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(*R*)-Methanandamide and Δ^9-tetrahydrocannabinol-induced operant rate decreases in rats are not readily antagonized by SR-141716A

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Abstract

The current study examined the interaction between the cannabinoid CB_1 receptor agonists Δ^9-tetrahydrocannabinol and (*R*)-methanandamide in combination with the cannabinoid CB_1 receptor antagonist SR-141716A (*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl) in rats responding for food on a fixed ratio (FR-10) schedule of food reinforcement. The study provided only limited evidence for antagonism by SR-141716A (at 1 mg/kg but not with 0.3, 3 and 10 mg/kg) of the rate suppressant effects induced by the cannabinoid CB_1 receptor agonist Δ^9-tetrahydrocannabinol (and only at the single dose of 5.6 mg/kg Δ^9-tetrahydrocannabinol). (*R*)-Methanandamide in combination with SR-141716A resulted in a greater rate suppression compared to that induced by (*R*)-methanandamide alone. Thus, SR-141716A augmented the rate-decreasing effects of (*R*)-methanandamide and only minimally altered the rate-decreasing effects of Δ^9-tetrahydrocannabinol. Additionally, high doses (10 and 30 mg/kg) of SR-141716 singly consistently suppressed the rate of responding. The current results coupled with our previous data examining combinations of Δ^9-tetrahydrocannabinol or (*R*)-methanandamide and SR-141716 (see text) underscore pharmacological/behavioral differences (whether quantitative or qualitative) between the cannabinoid CB_1 agonists (*R*)-methanandamide and Δ^9-tetrahydrocannabinol revealed by their interactions with the cannabinoid CB_1 antagonist SR-141716.

Keywords: Cannabinoid; (*R*)-Methanandamide; Δ^9-Tetrahydrocannabinol; SR-141716A; Behavior, schedule controlled; (Rat)

1. Introduction

Our understanding of the biological mechanisms responsible for the psychotropic effects of cannabis has expanded rapidly since the discovery of endocannabinoids and two receptors through which these endocannabinoids and drugs like Δ^9-tetrahydrocannabinol, the primary active constituent of cannabis, are thought to act (e.g., Goutopoulos and Makriyannis, 2002; Palmer et al., 2000; Pertwee, 1999). Endocannabinoids, such as anandamide, have effects that substantially overlap the actions of tetrahydrocannabinol-like drugs. However, there are important examples of divergence between these two classes of compounds. Similarly, while many of the central actions of these compounds appear to be mediated by the cannabinoid CB_1 receptor, there are important examples of cannabinoid action that are not easily understood by reference to currently known cannabinoid CB1 receptor mechanisms (Breivogel et al., 2001; Di Marzo et al., 2000; Martin et al., 1999; Mechoulam et al., 1998). As an example, Monory et al. (2002) recently reported that binding occurred for anandamide to a site in the cerebellum of CB_1 knock-out mice that did not bind Δ^9-tetrahydrocannabinol.

One particularly important set of observations about the correspondence and divergence of the actions of anandamide-like compounds and tetrahydrocannabinol-like compounds comes from studies in animals trained to discriminate and thus recognize the effects of cannabinoids. In a drug discrimination procedure, the subject is reinforced for making one response following administration of the

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training drug and for making another response following administration of drug vehicle. Drug discrimination procedures are exquisitely specific and appear to parallel the ability of humans to identify drugs as belonging to different pharmacological classes (Lamb and Henningfield, 1994). Studies in rats trained to discriminate between Δ^9-tetrahydrocannabinol and vehicle have shown that exposure to cannabis smoke, but not placebo smoke, occasions Δ^9-tetrahydrocannabinol appropriate responding in rodents (summarized in Järbe and Mathis, 1992). Thus, the Δ^9-tetrahydrocannabinol discrimination in all likelihood measures the psychoactive effects of cannabis that are associated with its recreational use and abuse (Balster and Prescott, 1992).

In rats trained to recognize the effects of Δ^9-tetrahydrocannabinol, anandamide generally does not occasion Δ^9-tetrahydrocannabinol-appropriate responding (Burkey and Nation, 1997; Järbe et al., 2001; Wiley, 1999). However, this may be a result of metabolic instability of anandamide rather than any major pharmacological difference between Δ^9-tetrahydrocannabinol and anandamide. When testing the more metabolically stable analog of anandamide, (*R*)-methanandamide (Abadji et al., 1994), complete Δ^9-tetrahydrocannabinol-appropriate responding occurred in rats trained with 1.8 or 3.0 mg/kg Δ^9-tetrahydrocannabinol, but not in rats trained with 5.6 mg/kg Δ^9-tetrahydrocannabinol (Järbe et al., 1998, 2000). There are several possible explanations for these observations. The assay involving the higher training dose of Δ^9-tetrahydrocannabinol potentially has a greater efficacy demand than the assays with the lower Δ^9-tetrahydrocannabinol training doses. This seems to be the case for rats trained with varying doses of opioids (Young, 1991). Thus, (*R*)-methanandamide might be a lower efficacy cannabinoid than Δ^9-tetrahydrocannabinol, and this may be the reason why (*R*)-methanandamide will substitute in the low-training dose groups, but not in the high-training Δ^9-tetrahydrocannabinol dose group.

Alternatively, these findings may result from differences in the mechanisms by which (*R*)-methanandamide and Δ^9-tetrahydrocannabinol decrease responding. Doses of (*R*)-methanandamide up to those that severely decreased responding were tested in the rats trained with 5.6 mg/kg Δ^9-tetrahydrocannabinol. Yet, even at these behaviorally disruptive doses, (*R*)-methanandamide did not fully substitute for Δ^9-tetrahydrocannabinol (maximum generalization $\approx$ 50%). However, if (*R*)-methanandamide's rate-decreasing effects were primarily a result of cannabinoid (non-)CB_1 receptor mediated activity, these cannabinoid (non-)CB_1 receptor actions might prevent testing doses of (*R*)-methanandamide needed to produce complete substitution in the high-dose training group. In other words, the rate-decreasing effects of (*R*)-methanandamide may have masked its potential to substitute for 5.6 mg/kg Δ^9-tetrahydrocannabinol. One way to examine if Δ^9-tetrahydrocannabinol and (*R*)-methanandamide decrease responding by the same mechanism is to examine if chronic administration of Δ^9-tetrahydrocannabinol that produces tolerance to the rate-decreasing effects of Δ^9-tetrahydrocannabinol also produces cross-tolerance to the rate-decreasing effects of (*R*)-methanandamide. In such a study, we found tolerance to the rate-decreasing effects of Δ^9-tetrahydrocannabinol and cross-tolerance to the rate-decreasing effects of (*R*)-methanandamide (Lamb et al., 2000; see also Fride, 1995, with regard to cross-tolerance between Δ^9-tetrahydrocannabinol and anandamide).

Another possibility is that Δ^9-tetrahydrocannabinol and (*R*)-methanandamide produce their discriminative and rate-decreasing effects through different sites or mechanisms of action. Thus, (*R*)-methanandamide might be more potent or efficacious at the rate-decreasing site, while the opposite might be true for Δ^9-tetrahydrocannabinol. One way of addressing this issue is to examine if both of these effects are similarly antagonized by drugs like the cannabinoid CB_1 receptor antagonist SR-141716 [(*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide); Rinaldi-Carmona et al., 1994]. Doses of 0.3 and 1.0 mg/kg SR-141716 produced large rightward shifts in the Δ^9-tetrahydrocannabinol and (*R*)-methanandamide dose–response curves for occasioning Δ^9-tetrahydrocannabinol- or (*R*)-methanandamide-appropriate responding (Järbe et al., 2001). In this study, we examine if SR-141716 can produce similar shifts in the Δ^9-tetrahydrocannabinol and (*R*)-methanandamide dose–response curves for decreasing responding.

2. Materials and methods

2.1. Subjects

Six experimentally naive male Sprague–Dawley rats (Taconic Farms, Germantown, NY), weighing between 275 to 300 g upon arrival to the laboratory, were used. Rats were individually housed in polycarbonate cages. The animals had unlimited access to water, but access to food was limited to 12–15 g/day (on Fridays, rats received 45 g of food for the weekend). This food allotment was provided shortly following experimental sessions, maintaining bodyweights at 285–355 g during the course of the experiment, and resulted in adequate motivation. The vivarium had a 12-h light/12-h dark illumination cycle, and rats were trained and tested during the light phase. All procedures were approved by the Animal Care and Use Committee of the MCP/Hahnemann University (formerly Allegheny University of the Health Sciences), Philadelphia, PA, USA. The "Principles of animal laboratory care" (NIH publication NO. 85–23, revised 1985) were followed.

2.2. Apparatus

Experimental sessions were conducted in operant chambers containing three levers. Only the left lever was utilized

in these experiments. Behavioral contingencies were controlled using Med-PC (Med-Associates, St. Albans, VT).

2.3. Procedures

Experimental sessions were conducted 5 days a week and consisted of four 5-min periods of access to food under an Fixed-Ratio (FR)-10 schedule of reinforcement. Each 5-min period of food availability was preceded by a 15-min time-out period in which responding had no programmed consequences. During each 5-min period of food availability, every tenth response (FR-10) on the left lever when the stimulus light above the lever was lighted resulted in the delivery of two 45-mg Noyes (Formula A) food pellets and the beginning of a 10-s time-out period. During this 10-s time-out period, the stimulus light above the lever was turned off, the house light was illuminated, the white noise generator turned on and responses had no programmed consequences. Following this 10-s time-out period, the FR-10 schedule was again in effect, the house-light and white noise turned off, and the stimulus light above the lever turned on, unless the 5-min period of food availability had expired. If the 5-min period of food availability had expired, then a 15-min time-out period occurred during which all lights were out and no white noise was provided, or if the fourth food availability period was just completed, the experimental session ended. An identical 15-min time-out period also preceded the first period of food availability.

2.4. Drugs

2.4.1. Cumulative dosing procedure

Drug doses of the agonists were administered in a cumulative fashion on test days (Fridays), preceded by administration of either vehicle or a given dose of SR-141716A (range 0–10 mg/kg). The lowest dose in the dose response curves for either Δ^9-tetrahydrocannabinol or (*R*)-methanandamide would be administered at the beginning of the time-out period preceding the second period of food availability. Following this period of food availability at the beginning of the third 15-min time-out period, the second lowest dose in the dose–response curve was administered. The third dose was administered at the beginning of the fourth 15-min time-out period. This sequence permitted the determination of a three-point dose–response curve in a single session. Doses are reported as the cumulative dose given, i.e., the dose reported is the sum of the dose given and the doses that were given before it. For example, if at the beginning of the second time-out period, a dose of 3 mg/kg was given, a dose of 7 mg/kg would be given at the beginning of the third time-out and the reported dose would be 10 mg/kg. However, the dose–response curve for SR-

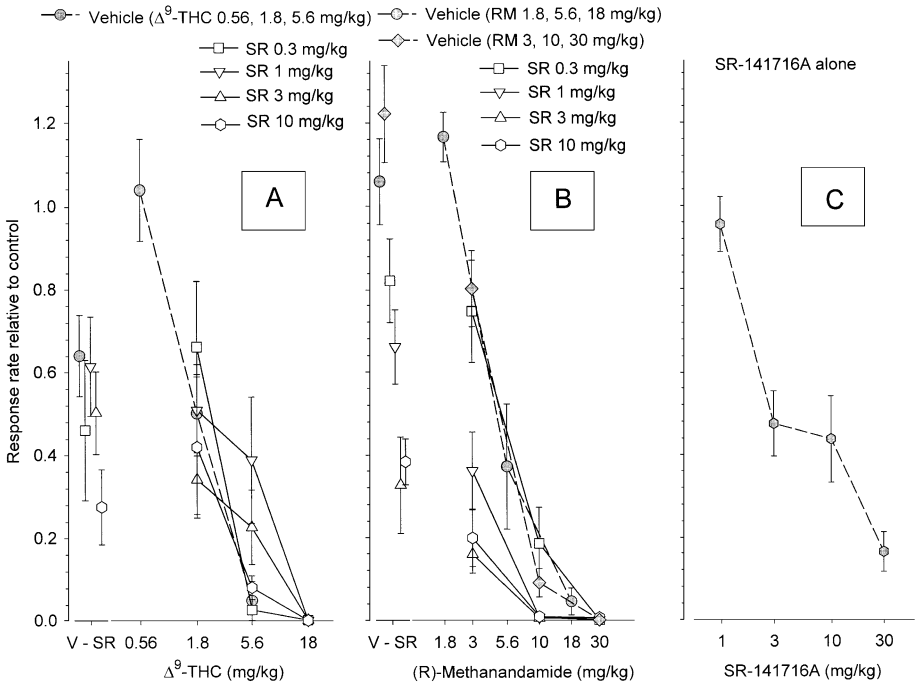

Fig. 1. Effects of Δ^9-tetrahydrocannabinol, Δ^9-THC, (A), (*R*)-methanandamide (B) and SR-141716A (C) on rates of lever pressing in rats maintained on an FR-10 schedule of food reinforcement. *Y*-axis, average number of responses per second (± S.E.M) expressed as the mean proportion of the rate of lever pressing between the immediately preceding vehicle session (Thursday) and the corresponding test session result (Friday). *X*-axis, drug dose in mg/kg. V = vehicle; SR = SR-141716A. A cumulative dosing procedure was used. Drug doses of the two agonists were administered in a cumulative fashion on test days (Fridays), preceded by administration of either vehicle or a given dose of SR-141716A (range 0–10 mg/kg). The dose–response curve for SR-141716A alone represents a four-point curve determined cumulatively in a single session. For reference, the average raw, untransformed scores of the rate of lever pressing (± S.E.M) on Thursdays vehicle sessions were: first 5-min period, 2.92 (0.14); 2nd period, 2.97 (0.14); 3rd period, 3.06 (0.14); and the 4th period, 2.73 (0.15) responses/s, respectively. Further details in Materials and methods.

141716A singly in Fig. 1C (see Results) represents a four-point curve determined cumulatively in a single session. That is, the first dose of SR-141716A was 1 mg/kg, followed by 2 mg/kg for the second cycle (i.e., total dose of 3 mg/kg), 7 mg/kg for the third cycle (i.e., total dose of 10 mg/kg) and 20 mg/kg for the final fourth cycle resulting in a total of 30 mg/kg SR-141716A. Dose–response curves were determined at weekly intervals on Fridays as noted above. Administration of drugs only once a week was adopted to minimize potential tolerance development. Two dose-effect determinations of (R)-methanandamide singly were run to ensure an adequate dose range of the compound.

2.4.2. Drug preparations

Δ^9-Tetrahydrocannabinol, dissolved in ethanol (200 mg/ml), was provided by the National Institute on Drug Abuse (NIDA), Bethesda, MD, and stored at −20 °C until used. (*R*)-Methanandamide [(*R*)-(+)-arachidonyl-1′-hydroxy-2′-propylamide] was synthesized according to Abadji et al. (1994) and sent to the site of behavioral evaluation in argon-capped vials. Upon arrival, (*R*)-methanandamide was dissolved in ethanol, appropriate amounts withdrawn, the ethanol evaporated under a stream of nitrogen, the residue then dissolved in a solution of propylene glycol and Tween-80 and stored at −20 °C. Shortly before being used, the solute was diluted with normal (0.9%) saline after the solute had been sonicated for 20–30 min. This procedure was followed for preparing suspensions of Δ^9-tetrahydrocannabinol as well. SR-141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl; provided by NIDA) was dissolved in a propylene glycol/Tween-80 mixture before being diluted with saline. Drugs were administered i.p. in volumes ranging between 2 and 4 ml/kg. The volume of vehicle injections on Thursdays corresponded to those used for the following Friday testing. Suspensions were used within 0.5 h after saline was added to the drug/propylene glycol (5%)/Tween-80 (3–5%) mixture. All doses are expressed in the forms indicated above.

2.5. Statistics

One-way (randomized block) and two-way (randomized block factorial) repeated measures analyses of variance (ANOVA; Kirk, 1968) were calculated using SigmaStat (version 2.0), run on an IBM 166-MHz PC. Subsequent post hoc analyses used Tukey's Honestly Significant Difference (HSD) test statistic with $\alpha = 0.05$, two-tailed, for the collection of comparisons (Kirk, 1968). To reduce the effect of individual variability, data are expressed as the mean proportion of the rate of lever pressing between the immediately preceding vehicle session (Thursday) and the corresponding test session result (Friday). Hence, data for each rat are expressed as a proportion of the immediately preceding Thursday vehicle response rate. To better meet the assumptions of homogeneity of error variance and normality of treatment-level distributions, all data were square-root transformed for statistical analysis (Kirk, 1968).

3. Results

As can be seen in Fig. 1, Δ^9-tetrahydrocannabinol (Panel A, shaded circles), (*R*)-methanandamide (Panel B, shaded circles and diamonds) and SR-141716A (Panel C, shaded circles) dose-dependently decreased responding when administered alone. The rate-decreasing effects of Δ^9-tetrahydrocannabinol and (*R*)-methanandamide when given alone are supported by main effects for both drugs in the ANOVA evaluating statistically the effects of these agonists in combination with SR-141716A [$F(1, 20) = 90.59$; $P = 0.001$ for Δ^9-tetrahydrocannabinol; and $F(1, 12) = 372.55$; $P = 0.001$ for (*R*)-methanandamide; $n = 4$ for the latter ANOVA because of missing observations]. Similarly, the decrease in response rate for SR-141716A alone (Panel C) was also statistically significant [$F(5, 15) = 15.95$; $P = 0.001$].

Separate ANOVAs of the effects of Δ^9-tetrahydrocannabinol in combination with SR-141716A suggested no changes with regard to 1.8 mg/kg Δ^9-tetrahydrocannabinol, i.e., the response rates were similar to those produced by this dose of Δ^9-tetrahydrocannabinol alone (Panel A). However, the comparison between 1 mg/kg SR-141716A together with 5.6 mg/kg Δ^9-tetrahydrocannabinol was significantly different from 5.6 mg/kg Δ^9-tetrahydrocannabinol alone ($P < 0.05$) according to Tukey's HSD.

Also, note that despite the 10 mg/kg dose of SR-141716A having significant (ANOVA followed by Tukey's HSD) rate-decreasing effects by itself (see point above 'V-SR'), the effects of this SR-141716A dose in combination with 1.8 and 5.6 mg/kg Δ^9-tetrahydrocannabinol were no different from the effects of those doses of Δ^9-tetrahydrocannabinol when tested alone. Thus, there appears to be only limited antagonism of the effects of Δ^9-tetrahydrocannabinol by SR-141716A and no additive rate-decreasing effect of the drug combination.

In contrast, the effects of (*R*)-methanandamide in combination with SR-141716A appeared greater than those produced by (*R*)-methanandamide alone (Panel B). Separate ANOVAs of the effects of (*R*)-methanandamide in combination with SR-141716A suggested that the comparisons between 3 mg/kg (*R*)-methanandamide alone and either 3 or 10 mg/kg SR-141716A plus (*R*)-methanandamide (3 mg/kg) were significantly different ($P < 0.05$) according to Tukey's HSD.

The furthermost left panel of graph B shows the results of vehicle and SR-141716A singly (i.e., the first 5-min period of the cumulative dosing test session). ANOVA indicated a significant effect between treatments [$F(5, 25) = 17.65$; $P = 0.001$). Post hoc analysis suggested that the doses of 3 and 10 mg/kg SR-141716A resulted in significantly lower rates of responding compared to the

treatments with the vehicle condition as well as when compared to the 0.3 mg/kg SR-141716A condition ($P < 0.05$).

4. Discussion

The current study provided at best limited evidence for antagonism by SR-141716 of the rate-suppressant effects induced by the cannabinoid CB_1 receptor agonist Δ^9-tetrahydrocannabinol in rats maintained on an FR-10 schedule of food reinforcement. With regard to (*R*)-methanandamide in combination with SR-141716, ANOVA suggested that the combination resulted in a greater rate depression compared to that produced by (*R*)-methanandamide alone. Apparently, this did not occur with the combination of Δ^9-tetrahydrocannabinol and SR-141716. Thus, SR-141716 augmented the rate-decreasing effects of (*R*)-methanandamide and did not profoundly change the rate-decreasing effects of Δ^9-tetrahydrocannabinol. This contrasts with our findings examining the discriminative stimulus effects of these compounds: SR-141716 produced substantial rightward shifts in the dose–response curves (generalization gradients) of Δ^9-tetrahydrocannabinol and (*R*)-methanandamide, although demonstration of surmountable antagonism was limited with regard to (*R*)-methanandamide. These shifts in the dose–response curves were, however, not associated with a normalization of the rate of lever pressing, especially so with regard to combinations of (*R*)-methanandamide and SR-141716 (Järbe et al., 2001). Moreover, our recent open-field studies with rats revealed that, short of antagonizing the (*R*)-methanandamide-induced ambulatory effect (distance traveled horizontally), the higher doses of SR-141716 (1, 3 and 5.6 mg/kg) actually acted in concert with (*R*)-methanandamide (10 and 18 mg/kg) to produce more suppression of ambulation compared to controls. A pattern similar to that for ambulation was also found for rearing, a vertical activity (personal observation). This did not occur with combinations of Δ^9-tetrahydrocannabinol and SR-141716 when examining these same open-field behaviors, i.e., measures became more normalized with the drug combination as compared to the effects induced by Δ^9-tetrahydrocannabinol alone (Järbe et al., 2002).

It is becoming increasingly clear that not all of the effects of cannabinoids are mediated by the two currently known cannabinoid receptors (Breivogel et al., 2001; Di Marzo et al., 1998, 2000; Monory et al., 2002). Furthermore, cannabinoid ligands may interact with the cannabinoid CB_1 receptor in distinctly different binding motifs. This in turn may result in a selective activation of different G proteins resulting in different cascades of events downstream (Bonhaus et al., 1998; Houston and Howlett, 1998; Mukhopadhyay and Howlett, 2001; Rubino et al., 1998; Thomas et al., 1998).

Our current data suggest a differential interaction for the two cannabinoid receptor agonists in the presence of SR-141716. Such differential interaction would seem to be in agreement with our finding that surmountable antagonism of the discriminative stimulus effects of Δ^9-tetrahydrocannabinol in the presence of 1 mg/kg SR-141716 could be demonstrated, whereas response suppression precluded testing high enough doses of (*R*)-methanandamide to produce a full dose response curve. A slight shift to the right of the dose response curve for (*R*)-methanandamide occurred in the presence of 0.3 mg/kg SR-141716 in animals trained to discriminate between (*R*)-methanandamide and vehicle. In contrast, response suppression precluded generating a full dose response curve for (*R*)-methanandamide in the presence of 0.3 mg/kg SR-141716 in animals trained with Δ^9-tetrahydrocannabinol (Järbe et al., 2001). Thus, in spite of apparent cross-tolerance and overlapping discriminative stimulus effects between Δ^9-tetrahydrocannabinol and (*R*)-methanandamide (Järbe et al., 2001; Lamb et al., 2000), components of the (*R*)-methanandamide pharmacological spectrum may involve targets other than the CB_1 cannabinoid receptor or may reflect differences in the binding motif. Similarly, some actions of anandamide also seem to involve targets other than the currently known cannabinoid receptor system(s) or binding motifs (Breivogel et al., 2001; Di Marzo et al., 2000, and references cited therein; Monory et al., 2002). As an example, changes in spontaneous activity, tail-flick response, rectal temperature and the ring catalepsy test induced by Δ^9-tetrahydrocannabinol are readily antagonized by the cannabinoid CB_1 receptor antagonist SR-141716 in mice (Compton et al., 1996); similar effects induced by anandamide in mice were not (Adams et al., 1998; see, however, Costa et al., 1999, for a different outcome in rats, although only a single dose of the agonist/antagonist interaction was examined). The involvement of this unidentified target/mechanism of action seems less pronounced for Δ^9-tetrahydrocannabinol as compared to (*R*)-methanandamide. Thus, co-administration of SR-141716 and (*R*)-methanandamide resulted in an augmented suppression of responding not seen with the combination of Δ^9-tetrahydrocannabinol and SR-141716.

In many instances, SR-141716 alone significantly reduced lever pressing in the current study (see graph C and the left panels of graphs A and B; see also Freedland et al., 2000; Järbe et al., 2001). In several other studies, SR-141716 has displayed intrinsic activity (e.g., Chaperon and Thiébot, 1999; Freedland et al., 2001; Williams and Kirkham, 2002). Additionally, it has been proposed that some effects more commonly associated with higher doses of SR-141716 may be the result of mechanisms not related to cannabinoid CB_1 receptor binding; or that SR-141716 interacts with the cannabinoid CB_1 receptor to produce inverse agonist as opposed to competitive antagonist effects (Sim-Selley et al., 2001). Inverse agonism, however, does not explain our data. Additionally, indirect activation of (non-)CB_1 neuronal receptor systems such as serotonergic, glutaminergic and tachykinin targets was proposed by Darmani and Pandya (2000) to explain SR-141716-induced

scratching and head-twitch responses in mice. These same transmitter systems presumably may also be involved for the SR-141716-induced scratching observed in rats (e.g., Arévalo et al., 2001; Järbe et al., 2002; Rubino et al., 1998). Thus, the net effect of higher doses of SR-141716 may involve activation of other systems in addition to its blockade of CB_1 receptor-mediated cannabinoid agonistic actions.

In summary, our data continue to suggest that there are differences in the pharmacological/behavioral profiles between tetrahydrocannabinols and anandamides, and that some effects induced by these agents may not be mediated through cannabinoid CB_1 receptor activation or, alternatively, cannabinergic ligands may interact differently at the recognition site leading to different cascades of events after receptor coupling/activation. Whatever the mechanism(s), the current results coupled with our previous data-examining combinations of Δ^9-tetrahydrocannabinol or (*R*)-methanandamide and SR-141716 (Järbe et al., 2001, 2002; personal observation) underscore pharmacological/behavioral differences between the cannabinoid CB_1 agonists (*R*)-methanandamide and Δ^9-tetrahydrocannabinol revealed by their interactions with the cannabinoid CB_1 antagonist SR-141716. Whether this reflects quantitative [no additive effects as regards Δ^9-tetrahydrocannabinol or synergism as regards (*R*)-methanandamide] or qualitative (different receptor mechanisms) differences will have to await additional research.

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