Task specificity of cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide analogs in mice

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Abstract

Relatively few studies have compared the effects of tetrahydrocannabinols and anandamide-like cannabinoids following repeated dosing. Whereas pronounced tolerance develops to many of the in vivo pharmacological effects of Δ^9-tetrahydrocannabinol with repeated dosing, tolerance to anandamide-induced effects is typically less noted. In the present study, we examined cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide-like compounds (anandamide, 2-methylanandamide, and O-1812) in a tetrad of in vivo tests sensitive to cannabinoid action, including spontaneous activity, tail flick, rectal temperature, and a ring immobility test of catalepsy. Six intraperitoneal injections of Δ^9-tetrahydrocannabinol 10 mg/kg over a period of 4 days resulted in the development of pronounced tolerance to all of its in vivo effects. In contrast, task specificity was observed in cross-tolerance to anandamide and its analogs: antinociception (all three compounds), suppression of spontaneous activity (2-methylanandamide and O-1812), catalepsy (O-1812), and hypothermia (none of the compounds). Furthermore, when it occurred, the magnitude of cross-tolerance was notably smaller. These results suggest that anandamide-like cannabinoids may have a unique pharmacology that only partially overlaps with that of Δ^9-tetrahydrocannabinol and other traditional cannabinoids. Although the basis for this unique pharmacology has not as yet been determined, it is possible that regional specificity of cannabinoid CB_1 receptor downregulation and endocannabinoid release induced by repeated dosing with Δ^9-tetrahydrocannabinol may play a role.
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1. Introduction

The discovery of arachidonylethanolamide (anandamide; Devane et al., 1992), an endogenous ligand for brain cannabinoid CB_1 receptors, prompted extensive exploration of similarities and differences between the pharmacology of anandamide and traditional plant-derived cannabinoids. In mice, Δ^9-tetrahydrocannabinol and other psychoactive constituents of the marijuana plant produce a profile of acute cannabimimetic effects in mice, including suppression of spontaneous activity, antinociception, hypothermia, and catalepsy (Martin et al., 1991). Whereas tetrahydrocannabinols are equipotent (across tests) and equally efficacious in producing these four pharmacological effects, differences in potencies and/or magnitudes of maximal effect in tests with anandamide-like cannabinoids have been observed. Anandamide and its analogs are more efficacious than Δ^9-tetrahydrocannabinol in producing catalepsy; however, their efficacy at reducing body temperature is only about half that of other classes of cannabinoids (Ryan et al., 1997; Seltzman et al., 1997; Smith et al., 1994). Furthermore, correlations between in vitro affinities of anandamide analogs for cannabinoid CB_1 receptors and their in vivo potencies are not as strong as for traditional, bicyclic, and indole-derived

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cannabinoids (Adams et al., 1995a,b). Other studies have shown that the mechanism through which anandamide produces spinal antinociception in mice may differ from that of traditional and bicyclic cannabinoids (Houser et al., 2000; Smith et al., 1994; Welch and Eads, 1999; Welch et al., 1998). Finally, Adams et al. (1998) reported that anandamide's pharmacological effects were not blocked by the cannabinoid CB_1 antagonist, SR 141716A, although SR 141716A blocked the cannabimimetic effects of a more stable anandamide analog, 2-methyl-2'-fluoroethylanandamide.

Although numerous studies have examined and compared the acute in vivo pharmacology of tetrahydrocannabinols and anandamide-like cannabinoids, relatively few have compared the effects of these cannabinoid classes following repeated dosing. With repeated administration, pronounced tolerance develops to many of the in vivo pharmacological effects of Δ^9-tetrahydrocannabinol, including hypomobility, antinociception, hypothermia, catalepsy, discriminative stimulus effects, operant response rate decreases, and reduced defecation (Fan et al., 1994; Fride, 1995; Lamb et al., 2000; Wiley et al., 1993). In addition, cross-tolerance between Δ^9-tetrahydrocannabinol and cannabinoids in the bicyclic and aminoalkylindole classes has been demonstrated for many of these effects (Fan et al., 1994). Similarly, tolerance to anandamide-induced suppression of spontaneous activity, antinociception, catalepsy, hypothermia, and the twich response in mouse vas deferens has also been reported (Costa et al., 2000; Fride, 1995; Pertwee et al., 1993; Welch, 1997; Welch et al., 1995), although the magnitude of tolerance tended to be lower than for Δ^9-tetrahydrocannabinol (Welch, 1997; Welch et al., 1995). In contrast, tolerance to anandamide-induced reduction in defecation has not been observed (Costa et al., 2000; Fride, 1995). Cross-tolerance between anandamide or one of its analogs and Δ^9-tetrahydrocannabinol in rodents tolerant to one of these drugs has also been reported for some of these effects (Fride, 1995; Lamb et al., 2000; Pertwee et al., 1993; Welch et al., 1995); however, the potencies of anandamide to induce hypothermia (Pertwee et al., 1993) and decreased defecation (Fride, 1995) were not affected in Δ^9-tetrahydrocannabinol-tolerant rodents, suggesting that a different population of receptors might be involved in producing these effects. As with the acute dosing studies, these results suggest that anandamide interaction with brain cannabinoid CB_1 receptors may differ from that of the tetrahydrocannabinols or that multiple brain cannabinoid receptors may exist. In the present study, we examined cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide and two of its metabolically stable analogs, 2-methylanandamide (O-680) and [(R)-(20-cyano-16,16-dimethyl docosa-cis-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propylamine] (O-1812; Fig. 1). To the extent that anandamide-like cannabinoids share mechanism(s) of
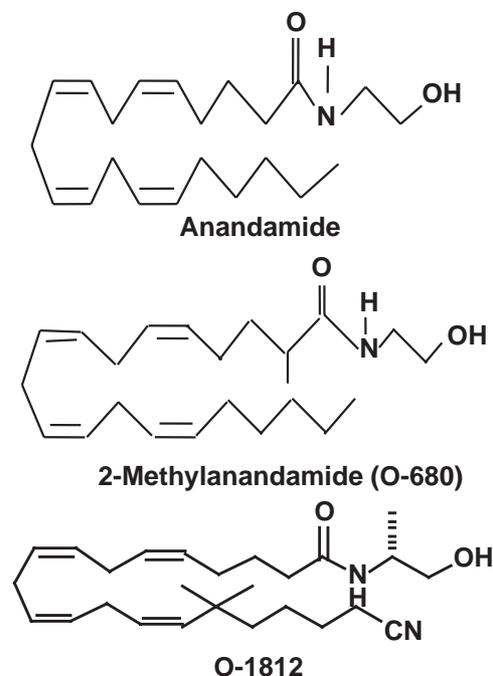

Fig. 1. Chemical structures of anandamide, 2-methylanandamide (O-680), and O-1812.

action with Δ^9-tetrahydrocannabinol, they would be expected to induce cross-tolerance in Δ^9-tetrahydrocannabinol-tolerant mice.

2. Materials and methods

2.1. Subjects

Male ICR mice (25–32 g), purchased from Harlan (Dublin, VA), were housed in groups of five. All animals were kept in a temperature-controlled (20–22 °C) environment with a 12-h light/dark cycle (lights on at 7 a.m.). Separate mice were used for testing each drug dose in the in vivo behavioral procedures. The mice were maintained on a 14:10 h light/dark cycle, and received food and water ad libitum. The studies reported in this manuscript were carried out in accordance with guidelines published in guide for the care and use of laboratory animals (National Research Council, 1996) and were approved by our Institutional Care and Use of Animals Committee.

2.2. 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.

2.3. Drugs

Δ^9-Tetrahydrocannabinol (National Institute on Drug Abuse, Rockville, MD), anandamide (synthesized in our laboratories, Woburn, MA), 2-methylanandamide (synthesized in our laboratories), and O-1812 (synthesized in our laboratories) were mixed in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ), and saline in a ratio of 1:1:18. Prior to all in vivo testing on Day 1 and Day 5, all drugs were administered to the mice intravenously in the tail vein. During the repeated dosing period, injections were administered subcutaneously. All drugs were injected at a volume of 0.1 ml/10 g.

2.4. Procedure

The Δ^9-tetrahydrocannabinol tolerance experiment was conducted over a 5-day period. On the morning of Day 1, separate groups of mice (*n*=5–6 mice per dose) were randomly assigned to receive a single dose of vehicle or Δ^9-tetrahydrocannabinol (0.1–30 mg/kg). Following this initial injection, each mouse was tested in all four tests of the tetrad as described below. After completion of the tests, mice were returned to the vivarium. On the afternoon of Day 1, they received an injection of 10 mg/kg Δ^9-tetrahydro-cannabinol. On Days 2 and 3, each mouse was injected twice daily (9 a.m. and 4 p.m.) with 10 mg/kg Δ^9-tetrahydrocannabinol. On Day 4, mice were given the morning injection only. On Day 5, mice were transported to the laboratory and injected with the same dose of vehicle or Δ^9-tetrahydrocannabinol that they received on Day 1. They were then re-tested in the tetrad tests. A second experiment was conducted using an identical protocol with the exception that mice received injections of vehicle during the repeated dosing period from the afternoon of Day 1 until the morning of Day 4. Data from this experiment served as a control to evaluate the effects of testing the mice with Δ^9-tetrahydrocannabinol in the tetrad twice, once on Day 1 and the second time on Day 5. Separate mice (*n*=11–12 per dose for all tests except ring immobility, *n*=5–6 mice per dose) were tested for each dose.

The protocol for cross-tolerance experiments with anandamide, 2-methylanandamide, and O-1812 was similar to that used for Δ^9-tetrahydrocannabinol, except that individual mice were only tested in two of the tetrad tests. This change was necessary due to the short duration of action of anandamide and other endogenous cannabinoids. Separate groups of mice (*n*=5–6 per dose) were injected with a single dose of drug on Day 1 and were tested in two of the tetrad tests (spontaneous activity and tail flick or rectal temperature and ring immobility). Different mice tested in the other two tests for each drug dose. Following initial tests on Day 1, mice received repeated injections with Δ^9-tetrahydrocannabinol as described above for the Δ^9-tetrahydrocannabinol tolerance experiment. On Day 5, mice were again injected with the same dose of drug and tested in the same two tests as on Day 1.

Prior to testing in any of the tetrad procedures, mice were acclimated to the experimental setting (ambient temperature 22–24 °C) for at least 1 h. Pre-injection control values were determined for rectal temperature and tail-flick latency (in seconds). For the Δ^9-tetrahydrocannabinol tolerance experiment and the associated vehicle control experiment, the following procedure was used for the tetrad tests. Five min after i.v. injection with Δ^9-tetrahydrocannabinol or vehicle, mice were placed in individual activity chambers and spontaneous activity was measured for 10 min. Activity was measured as total number of interruptions of 16 photocell beams per chamber during the 10-min test and expressed as % inhibition of activity of the vehicle group. Tail-flick latency was measured at 20 min post-injection. Maximum latency of 10 s was used. Antinociception was calculated as percent of maximum possible effect {% maximal possible effect=[(test−control latency)/ (10−control)]×100}. Control latencies typically ranged from 1.5 to 4.0 s. At 30 min post-injection, rectal temperature was measured. This value was expressed as the difference between control temperature (before injection) and temperatures following drug administration (Δ°C). Ring immobility was evaluated for 5 min beginning at 40 min post-injection. During placement on the ring immobility apparatus, 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 percent immobility rating. The criterion for ring immobility was the absence of all voluntary movement, including snout and whisker movement.

For the cross-tolerance experiments with anandamide and its analogs, each mouse was tested in two procedures (either spontaneous activity and tail flick or rectal temperature and ring immobility). Tail-flick latency or rectal temperature was measured at 4 min after the last injection. One min after measurement of antinociception or rectal temperature, mice were placed in individual activity chambers where spontaneous activity was measured for 10 min or they were placed on the ring immobility apparatus for 5 min. Dependent measures were calculated in the same manner as described above for the Δ^9-tetrahydrocannabinol tolerance experiment.

2.5. Data analysis

Data for each measure within each tolerance/cross-tolerance experiment were separately analyzed with two-way (dose×time) analysis of variance (ANOVA). When ANOVAs were significant, Tukey post hoc tests (α=0.05) were used to analyze the data further. In addition, in order to estimate degree of tolerance/cross-tolerance, ED_{50}s (defined as the dose at which half maximal effect occurred) were

calculated separately using least-squares linear regression on the linear part of the dose-effect curve for each measure in the mouse tetrad, plotted against $\log_{10}$ transformation of the dose. Based on data obtained from numerous previous studies with cannabinoids (for review see Compton et al., 1993; Martin et al., 1991), maximal cannabinoid effects in each procedure were estimated as follows: 100% inhibition of spontaneous activity and 100% maximal possible effect in the tail flick procedure. Maximal change in rectal temperature was estimated at -6 °C for Δ^9-tetrahydrocannabinol and at -3 °C for anandamide and its analogs (Ryan et al., 1997; Seltzman et al., 1997). Estimated maximal percentage ring immobility was 60% for Δ^9-tetrahydrocannabinol and anandamide and 100% for the two anandamide analogs.

3. Results

On Day 1, Δ^9-tetrahydrocannabinol produced dose-dependent antinociception, catalepsy, and decreases in spontaneous activity and rectal temperature in the Δ^9-tetrahydrocannabinol tolerance experiment (Fig. 2) and the associated vehicle control experiment (Fig. 3). Initial ED_{50} values for Δ^9-tetrahydrocannabinol ranged from 1.39 to 6.32 mg/kg (Table 1). In the vehicle control experiment, a significant main effect for dose was observed for each test. Post hoc analysis revealed that Δ^9-tetrahydrocannabinol

significantly and dose-dependently decreased spontaneous activity and body temperature and increased antinociception and catalepsy. A significant interaction was not demonstrated for any of the measures after repeated vehicle injection nor was a significant main effect of time shown for locomotion, temperature or catalepsy; hence, no significant tolerance or sensitization was observed with these latter three measures. This finding was also supported by the observation that the 95% confidence limits of the before and after ED_{50} values did not overlap. In contrast, a significant main effect for time was observed for antinociception such that less antinociception was seen after repeated vehicle injection than before. ED_{50} values indicated that vehicle control mice exhibited a maximum of 2-fold tolerance to antinociceptive effects of Δ^9-tetrahydrocannabinol upon the second injection. In contrast with the results obtained after repeated dosing with vehicle, repeated dosing with Δ^9-tetrahydrocannabinol produced pronounced tolerance across all four measures. The magnitude of tolerance to the cataleptic and antinociceptive effects of Δ^9-tetrahydrocannabinol ranged from 10- to 14-fold, respectively. Furthermore, efficacy of the highest Δ^9-tetrahydrocannabinol dose (30 mg/kg) to induce reduction of spontaneous activity and hypothermia decreased by about 50% such that calculation of exact ED_{50} values were not possible for these measures. For each measure, dose and time main effects, as well as interactions, were significant. Post hoc analysis of the interactions showed that Δ^9-tetrahydrocannabinol doses
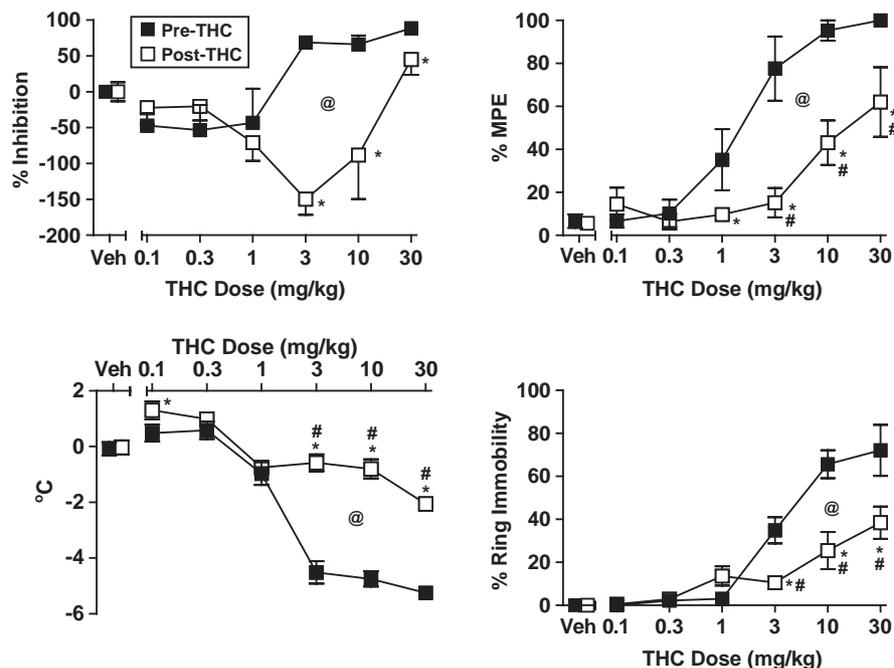

Fig. 2. Effects of i.v. Δ^9-tetrahydrocannabinol on percent (%) inhibition of spontaneous activity (upper left panel), percent (%) maximal possible antinociceptive effect (upper right panel), change in rectal temperature (lower left panel), and percent (%) time of ring immobility (lower right panel) before (□) and after (■) six repeated dosings with 10 mg/kg Δ^9-tetrahydrocannabinol s.c. over a period of 4 days. Each point represents the mean ($\pm$S.E.M.) of data from 4 to 6 mice. [#]A significant main effect for dose; [@]A significant main effect for time; *A significant interaction and that the pre- and post-Δ^9-tetrahydrocannabinol points differ.
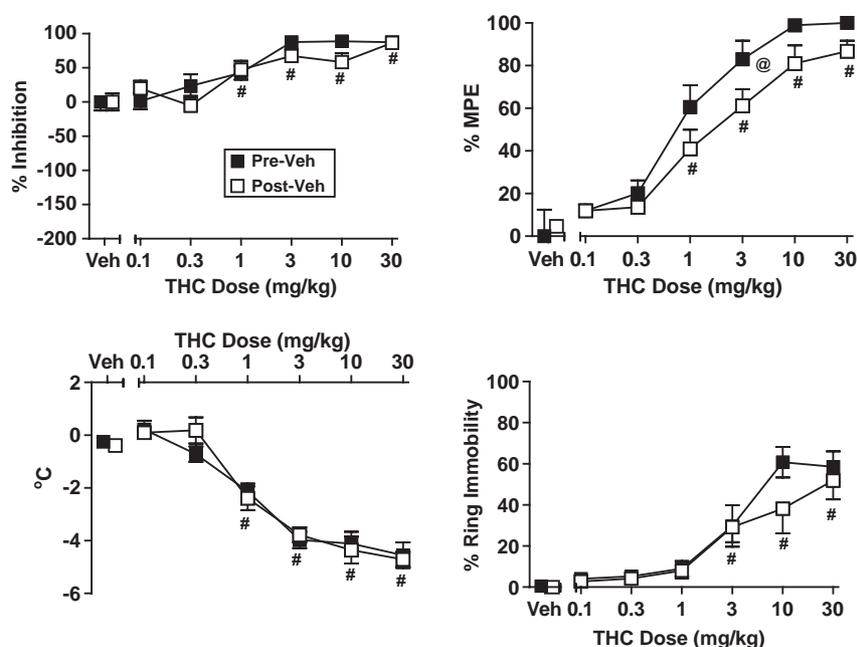
Fig. 3. Effects of i.v. Δ^9-tetrahydrocannabinol on percent (%) inhibition of spontaneous activity (upper left panel), percent (%) maximal possible antinociceptive effect (upper right panel), change in rectal temperature (lower left panel), and percent (%) time of ring immobility (lower right panel) before (□) and after (■) six repeated dosings with vehicle (1:1:18 ratio of emulphor/ethanol/saline) s.c. over a period of 4 days. Each point represents the mean (±S.E.M.) of data from 6 to 12 mice. #A significant main effect for dose; @A significant main effect for time.

within the range of 1–30 mg/kg produced more prominent cannabinoid effects in the tests before repeated dosing with Δ^9-tetrahydrocannabinol than after (see Fig 2 for indication of the exact simple effects that were significant).

Unlike with Δ^9-tetrahydrocannabinol, pronounced cross-tolerance to anandamide-induced effects did not develop after repeated dosing with Δ^9-tetrahydrocannabinol (Fig. 4). Anandamide ED_{50} values for hypomobility and hypothermia were approximately equal for Days 1 and 5. A

significant main effect for dose was observed for these two measures. Although a significant main effect for time and a significant interaction were also seen for hypothermia, post hoc analysis revealed that these effects were produced by before-after differences in the vehicle group as well as overall low variability for this measure. The ED_{50} for catalepsy was lower after dosing with Δ^9-tetrahydrocannabinol than before; however, variability with this measure was high as indicated by the large and overlapping

Table 1

Potencies of cannabinoids before and after repeated dosing with Δ^9-tetrahydrocannabinol

| ED_{50} values in mg/kg (95% CL)				
Drug	Hypoactivity	Antinociception	Hypothermia	Ring immobility
Vehicle control *(repeated dosing with s.c. vehicle)*				
Day 1: Δ^9-THC, i.v.	1.29 (0.77–2.15)	0.84 (0.58–1.21)	2.92 (1.99–4.30)	2.08 (1.25–3.44)
Day 5: Δ^9-THC, i.v.	2.34 (1.13–4.85)	1.83 (1.27–2.64)	2.93 (1.99–4.31)	3.96 (1.81–8.69)
Δ^9-Tetrahydrocannabinol *(repeated dosing with s.c. Δ^9-THC)*				
Day 1: Δ^9-THC, i.v.	6.32 (2.83–14.1)	1.39 (0.92–2.12)	2.85 (2.01–4.04)	1.78 (1.18–2.67)
Day 5: Δ^9-THC, i.v.	~30	19.44 (6.98 to out of range)	>30	18.56 (3.36 to out of range)
Anandamide *(repeated dosing with s.c. Δ^9-THC)*				
Day 1: Anandamide, i.v.	29.22 (6.05 to out of range)	3.04 (1.71–5.40)	32.38 (15.86 to out of range)	18.04 (9.06–35.94)
Day 5: Anandamide, i.v.	33.39 (5.24 to out of range)	13.05 (5.10–33.38)	~ 30	7.18 (4.91–10.50)
2-Methylanandamide *(repeated dosing with s.c. Δ^9-THC)*				
Day 1: 2-Methylanandamide, i.v.	2.44 (1.62–3.69)	2.03 (1.21–3.42)	~1	13.93 (10.31–18.82)
Day 5: 2-Methylanandamide, i.v.	22.95 (13.06–40.32)	6.10 (4.73–7.87)	2.05 (1.22–3.45)	10.49 (8.28–13.27)
O-1812 *(repeated dosing with s.c. Δ^9-THC)*				
Day 1: O-1812, i.v.	0.03 (0.02–0.06)	0.03 (0.02–0.05)	no hypothermia (>1)	0.22 (0.15–0.32)
Day 5: O-1812, i.v.	stimulation only	0.38 (0.24–0.60)	no hypothermia (>1)	1.39 (1.22–1.58)
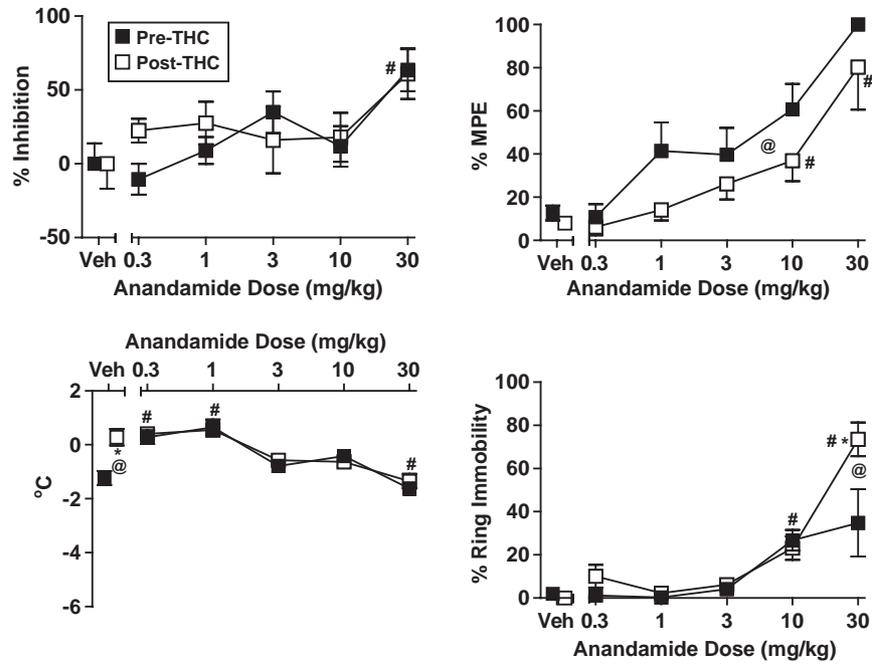
Fig. 4. Effects of i.v. anandamide on percent (%) inhibition of spontaneous activity (upper left panel), percent (%) maximal possible antinociceptive effect (upper right panel), change in rectal temperature (lower left panel), and percent (%) time of ring immobility (lower right panel) before (□) and after (■) six repeated dosings with 10 mg/kg Δ^9-tetrahydrocannabinol s.c. over a period of 4 days. Each point represents the mean (±S.E.M.) of data from 4 to 6 mice. [#]A significant main effect for dose; [@]A significant main effect for time; *A significant interaction and that the pre- and post-Δ^9-tetrahydrocannabinol points differ.

confidence intervals. Post hoc analysis of the significant interaction for this measure showed that a before-after difference in score was seen only at the 30 mg/kg dose of anandamide. A modest degree (4-fold) of cross-tolerance

between anandamide and Δ^9-tetrahydrocannabinol antinociceptive effects was indicated, albeit confidence limits showed a slight overlap. This cross-tolerance was also evidenced by a significant main effect of time for this
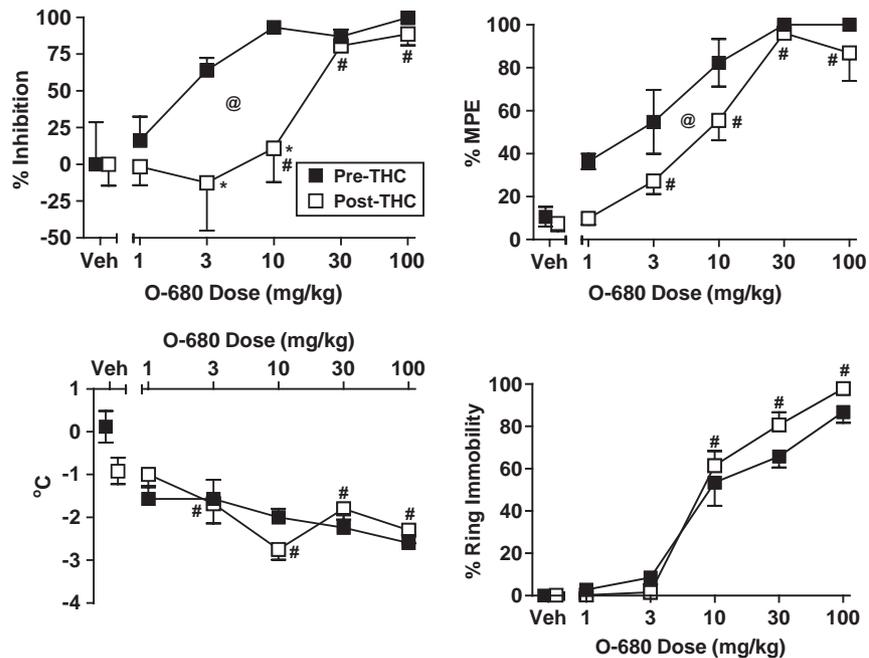

Fig. 5. Effects of i.v. 2-methylanandamide (O-680) on percent (%) inhibition of spontaneous activity (upper left panel), percent (%) maximal possible antinociceptive effect (upper right panel), change in rectal temperature (lower left panel), and percent (%) time of ring immobility (lower right panel) before (□) and after (■) six repeated dosings with 10 mg/kg Δ^9-tetrahydrocannabinol s.c. over a period of 4 days. Each point represents the mean (±S.E.M.) of data from 4 to 6 mice. [#]A significant main effect for dose; [@]A significant main effect for time; *A significant interaction and that the pre- and post-Δ^9-tetrahydrocannabinol points differ.

measure (i.e., less antinociception was observed following repeated dosing with Δ^9-tetrahydrocannabinol than before).

The maximal degree of cross-tolerance between Δ^9-tetrahydrocannabinol and the two anandamide analogs, 2-methylanandamide (Fig. 5) and O-1812 (Fig. 6), was greater than for anandamide; however, similar to anandamide, cross-tolerance between Δ^9-tetrahydrocannabinol and these anandamide analogs was not consistent across all measures. 2-Methylanandamide was 9-fold less potent at reducing spontaneous activity after repeated dosing with Δ^9-tetrahydrocannabinol; analysis of significant main effects for dose and time and the significant interaction revealed that 3 and 10 mg/kg 2-methylanandamide produced less inhibition of locomotor activity after repeated Δ^9-tetrahydrocannabinol administration than before. Maximal locomotor suppression was observed at the 30 and 100 mg/kg doses at both time points; hence, a ceiling effect may have prevented observation of differences at these doses. In contrast, the degree of tolerance (if any) to the antinociceptive, hypothermic, and cataleptic effects of 2-methylanandamide was less pronounced. A 3-fold rightward shift in potency for antinociception was observed, as confirmed by a significant main effect for time for this measure. Before and after potencies for hypothermia were 2-fold or less and neither the time main effects nor the interactions were statistically significant, suggesting that significant cross-tolerance did not develop for these measures. Similarly, O-1812 was 13-fold less potent at inducing antinociception at Day 5; however, it was also 6-fold less potent at producing catalepsy. Signifi-

cant dose and time main effects and interactions were observed for these measures. Post hoc analysis of the interaction showed that antinociceptive and cataleptic effects at the higher O-1812 doses were significantly lower after repeated Δ^9-tetrahydrocannabinol dosing than before. Furthermore, a significant main effect for time was seen for the locomotor activity measure. Although O-1812 initially produced only decreases in spontaneous activity, it produced only stimulation of activity after repeated dosing with Δ^9-tetrahydrocannabinol. Typically, stimulation is induced only by lower doses of cannabinoid agonists or by cannabinoid antagonists (Bass et al., 2002; Sañudo-Peña et al., 2000). Tolerance to hypothermia could not be evaluated, as O-1812 did not produce a significant degree of hypothermia over the range of doses during either test, although it had done so in previous experiments (Di Marzo et al., 2001).

4. Discussion

In the present study, pronounced tolerance to the tetrad of in vivo pharmacological effects of Δ^9-tetrahydrocannabinol developed after as few as six supplemental injections over a 4-day period. Indeed, a small (~2-fold), but significant, shift of the Δ^9-tetrahydrocannabinol dose–effect curve for antinociception was observed in mice injected for only the second time with Δ^9-tetrahydrocannabinol after an intervening period of 3.5 days during which they received vehicle injections. These results are consistent with numerous
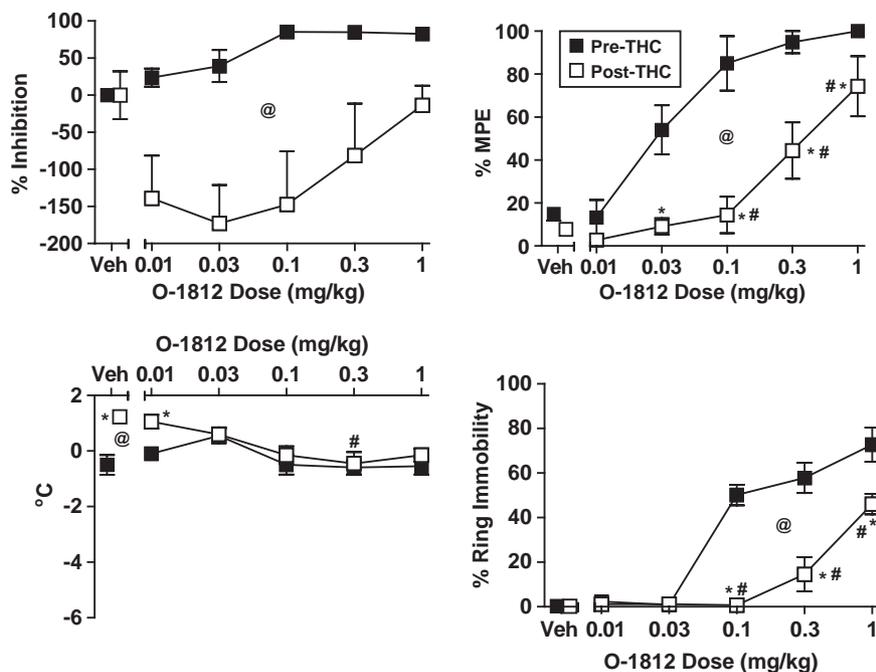

Fig. 6. Effects of i.v. O-1812 on percent (%) inhibition of spontaneous activity (upper left panel), percent (%) maximal possible antinociceptive effect (upper right panel), change in rectal temperature (lower left panel), and percent (%) time of ring immobility (lower right panel) before (□) and after (■) six repeated dosings with 10 mg/kg Δ^9-tetrahydrocannabinol s.c. over a period of 4 days. Each point represents the mean (±S.E.M.) of data from 4 to 6 mice. #A significant main effect for dose; @A significant main effect for time; *A significant interaction and that the pre- and post-Δ^9-tetrahydrocannabinol points differ.

previous studies which have shown that tolerance develops to many of the pharmacological effects of Δ^9-tetrahydrocannabinol and in several species, including mice, rats, monkeys, and dogs (for review, see Compton et al., 1990). Furthermore, the time course of tolerance development parallels that seen in a previous study in which maximal tolerance occurred with supplemental injections over a period of 3.5 days (Bass and Martin, 2000). These behavioral changes were associated with a loss of brain cannabinoid CB_1 cannabinoid receptors in specific brain areas such as the hippocampus and cerebellum (Breivogel et al., 1999); alteration of cannabinoid receptor B_{max} was not observed in whole brain assays (Abood et al., 1993). In addition to inducing tolerance to its own effects, chronic administration of Δ^9-tetrahydrocannabinol also produces cross-tolerance to the effects of other tricyclic, bicyclic, and aminoalkylindole cannabinoids (Fan et al., 1994; Pertwee et al., 1993).

In contrast to full tolerance produced by Δ^9-tetrahydrocannabinol to itself across all assays, the degree of cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide-like cannabinoids observed in the present study depended upon the specific drug and measure. The effects of repeated dosing with anandamide-like cannabinoids on spontaneous activity were variable across compounds. Whereas pronounced cross-tolerance was observed with 2-methylanandamide, cross-tolerance did not occur with anandamide. With O-1812, only stimulation of activity was recorded during the Day 5 test. Typically, stimulation is observed with lower doses of traditional cannabinoids (Sañudo-Peña et al., 2000), but was not seen with any dose of O-1812 on Day 1. Nevertheless, since stimulation is characteristically a low-dose cannabinoid effect, it may represent cross-tolerance, as cross-tolerance implies that higher doses are required to produce an effect that is usually produced by lower doses. On the other hand, stimulation has also been reported following administration of the cannabinoid CB_1 antagonist SR141617A (Bass et al., 2002; Compton et al., 1996), albeit this effect is not believed to be mediated via interaction with the cannabinoid CB_1 receptor (Bass et al., 2002).

Unlike for spontaneous activity, cross-tolerance to the antinociceptive effects of Δ^9-tetrahydrocannabinol was robust and occurred after repeated injections of all three anandamide-like cannabinoids, although its magnitude varied across compounds from 3-fold for 2-methylanandamide, 4-fold for anandamide, and 13-fold for O-1812. Antinociception was also the measure to which Δ^9-tetrahydrocannabinol showed greatest degree of tolerance and the magnitude of cross-tolerance with O-1812 for antinociception approximated the degree of tolerance produced by Δ^9-tetrahydrocannabinol to itself. In contrast with O-1812, the magnitude of cross-tolerance with 2-methylanandamide and anandamide was notably small, particularly given that a 2-fold rightward shift of the Δ^9-tetrahydrocannabinol dose–effect curve for antinociception

occurred in the vehicle control group. Previous studies have also reported cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide for antinociception (Fride, 1995; Welch, 1997), as well as tolerance to this effect following repeated dosing with anandamide (Fride, 1995; Welch et al., 1995). As with 2-methylanandamide and anandamide in the present study, however, the magnitude of cross-tolerance tended to be small (e.g. 2- to 4-fold; Welch, 1997).

In contrast to cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide-like cannabinoids for their antinociceptive effects, little cross-tolerance for hypothermia was observed with anandamide or either of its analogs. Although a previous study with mice also failed to find cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide for hypothermia (Pertwee et al., 1993), other studies have reported development of tolerance and/or cross-tolerance to anandamide's hypothermic effects (Costa et al., 2000; Fride, 1995). One difference between these latter studies and the present study was the duration of tolerance induction was considerably longer in the previous studies (2 weeks as compared to 4 days). It is possible that a longer period of Δ^9-tetrahydrocannabinol exposure is required for the receptor alterations necessary for cross-tolerance to anandamide's hypothermic effects to occur than for those necessary for cross-tolerance to its antinociceptive or locomotor effects.

Cross-tolerance to the cataleptic effects of Δ^9-tetrahydrocannabinol occurred with O-1812, but not with anandamide or 2-methylanandamide. Hence, of the three anandamide-like analogs, O-1812 produced the greatest degree of cross-tolerance across measures (providing stimulation of activity is considered a "cross-tolerance" effect). Interestingly, of the four cannabinoids tested in this study, O-1812 was the most potent in vivo and had the greatest affinity for cannabinoid CB_1 receptors (Ki=4.6 nM for O-1812; compared to 89 nM for anandamide, 53 nM for 2-methylanandamide, and 41 nM for Δ^9-tetrahydrocannabinol, with phenylmethylsulfonyl fluoride added to binding assay for all anandamide-like compounds; Adams et al., 1995b; Di Marzo et al., 2001). Furthermore, the cannabinoid CB_1 receptor binding affinity for O-1812 was not significantly altered when measured without phenylmethylsulfonyl fluoride (Ki=3.4 nM) whereas those of anandamide and 2-methylanandamide were changed dramatically (Ki=5400 and 137 nM, respectively), suggesting that O-1812 may be more resistant to metabolic degradation. Hence, pharmacokinetics could play a potential role in O-1812's greater degree of cross-tolerance across measures. In addition, an association between high affinity and greater magnitude of tolerance development has been observed previously with Δ^9-tetrahydrocannabinol and CP 55,940; i.e., the magnitude of tolerance development in the tetrad tests following repeated injection with CP 55,940 is approximately 100-fold versus only 4-fold after repeated treatment with Δ^9-tetrahydrocannabinol (Fan et al., 1994). Finally, in acute dosing studies, anandamide analogs have exhibited less

correspondence between measures of binding affinity and pharmacological potency in these assays as compared to that which has been reported for classical cannabinoids (Adams et al., 1995a,b; Compton et al., 1993), suggesting that other factors besides cannabinoid CB_1 receptor affinity may contribute to the potency of anandamide analogs in these assays.

Perhaps the most notable finding in the present study, however, was the task specificity of cross-tolerance between anandamide-like analogs and Δ^9-tetrahydrocannabinol. Whereas repeated dosing with Δ^9-tetrahydrocannabinol induced tolerance to all four of its own effects, cross-tolerance with anandamide analogs was observed for antinociception (all three compounds), suppression of spontaneous activity (2-methylanandamide and O-1812), catalepsy (O-1812), but not for hypothermia. Pharmacodynamic factors undoubtedly are involved in this task specificity, given that differences in cross-tolerance among cannabinoids also occurs in an isolated tissue preparation (Pertwee et al., 1993). A couple of pharmacodynamic factors are likely candidates. First, although each of these tasks is mediated by cannabinoid CB_1 receptors (Compton et al., 1993, 1996), the specific receptors mediating each effect are probably located in different areas of the brain and/or spinal cord. In vitro analysis of rat brain following repeated treatment with Δ^9-tetrahydrocannabinol has shown that down-regulation and desensitization of cannabinoid CB_1 receptors is both time- and region-dependent (Breivogel et al., 1999; Romero et al., 1998), suggesting that tolerance/cross-tolerance in tasks mediated by some brain areas may occur at different rates than tolerance/cross-tolerance in tasks mediated by other brain areas. Of course, this factor would be likely to affect both tolerance and cross-tolerance across the tasks; yet, only cross-tolerance was task specific with the dosing regimen employed. A second factor that may play a role in task specificity is the regional level of endogenous cannabinoids. Di Marzo et al. (2000a) has shown that the above-mentioned cannabinoid CB_1 receptor down-regulation/desensitization following repeated dosing with Δ^9-tetrahydrocannabinol is accompanied by changes in regional endocannabinoid contents. Furthermore, these changes were independent of alterations in cannabinoid CB_1 receptors and, hence, provide a possible mechanism through which task specificity for cross-tolerance to anandamide analogs could develop in the absence of task specificity for tolerance to Δ^9-tetrahydrocannabinol itself. Third, it is possible that differences in the degree of cross-tolerance across task may be related to differences in the degree to which other cannabinoid receptor subtypes (e.g., the putative cannabinoid CB_3 receptor) might be involved in producing the effect. Primary lines of evidence in support of the existence of additional cannabinoid receptors include observed differences in brain levels of anandamide and its in vivo pharmacology in CB_1 knockout and CB_1/CB_2 double knockout mice (Di Marzo et al., 2000b; Járai et al., 1999; see Wiley and Martin, 2002 for a review).

In summary, Δ^9-tetrahydrocannabinol exhibited pronounced tolerance to itself in all four in vivo assays. In contrast, cross-tolerance with anandamide and two anandamide analogs, 2-methylanandamide and O-1812, was task-specific. These results suggest that anandamide-like cannabinoids may have a unique pharmacology that only partially overlaps with that of Δ^9-tetrahydrocannabinol and other traditional cannabinoids. Although the basis for this unique pharmacology has not as yet been determined, pharmacodynamic factors are certain to play a role, as differences between Δ^9-tetrahydrocannabinol and anandamide have been observed in vitro as well as in vivo. Regional specificity of cannabinoid CB1 receptor downregulation and endocannabinoid release induced by repeated dosing with Δ^9-tetrahydrocannabinol may contribute to task specificity of cross-tolerance observed in the present study, as may differential activation of putative cannabinoid CB_3 receptors.

Acknowledgements

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