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European Journal of Pharmacology 357 (1998) 139–148

ejp

Effects of SR 141716A after acute or chronic cannabinoid administration in dogs

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Received 26 January 1998; revised 10 July 1998; accepted 24 July 1998

Abstract

The effects of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A), a specific cannabinoid receptor antagonist, were assessed in the dog static ataxia test after either acute treatment with two cannabinoid receptor agonists, Δ^9-tetrahydrocannabinol and arachidonylethanolamide (anandamide), or chronic treatment with Δ^9-tetrahydrocannabinol. As previously reported, acute intravenous (i.v.) injected Δ^9-tetrahydrocannabinol produced dose-dependent cannabinoid effects, including marked static ataxia, prancing, loss of muscle tone, and incoordination. The behavioral profile of anandamide was distinctly different in that it produced a loss of muscle tone and considerable sedation with little static ataxia, prancing, or incoordination. Despite these qualitative differences between the two agonists, SR 141716A blocked the acute behavioral effects of both drugs indicating a cannabinoid receptor mechanism of action. Interestingly, SR 141716A was able to precipitate a withdrawal syndrome in Δ^9-tetrahydrocannabinol-tolerant dogs, but failed to produce any observable effects in dogs receiving chronic vehicle injections. Acute toxicity caused by anandamide, which was not blocked by SR 141716A, precluded conducting dependence studies with this drug. The Δ^9-tetrahydrocannabinol precipitated withdrawal syndrome included diarrhea, vomiting, excessive salivation, decreases in social behavior, and increases in restless behavior and trembling. This is the first demonstration of a precipitated withdrawal syndrome in a non-rodent species.

Keywords: Cannabinoid; Anandamide; δ^9-Tetrahydrocannabinol; SR 141716A; Withdrawal precipitated; Dependence; Static ataxia

1. Introduction

The development of the cannabinoid receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR 141716A) (Rinaldi-Carmona et al., 1994), has provided a powerful tool for the investigation of cannabinoid pharmacology. SR 141716A has been reported to block many of the acute behavioral effects of the cannabinoids in rodents including antinociception, locomotor suppression, hypothermia (Rinaldi-Carmona et al., 1994; Compton et al., 1996) as well as the hypotensive (Varga et al., 1995), memory impairing (Brodkin and Moerschbaecher, 1997; Lichtman and Martin, 1997), and discriminative stimulus cues of these drugs (Wiley et al., 1995b,c). Following chronic Δ^9-tetrahydrocannabinol administration, SR 14716A has been found to precipitate a withdrawal syndrome in rats (Aceto et al., 1995; Tsou et al., 1995) and mice (Cook et al., 1998).

The presence of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in brain (Devane et al., 1992) suggests the existence of an endogenous cannabinoid system. Anandamide can be formed in neurons (Marzo et al., 1994), binds to both cannabinoid receptor subtypes, CB_1 (Devane et al., 1992) and CB_2 (Showalter et al., 1996), inhibits forskolin-stimulated adenylyl cyclase (Vogel et al., 1993) as well as Ca^{2+} currents (Mackie et al., 1993), and when given exogenously can produce similar pharmacological effects as other cannabinoid receptor agonists in mice (Fride and Mechoulam, 1993; Smith et al., 1994a). However, a variety of differences exist between anandamide and other cannabinoid receptor ago-

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0014-2999/98/$19.00
PII: S0014-2999(98)00558-5

nists. Most notably, SR 141716A failed to block the antinociceptive, cataleptic, locomotor suppressant, and hypothermic actions of anandamide in mice (Adams et al., 1998). However, the antinociceptive and locomotor suppressant effects of a metabolically stable analog of anandamide, 2-methyl-2′-fluoroethylanandamide, were antagonized by SR 141716A. In addition, SR 141716A has been reported to antagonize the bradycardic and prolonged depressor effects of anandamide in rats (Lake et al., 1997) as well as anandamide-induced inhibition of long term potentiation in rat hippocampal slices (Terranova et al., 1995). Unlike Δ^9-tetrahydrocannabinol, which is active after either intrathecal or intracerebroventricular administration in mice (Welch et al., 1995b) and rats (Lichtman and Martin, 1991; Lichtman et al., 1996), anandamide failed to produce antinociception, catalepsy, or hypothermia after intracerebroventricular administration in rats (Lichtman et al., 1996), though it was pharmacologically active in mice after intrathecal administration (Smith et al., 1994a). Finally, the spinal mechanisms underlying the antinociceptive effects of anandamide appear to differ from those of other cannabinoids. Intrathecal administration of the κ-opioid receptor antagonist *nor*-binaltorphimine as well as two modulators of c-AMP, Cl-c-AMP and forskolin, blocked the antinociceptive effects of Δ^9-tetrahydrocannabinol, but not those of anandamide (Smith et al., 1994a,b; Welch et al., 1995a).

A major goal of the present research was to compare the pharmacological actions of Δ^9-tetrahydrocannabinol, a prototypical cannabinoid, and anandamide in the dog static ataxia test. Dogs are known to be particularly sensitive to the psychoactive effects of cannabinoids, and this assay has been found to be highly predictive of cannabinoid activity since its first use, over 60 years ago (Walton et al., 1938). In this paradigm, intravenous (i.v.) administration of a cannabinoid agonist produces a behavioral syndrome marked by a combination of sedation, excitation, prancing, and motor incoordination that is unique for this class of compounds. Importantly, a structure-activity relationship has been demonstrated between cannabinoids and the behavior assessed in the dog static ataxia test (Wilson and May, 1974; Martin et al., 1976; Wilson and May, 1979; Martin et al., 1984). Consequently, this model provides additional means for verifying the cannabinoid properties shared by anandamide and Δ^9-tetrahydrocannabinol. In order to determine the involvement of cannabinoid receptors, the dogs were also pretreated with SR 141716A prior to each agonist.

Another issue that has received considerable attention recently is whether physical withdrawal symptoms occur in chronic cannabis users following drug cessation. Although the cannabis dependence syndrome is not characterized in terms of physical symptoms (DSM-IV, 1994), an abrupt withdrawal syndrome has been described after prolonged marijuana exposure (Jones and Benowitz, 1976) or Δ^9-tetrahydrocannabinol administration (Jones et al., 1981) to humans. Similarly, abrupt withdrawal from chronic Δ^9-tetrahydrocannabinol in rhesus monkeys has been reported to disrupt operant responding (Beardsley et al., 1986) all well as induce a variety of behavioral effects including hyperirritability, tremors, and anorexia (Kaymakcalan and Deneau, 1972). Still, other studies failed to observe abrupt withdrawal effects following chronic Δ^9-tetrahydrocannabinol administration in dogs (McMillan et al., 1971) or rats (Leite and Carlini, 1974; Aceto et al., 1996). Following the availability of a cannabinoid receptor antagonist, a striking precipitated withdrawal syndrome that included wet dog shakes, forepaw fluttering, and increases in grooming, scratching, and overall activity that occurred upon challenge with SR 141716A was characterized in rats treated chronically with Δ^9-tetrahydrocannabinol (Aceto et al., 1995; Tsou et al., 1995; Aceto et al., 1996). A similar precipitated cannabinoid withdrawal syndrome has been recently described in mice (Cook et al., 1998). The availability of the cannabinoid receptor antagonist also afforded us the opportunity to determine whether SR 141716A can precipitate a withdrawal syndrome in dogs. One of the initial goals of this study was to compare the effects of SR 141716A challenge in dogs treated chronically with Δ^9-tetrahydrocannabinol and anandamide. However, toxicity was observed to occur after an acute anandamide injection, thus precluding the administration of chronic anandamide. In the present study, dogs made tolerant to Δ^9-tetrahydrocannabinol or treated chronically with vehicle were challenged with SR 141716A and observed for behavioral alterations.

2. Materials and methods

2.1. Subjects

Ten female beagles weighing between 9 and 11 kg were individually housed in a temperature controlled (20–22°C) environment with a 12-h light/dark cycle. The dogs were fed approximately 25–45 g kg^{-1} day^{-1} of Teklad 25% Lab Dog Diet (Harlan, Madison, WI, USA) and water was available ad libitum. All procedures were approved by the Virginia Commonwealth University Animal Care and Use Committee.

2.2. Drugs

Δ^9-tetrahydrocannabinol was provided by the National Institute on Drug Abuse, SR 141716A was provided by Pfizer (Groton, CT, USA), and anandamide was supplied by Organix (Woburn, MA, USA). Each drug was dissolved in a 1:1 mixture of absolute ethanol and Emulphor-620 (Rhone-Poulenc, Princeton, NJ, USA) and diluted with saline to form a final vehicle mixture of ethanol: emulphor:saline (1:1:18). Intravenous injections were given

into the dorsal side of a leg in a volume of 1 ml/5 kg. As we anticipated needing relatively high concentrations of anandamide, the injection volume was 1.65 ml/5 kg in the initial anandamide experiment. However, because of the anandamide toxicity observed following a dose of 3 mg kg^{-1}, the injection volume used in the SR 141716A antagonism study was 1 ml/5 kg.

2.3. Acute studies

Each dog was weighed and singly brought into the test room (6.4 × 2.6 m) where it was allowed a few minutes to acclimate. In the initial studies, dogs were administered either vehicle, Δ^9-tetrahydrocannabinol (0.1, 0.2, 0.4 or 0.6 mg kg^{-1}) or anandamide (0.3, 1, or 3 mg kg^{-1}). In the antagonism studies, subjects were pretreated with vehicle or 1 mg kg^{-1} SR 141716A and then treated with either vehicle, Δ^9-tetrahydrocannabinol (0.6 or 2 mg kg^{-1}) or 3 mg kg^{-1} anandamide. In order to limit the incidence of tolerance, dogs were tested no more than twice per week.

A minimum of three observers, blind to the drug condition, scored the behavior of each dog using a slight modification (Martin et al., 1976) of the ordinal scale (Table 1) developed by Walton et al. (1938) at 5, 15, and 30 min after the injection and at these same time points following the first injection in the antagonism experiments. Based on the entire 30-min observation period, each observer assigned an overall rating to each animal. During the observation period, the observers were located in the perimeter of the room seated on the floor and leaning against the walls. In order to provide an ordinal measure of static ataxia, subjects were assigned scores of either 0 which indicated none observed, 1 which indicated mild static ataxia as manifested by swaying movements after the dog stood in one position between three and five min, 2 which indicated moderate static ataxia as manifested by swaying movements after the dog stood in one position between one and three min, or 3 which indicated marked static ataxia in which the dog swayed forward and backward as well as from side to side after standing in one position for less than 1 min. Finally, behavioral depression was defined as a decrease in activity from preinjection activity levels.

2.4. Chronic Δ^9-tetrahydrocannabinol administration and precipitated withdrawal

Dogs used in the acute studies served as subjects to examine whether SR 141716A would precipitate withdrawal after chronic treatment with Δ^9-tetrahydrocannabinol. Subjects were given two daily i.v. injections of Δ^9-tetrahydrocannabinol ($n = 6$) or vehicle ($n = 8$) in the test room, at approximately 0900 and 1600 h, for nine days. On days 1 and 2, subjects were given 0.6 mg kg^{-1} per injection Δ^9-tetrahydrocannabinol and the dose was escalated to 1.0 mg kg^{-1} per injection from days 3 to 9; the control dogs received two daily i.v. injections of vehicle. To assess tolerance, the dogs were observed and behavioral effects were scored immediately after the morning injection on days 1, 3, 5, 7 and 9. This high dose regimen was employed to ensure the likelihood of precipitating a withdrawal syndrome in Δ^9-tetrahydrocannabinol-tolerant dogs. On Day 10, each dog was challenged with 1.0 mg kg^{-1} SR 141716A approximately 2 h after its respective morning injection and observed for 30 min. A cross-over design was employed in which each subject was given a 10 day hiatus following the SR 141716A challenge prior to receiving the counter-balanced treatment. Each dog was observed for the following 17 behaviors: (1) decreased social behavior, exemplified by a withdrawal from human contact compared to non-drug days, (2) restless behavior, (3) restless circling, (4) trembling, shaking, or shivering, (5) hyper-responsiveness, an exaggerated response to auditory or visual stimuli typically present, (6) excessive salivation, (7) diarrhea, (8) vomiting, (9) exaggerated startle reflex to a swinging hand, (10) full body shakes, (11) head shakes, (12) yawning, (13) whining, (14) solid defecation, (15) urination, (16) genital licking and (17) sneezing. Half of the dogs were tested with chronic vehicle first and the other half received chronic Δ^9-tetrahydrocannabinol.

2.5. Statistical analysis

All ordinal data were analyzed using either the Kruskal–Wallis one-way analysis of variance or the Mann–Whitney *U*-test. Comparisons between each experi-

Table 1
Rating scale of cannabinoid behavioral effects in dogs[a]

Score	Behavioral effects
0	No effect
1	Slight depression of activity, slight static ataxia after standing in one position for 3–5 min
2	Walks with prance-like placement of feet, exaggerated reflex to a swinging hand, static ataxia after standing in one position for 2–3 min
3	Tail is often tucked, some loss of tone in hind legs, static ataxia more pronounced and seen after standing in one position for 1–2 min
4	Marked static ataxia, sways forward and backward and/or side to side, almost falls after standing in one position for a minute
5	Cannot stand for longer than 30 s without falling, frequently plunges about
6	Lies prostrate on the floor

[a]Rating scale based on a modified version of the Walton Static Ataxia Scale (Martin et al., 1976).

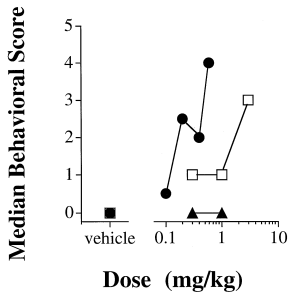

Fig. 1. The acute behavioral effects of an i.v. injection of Δ^9-tetrahydrocannabinol (–●–), anandamide (– □ –), and SR 141716A (-▲-) in dogs. The rating scale is shown in Table 1 and each rating is based on the dog's overall behavior throughout the 30-min observation period. The median behavioral score following the 3 mg kg^{-1} dose of anandamide reflects behavioral depression and loss of tone in the hind legs while the scores following Δ^9-tetrahydrocannabinol mainly reflect static ataxia.

Table 3
Comparison of the static-ataxia produced by i.v. injections of Δ^9-tetrahydrocannabinol and anandamide

Treatment	*n*	Static ataxia			
		No effect	Slight	Moderate	Marked
Vehicle	10	8	2	0	0
Δ^9-THC					
0.1 mg kg^{-1}	4	3	1	0	0
0.2 mg kg^{-1}	4	1	1	0	2
0.4 mg kg^{-1}	9	0	5	0	4
0.6 mg kg^{-1}	5	0	1	0	4
Anandamide					
0.3 mg kg^{-1}	5	4	1	0	0
1.0 mg kg^{-1}	5	3	2	0	0
3.0 mg kg^{-1}	3	2	1	0	0

Numbers reflect the number of dogs rated at each ordinal measure.

mental condition and the vehicle control group were conducted when the Kruskal–Wallis test achieved statistical significance. The significance was set at $P < 0.05$ for all analyses.

In the SR 141716A challenge study following chronic treatment with vehicle or Δ^9-tetrahydrocannabinol, each dog was observed for the 17 behavioral signs. Each behavioral sign that failed to occur was scored as a 0 and each behavioral sign that occurred was scored as a 1. The number of observed signs was summed for each dog. The data are presented as mean number of signs $\pm$ S.E. and analyzed using the Mann–Whitney *U*-test.

3. Results

The cannabinoid effects of Δ^9-tetrahydrocannabinol, anandamide, and SR 141716A are presented in Fig. 1. Δ^9-Tetrahydrocannabinol produced a significant increase in cannabinoid behavioral effects (χ^2 (4) = 14.8, $P < 0.05$), with the 0.6 mg kg^{-1} and 0.4 mg kg^{-1} Δ^9-tetrahydrocannabinol doses producing significantly greater effects than the vehicle. SR 141716A (0.3 or 1.0 mg kg^{-1}) given alone failed to produce any overt behavioral effects throughout the entire 30-min observational period. Although anandamide failed to affect significantly the overall behavioral score for the entire 30-min observation period ($P = 0.07$), a significant effect was found at the five min behavioral observation period (χ^2 (4) = 8.0, $P < 0.05$). At the 5-min observational period, 3 mg kg^{-1} of anandamide produced significantly greater cannabinoid behavioral effects than the vehicle treatment. It is unlikely that the lack of significance for the overall assessment resulted from a short time course of action because the median values were virtually identical between the overall assessment and the 5-min observational period. Instead, this lack of statistical significance is likely to be a consequence of increased variability in the vehicle and 1 mg kg^{-1} anandamide groups for the overall assessment compared to the 5-min observational period. Doses of anandamide higher than 3 mg kg^{-1} were not assessed because an unantici-

Table 2
Cannabinoid-sensitive behaviors in dogs following i.v. injections of either Δ^9-tetrahydrocannabinol or anandamide

Treatment	*n*	Prancing	Exaggerated reflex	Tail tucked	Loss of tone	Swaying	Plunges about	Lies prostrate
Vehicle	10	0	0	0	1	0	0	0
Δ^9-THC								
0.1 mg kg^{-1}	4	3	2	0	0	0	0	0
0.2 mg kg^{-1}	4	2	1	1	2	1	2	0
0.4 mg kg^{-1}	9	8	5	2	4	1	3	0
0.6 mg kg^{-1}	5	3	3	0	4	2	3	0
Anandamide								
0.3 mg kg^{-1}	5	1	0	0	2	0	0	0
1.0 mg kg^{-1}	5	2	0	0	3	0	0	0
3.0 mg kg^{-1}	3	0	0	0	2	0	0	0

Numbers reflect the number of dogs exhibiting each specific behavior.

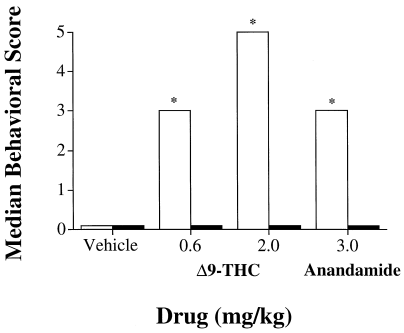

Fig. 2. A 10-min pretreatment with i.v. administered SR 141716A (1 mg kg^{-1}) completely blocked the acute behavioral effects of Δ^9-tetrahydrocannabinol (0.6 and 2.0 mg kg^{-1}) and anandamide (3 mg kg^{-1}). * Indicates significantly more behavioral effects were observed in the dogs pretreated with vehicle compared to the SR 141716A pretreatment for each respective dose of agonist ($P < 0.05$). Hollow bars indicate animals receiving vehicle pretreatment and speckled bars indicate pretreatment with SR 141716A. The median behavioral score following anandamide injection in the vehicle pretreated dogs reflects behavioral depression and loss of tone in the hind legs while the scores following Δ^9-tetrahydrocannabinol in the vehicle pretreated dogs mainly reflect static ataxia.

pated toxicity occurred following this dose that included vomiting and diarrhea accompanied with a bloody, mucous-like discharge from the rectum of one dog, vomiting alone in a second dog, and diarrhea accompanied with a bloody, mucous-like discharge from the rectum of a third dog. These dogs were given veterinary care and allowed at least a four day period to recover before testing resumed.

While the data in Fig. 1 represent the composite scores outlined in Table 1, it was apparent that these scores did not represent identical behaviors for anandamide and Δ^9-tetrahydrocannabinol. In order to examine qualitative differences between these two drugs, the specific behaviors that were used to derive a score are presented in Tables 2 and 3. Depicted in Table 2 is the number of dogs in each condition that exhibited various cannabinoid-sensitive behaviors. Δ^9-Tetrahydrocannabinol produced dose-dependent cannabinoid effects, including marked static ataxia, prancing, loss of muscle tone, and incoordination. The behavioral profile of anandamide was distinctly different in that it produced loss of muscle tone with little static ataxia, prancing, or incoordination. The 3 mg kg^{-1} dose of anandamide resulted in a median behavioral score of 3 because of this loss of muscle tone. In addition, both drugs produced behavioral depression as exhibited by a decrease in activity from preinjection activity levels. Shown in Table 3 is the ordinal assessment of static ataxia for each dog following i.v. administration of Δ^9-tetrahydrocannabinol or anandamide. Whereas Δ^9-tetrahydrocannabinol produced a dose-dependent increase in the degree of static ataxia, anandamide failed to produce any ataxia in half the dogs tested and produced only mild ataxia in the other half.

Pretreatment with 1 mg kg^{-1} SR 141716A completely blocked the behavioral effects of 0.6 and 2.0 mg kg^{-1} Δ^9-tetrahydrocannabinol ($z = 2.4$, $P < 0.05$ for both) as well as 3 mg kg^{-1} anandamide ($z = 2.5$, $P < 0.05$) compared to the dogs pretreated with vehicle (Fig. 2). In order to make a qualitative comparison between the behavioral effects of Δ^9-tetrahydrocannabinol and anandamide, various indices that were used to assign the behavioral score are shown in Tables 4 and 5. SR 141716A antagonized the various cannabinoid-sensitive effects, though one dog pretreated with SR 141716A prior to 2.0 mg kg^{-1} Δ^9-tetrahydrocannabinol exhibited prancing and an exaggerated reflex to a swinging hand (Table 4). In addition, SR 141716A blocked the profound static ataxia produced by 0.6 or 2.0 mg kg^{-1} of Δ^9-tetrahydrocannabinol (Table 5). Once again, the median rating of a 3 assigned to the dogs pretreated with vehicle prior to anandamide in Fig. 2 reflected behavioral depression and a lack of muscle tone in the hind legs. Two of the anandamide-treated dogs exhibited a lack of muscle tone, one of which also plunged about (Table 4). Pretreatment with SR 141716A resulted in only one anandamide-treated subject exhibiting a loss of muscle tone. Anandamide produced mild static ataxia in two of the three dogs pretreated with vehicle, while SR

Table 4
SR 141716A antagonism of cannabinoid-sensitive behavioral effects of acute i.v. injections of Δ^9-tetrahydrocannabinol and anandamide

Pretreatment	Treatment	*n*	Prancing	Exaggerated reflex	Tail tucked	Loss of tone	Swaying	Plunges about	Lies prostrate
Vehicle	Vehicle	3	0	0	0	0	0	0	0
SR 141716A	Vehicle	3	0	0	0	0	0	0	0
	Δ^9-THC								
Vehicle	0.6 mg kg^{-1}	3	2	3	0	1	0	1	0
SR 141716A	0.6 mg kg^{-1}	3	0	0	0	0	0	0	0
Vehicle	2.0 mg kg^{-1}	3	3	1	0	1	1	3	0
SR 141716A	2.0 mg kg^{-1}	3	1	1	0	0	0	0	0
	Anandamide								
Vehicle	3.0 mg kg^{-1}	3	0	0	0	2	0	1	0
SR 141716A	3.0 mg kg^{-1}	4	0	0	0	1	0	0	0

Numbers reflect the number of dogs exhibiting each specific behavior.

Table 5
SR 141716A antagonism of Δ^9-tetrahydrocannabinol- and anandamide-induced static ataxia

Pretreatment	Treatment	*n*	Static ataxia			
			No effect	Slight	Moderate	Marked
Vehicle	Vehicle	3	3	0	0	0
SR 141716A	Vehicle	3	3	0	0	0
	Δ^9-THC					
Vehicle	0.6 mg kg^{-1}	3	0	1	0	2
SR 141716A	0.6 mg kg^{-1}	3	3	0	0	0
Vehicle	2.0 mg kg^{-1}	3	0	0	0	3
SR 141716A	2.0 mg kg^{-1}	3	3	0	0	0
	Anandamide					
Vehicle	3.0 mg kg^{-1}	3	1	2	0	0
SR 141716A	3.0 mg kg^{-1}	4	4	0	0	0

Numbers reflect the number of dogs rated at each ordinal measure.

141716A pretreatment completely blocked this effect (Table 5). SR 141716A also completely blocked anandamide-induced behavioral depression. However, SR 141716A did not appear to block the visceral effects induced by anandamide. Anandamide administration elicited vomiting in all the dogs pretreated with vehicle and diarrhea in two of these animals. SR 141716A given prior to anandamide administration resulted in vomiting in three of the four dogs and diarrhea in the fourth animal. No apparent behavioral effects were observed following i.v. administration of SR 141716A in the vehicle-treated dogs.

As can be seen in Fig. 3, tolerance developed to the behavioral effects of Δ^9-tetrahydrocannabinol (χ^2 (4) = 15.6, $P < 0.05$). The first Δ^9-tetrahydrocannabinol injection of 0.6 mg kg^{-1} elicited a median behavioral score of 3.5; however, the median scores by day 5, after the dogs had received 1 mg kg^{-1} Δ^9-tetrahydrocannabinol, no longer differed from those in the vehicle condition. Nonetheless, four of the six dogs continued to exhibit prancing behavior even on day 9. In contrast, chronic vehicle treatment failed

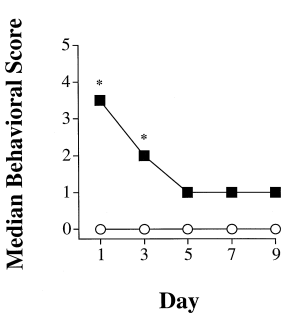

Fig. 3. Chronic behavioral effects of vehicle (– ○ –) or Δ^9-tetrahydrocannabinol (– ■ –) treatment. The Δ^9-tetrahydrocannabinol -treated dogs were administered 0.6 mg kg^{-1} of drug for each injection on days 1 and 2, and were administered 1.0 mg kg^{-1} of drug for each injection from days 3 to 9. Subjects were evaluated every other day for 15 min following the morning injection. * Indicates significantly greater effects were observed following Δ^9-tetrahydrocannabinol compared to the corresponding vehicle treatment ($P < 0.05$).

to elicit any observable behavioral effects other than non-specific scratching and facial edema immediately following the injection.

Challenge with SR 141716A elicited a qualitative change in behavior in Δ^9-tetrahydrocannabinol-tolerant dogs compared to the chronic vehicle treatment (Table 6). The number of behavioral signs that was exhibited by each dog was summed and the mean ± S.E. number of these signs for both groups is presented in Fig. 4. A significantly greater number of behavioral signs was elicited by SR 141716A in the chronic Δ^9-tetrahydrocannabinol group than in the chronic vehicle group ($z = 3.0$, $P < 0.05$). One of the most dramatic withdrawal effects was decreased social behavior, exemplified by a withdrawal from human contact compared to the chronic vehicle group. Whereas many of the subjects would typically seek close physical contact and interact playfully with the experimenters, SR 141716A treatment led to an avoidance of the experimenters, increased restless or nervousness accompanied by an uncharacteristic restless circling behavior. In dogs given chronic injections of Δ^9-tetrahydrocannabinol, SR 141716A also elicited trembling, shaking, or shivering as well as hyper-responsiveness or an exaggerated response to auditory or visual stimuli that typically occurred in the testing room. Precipitated withdrawal also elicited profound visceral effects including excessive salivation, vomiting, and diarrhea. On the other hand, the percentage of dogs defe-

Table 6
Cannabinoid precipitated withdrawal syndrome in dogs

Behavior	Chronic vehicle	Chronic Δ^9-THC
Precipitated behaviors		
Decreased social behavior	0	100
Restless behavior	0	83
Restless circling	38	100
Excessive salivation	25	83
Diarrhea	25	83
Vomiting	0	83
Hyper-responsiveness	12	83
Trembling/shaking	38	83
Non-precipitated behaviors		
Exaggerated reflex	38	67
Full body shakes	50	83
Head shakes	12	17
Yawning	63	83
Whining	38	67
Solid defecation	50	50
Urination	12	17
Genital licking	25	17
Sneezing	0	0

Percentages of dogs given chronic injections of either vehicle ($n = 8$) or Δ^9-tetrahydrocannabinol ($n = 6$) exhibiting specific behaviors following a challenge of 1 mg kg^{-1} SR 141716A. The measures in the top portion reflect behaviors that occurred at a higher incidence in the Δ^9-tetrahydrocannabinol tolerant group than the chronic vehicle group while the measures in the bottom portion did not distinguish the two chronic conditions.

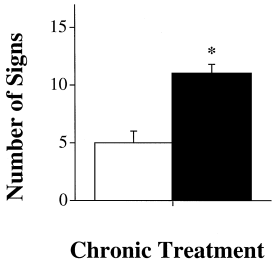

Fig. 4. SR 141716A (1 mg kg^{-1}) challenge produced significantly more behavioral signs in dogs chronically treated with Δ^9-tetrahydrocannabinol than those treated with chronic vehicle. The data are expressed as mean number of signs ± S.E.M. $* P < 0.05$.

cating or urinating after SR 141716A challenge did not appear different between the two chronic treatments.

Finally, a brief period of intense generalized scratching behavior as well as edema around the dog's face, regardless of injection condition, was observed immediately following almost every i.v. injection which lasted less than five min in duration. This reaction generally terminated before the onset of any observable drug effects. No necrosis was evident at the injection sites.

4. Discussion

A primary goal of this research was to examine the acute pharmacological actions of anandamide in the dog static ataxia test and to compare these effects to those produced by Δ^9-tetrahydrocannabinol. As previously reported (Dewey et al., 1972; Martin et al., 1976), Δ^9-tetrahydrocannabinol produced cannabinoid behavioral effects, including dose-related increases in static ataxia. Although the existence of a structure-activity relationship of cannabinoids in the static ataxia test (Martin et al., 1984; Little et al., 1989) is consistent with a receptor mechanism of action, the availability of the cannabinoid receptor antagonist, SR 141716A, afforded the first opportunity to determine the direct involvement of cannabinoid receptors. Whereas SR 141716A failed to induce any observable effects on its own, its complete blockade of Δ^9-tetrahydrocannabinol-induced motor dysfunction indicates the involvement of cannabinoid CB_1 receptors.

While anandamide very clearly produced effects that were cannabinoid as evidenced by some similarity to Δ^9-tetrahydrocannabinol, it was distinguishable from Δ^9-tetrahydrocannabinol in its lack of ability to produce a marked degree of static ataxia, the most distinguishing characteristic feature of the model. Indeed, previous structure-activity relationship studies relied heavily on the coincidence of static-ataxia and marked behavioral depression (Razdan, 1986). The ability of anandamide to produce one effect without the other raises the possibility of multiple receptors, one of which anandamide fails to activate. The fact that SR 141716A blocked the depressive effects of anandamide as well as produced an apparent reduction in the incidence of plunging about is consistent with a receptor mechanism of action. Conversely, anandamide may not be capable of activating the cannabinoid CB_1 receptor in such a manner that it is capable of interacting with the full complement of second messenger systems. It has recently been proposed that single receptors may be coupled to multiple second messenger systems (Leff et al., 1997).

The apparent limited ability of anandamide in producing static ataxia in dogs is consistent with its action in a variety of other tests. In the drug discrimination paradigm, anandamide failed to produce generalization from Δ^9-tetrahydrocannabinol in rhesus monkeys (Wiley et al., 1997); and in rats, anandamide (Wiley et al., 1995a) and the stable analog methanandamide (Burkey and Nation, 1997) only generalized to Δ^9-tetrahydrocannabinol at doses that also suppressed response rates. Anandamide also failed to disrupt performance in a variety of memory tasks (Crawley et al., 1993; Lichtman et al., 1995; Brodkin and Moerschbaecher, 1997). However, anandamide had significant, but small, effects in a nonmatch-to-position memory task in rats pretreated with the protease inhibitor phenylmethylsulfonyl fluoride (Mallet and Beninger, 1996). Similarly, methanandamide, a stable analog of anandamide, impaired memory as assessed in a repeated acquisition procedure (Brodkin and Moerschbaecher, 1997). In addition, anandamide is of lower efficacy than other cannabinoid receptor agonists in inhibiting either adenylyl cyclase (Vogel et al., 1993; Childers et al., 1994) or Ca^{2+} currents (Mackie et al., 1993). Finally, both anandamide and Δ^9-tetrahydrocannabinol have been proposed to be partial agonists because of their decreased ability to stimulate G protein activity compared to (*R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1,2,3-de-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) (WIN 55,212-2) (Burkey et al., 1997). Although our results indicate that Δ^9-tetrahydrocannabinol acted as a full agonist in the dog static ataxia test, the apparent inability of anandamide to produce marked static ataxia in dogs is consistent with this proposal. On the other hand, we cannot rule out the possibility that anandamide is a full agonist in the dog static ataxia test because its toxicity precluded testing of higher doses. These data indicate that, at the least, anandamide is markedly less potent than Δ^9-tetrahydrocannabinol in the dog static ataxia test.

Unlike Δ^9-tetrahydrocannabinol, a relatively high dose of anandamide produced vomiting and diarrhea. The failure of SR 141716A to block these visceral effects suggests a mechanism of action other than the involvement of cannabinoid CB_1 receptors. Anandamide is known to rapidly degrade to arachidonic acid and other polar metabolites in cells and tissues (Deutsch and Chin, 1993) as well as in whole animals (Willoughby et al., 1997). It is likely that the visceral effects were caused by arachidonic acid or other metabolites, since diarrhea and vomiting are common side effects of prostaglandins (Campbell and

Halushka, 1996). Unfortunately, this toxicity precluded administering anandamide chronically to assess tolerance and dependence.

In contrast to a recent report of inverse agonistic activity of SR 141716A (Landsman et al., 1997), we observed no obvious effects that would be consistent with such a notion. Although it is possible that increasing the dose of SR 141716A may have produced behavioral effects in the dogs, the dose employed in the present study of 1 mg kg^{-1}, which completely blocked Δ^9-tetrahydrocannabinol-induced static ataxia, failed to elicit any observable effects when given in the absence of an agonist. In fact, SR 141716A has been found to stimulate locomotor activity in mice albeit at high doses (Compton et al., 1996). Specifically, SR 141716A's ED50 in stimulating locomotor activity was approximately 39-fold greater than its AD50 in antagonizing Δ^9-tetrahydrocannabinol-induced hypoactivity.

Another aim of this research was to evaluate whether SR 141716A would precipitate a withdrawal syndrome in Δ^9-tetrahydrocannabinol-tolerant dogs. As previously reported (Dewey et al., 1972; Martin et al., 1976), the motor dysfunction caused by high doses of Δ^9-tetrahydrocannabinol underwent rapid tolerance with repeated drug administration. Moreover, we found that challenge with SR 141716A precipitated a withdrawal syndrome in the dogs that included excessive salivation, vomiting, diarrhea, decreases in social behavior, and increases in restless behavior and trembling. Several symptoms similar to these, including restlessness, nausea, and loose stools have been reported in humans undergoing abrupt withdrawal from chronic Δ^9-tetrahydrocannabinol administration (Jones et al., 1981). These similarities lend credence to the validity of this precipitated cannabinoid withdrawal model in dogs. The occurrence of a precipitated withdrawal syndrome in rats (Aceto et al., 1995; Tsou et al., 1995), mice (Cook et al., 1998), and now dogs offers several animal species to investigate cannabinoid physical dependence.

Although it is difficult to make direct comparisons between dose of Δ^9-tetrahydrocannabinol administered to laboratory animals and those self-administered via inhalation in humans, the dosing regimen used in the present study would be relevant for chronic heavy cannabis smokers. On the other hand, it is unknown whether the syndrome would occur using a lower dose regimen of drug than that used in the present study. The high prevalence of marijuana use in the United States (Johnston et al., 1995) and the strong association between marijuana use and marijuana dependency (Chen et al., 1997) suggest that physical withdrawal effects should be considered when cannabinoid use is discontinued.

The observation that behavioral tolerance to Δ^9-tetrahydrocannabinol can continue from 1 to 3 weeks following cessation of drug use (Dewey et al., 1972) suggests the possibility that cannabinoid dependence may be of a long duration. The results in the present study are consistent with this prospect. In our experimental design, each dog was challenged with SR 141716A twice, once after chronic treatment with vehicle and once after chronic Δ^9-tetrahydrocannabinol treatment. The chronic treatments were given in a counter-balanced fashion that included a ten day hiatus between the two regimens. SR 141716A challenge failed to produce restless circling, trembling, excessive salivation, or diarrhea in any of the four dogs first given chronic vehicle. In the subjects treated second with chronic vehicle, however, SR 141716A elicited circling behavior and trembling in three of four dogs and diarrhea and excessive salivation in two of the four animals. In contrast to a recent report in which SR 141716A (3 mg kg^{-1}) produced mild withdrawal effects in rats (Rodriguez de Fonseca et al., 1997), no overt effects were observed when SR 141716A was administered alone in the acute dog studies.

In conclusion, the finding that SR 141716A antagonized the motor disruptive effects of Δ^9-tetrahydrocannabinol and anandamide clearly indicates that both drugs produce their motor effects through stimulation of cannabinoid CB_1 receptors. Consistent with other reports in the literature, the potency and possibly efficacy were lower for anandamide than for Δ^9-tetrahydrocannabinol. The failure of SR 141716A to block the visceral effects of anandamide indicates a noncannabinoid mechanism of action, possibly an effect of its metabolic by-product arachidonic acid on the gastrointestinal tract. Finally, the results presented in this paper clearly indicate that SR 141716A precipitates a withdrawal syndrome in Δ^9-tetrahydrocannabinol-tolerant dogs. The occurrence of a precipitated cannabinoid withdrawal syndrome in rodent and canine species may provide a model to investigate potential treatments for marijuana users that are physically dependent on cannabis.

Acknowledgements

The authors thank Steven Brigido for his valuable and dependable assistance in behavioral assessment, Renee Jefferson for her technical assistance, and Mary Ellen White, Patricia Gerber, Dr. Paul Nicolaysen, and Dr. William Henry for excellent veterinary care. In addition, we would like to thank John Lowe and Pfizer Pharmaceutical for the generous gift of SR 141716A, NIDA for Δ^9-tetrahydrocannabinol, and Organix for anandamide. This research was supported by NIDA Grants DA-03672, DA-08387, and DA-09789.

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