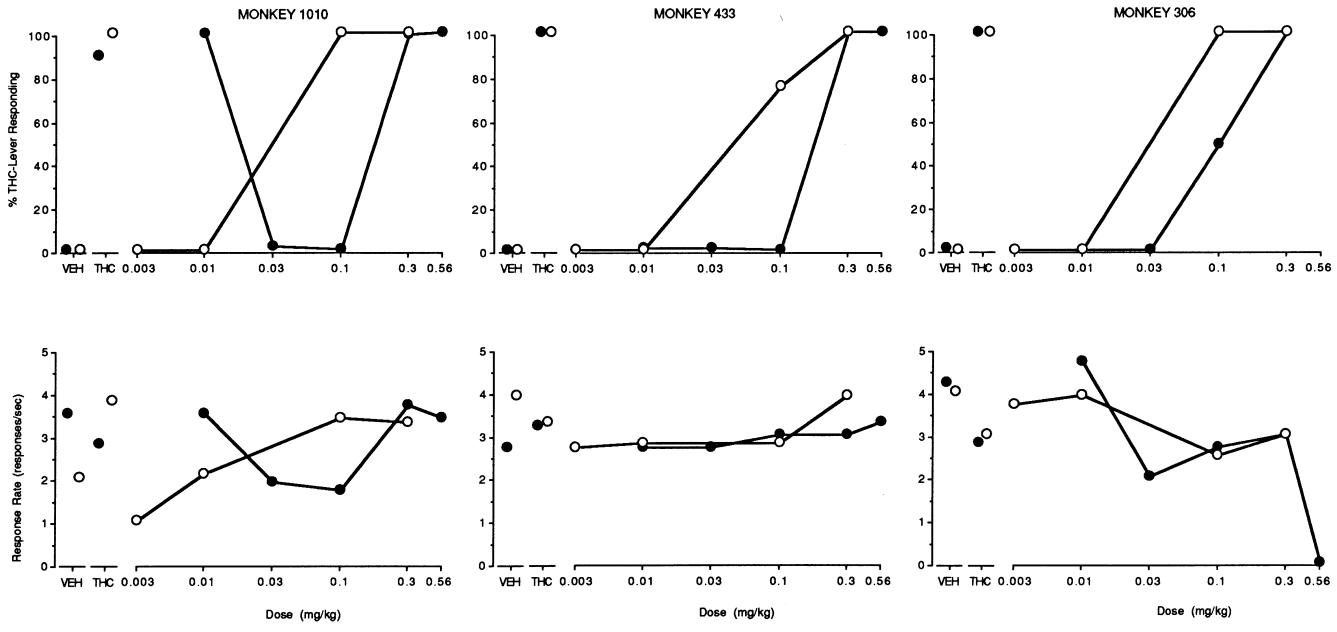


FIG. 3. Effects of Δ^9-THC (filled circles) and methylated fluoroanandamide (open circles) on percentage of Δ^9-THC-lever responding (top panel) and response rates (bottom panel) in three rhesus monkeys trained to discriminate 0.3 mg/kg Δ^9-THC from vehicle. Points above VEH and THC represent the results of control tests with vehicle and the training dose of Δ^9-THC conducted before each dose–effect curve determination.

similar with (K_i = 6 + 1 nM) and without (K_i = 8 + 2 nM) the addition of the amidase inhibitor PMSF to the binding medium. These results suggest that methylated fluoroanandamide would not be subject to rapid metabolism by amidases, as is anandamide. Consistent with this hypothesis, the discriminative stimulus effects of methylated fluoroanandamide differed from those of anandamide in the present study. Methylated fluoroanandamide produced a dose-dependent substi-tution for Δ^9-THC in the monkeys without accompanying changes in response rates, a pattern that was similar to that produced by Δ^9-THC itself. Further, consistent with its greater binding affinity for CB1 receptors (compared to Δ^9-THC; 8 vs. 40 nM), methylated fluoroanandamide was approximately threefold more potent in producing cannabimimetic effects than was Δ^9-THC. The present results with the more metabolically stable methylated fluoroanandamide suggest that anandamide failed to substitute for Δ^9-THC in rhesus monkeys due to its rapid metabolism in this species; however, because anandamide metabolism has not been studied directly in this species, firm conclusions await further research.

In summary, whereas anandamide produced cannabimimetic effects, it did not substitute for Δ^9-THC in rhesus monkeys trained to discriminate Δ^9-THC from vehicle nor did it produce decreases in response rates. In contrast, methylated fluoroanandamide, a metabolically more stable analog, dose dependently substituted for Δ^9-THC. The present results provide further support for the role of anandamide as an endogenous cannabinoid ligand and suggest that metabolically more stable analogs may aid in discovery of the functional properties of the endogenous cannabinoid system.

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