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Pharmacology, Biochemistry and Behavior 67 (2000) 265–270

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding

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Received 22 December 1999; received in revised form 2 May 2000; accepted 17 May 2000

Abstract

Previous reports have indicated that administration of the central cannabinoid receptor (CB_1) antagonist SR141716A decreases intake of highly palatable food and drink. Disruption of normal food intake has been reported only at high doses known to disrupt spontaneous behaviors. The present study was designed to determine if rates of responding for normal food were sensitive to the effects of cannabinoid receptor blockade. Adult, male Sprague–Dawley rats were trained to lever press for normal food pellets under a fixed-ratio 15 (FR 15) schedule of reinforcement. SR141716A (0.3–3.0 mg/kg) produced dose-dependent reductions in response rate. WIN 55,212-2 (0.3 mg/kg), a high efficacy cannabinoid agonist, given as a pre-treatment to SR141716A, significantly attenuated the rate-suppressing effects of SR141716A, suggesting a principal role of CB_1 receptors in mediating these behavioral effects. These data indicate that high palatability is not necessary to observe an anorectic effect of SR141716A.

Keywords: CB_1; SR141716A; WIN 55,212-2; Food-maintained responding; Fixed-ratio schedule; FR 15; Isobologram

1. Introduction

The biochemical and physiologic actions of cannabis and cannabinomimetic substances have been the subject of intensive study for decades. The identification [23] and subsequent localization [15] of the central cannabinoid receptor (CB_1) provided a specific receptor target for the actions of cannabinoids. CB_1 receptors have seven putative membrane-spanning domains and are coupled to an inhibitory G-protein which inhibits adenylyl cyclase [16,17] and a pertussis toxin-sensitive G-protein which regulates calcium currents [6,21]. Autoradiographic experiments have demonstrated a heterogeneous distribution of CB_1 receptors in brain with high levels in molecular layer of cerebellum, substantia nigra, hippocampus, and cingulate cortex [15], brain regions which correspond well with the known effects of cannabinoids in vivo. The recent discovery [11,33] and behavioral characterization [29,30] of endogeneous cannabinoids, e.g. anandamide and 2-acetyl-glycerol (2-AG), as candidate neurotransmitters for CB_1 receptors suggests a role for CB_1 receptors in normal brain function.

Understanding of the physiologic relevance of CB_1 receptor action has been greatly increased by the development of the selective CB_1 antagonist SR141716A [25]. SR141716A has been shown to block the effects of various cannabinoids in long-term potentiation [7], cardiovascular function [36], and a number of behavioral assays [10,25] including rodent drug discrimination [38] and memory tasks [20]. The in vivo effects of SR141716A have been investigated in both cannabinoid-naïve and cannabinoid-tolerant animals. In tolerant animals, treatment with SR141716A precipitates a withdrawal-like state, characterized by intense scratching, grooming, and wet dog shakes, as well as increased locomotor activity and defensive withdrawal behavior [1,26,35]. Similar findings have also been reported in cannabinoid-naïve animals [24] suggesting that there may be high levels of endogeneous tone in the cannabinoid system.

The potential involvement of cannabinoids in feeding has been suggested for many years in anecdotal reports involving humans. Controlled studies of this phenomenon have supported these claims. For example, Trojiniar and Wise [34] reported that Δ^9-THC significantly reduced electrical brain stimulation thresholds for hypothalamically stimulated feeding in satiated rats. Recently, it has been demonstrated

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0091-3057/00/$ – see front matter
PII: S0091-3057(00)00359-2

that administration of either Δ^9-THC or the endogenous cannabinoid anandamide produces hyperphagia in satiated animals [39,40]. Furthermore, it appears likely that cannabinoid agonists stimulate feeding through a specific CB_1-mediated mechanism, given that the hyperphagic response to the endogeneous cannabinoid, anandamide in this paradigm was dose-dependently antagonized by SR141716A pre-treatment. Similarly, the high potency cannabinoid agonist CP-55,940 dose-dependently increased break points for animals responding for beer, ''near-beer'', and sucrose solutions under a progressive-ratio schedule of reinforcement [14]. This effect on break point was also blocked by pre-treatment with SR141716A. Taken together, these findings suggest a general stimulatory role for CB_1 receptors in ingestive behaviors.

In contrast to the stimulation of feeding behaviors by cannabinoid agonists, cannabinoid antagonists may possess anorectic effects [2,9,13,32]. Results of studies with SR141716A indicate that intake of highly palatable or rewarding food and drink may be more sensitive to the effects of CB_1 receptor blockade than intake of normal food and drink. For example, suppression of ethanol intake has been observed at doses which do not significantly alter intake of normal food or water [9]. Similar results have been reported using other paradigms and highly palatable food rewards, however, the doses required to suppress intake of normal food are in a range known to produce significant behavioral disruption [27,28]. These findings have led to the hypothesis that central cannabinoid receptors are involved in mediating the appetitive value of ingested substances. The present series of experiments was designed to determine if high palatability is required to observe an anorectic effect of SR141716A. Animals were trained under a fixed-ratio 15 (FR 15) schedule of normal food presentation and the effects of SR141716A on rates of food-maintained responding were assessed.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were maintained (Harlan Laboratories, Indianapolis, IN, USA) at 85–90% of their free-feeding weight for all experiments. Water was available ad libitum. Rats were housed in a temperature- and humidity-controlled room with a 12-h light–dark cycle (lights on at 07:00 h). All procedures were carried out in accordance with established practices as described in the NIH Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Wake Forest University School of Medicine.

2.2. Drugs

SR141716A was generously provided by Pfizer (Groton, CT). WIN 55,212-2 was purchased from RBI (Natick, MA). All injections were given intraperitoneally in a volume of 1 ml/kg body weight. All drugs were suspended in a pluronic acid vehicle. Preparation of the pluronic vehicle has previously been described [31]. Briefly, drug (SR141716A or WIN 55,212-2) was dissolved in ethanol. The ethanol solution was then suspended in a 1:4:1 ratio with Pluronic F-68 detergent, in ethanol and saline, and the ethanol was evaporated under a stream of nitrogen. Drugs were then diluted to proper concentrations with saline for injection.

2.3. Schedule-controlled responding

Rats were trained to lever press in standard two-lever operant chambers in daily 30-min sessions ($N=8$). Rats began on a fixed-ratio 1 (i.e. FR 1) schedule and progressed to an FR 15 schedule. Stable baseline rates of responding on the FR 15 schedule were defined as no more than 10% variation from the mean of three consecutive training sessions. Animals were required to recover a stable baseline following testing before the next test session was conducted. On test days, SR141716A and WIN 55,212-2 were given 60 and 15 min, respectively, before behavioral testing. On days when combination treatments were tested, WIN 55,212-2 was given as a 15-min pre-treatment before administration of SR141716A. All operant test sessions were conducted Monday–Friday during the animals' light cycle.

2.4. Locomotor behavioral testing

Locomotor activity was measured in Plexiglas® test chambers ($42 \times 42 \times 30$ cm) by electronic counters that detected interruptions of eight independent infrared photocell beams (Omnitech, Columbus, OH, USA). Photocell counts were recorded and stored in 10-min bins. Rats were habituated to experimental procedures for 2 days prior to testing. On these days, rats were injected with saline and placed in the locomotor chambers for 1 h. On each test day, animals were injected with SR141716A (1.0 or 3.0 mg/kg) or vehicle intraperitoneally and placed immediately in the photocell chambers. Locomotor activity was then monitored for 4 h. Animals were randomly assigned to test groups ($N=6$/group).

2.5. Data analysis

All response rate data were analyzed using a within-subjects design. Data were analyzed using a one-way ANOVA with repeated measures to determine main effects of treatment. Student–Newman–Keuls post-hoc analysis was used to identify between-treatment differences. Spontaneous activity data were analyzed using a one-way ANOVA with Dunnett's post-hoc analysis. For each dose–effect curve, an estimate of the dose that decreased response rate by 50% (i.e. ED_{50} value) was computed by log linear interpolation (least squares method) using the descending portion of the dose–effect curve. To assess the nature of the

drug–drug interaction in the combination studies, ED_{50} values for each drug alone and when given in combination are plotted as an isobologram [37,42].

3. Results

3.1. Food-maintained responding

Treatment with SR141716A produced a statistically significant, dose-dependent decrease in food-maintained rates of responding under an FR 15 schedule of food presentation ($F_{4,39}=23.75$, $P<0.0001$). These data are shown in Fig. 1. Post-hoc analyses revealed significant suppression of response rate at the 1.0 (37%) and 3.0 (53%) mg/kg doses of SR141716A when compared to control rates (Fig. 1).

To determine if the effects of SR141716A on response rate were specifically mediated by CB_1 receptors, SR141716A (0.3–3.0 mg/kg) was given in combination with the high efficacy cannabinoid agonist WIN 55,212-2. When administered alone, WIN 55,212-2 produced dose-dependent decreases in response rate vs. control injections ($F_{3,31}=71.19$, $P<0.001$) (Fig. 1). Based on the WIN 55,212-2 dose–response curve, 0.3 mg/kg WIN 55,212-2 was chosen for combination treatment studies as this dose had no rate-suppressing effects on its own. Pre-treatment with WIN 55,212-2 (0.3 mg/kg) significantly attenuated the rate-suppressing effects of SR141716A ($F_{5,47}=14.81$, $P<0.001$) (Fig. 2A) as indicated by a rightward shift in the dose–effect curve. WIN 55,212-2 (0.3 mg/kg) pre-treatment completely reversed the rate-suppressing effects of 1.0 mg/kg SR141716A and produced an attenuation (i.e. 40%) of rate-suppressing effects of 3.0 mg/kg SR141716A.

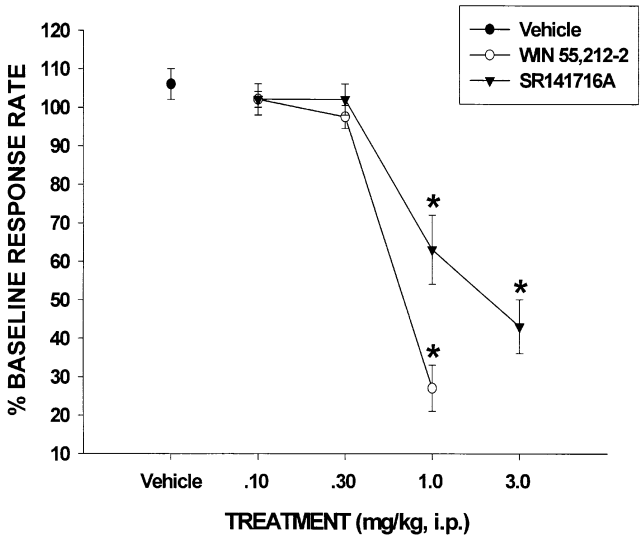

Fig. 1. Dose-dependent effects of SR141716A (0.1–3.0 mg/kg) and WIN 55,212-2 (0.1–1.0 mg/kg) on food-maintained responding. Data are presented as percentage of baseline rate with each point double-determined. Data were analyzed using a one-way ANOVA with repeated measures and Student–Newman–Keuls post-hoc analysis. * denotes significant difference from vehicle ($P<0.05$).

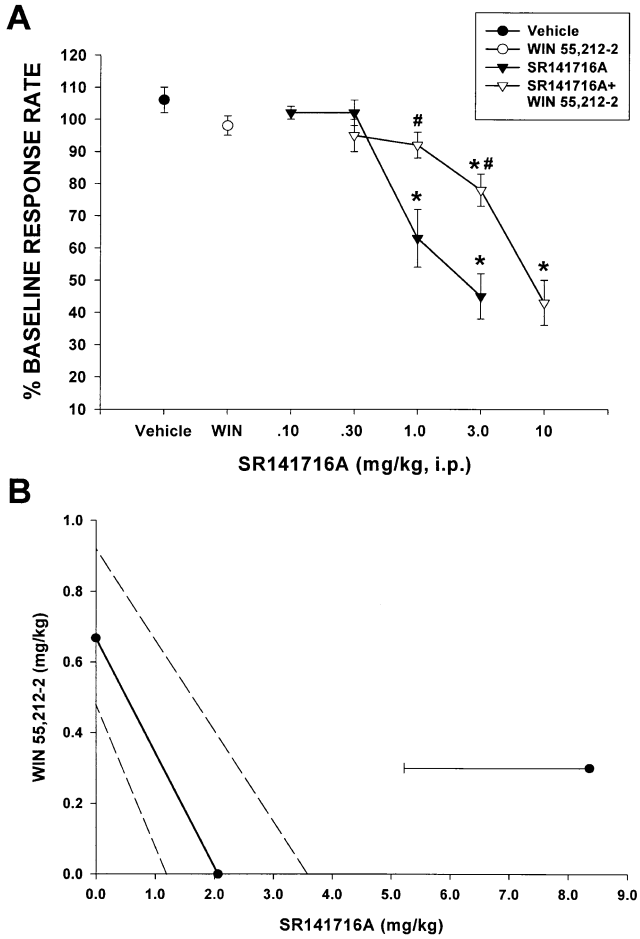

Fig. 2. (A) Effects of WIN 55,212-2 (0.3 mg/kg) pre-treatment on SR141716A (0.3–3.0 mg/kg) induced reductions in food-maintained response rate. Data are presented as percentage of baseline rate with each point double-determined. Data were analyzed using a one-way ANOVA with repeated measures and Student–Newman–Keuls post-hoc analysis. * denotes significant difference from vehicle ($P<0.05$). # denotes significant difference from SR141716A alone ($P<0.05$). (B) Isobolographic analysis of the effects of SR141716A alone and in combination with WIN 55,212-2 on rates of food-maintained responding.

Given that both the cannabinoid agonist and antagonist suppressed response rate, isobolograms were constructed to characterize the nature of the interaction of these drugs on rate. ED_{50} values for response rate suppression for each drug alone and in combination were used in this analysis (Fig. 2B). The ED_{50} values for SR141716A alone and in combination with WIN 55,212-2 were 2.06 mg/kg (1.19–3.58, 95% confidence intervals) and 8.36 mg/kg (5.22–13.40, 95% confidence intervals), respectively. The results of the isobolographic analysis indicate that SR141716A and WIN 55,212-2 are acting in an antagonistic manner [37,42], as indicated by the lack of overlap in the calculated 95%

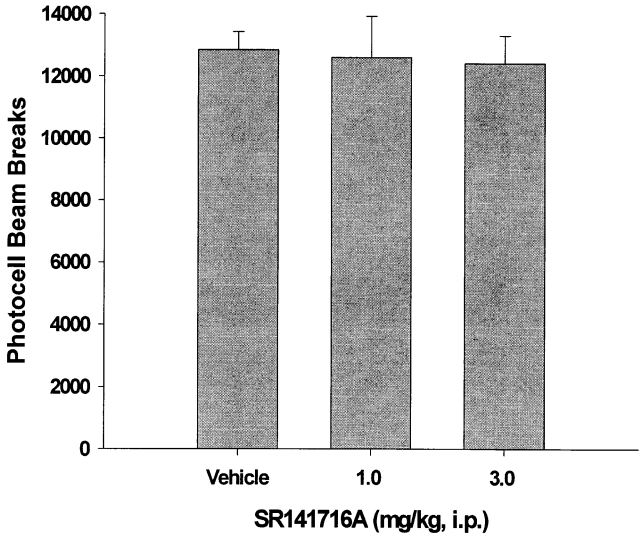

Fig. 3. Effects of SR141716A (1.0 and 3.0 mg/kg) on horizontal locomotor activity. Data were analyzed using a one-way ANOVA and Dunnett's post-hoc analysis.

confidence limits for SR141716A alone and in combination with WIN 55,212-2.

3.2. Spontaneous behavior

In order to assess potential non-specific effects of SR141716A on behavior, the effects of SR141716A (1.0 and 3.0 mg/kg) on spontaneous locomotor activity were investigated. Treatment with 1.0 or 3.0 mg/kg SR141716A did not significantly alter either horizontal activity ($F_{2,15} = 0.020$, $P = 0.980$) (Fig. 3) or stereotypy ($F_{2,15} = 0.421$, $P = 0.665$; data not shown) as compared to vehicle treatment in a 4-h locomotor test session.

4. Discussion

In the present study, administration of the CB_1 receptor antagonist SR141716A (0.1–3.0 mg/kg) dose-dependently decreased rates of responding for normal food under an FR 15 schedule of reinforcement. This rate suppression occurred in the absence of gross behavioral disruption (Fig. 3), indicating that the motor behavior of the animals was not impaired. Pre-treatment with the highest dose of WIN 55,212-2, which did not significantly suppress response rates when administered alone (i.e. 0.3 mg/kg), significantly attenuated the rate-decreasing effects of SR141716A, demonstrating a primary role for CB_1 receptors in the observed rate effects. These data demonstrate that intake of normal food is sensitive to cannabinoid receptor blockade in a dose range which does not disrupt other normal behaviors. Moreover, these data establish that high palatability is not required to observe an anorectic effect of SR141716A administration.

Previous studies investigating the effects of SR141716A on normal food intake have reported anorectic effects at doses in a range known to produce gross behavioral alterations [8,27,28]. In contrast, intake of highly palatable food and drink has been shown to be more sensitive to the effects of cannabinoid receptor blockade. For example, SR141716A (0.3–3.0 mg/kg) decreases sucrose and ethanol intake in rats [2], decreases intake of highly palatable foods in marmosets [32], decreases alcohol consumption in alcohol-preferring rats [9], and reduces breakpoints for beer and "near-beer" in animals responding under a progressive ratio schedule of reinforcement [14]. The range of effective doses in these studies of highly palatable food rewards is similar to the range of doses which produced significant decreases in response rate for normal food in the present study. In addition, the effects seen in the present study were observed in the absence of any influence on spontaneous activity, suggesting that the observed effects on rate reflect a direct effect on feeding behavior rather than a non-specific effect on responding in general. These data, then, demonstrate that normal food intake is sensitive to central cannabinoid receptor blockade and can be influenced by doses of SR141716A which do not significantly alter other normal behaviors.

The absence of an increase in food-maintained rates of responding following WIN 55,212-2 treatment contrasts with reports of hyperphagia following administration of other cannabinoid agonists such as Δ^9-THC [40] and anandamide [39]. This apparent discrepancy may simply reflect paradigmatic differences. In these previous studies, the facilitatory effects of agonist administration on feeding were demonstrated in satiated rats, while the present study utilized rats under mild food restriction. Moreover, these previous studies characterized the hyperphagic response of cannabinoid agonists under free access conditions while the present study utilized a response-contingent schedule of food presentation. In support of this assertion, Carriero et al. [5] demonstrated that administration of a variety of cannabinoid agonists (e.g. CP 55,940, WIN 55,212-2, Δ^8-THC, and AM 356) under a fixed-ratio 5 (FR 5) schedule of food presentation, not unlike the schedule employed in the present study, did not produce hyperphagic responses. Further research is necessary to determine the exact nature that contribute to these differences in the effects of some agonists and the antagonists.

Interestingly, the effects of SR141716A on rates of food-maintained responding are similar to previous reports on the effects of spontaneous cannabinoid withdrawal. In animals treated chronically with Δ^9-THC, cessation of cannabinoid administration markedly reduced rates of food-maintained responding [3] and disrupted the induction of complex operant tasks [4]. These behavioral disruptions are analogous to reports in the human literature of depression, appetite loss, anhedonia, and cognitive disruption following cessation of chronic cannabis use [12,18,41]. The ability of SR141716A to suppress normal food intake in cannabinoid-

naïve animals is consistent with the presence of a high level of endogenous cannabinoid tone within the rat CNS and suggests that this tone may play a principal role in mediating ingestive behaviors.

The ability of SR141716A to disrupt behavior in cannabinoid-naïve animals through a CB_1 receptor-specific mechanism could be accounted for by at least two distinct pharmacological interactions between SR141716A and CB_1 receptors. SR141716A was initially reported to be an antagonist at CB_1 receptors [25]. Recent studies have suggested, however, that SR141716A may possess inverse agonist activity in vitro [19,22]. The recent identification and characterization of two candidate neurotransmitters for CB_1 receptors, anandamide and 2-AG, indicates that there may be a significant level of endogeneous cannabinoid tone in the brain. If SR141716A is acting as a silent antagonist, it may be disrupting behavior by blocking this endogeneous cannabinoid activity. If, however, SR141716A is acting as an inverse agonist, it could produce these effects in the absence of endogenous tone. Whether the functional consequences of these distinct actions at CB_1 receptors would result in different behavioral outcomes, however, is not discernable from the present data. In fact, the actions of an inverse agonist and antagonist at CB_1 receptors would be predicted to have very similar functional outcomes.

In summary, SR141716A administration produces disruptions in rates of food-maintained responding under a fixed-ratio schedule of reinforcement for normal food through a CB_1 receptor-specific mechanism in cannabinoid-naïve animals. The decreases in response rate occurred at doses which did not significantly affect spontaneous behaviors and previously have been shown to selectively affect intake of highly palatable foods. These data indicate that high palatability or appetitive value is not required to observe an anorectic effect of SR141716A.

Acknowledgments

The authors would like to thank Dr. Robert S. Mansbach for his assistance with this project. The authors would also like to thank Charlie Cook and Dr. Drake Morgan for their assistance with the isobolographic analysis and Hilary R. Smith and Stephanie L. Hart for their editorial comments. This work was supported by the National Institute on Drug Abuse Grants F31DA05940 (CSF) and P50DA06634 (LJP).

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