Psychopharmacology (1998) 140:519–522

ORIGINAL INVESTIGATION

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Δ^9-THC training dose as a determinant for (*R*)-methanandamide generalization in rats

Received: 9 May 1998 / Final version: 28 May 1998

Abstract The objective of this study was to examine if (*R*)-methanandamide, a metabolically stable chiral analog of the endogenous ligand anandamide, is a cannabimimetic with a lower efficacy than Δ^9-THC. Employing a two-lever choice drug discrimination procedure, rats were trained to discriminate between 1.8, 3.0, or 5.6 mg/kg Δ^9-tetrahydrocannabinol (Δ^9-THC) and vehicle. Different training doses were used in order to create assays with different efficacy demands. Generalization tests with 18 mg/kg (*R*)-methanandamide yielded around 90% Δ^9-THC responses in the two lower Δ^9-THC training dose conditions. However, only around 60% Δ^9-THC responses occurred in the 5.6 mg/kg Δ^9-THC training dose condition in tests with 18 mg/kg (*R*)-methanandamide; a higher dose (30 mg/kg) produced even fewer Δ^9-THC-appropriate responses in this group. Morphine did not substitute for Δ^9-THC. In conclusion, the data with Δ^9-THC and (*R*)-methanandamide indicate that cannabinoid agonists can have varying degrees of intrinsic activity at a receptor site, or may produce their behavioral actions through multiple mechanisms, or both.

Key words Δ^9-THC · Cannabinoids · (*R*)-methanandamide · Anandamides · Efficacy · Drug discrimination · Rat

Introduction

Anandamide (20:4, *n*-6; *N*-arachidonylethanolamide) is a putative endogenous ligand for the CB1 receptor (Devane et al. 1992). However, anandamide is biologically unstable (e.g., Sheskin et al. 1997), making its use in certain biologic assays difficult. With the objective of enhancing biologic stability, (*R*)-methanandamide [*R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide; AM 356] was synthesized (Abadji et al. 1994). This chiral analog is not only resistant to hydrolytic cleavage by anandamide amidase, but is also more potent and selective for the CB1 receptor than anandamide in in vitro assays (Khanolkar et al. 1996) and in vivo assays (e.g., Romero et al. 1996; Burkey and Nation 1997).

Despite their similarities, the effects Δ^9-THC and (*R*)-methanandamide may differ qualitatively. Following Δ^9-THC administration, but not (*R*)-methanandamide, circling occurs in an open-field test (Järbe et al. 1998). Three possible explanations for this difference were considered: (i) circling was not a cannabinoid receptor mediated effect; (ii) circling represented an assay with a high efficacy demand and (*R*)-methanandamide was a low efficacy agonist; or (iii) circling was mediated by a receptor site where (*R*)-methanandamide was not an effective agonist. The first explanation seems incorrect because, (i) other cannabinoids produce circling, and (ii) the CB1 receptor antagonist SR 141716 eliminated the Δ^9-THC-induced circling (Järbe et al. 1998). Thus, circling appears to be a cannabinoid receptor mediated effect shared by classical cannabinoid cannabimimetics. In this study, we investigated the second of these possibilities, i.e., (*R*)-methanandamide is a cannabimimetic with a lower efficacy than Δ^9-THC, by comparing the effects of (*R*)-methanandamide in Δ^9-THC discrimination assays with different efficacy demands.

Studies with opioids show differences in the generalization pattern between various types of opioids can result not only from qualitative differences but also from differences along quantitative dimensions. For instance, mixed opioid agonists with low efficacy at the mu receptor will substitute in animals trained with a low dose of a high-efficacy mu agonist such as morphine whereas generalization is at best partial when such opioids are examined in animals trained with a high dose of morphine (see Young 1991). Studies now make clear that these results most likely depend on quantitative rather than qualitative differences between such opioid drugs (e.g., Koek and Woods 1989; Picker et al. 1993, 1996).

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Materials and methods

Apparatus

Drug discrimination training and testing were conducted in eight operant chambers (ENV-001, Med. Associates, St Albans/Georgia, Vt., USA), constructed of Plexiglas and aluminum, equipped with two response levers, house-and lever lights, and a grid floor. Each chamber was enclosed within sound- and light-attenuating boxes equipped with an exhaust fan. These chambers were connected to an IBM compatible PC.

Animals

Adult male Sprague-Dawley rats (*n*=16; Taconic Farms, Germantown, N.Y., USA) were individually housed in a colony room with an average temperature of 20°C and a 12-h light/dark cycle (rats were trained and tested during the light phase). Purina Rat Chow was restricted to approximately 12 g/day, thus maintaining body weights between 350 and 400 g.

Training

Rats were magazine trained, and shaped to lever press for food reinforcement until they responded 10 times for each reinforcer (FR 10). Each reinforcement consisted of two 45 mg Noyes pellets. The rats were then trained in a two-choice task to respond on drug- or vehicle-appropriate levers once daily. The position of drug-appropriate levers was randomly assigned among subjects so that it was to the right of the food cup for half the subjects. Animals were administered Δ^9-THC or vehicle (2 ml/kg) IP 30 min before session onset. Throughout the session, an FR-10 schedule was in effect so that ten presses on the appropriate lever delivered two food pellets. Presses on the wrong lever were recorded, but had no programmed consequences. The schedule of drug (D) or saline (N) administrations was non-systematic, with no more than two consecutive D or N trials. To avoid the influence of odor cues left in a chamber by a preceding subject (see Extance and Goudie 1981), the order in which D and N training sessions were conducted for animals trained in the same chamber was randomized. Training sessions were conducted Monday through Friday, and lasted for 20 min. Training continued until animals reached the acquisition criterion of selecting the state-appropriate lever on at least eight out of ten consecutive training days. Correct selection was defined as total presses before the first reinforcement being equal to, or less than 14 (i.e., an animal did not press the wrong lever more than 4 times before pressing 10 times on the appropriate lever).

Testing

After animals reached acquisition criterion, test sessions were conducted on average 3 times every 2 weeks; on interim days, training sessions were conducted. A drug training session preceded half the test sessions; the other half was preceded by a vehicle session. Tests were conducted only if responding during the preceding training sessions had been correct. During testing, animals were reinforced for ten presses on either lever until 20 min had elapsed or six reinforcers had been delivered, whichever occurred first. There was one session per test day. For each dose tested, the percentage of responding on the drug-appropriate lever was calculated from the ratio of the number of presses on the Δ^9THC-associated lever to the total number of presses in a test session. Only data for animals receiving at least one reinforcer during the test session were considered for this measure. Additionally, response rate (responses per second) was calculated. This measure is based on the performance of all animals, including non-responders.

In our study, rats were trained to discriminate between Δ^9-THC and vehicle using 1.8, 3.0, or 5.6 mg/kg Δ^9-THC. All animals (*n*=16) were originally trained with the 3 mg/kg dose of Δ^9-THC, and tested with Δ^9-THC (*n*=8) or (*R*)-methanandamide (*n*=8). Thereafter, the animals were split into two equally sized, counterbalanced groups and retrained with 1.8 (*n*=8) and 5.6 (*n*=8) mg/kg Δ^9-THC, followed by additional tests with Δ^9-THC and (*R*)-methanandamide.

Table 1 ED_{50} values (mg/kg) for Δ^9-THC and (*R*)-methanandamide for the three training conditions of Δ^9-THC (S^D 1.8, 3.0 and 5.6 mg/kg) employed in the present investigation. Doses were ln transformed and data subjected to a least square linear regression model

	Δ^9-THC	(*R*)-methanandamide
S^D 1.8	0.34	5.41
S^D 3.0	0.46	5.10
S^D 5.6	0.64	N/A

Results and discussion

As can be seen in Fig. 1, (*R*)-methanandamide dose-dependently occasioned Δ^9-THC appropriate responding in rats trained to discriminate between 3 mg/kg Δ^9-THC and vehicle. This was also the case for animals retrained with 1.8 mg/kg Δ^9-THC (ED_{50} values are shown in Table 1). These results are consistent with Burkey and Nation's (1997) data showing that (*R*)-methanandamide occasioned Δ^9-THC appropriate responding in rats trained with a dose of 2 mg/kg Δ^9-THC. These results are also consistent with the finding that another putatively more metabolically stable analog of anandamide (2-methyl-arachidonyl-2'-fluoroethylamide) substituted for Δ^9-THC in rhesus monkeys, whereas anandamide did not (Wiley et al. 1997). However, (*R*)-methanandamide did not fully substitute for Δ^9-THC over the dose-range that could be tested in rats retrained with 5.6 mg/kg Δ^9-THC (six and three rats out of eight tested with doses of 18 and 30 mg/kg (*R*)-methanandamide, respectively, responded sufficiently to calculate percentage of THC appropriate responding).

(*R*)-Methanandamide reduced response rate under all three training conditions. Burkey and Nation (1997) also reported a dose-dependent suppression of response rate by (*R*)-methanandamide such that only roughly half the rats tested with the highest dose of (*R*)-methanandamide responded enough to receive reinforcement.

One interpretation of our results is that (*R*)-methanandamide is a lower efficacy cannabinoid than Δ^9-THC. As described earlier, studies with opioids indicate that discriminations using higher training doses of a full agonist generally have higher efficacy demands than discriminations trained using a lower dose of the agonist. One should note that higher doses of (*R*)-methanandamide could not be tested because responding was severely depressed at the highest dose tested. Additional tests (not shown) with 18 mg/kg (*R*)-methanandamide 15 min (rather than 30 min) post-injection also had profound rate-decreasing effects in the groups trained with 3.0 and 1.8 mg/kg. In the latter tests, three out of eight and two

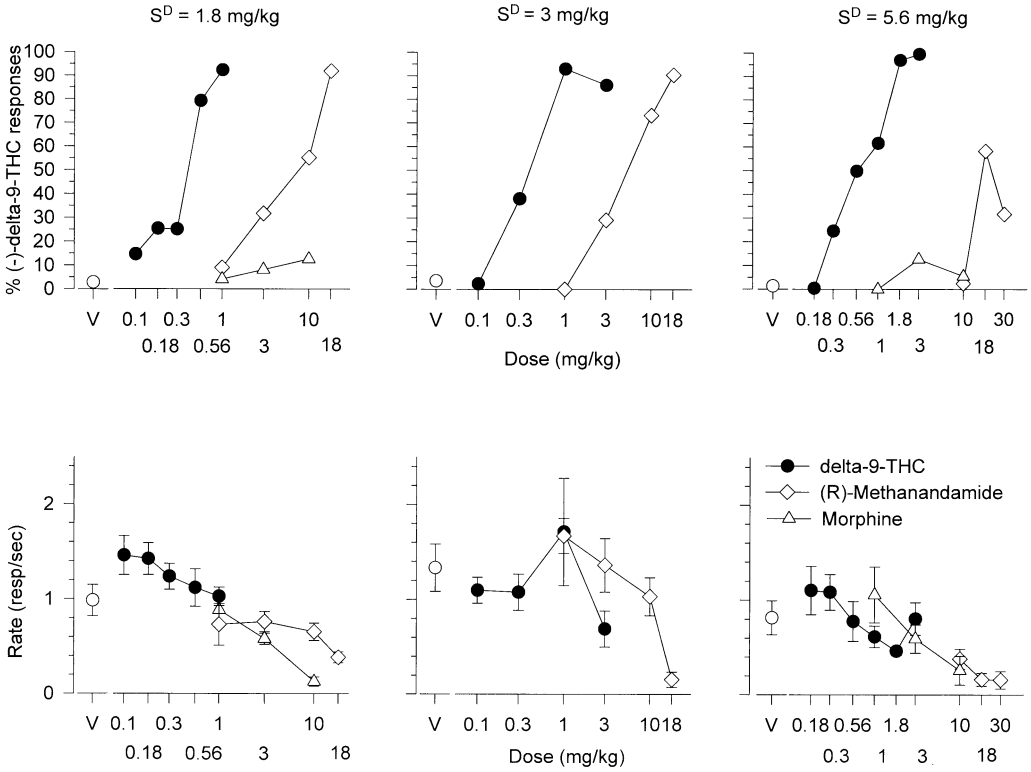

Fig. 1 Generalization test results (*top*) and response rate data (*bottom*) for rats trained to discriminate between (–)-Δ^9-tetrahydrocannabinol (Δ^9-THC) and vehicle using different training doses (S^D) of Δ^9-THC: 1.8 mg/kg (*left*), 3 mg/kg (*middle*), and 5.6 mg/kg (*right*). The generalization test results (*top*) represent the mean percentage of lever presses on the Δ^9-THC appropriate lever out of the total number of lever presses emitted during a six-trial test probe (*y-axis*); doses examined in mg/kg (*x-axis*). Rate (*bottom*) refers to the mean (±SEM) number of lever presses per second emitted during a six-trial test probe (*y-axis*); doses examined in mg/kg (*x-axis*). For S^D=1.8 mg/kg, data points are based on two observations each with *n*=7–8, with the exception of 3 mg/kg morphine where *n*=6; vehicle (*V*) tests represent the average of one test with the Δ^9-THC vehicle (2 ml/kg) and one test with the (*R*)-methanandamide vehicle (3 ml/kg). The vehicles consisted of 3% Tween-80, 5% propylene glycol, and 92% normal saline except for 18 and 30 mg/kg (*R*)-methanandamide, where 4% Tween-80 was used at the expense of saline. For the two other training conditions (S^D=3 mg/kg and S^D=5.6 mg/kg), the data points are based on one observation each with *n*=7–8; vehicle (*V*) tests represent the average of one test with the Δ^9-THC vehicle. Data points were obtained on separate test days. Δ^9-THC and (*R*)-methanandamide were administered IP 30 min and morphine SO_4 20 min prior to session onset; the vehicle for morphine was normal saline (1 ml/kg). Suspensions were prepared fresh daily. ● Δ^9-THC, ◇ (*R*)-methanandamide, △ morphine

out of seven rats earned at least one reinforcer, resulting in 88% and 95% Δ^9-THC appropriate responding in the groups trained with 3.0 and 1.8 mg/kg, respectively. Such data serve to illustrate that a high level of generalization is obtainable under conditions where response output is markedly reduced.

Alternatively, it could be that (*R*)-methanandamide has equal efficacy in producing discriminative effects, but (*R*)-methanandamide's rate-decreasing effects mask the detection of these discriminative effects. Thus, it is possible that the rate-decreasing and discriminative effects of cannabinoids are mediated through different receptors or receptor states. Sorting out these possibilities will require systematic structure activity relationship studies and quantitative antagonism research.

A potential limitation of this study is the possibility of carry-over effects because all animals initially had been trained with the middle Δ^9-THC dose before being retrained with the lower and higher Δ^9-THC maintenance doses. Thus, it is not known if the prior Δ^9-THC experience with the middle reference dose influenced the functional value of the retrained conditions. To the best of our knowledge, this issue does not seem to have been directly addressed in the existing literature. However, one might suspect that the main effect would be to increase THC appropriate responding in tests following (*R*)-methanandamide administration in the group retrained with 5.6 mg/kg Δ^9-THC.

Finally, the lack of substitution with morphine in the groups trained with 1.8 and 5.6 mg/kg is in agreement with previous examinations of morphine in rats trained with 3 mg/kg Δ^9-THC (Balster and Prescott 1992). Although limited to only one test agent, the lack of generalization with morphine lends support for pharmacological specificity for our Δ^9-THC discrimination assays.

Thus, our data indicate that cannabinoid agonists either have varying degrees of intrinsic activity at a receptor site or may produce their behavioral actions via different mechanisms, or both. This adds fuel to recent speculations by other researchers about the possibility of receptor heterogeneity or differences in binding modes to explain emerging test results derived from structure activity relationship studies (Seltzman et al. 1997; Tho-

mas et al. 1998). Those speculations arose from different results (rank order affinities) obtained in displacement studies using agonists and antagonists as probes, respectively.

Acknowledgements We thank NIDA for free supplies of (–)-Δ9-THC (batch 7074-91) and morphine. Michelle Harris provided excellent technical assistance. We also thank Drs. Alice Young and Michael Picker for helpful comments concerning different training strategies in drug discrimination studies. The Animal Care and Use Committee of the Allegheny University of the Health Sciences, Philadelphia, Pa., approved all procedures. This study was supported by NIDA grants DA 09064, and DA 00253 (Philadelphia); DA 03801, DA 9158, DA 7215 and DA 00152 (Storrs).

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