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The cannabinoid receptor antagonist SR-141716 does not readily antagonize open-field effects induced by the cannabinoid receptor agonist (R)-methanandamide in rats

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Abstract

This study examined the effects of the cannabinoid CB_1 receptor agonist (R)-methanandamide and the CB_1 receptor antagonist SR-141716 on open-field behaviors in rats. Animals were examined after administration of (R)-methanandamide (dose range 10 to 30 mg/kg) plus vehicle, and the two drugs in combination; the dose range of SR-141716 was 0.3 to 5.6 mg/kg. Injections were given intraperitoneally 20 min prior to initial testing. Additional exposures to the open-field arena occurred for the groups treated with 30 mg/kg (R)-methanandamide at 60 and 120 min post administration. There was a dose-related suppression of ambulation (horizontal activity) and rearing (vertical activity) after (R)-methanandamide administration. Coadministration of SR-141716 did not counteract the suppression induced by 10 and 18 mg/kg (R)-methanandamide but rather tended to augment it, especially with regard to ambulation using SR-141716 doses of 1 mg/kg and up. The latency to leave the starting area in the center of the field was significantly elevated by 30 mg/kg (R)-methanandamide. This effect was completely blocked by SR-141716. With increasing doses of SR-141716, there was an increase in grooming and scratching. Generally, the strongest effects occurred 20 min post administration with the exception of grooming, which reached maximum at 60 min post. Differences in the number of circlings, vocalizations, urination, and defecation generally did not differ clearly among treatments. These results coupled with previous open-field data examining combinations of Δ^9-tetrahydrocannabinol (Δ^9-THC) and SR-141716 [Pharmacol. Biochem. Behav. 73 (2002) 911] underscore pharmacological differences between (R)-methanandamide and Δ^9-THC revealed by their interactions with SR-141716.

Keywords: (R)-Methanandamide; Cannabinoid agonist; SR-141716; Cannabinoid antagonist; Open-field; Rats

1. Introduction

The discovery of specific receptors as the cellular targets for natural cannabinoids (constituents in hashish and marijuana preparations) as well as more recently identified endogenous ligands such as anandamide (Devane et al., 1992) and other endogenous cannabinoids (Hanûs et al., 1993, 2001; Mechoulam et al., 1995; Sugiura et al., 1995), has propelled research into the biological functions of an endocannabinoid signaling/neuromodulatory system. These efforts have resulted in the development of a great number of ligands able to activate the currently known cannabinoid receptor sites, CB_1 localized primarily within the CNS, and the cannabinoid CB_2 receptor in immune tissue. Selective antagonists of the cannabinoid CB_1 (Rinaldi-Carmona et al., 1994) and CB_2 (Rinaldi-Carmona et al., 1998) receptor sites have been developed as well as agents affecting the cellular transport mechanism and the enzymatic degradation of cannabinoid ligands such as anandamide (Goutopoulos and Makriyannis, 2002; Palmer et al., 2000). Recently, a third cannabinoid receptor site (CB_3?) has been postulated seemingly having a distribution in the CNS different from the initially discovered cannabinoid CB_1 receptor (Breivogel et al., 2001; Di Marzo et al., 2000; see also Howlett et al., 2002; Pertwee and Ross, 2002). Using CB_1 knockout mice, Monory et al. (2002) reported on a non-CB_1/CB_2 site

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in the cerebellum binding anandamide but not Δ^9-tetrahydrocannabinol (Δ^9-THC).

Although cannabinoid receptor agonists such as Δ^9-THC and anandamide produce many effects in common there are also key differences in the pharmacological/behavioral spectrum between these agents (e.g., Adams et al., 1998; Smith et al., 1998; see also Fride, 2002). A complication in comparing behavioral effects of Δ^9-THC and anandamide concerns the short duration of action of the endogenous ligand. With the objective of enhancing metabolic stability, Abadji et al. (1994) developed (R)-methanandamide, a chiral analog of anandamide exhibiting a longer duration of action than the parent compound both in vitro (Abadji et al., 1994; Goutopoulos et al., 2001) and in vivo (e.g., Järbe et al., 1998b, 2001; Romero et al., 1996). There is cross-tolerance between Δ^9-THC and (R)-methanandamide with regard to, e.g., operant food maintained lever pressing for rats receiving a daily dose of 18 mg/kg Δ^9-THC (Lamb et al., 2000) and overlapping discriminative stimulus effects (Burkey and Nation, 1997; Järbe et al., 1998a, 2000, 2001) as well as cross-tolerance between anandamide and Δ^9-THC for motor activity, catalepsy, hypothermia and analgesia in anandamide (20 mg/kg) tolerant mice (Fride, 1995). However, other experimental situations have revealed differences between the two cannabinoid agonists. For example, even though surmountable antagonism occurred between Δ^9-THC and SR-141716 (and to some extent also between (R)-methanandamide and SR-141716) when assessing the discriminative stimulus effects in rats, higher doses of the agonists in the presence of SR-141716 resulted in reduced rates of lever pressing, especially so for (R)-methanandamide (Järbe et al., 2001). More recently we examined the antagonistic effects of SR-141716 (dose range: 0.3 to 10 mg/kg) on Δ^9-THC and (R)-methanandamide induced effects in rats maintained on a fixed-ratio (FR-10) schedule of food reinforcement. We observed limited antagonism of the Δ^9-THC-induced decreases of lever pressing and no antagonism of the (R)-methanandamide-induced decreases in operant responding. Rather the combinations of SR-141716 and (R)-methanandamide produced additive effects, resulting in an even more reduced response output than either drug alone (Järbe et al., 2003).

Furthermore, circling in rats (see Methods for definition) is commonly observed after treatments with higher doses of more traditional tricyclic agonists such as Δ^9-THC, Δ^8-THC, cannabinol (CBN) and HU-210, the dimethylheptyl homologue of (−)-11-OH-Δ^8-THC [i.e., (−)-11-OH-Δ^8-THC-DMH], but circling was nonsignificant after (R)-methanandamide administration (Järbe et al., 1998b). Δ^9-THC-induced circling was blocked by SR-141716 although a fairly high dose of SR-141716 (5.6 mg/kg) was required for complete reversal (Järbe et al., 1998b, 2002).

Given previously observed differences between anandamides, including (R)-methanandamide, and the *Cannabis sativa* constituent Δ^9-THC, the current study revisited the open-field test to examine more closely the interaction between the cannabinoid CB_1 receptor agonist (R)-methanandamide and the cannabinoid CB_1 receptor antagonist SR-141716. Three doses of (R)-methanandamide (10, 18 and 30 mg/kg) in combination with four doses of SR-141716 (0.3, 1, 3 and 5.6 mg/kg) were examined 20 min post administration. Additionally, the effects of 30 mg/kg of (R)-methanandamide were followed by two more recordings occurring 1 and 2 h post drug administration. This extended time course examination was prompted by a previous report (Romero et al., 1995) suggesting that the endogenous ligand anandamide might induce behavioral/pharmacological changes in a protracted fashion not necessarily neuropharmacologically directly related to the initial effect spectrum (see also Willoughby et al., 1997). The open-field test was chosen because it generates several exploratory, novelty behaviors sensitive to drug manipulation.

2. Methods

2.1. Animals

A total of 180 adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY) being between 2.5 and 3 months old upon arrival were used. Upon arrival to the Temple quarters, the animals were quarantined for 1 week. Thereafter the animals were handled each weekday for 2 weeks and also given sham injections prior to beginning testing. Rats were individually housed with free access to food and water under a 12-h light/dark cycle (lights on at 7 a.m.). The Animal Care and Use Committee of Temple University, Philadelphia, PA, approved all procedures. The "Principles of animal laboratory care" (NIH publication No. 85-23, revised 1985) were followed.

2.2. Treatments

Twenty minutes prior to testing in the open-field arena, rats were given two intraperitoneal injections on either side of the peritoneal midline. Groups of rats ($n = 10$) were given either (R)-methanandamide or vehicle or SR-141716, or vehicle and vehicle (vehicle controls). The doses of (R)-methanandamide were 10, 18 or 30 mg/kg, and the doses of SR-141716 were 0.3, 1, 3, and 5.6 mg/kg.

As a precaution aimed at counterbalancing for the possible influence of length of stay in the vivarium prior to testing, treatments (i. e., various combinations of drugs and dosages) were staggered. Open-field sessions occurred during the lighted portion of the light/dark cycle (1–4 p.m.) during weekdays. No sessions were run on the first day after holidays or weekends.

2.3. Open-field test apparatus

The open-field arena is a gray painted box (60 × 60 × 45 cm) with an open top and a white floor divided into 16

squares (15 × 15 cm) and a circle (19 cm in diameter) marked in the center of the field. The floor was covered with a piece of acrylic, which was cleaned between sessions. This is the same open-field arena as that used to examine open-field behaviors after cannabinoid administration in previous studies from our laboratory (Järbe et al., 1998b, 2002, and references cited therein). A video camera was mounted 1.5 m above the floor of the open-field arena, such that the whole arena was visible on camera. The entire apparatus was centered in an otherwise empty room measuring 2 × 2.4 m in the Temple University Department of Psychology vivarium. Overhead fluorescent lights and two clips-on incandescent lamps with 40-W bulbs provided lighting mounted about 2 m above the box floor.

Sessions began by placing the rat in the center circle and ended after 5 min. The entire session was recorded on videotape and scored later.

2.4. Behavioral measures

The behavioral measures recorded were (i) ambulation (the number of squares crossed with all four feet); (ii) rearing frequency (the number of times the rat stood erect on its hind-legs); (iii) latency (the time in seconds to leave the starting area, the circle in the center of the field); (iv) circling (the number of times the animals turned around its vertical axis, 0.5 point given for each 180° turn); we also considered whether the potential circling (or turning behavior) consistently was directed to the left or right and whether it shifted during a single open-field exposure; (v) grooming episodes (the number of cleaning bouts); as well as (vi) grooming duration (i.e., the total time in seconds spent grooming); it has been argued that total duration time rather than just frequency of grooming is a more revealing measure (Eilam et al., 1992), though frequency is the more commonly used measure. We also kept record of (vii) scratching frequency [defined according to Darmani and Pandya (2000) as "A scratching episode produced by a particular hind limb consisted of one or more repetitive scratches with less than 2 seconds in between. If the interval between consecutive scratches by a particular hind limb was greater than 2 seconds, the scratches were considered as separate episodes. If the scratches were produced by alternative hind legs, then each scratch was considered as a separate episode"]; (viii) urination; and (ix) defecation (the number of urination spots and fecal boli deposited during the 5-min observation period). Also, the presence and absence of vocalization (squeaking) were noted when the rat was lifted up for placement into the open-field arena ("vocalization before"), as well as when the rat was lifted up for removal from the open-field arena ("vocalization after").

2.5. Drugs

(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 5% propylene glycol and 3% (10 mg/kg), 4% (18 mg/kg), or 5% (30 mg/kg) 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. The increase in the amount of Tween-80 occurred at the expense of saline. (R)-Methanandamide was injected intraperitoneally in volumes of 3 ml/kg (10 and 18 mg/kg) and 5 ml/kg (30 mg/kg) (see Järbe et al., 2001). SR-141716, as base [*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 H-pyrazole-3-carboxamide], was dissolved in a propylene glycol (5%)/Tween-80 [3% (0.3 to 3 mg/kg), or 4% (5.6 mg/kg)] mixture before being diluted with saline. All SR-141716 doses were administered intraperitoneally in a volume of 2 ml/kg. SR-141716 was kindly provided by the National Institute on Drug Abuse (NIDA), Bethesda, MD.

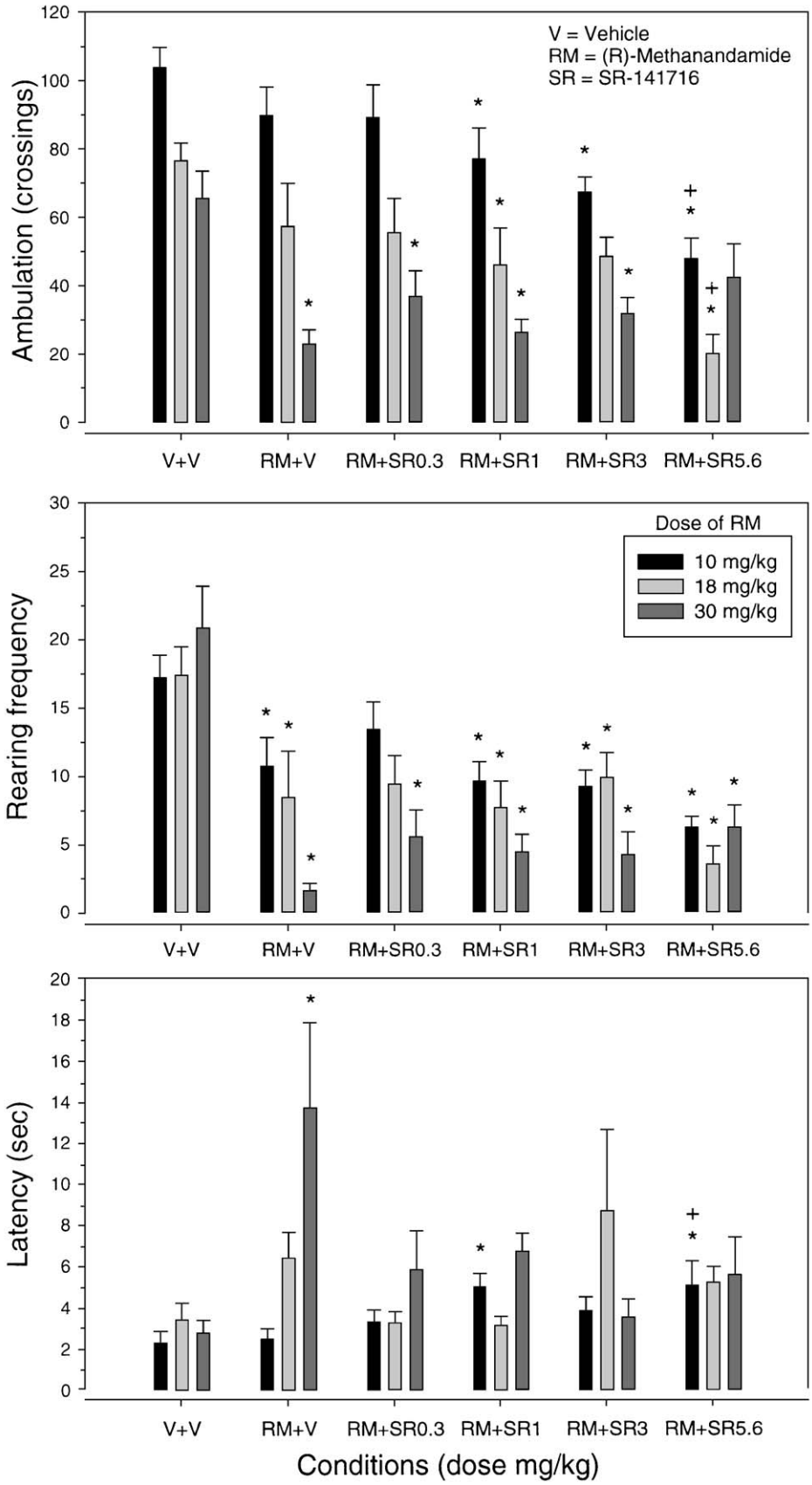
2.6. Statistics

Completely randomized one- and two-way ANOVAs as well as repeated measures two-way ANOVA (Kirk, 1968) were calculated using SigmaStat (version 2.0), run on an IBM PC. Subsequent post hoc analyses used the Tukey's Test for all pair-wise comparisons and the Dunnet's Test involving a control mean with α=.05, two-tailed, for the collection of comparisons (Kirk, 1968). To better meet the assumptions of homogeneity of error variances and normality of treatment-level distributions, all data except presence/absence of vocalization were square-root transformed for statistical analysis (Kirk, 1968).

3. Results

3.1. (R)-Methanandamide (RM) and SR-141716 (SR) in combination 20 min post (Study 1)

Fig. 1 shows the effects of (R)-methanandamide (10, 18 and 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3 and 5.6 mg/kg) for ambulation (top graph), rearing (middle graph), and the latency to leave the middle circle in the open-field arena (bottom graph). The two-way ANOVA results for ambulation were: [RM factor, [$F(2,105) = 32.96$; $P < .001$; SR factor, $F(4,105) = 2.36$; P=.058]; the interaction (RM × SR) was significant [$F(8, 05) = 2.32$; P=.025]. The ANOVA outcome for rearing was: [RM factor, $F(2,105) = 15.45$; $P < .001$; SR factor, $F(4,105) = 1.22$; $P > .05$]; the interaction was nonsignificant ($P > .05$). In the case of latency, there were no significant main effects but the interaction (RM × SR) was significant [$F(8,105) = 3.11$; P=.003]. Given that two recordings per cell were lost due to apparatus failure during testing involving 18 mg/kg (R)-


V = Vehicle
RM = (R)-Methanandamide
SR = SR-141716
Ambulation (crossings)
Rearing frequency
Latency (sec)
Dose of RM
10 mg/kg
18 mg/kg
30 mg/kg
V+V
RM+V
RM+SR0.3
RM+SR1
RM+SR3
RM+SR5.6
Conditions (dose mg/kg)

methanandamide, for this two-way ANOVA analysis all cells contained eight observations. Thus, two recordings from all the other treatment conditions were dropped randomly to achieve a balanced design for this statistical analysis.

Subsequent main effect analysis suggested that ambulatory activity was markedly suppressed by 30 mg/kg (R)-methanandamide in comparison to both 10 and 18 mg/kg (R)-methanandamide irrespective of the SR-141716 dose (Tukey's Test), as well as when compared to the vehicle condition according to one-way ANOVA followed by Dunnet's Test for multiple comparisons vs. a control (see Fig. 1, upper graph). This suppression of ambulation was not significantly attenuated by the addition of SR-141716. Furthermore, 18 mg/kg (R)-methanandamide suppressed ambulation significantly more than 10 mg/kg, emphasizing the dose dependent character of the drug effect. Addition of SR-141716 to 18 mg/kg (R)-methanandamide did not increase ambulatory activity but rather ambulatory activity decreased with increasing doses of SR-141716. Thus, 5.6 mg/kg SR-141716 produced effects significantly different from (R)-methanandamide (18 mg/kg) alone, i.e., zero SR-141716, as well as 0.3 mg/kg SR-141716 [in combination with (R)-methanandamide]. This pattern of results is also evident from the analysis involving 10 mg/kg SR-141716. Although 10 mg/kg (R)-methanandamide did not significantly suppress ambulatory activity compared to vehicle controls [as was the case also for 18 mg/kg (R)-methanandamide], increasing doses of SR-141716 were associated with decreasing ambulatory activity as suggested by both the one- and two-way ANOVAs. Thus, doses of 1 mg/kg SR-141716 and up together with 10 mg/kg (R)-methanandamide resulted in significantly less ambulation than the vehicle controls. Also, within SR-141716 dose comparisons at the level of 10 mg/kg (R)-methanandamide showed that 5.6 mg/kg SR-141716 was significantly different from either the zero [i.e., 10 mg/kg (R)-methanandamide together with vehicle], as well as the 0.3 and 1 mg/kg SR-141716 plus (R)-methanandamide treatment conditions.

A similar pattern of outcome emerged also for rearing. Thus, rearing was suppressed below vehicle levels by all three (R)-methanandamide doses in a dose-dependent fashion (see Fig. 1, middle graph). Specifically, rearing was significantly more suppressed by 30 mg/kg as compared to 18 mg/kg (R)-methanandamide, and the latter dose significantly decreased rearing more than 10 mg/kg (R)-methanandamide. Also note that according to the Dunnet's Test, the two treatment conditions not significantly different from the corresponding vehicle controls were 10 and 18 mg/kg (R)-methanandamide together with 0.3 mg/kg SR-141716, suggestive perhaps of some limited antagonism by this dose of SR-141716 of the (R)-methanandamide produced decreases in rearing. On the other hand, none of the comparisons between dose of (R)-methanandamide alone and when the agonist was combined with SR-141716 were significant (Dunnet's Test).

Latency to leave the circle in the center of the open-field floor was significantly elevated by 30 mg/kg (R)-methanandamide compared to both the vehicle controls and 10 mg/kg (R)-methanandamide; the 10 and 18 mg/kg (R)-methanandamide treatment conditions did not differ significantly from one another (see Fig. 1, lower graph). Furthermore, the latency associated with 30 mg/kg (R)-methanandamide alone was significantly different from the latencies observed when 30 mg/kg (R)-methanandamide was combined with both 0.3 and 3 mg/kg SR-141716, suggesting antagonism. Yet, 10 mg/kg (R)-methanandamide together with 5.6 mg/kg SR-141716 produced longer latencies than 10 mg/kg (R)-methanandamide alone (reflecting, if anything, additive effects, not antagonism). No statistically significant changes occurred for the parameter "Latency" in tests involving 18 mg/kg (R)-methanandamide.

Fig. 2 shows the effects of (R)-methanandamide (10, 18 and 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3 and 5.6 mg/kg) for scratching frequency [RM factor, $F(2,105)=8.69$; $P<.001$; SR factor, $F(4, 105)=33.74$; $P<.001$], grooming episodes [RM factor, $F(2,105)=5.65$; $P=.005$; SR factor, $F(4,105)=8.75$; $P<.001$], as well as grooming duration [RM factor, $F(2,105)=4.62$; $P=.012$; SR factor, $F(4,105)=8.57$; $P<.001$]. For comparative purposes, grooming duration for controls and three doses (1, 3 and 5.6 mg/kg) of SR-141716 alone is also shown (data rescored from Järbe et al., 2002). There was a dose-related increase in grooming duration. One-way ANOVA yielded significance [$F(3,36)=7.15$; $P<.001$], and Dunnet's Test suggested that 5.6 mg/kg SR-141716 resulted in a significantly longer duration of grooming compared to the vehicle controls.

Subsequent analyses of the two-way ANOVA indicated that scratching occurred significantly more often for 10 mg/kg (R)-methanandamide as compared to either 18 or 30 mg/kg (R)-methanandamide; the latter two doses of (R)-methanandamide were not significantly different from one another. Additionally, scratching associated with 3 mg/kg SR-141716 was significantly more frequent compared to either

Fig. 1. The effects of (R)-methanandamide (RM, 10, 18 and 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3, and 5.6 mg/kg) on ambulation (top), rearing (middle), and latency (bottom) in different groups of Sprague–Dawley rats [$n=10$, except for (R)-methanandamide 18 mg/kg alone and in combination with SR-141716 as well as the corresponding vehicle control group, i.e., the middle bar above V+V, where $n=8$ because of lost recordings]. (R)-Methanandamide and SR-141716 injections were given intraperitoneally 20 min prior to session onset; controls received two vehicle injections (V+V). The left bar above "V+V" constitutes the vehicle control condition pertaining to the interaction study involving 10 mg/kg (R)-methanandamide (3+2 ml/kg), the middle bar pertains to 18 mg/kg (R)-methanandamide (3+2 ml/kg), and the right hand bar refers to the examination involving 30 mg/kg (R)-methanandamide (5+2 ml/kg). The data points represent the means (±S.E.M.) during a 5-min observation period in an open-field arena. Significantly ($P<.05$) different (Dunnet's Test involving a comparison/control mean) from *(R)-methanandamide/SR-141716 vehicle (V+V) controls (the three bars furthermost to the left in each graph) and $^{+}$(R)-methanandamide/SR-141716 vehicle, i.e., agonist alone (RM-V). Other details in text.

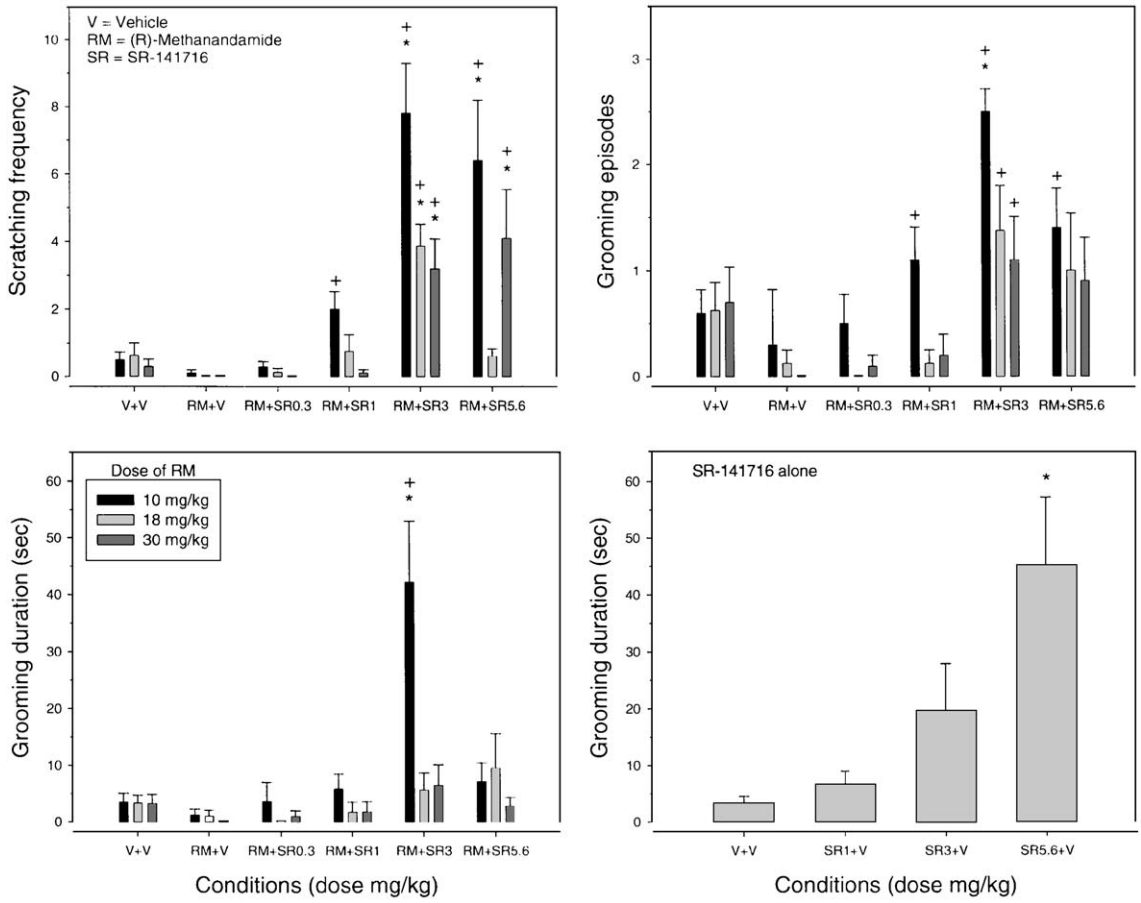
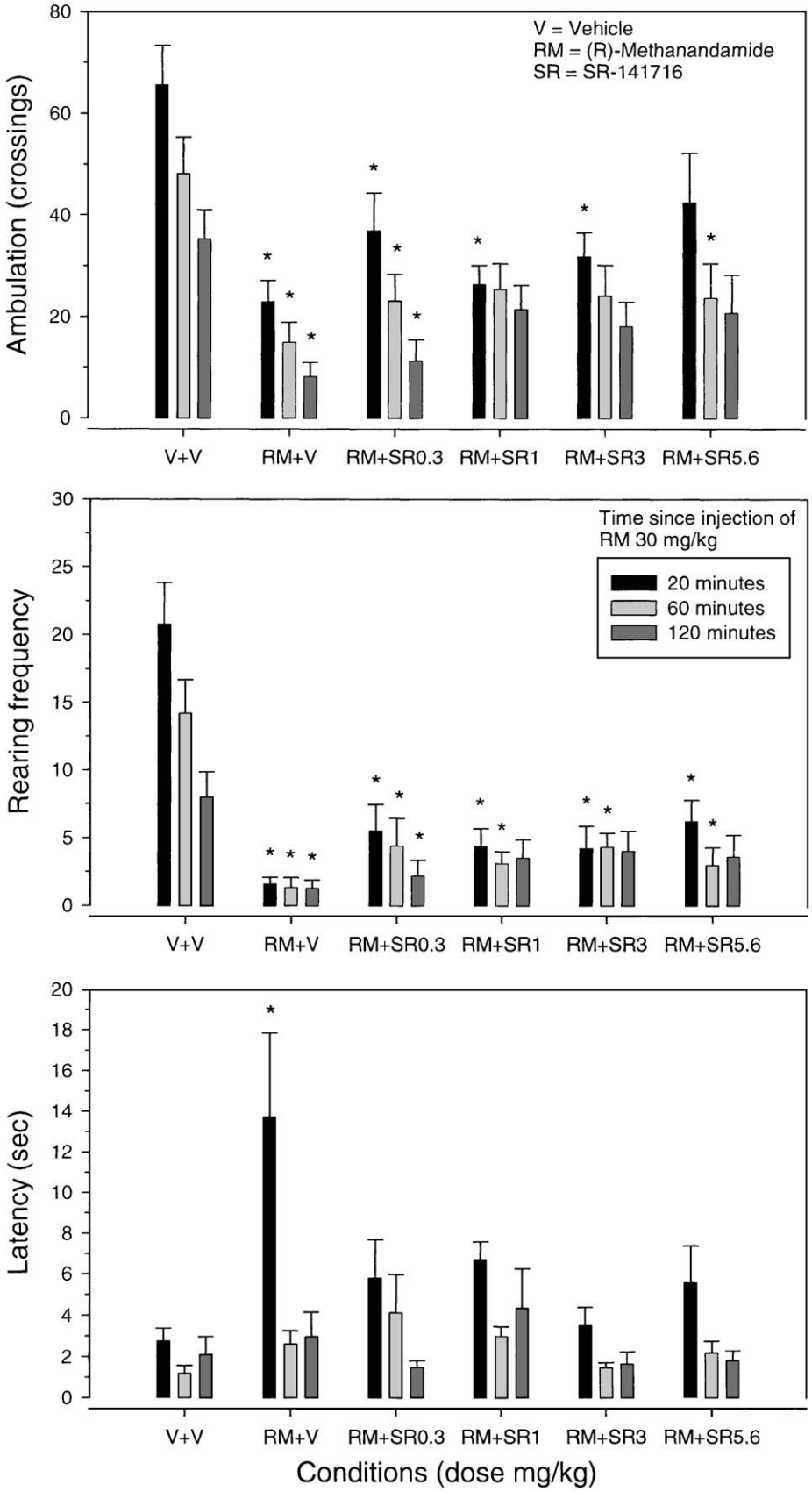


Fig. 2. The effects of (R)-methanandamide (RM, 10, 18 and 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3, and 5.6 mg/kg) on scratching frequency (top, left), grooming episodes (top, right), grooming duration (bottom, left), and grooming duration with SR-141716 (together with vehicle, 2 ml/kg) alone in different groups of Sprague–Dawley rats [$n=10$, except for (R)-methanandamide 18 mg/kg alone and in combination with SR-141716 as well as the corresponding vehicle control group, i.e., the middle bar above V + V, where $n=8$ because of lost recordings]. (R)-Methanandamide and SR-141716 injections were given intraperitoneally 20 min prior to session onset; controls received two vehicle injections (V + V). With regard to grooming duration for SR-141716 alone, the drug was injected intraperitoneally 30 min prior to testing (data rescored from Järbe et al., 2002). The left bar above "V + V" constitutes the vehicle control condition pertaining to the interaction study involving 10 mg/kg (R)-methanandamide (3 + 2 ml/kg), the middle bar pertains to 18 mg/kg (R)-methanandamide (3 + 2 ml/kg), and the right hand bar refers to the examination involving 30 mg/kg (R)-methanandamide (5 + 2 ml/kg). The data points represent the means (± S.E.M.) during a 5-min observation period in an open-field arena. Significantly ($P<.05$) different (Dunnet's Test involving a comparison/control mean) from *(R)-methanandamide/SR-141716 vehicle (V + V) controls (the three bars furthermost to the left in each graph) and $^{+}$(R)-methanandamide/SR-141716 vehicle, i.e., agonist alone (RM-V). Other details in text.

zero [i.e., (R)-methanandamide together with the SR-141716 vehicle] or 0.3 mg/kg SR-141716 [together with (R)-methanandamide]. One-way ANOVA followed by Dunnet's Test suggested that all three conditions associated with 3 mg/kg SR-141716 and two of the three conditions associated with 5.6 mg/kg SR-141716 exhibited a significantly higher degree of scratching compared to their respective vehicle controls. A similar pattern emerged when using (R)-methanandamide dose as the reference/comparison condition (see Fig. 2).

Grooming episodes were significantly more predominant after treatment with 10 mg/kg (R)-methanandamide as compared to 18 or 30 mg/kg (R)-methanandamide in combination with SR-141716; the latter two (R)-methanan-

Fig. 3. The effects of (R)-methanandamide (RM, 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3, and 5.6 mg/kg) at three intervals (20-, 60-, and 120-min) post injection on ambulation (top), rearing (middle), and latency (bottom) in different groups of Sprague–Dawley rats ($n=10$). Controls received two vehicle injections (V + V). The left bar above "V + V" constitutes the vehicle control condition pertaining to the interaction study carried out 20-min post injection (data reproduced from Fig. 1), the middle bar pertains to 60-min post injection, and the right hand bar refers to the examination carried out 120-min post injection (5 + 2 ml/kg). The data points represent the means (± S.E.M.) during three 5-min observation periods in an open-field arena following one administration of vehicles or drugs. * Significantly ($P<.05$) different (Dunnet's Test involving a control mean) from (R)-methanandamide/SR-141716 and vehicle (V + V) controls (the three bars furthermost to the left in each graph); no comparisons between (R)-methanandamide alone (RM + V) and the other conditions were significant ($P>.05$). Other details in text.


V = Vehicle
RM = (R)-Methanandamide
SR = SR-141716
Ambulation (crossings)
Time since injection of
RM 30 mg/kg
20 minutes
60 minutes
120 minutes
Rearing frequency
Latency (sec)
V+V
RM+V
RM+SR0.3
RM+SR1
RM+SR3
RM+SR5.6
Conditions (dose mg/kg)

damide plus SR-141716 conditions were not significantly different from one another. The higher SR-141716 doses (3 and 5.6 mg/kg) were significantly different from both the zero (i.e., vehicle plus agonist) and the 0.3 mg/kg SR-141716 conditions. The ANOVA of the related measure "Grooming duration" (in seconds) suggested that 10 mg/kg (R)-methanandamide was significantly different from either 18 or 30 mg/kg (R)-methanandamide and that the two latter doses of (R)-methanandamide did not differ significantly from one another. Furthermore, 3 mg/kg SR-141716 was associated with significantly longer duration of grooming than either of the SR-1417161 doses of 0.3 or zero mg/kg [together with(R)-methanandamide]. Apparently, the outcome with regard to grooming episodes and duration parallels that of scratching frequency.

Two-way ANOVA suggested no significance with regard to fecal boli, urination, circling, or "vocalization after", i.e., vocalization occurring after the open-field session ($P>.05$). "Vocalization before", however, was significant indicating that squeaking occurred more often in the (R)-methanandamide plus vehicle conditions vis-a-vis when (R)-methanandamide was combined with 5.6 mg/kg SR-141716 (Tukey's Test) [$F(4,105)=3.73$; $P=.007$; the means being 0.58 vs. 0.17]. The only other significant one-way ANOVAs for these measures appeared for the 10 mg/kg (R)-methanandamide treatment conditions for defecation [$F(5,54)=9.82$; $P<.001$], and urination [$F(5,54)=5.08$; $P<.001$]. Dunnet's Test indicated that the vehicle controls deposited more fecal boli than any of the other groups and that the controls urinated more than the 10 mg/kg (R)-methanandamide plus vehicle group (not shown).

3.2. (R)-Methanandamide and SR-141716 in combination—time course (Study 2)

Fig. 3 shows the effects of 30 mg/kg (R)-methanandamide in combination with SR-141716 (vehicle, 0.3, 1, 3 and 5.6 mg/kg) for ambulation (top graph), rearing (middle graph), and the latency to leave the middle circle in the open-field arena (bottom graph) at 20, 60 and 120 min post administration. There were two missing observations at the 60-min interval because of unintended external noise. The statistical program estimated these two missing values (general linear model).

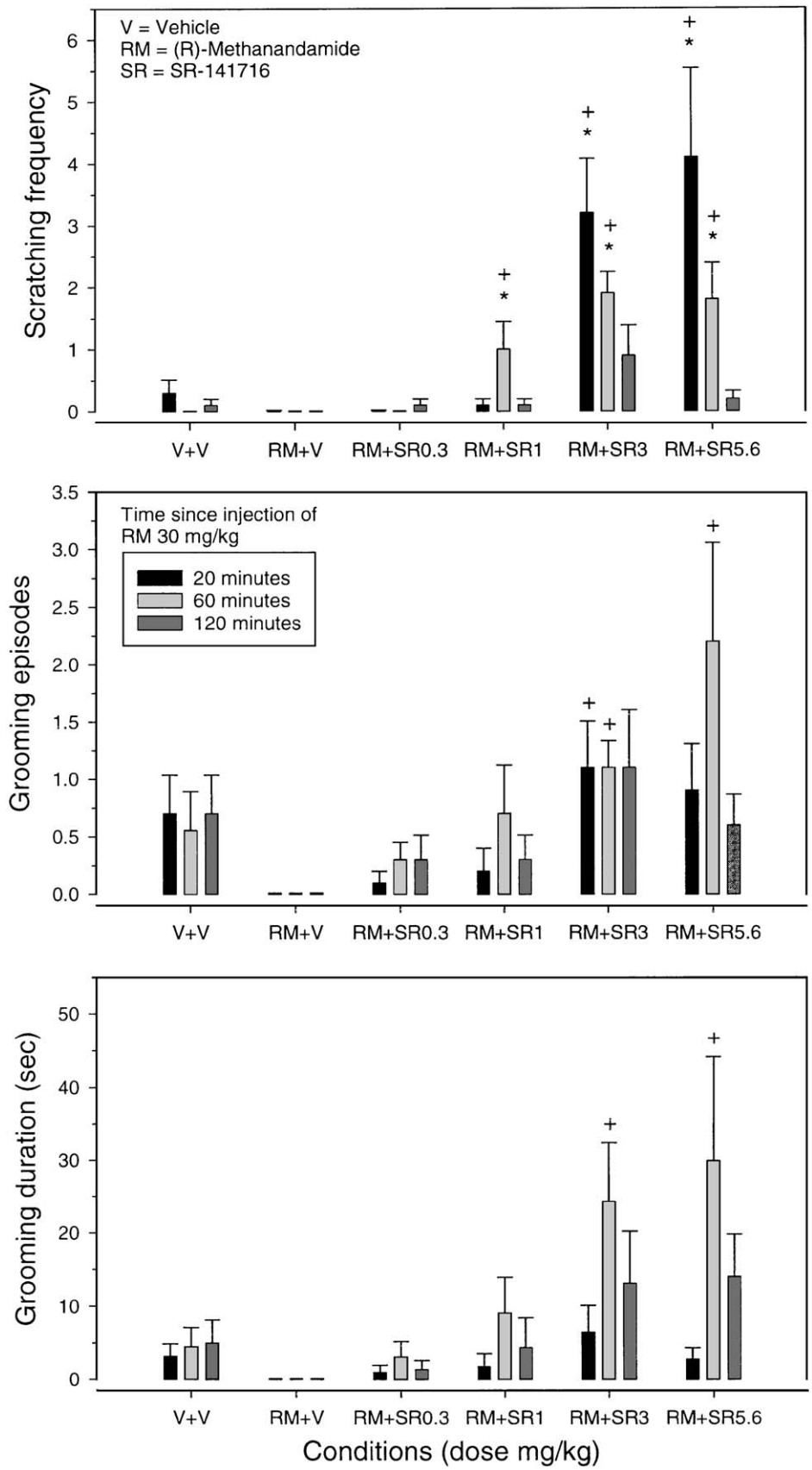
The two-way repeated measures ANOVA suggested a main effect of "Time" for ambulation [$F(2,89)=13.74$; $P<.001$], meaning that the animals ambulated less as a function of repeated exposures to the open-field arena. One-way ANOVAs followed by Dunnet's Test suggested that animals given 30 mg/kg (R)-methanandamide alone or together with 0.3 mg/kg SR-141716 ambulated less than the respective vehicle controls irrespective of time period (i.e., 20, 60 or 120 min post administration). With one exception, the lack of significance between vehicle controls and animals given (R)-methanandamide together with 1 to 5.6 mg/kg of SR-141716 might suggest some restoration of ambulation by these doses at the later time intervals. Note though that this statistical outcome is facilitated by the reduced ambulation in the vehicle controls as a result of repeated exposures to the open-field arena.

The ANOVA outcome for rearing was similar to that of ambulation with the factor "Time" being significant [$F(2,89)=4.24$; $P=.017$], suggesting that the amount of rearing declined over time. The amount of rearing at 20 min post was significantly higher than the amount of rearing expressed at 120 min post administration. As for ambulation, most comparisons with vehicle controls were significant with the exception of doses of 1–5.6 mg/kg SR-141716 120 min post administration (Dunnet's Test). Note though that levels of rearing at this time point were low among the vehicle controls. Thus, there was a decline in rearing for the vehicle controls from the first to the second and third exposure to the open field; the rearing frequency remained very low for the (R)-methanandamide (30 mg/kg) plus SR-141716 vehicle condition throughout the three time periods.

For latency, there were significant main effects for SR-141716 dose [$F(4,89)=3.07$; $P=.025$], and the factor "Time" [$F(2,89)=20.03$; $P<.001$], but the interaction was not significant. Thus, the latency to leave the center circle was significantly higher for the 20-min condition as compared to both the 60- and 120-min conditions. Additionally, the latency associated with 30 mg/kg (R)-methanandamide was significantly longer than the vehicle controls. This increased latency after 30 mg/kg (R)-methanandamide administration was counteracted by all doses of SR-141716 (see Fig. 3).

The statistical analysis of scratching frequency suggested that SR-141716 dose [$F(4,89)=15.72$; $P<.001$], "Time" [$F(2,89)=11.74$; $P<.001$], as well as the interaction between "SR dose" and "Time" [$F(8, 89)=5.11$; $P<.001$], were all significant. Thus, there were more scratching occurring 20- and 60-min post administration compared to scratching occurring 120 min after injections (see Fig. 4). Scratching was significantly higher for 3 and 5.6 mg/kg SR-

Fig. 4. The effects of (R)-methanandamide (RM, 30 mg/kg) in combination with SR-141716 (0, 0.3, 1, 3, and 5.6 mg/kg) at three intervals (20-, 60-, and 120-min) post injection on scratching frequency (top), grooming episodes (middle), and grooming duration (bottom) in different groups of Sprague–Dawley rats ($n=10$). Controls received two vehicle injections (V + V). The left bar above "V + V" constitutes the vehicle control condition pertaining to the interaction study carried out 20-min post injection (data reproduced from Fig. 2), the middle bar pertains to 60-min post injection, and the right hand bar refers to the examination carried out 120-min post injection (5 + 2 ml/kg). The data points represent the means (± S.E.M.) during three 5-min observation periods in an open-field arena following one administration of vehicles or drugs. Significantly ($P<.05$) different (Dunnet's Test involving a comparison/control mean) from *(R)-methanandamide/SR-141716 vehicle (V + V) controls (the three bars furthermost to the left in each graph) and $^{+}$(R)-methanandamide/SR-141716 vehicle, i.e., agonist alone (RM-V). Other details in text.


V = Vehicle
RM = (R)-Methanandamide
SR = SR-141716
Scratching frequency
Time since injection of
RM 30 mg/kg
20 minutes
60 minutes
120 minutes
Grooming episodes
Grooming duration (sec)
V+V
RM+V
RM+SR0.3
RM+SR1
RM+SR3
RM+SR5.6
Conditions (dose mg/kg)

141716 as compared to (R)-methanandamide plus vehicle as well as in combination with the lowest dose (0.3 mg/kg) of SR-141716. One-way ANOVA followed by Dunnet's Test indicated that 20 min post administration, scratching occurring with combinations of 30 mg/kg (R)-methanandamide in combination with SR-141716 doses 1, 3 and 5.6 mg/kg were significantly elevated compared to the vehicle controls as well as when compared to (R)-methanandamide alone. At 60 min this also included 1 mg/kg SR-141716. At 120 min post administration there was very little scratching irrespective of treatment condition.

With regard to grooming episodes, dose of SR-141716 [$F(4,89) = 7.41$; $P < .001$] and the "Time" factor [$F(2,89) = 3.32$; $P = .041$] were significant and there was no significant interaction between the two factors. Hence, (R)-methanandamide combined with 3 and 5.6 mg/kg SR-141716 resulted in more grooming episodes compared to (R)-methanandamide alone and in combination with 0.3 mg/kg SR-141716. There were no significant differences between the vehicle controls and the separate drug conditions at any time point for this behavior. However, there were a few instances where scratching was elevated when compared to (R)-methanandamide alone (Fig. 4).

The related variable "Grooming duration" disclosed a similar pattern. Thus, dose of SR-141716 [$F(4,89) = 5.45$; $P = .001$], and the "Time" factor [$F(2,89) = 7.33$; $P = .001$] were significant and there was no significant interaction between the two factors. Further analysis showed that the 3 and 5.6 mg/kg SR-141716 conditions [together with (R)-methanandamide] resulted in a longer grooming duration compared to (R)-methanandamide together with the SR-141716 vehicle; there was also a significant difference between 0.3 and 3 mg/kg SR-141716. With regard to the "Time" factor, grooming duration was significantly longer at 60 min compared to 20 min post administration. As suggested by the data shown in Fig. 4, grooming is on the decline 120 min post administration. Thus, 60 min post administration appears to represent the peak activity for this intrinsic activity of SR-141716 in our study [when combined with 30 mg/kg (R)-methanandamide].

The repeated measures two-way ANOVA was significant for circling [$F(2,89) = 7.20$; $P = .001$], and Tukey's Test indicated that the circling score (mean = 0.22) was higher 20 min after administration compared to 60 min (mean = 0.05), as well as 120 min (mean: 0.06) post administration. One-way ANOVA suggested no significant differences between the vehicle controls and the other treatment conditions. Two- and one-way ANOVAs for defecation, urination, vocalization (before and after) were all nonsignificant in Study 2 (not shown).

4. Discussion

The current study examined behavioral/pharmacological interactions between (R)-methanandamide (Abadji et al., 1994), a chiral analog of the endocannabinoid ligand anandamide, and the cannabinoid CB_1 receptor antagonist SR-141716 (Rinaldi-Carmona et al., 1994) in an open-field test. The over-all impression from this study is that antagonism between (R)-methanandamide and SR-141716 was limited. Indeed, with regard to the measure "ambulation" (distance traveled horizontally), short of antagonizing the (R)-methanandamide induced ambulatory effect, the higher doses of SR-141716 actually acted in concert with (R)-methanandamide (10 and 18 mg/kg) to produce more suppression of ambulation. As an illustration, 10 mg/kg (R)-methanandamide by itself did not suppress ambulation below levels of the vehicle control rats but doses of 1 mg/kg and up of SR-141716 [together with 10 mg/kg (R)-methanandamide] significantly reduced ambulatory activity below vehicle control levels. A similar outcome was observed also for the 18 mg/kg (R)-methanandamide condition, whereas for the conditions associated with 30 mg/kg (R)-methanandamide ambulation remained at low levels irrespective of SR-141716 dose (0.3, 1, 3 and 5.6 mg/kg), and time interval examined (20, 60 and 120 min post administration). There was no further reduction in ambulation when SR-141716 was combined with 30 mg/kg (R)-methanandamide. At 60 and 120 min post administration, however, ambulation was not significantly different from the vehicle controls (with one exception) indicative perhaps of some antagonism by SR-141716 (1 to 5.6 mg/kg) of 30 mg/kg (R)-methanandamide (Study 2). A pattern similar to that for ambulation was found also for rearing. As for ambulation, there was no further reduction in rearing when SR-141716 was combined with 30 mg/kg (R)-methanandamide. Also for rearing, the higher SR-141716 doses (1 to 5.6 mg/kg) seemed to attenuate the suppression by 30 mg/kg (R)-methanandamide at 2 h post but not at 1 h post (Study 2). Note though that for both ambulation and rearing, the vehicle controls exhibited reduced activity at 1 and 2 h post vehicle administration in comparison to the levels of ambulation and rearing displayed during the first encounter of the open-field arena 20 min post administration (Study 2). When we examined SR-141716 alone, only the highest dose (5.6 mg/kg) administered 30 min prior to open-field testing significantly suppressed ambulation but not rearing. The latency to leave the center circle in the open-field arena after SR-141716 treatment was not significantly different from the vehicle controls (Järbe et al., 2002). Furthermore, with increasing doses of SR-141716, scratching and grooming emerged, effects intrinsically linked to administration of SR-141716 itself (e.g., Aceto et al., 1996; Cook et al., 1998; Darmani and Pandya, 2000; Järbe et al., 2002; Rubino et al., 1998).

Of course, our current finding(s) is/are most reminiscent of earlier failures to reverse THC-like effects induced by anandamide with SR-141716. For example, Smith et al. (1998) did not observe clear-cut antagonism of the antinociceptive effects of anandamide by SR-141716. Yet, the antinociceptive effects of Δ^9-THC were blocked by this antagonist. Similarly, Adams et al. (1998) failed to reverse

the so-called cannabinoid-induced tetrad effect in anandamide-treated mice with SR-141716. The effects of Δ^9-THC in this tetrad test battery (antinociception, hypothermia, hypomotility/catalepsy) were antagonized by SR-141716 (Compton et al., 1996). At the time (Adams et al., 1998) it was speculated that the short duration of action of anandamide might at least partly be responsible for this lack of antagonism and that metabolites rather than the parent compound could be responsible for the outcome [later deemed unlikely by Wiley et al. (2000)]. One argument for this position originally was that the effects of the more metabolically stable anandamide analog 2-methyl-2′-fluoroethylanandamide seemed readily blocked by SR-141716 (Adams et al., 1998). The basic assumption for this reasoning presumably is that this longer acting analog retained the pharmacological profile of anandamide, an assumption that may not be tenable. Irrespective, the effects of (R)-methanandamide are fairly long lasting both when assessed in open-field testing (Romero et al., 1996; current study), as well as in a drug discrimination procedure (Järbe et al., 2001).

The drug interaction profile described here for ambulation and rearing appears to also have its counterpart in results from a study designed to examine the antagonistic effects of SR-141716 (dose range: 0.3–10 mg/kg) on Δ^9-THC- and (R)-methanandamide-induced rate changes for rats maintained on a fixed-ratio (FR-10) schedule of food reinforcement. We observed limited antagonism of the Δ^9-THC-induced decreases of lever pressing and no antagonism of the (R)-methanandamide-induced decreases in operant responding. Rather the combinations of SR-141716 and (R)-methanandamide produced additive effects, resulting in an even more reduced response output than either of these two drugs alone (Järbe et al., 2003; see also Järbe et al., 2001). Collectively, it would appear that although Δ^9-THC and (R)-methanandamide as well as anandamide have effects in common, their interaction with the CB_1 receptor antagonist SR-141716 reveals intricate differences in their pharmacological/behavioral profiles.

Previously we used the open-field test (Järbe et al., 2002) to evaluate two doses of Δ^9-THC (3 and 5.6 mg/kg) and three doses of SR-141716 (1, 3 and 5.6 mg/kg) singly and in combination 30 min post administration. We found that the suppressed ambulatory, horizontal activity was counteracted by coadministration of SR-141716 but not in a dose-dependent fashion. Thus, all three doses of SR-141716 (1, 3 and 5.6 mg/kg) appeared equally effective in this regard. The effect of 5.6 mg/kg Δ^9-THC seemed less normalized by SR-141716 than did the suppression induced by 3 mg/kg Δ^9-THC. A pattern similar to that of ambulation emerged also for rearing. However, after administration of 5.6 mg/kg Δ^9-THC, none of the three doses of SR-141716 employed were able to fully restore rearing to levels comparable to the corresponding vehicle control values. Again, all three doses of SR-141716 behaved in much the same way, producing essentially a flat dose–response curve. It is noteworthy that there was no instance where the drug combination (Δ^9-THC and SR-141716) produced additive effects, only antagonism was observed albeit to varying degrees.

In both the current and the previous open-field study examining interactions between Δ^9-THC and SR-141716 (Järbe et al., 2002), the increased latency to leave the circle was generally counteracted by SR-141716. Additionally, unlike the flat antagonism curves described above for ambulation and rearing, 1 mg/kg SR-141716 clearly was less effective in reducing the incidence of Δ^9-THC produced circling than was 5.6 mg/kg SR-141716 (Järbe et al., 2002), confirming previous observations to such an effect (Järbe et al., 1998b). In agreement with Järbe et al. (1998b), the category circling did not clearly differentiate between the various (R)-methanandamide conditions in the current study. Although we found a significant effect for ‘‘Time’’ in the two-way repeated measures ANOVA for circling in Study 2, the absolute (mean) scores were very low (0.22 turns at 20 min; 0.05 turns at 60 min; and 0.06 turns at 120 min post administration). The degree of circling after (R)-methanandamide administration was very low also in our initial assessment (Järbe et al., 1998b). In addition to Δ^9-THC, circling has been observed after administration of other classical, tricyclic agonist cannabinoids such as Δ^8-THC (Sjödén et al., 1973), CBN (Järbe and Hiltunen, 1987), as well as HU-210 (Ferrari et al., 1999). Thus, circling may reflect a motor disturbance that separates the pharmacological spectrum between (R)-methanandamide and more classical tricyclic cannabimetics in rats.

Treatments with SR-141716 were associated with increases in the number of grooming episodes as well as duration of grooming and frequency of scratching. Similar results were previously obtained after treatment with SR-141716 alone or when combined with Δ^9-THC (Järbe et al., 2002; see also Rubino et al., 1998). These increases in grooming and scratching seemed dampened at least partially by coadministration of Δ^9-THC in comparison to animals that received SR-141716 singly. Janoyan et al. (2002) observed that high doses of the potent cannabinoid agonists WIN 55,212-2, CP 55940 and HU-210 afforded essentially complete blockade of the scratching induced by 2.5 mg/kg SR-141716 in mice. The naturally occurring cannabinoid agonists Δ^8-THC and Δ^9-THC (highest dose tested was 20 mg/kg for each drug) also afforded protection against the SR-141716-induced scratching in mice, but the degree of blockade appeared less than that for the above mentioned synthetic cannabinoid agonists. Using relatively site selective antagonists, Darmani and Pandya (2000) suggested that the SR-141716-induced scratching in mice may ‘‘involve indirect potentiation of serotonergic, glutamatergic and tachykinin neurotransmitter systems.’’ Unlike the current studies and those of Arévalo et al. (2001) as well as Costa and Colleoni (1999; see also Costa et al., 1999), there was no significant change in the grooming score compared to

vehicle controls after SR-141716 treatment in the study employing mice (Darmani and Pandya, 2000). Scoring "Grooming duration" in the current study suggested that (R)-methanandamide generally reduced the time (duration) spent grooming associated with SR-141716 administration (comparative data are not available for Δ^9-THC). Although both grooming and scratching are associated with SR-141716 administration, the time course may differ slightly with scratching peaking earlier than grooming (Study 2). However, we probably have not identified all the determinants for the expression of these behaviors because of some anomalies in the outcomes concerning the interaction between SR-141716 and (R)-methanandamide. For example, 10 mg/kg (R)-methanandamide in combination with 3 mg/kg SR-141716 resulted in higher scores than when this dose of the agonist was combined with 5.6 mg/kg SR-141716 (Study 1). On the other hand, when SR-141716 was examined singly there clearly was a dose-related increase in scratching frequency and grooming episodes (Järbe et al., 2002), as well as in grooming duration as described in the current report.

The categories fecal boli, urination, and vocalization typically did not differentiate between the treatment conditions in the current study. That was generally also the case in our more recent open-field study examining Δ^9-THC and SR-141716, where also previous findings and reasons for examining these behaviors were described (Järbe et al., 2002).

These studies indicate that SR-141716 interacts with (R)-methanandamide in a manner that seems different than its interaction with Δ^9-THC examining the same open-field behaviors. This parallels previous observations that some of the effects induced by anandamide are not antagonized by SR-141716 whereas similar effects induced by Δ^9-THC are (e.g., Adams et al., 1998; Compton et al., 1996; Järbe et al., 2002; current study; Smith et al., 1998). We also observed such differential interaction(s) between SR-141716 and Δ^9-THC or (R)-methanandamide in rats trained to lever press for food as described earlier in this report (Järbe et al., 2003). Evidence is mounting 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; Lutz, 2002). For example, Monory et al. (2002) described an anandamide responsive site (non-CB_1/CB_2) in the cerebellum of CB_1 knockout mice that did not bind Δ^9-THC. Additionally, cannabinoid ligands may interact with the cannabinoid CB_1 receptor in distinctly different binding motifs which 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; Thomas et al., 1998). The intrinsic activity of SR-141716 (increases in grooming and scratching) described here and elsewhere complicates interpretation. Darmani and Pandya (2000) suggested the involvement of nonendocannabinoid transmitter systems for scratching (grooming was not changed in the mice study). Nonetheless, current results coupled with our previous data examining combinations of SR-141716 and Δ^9-THC or (R)-methanandamide (Järbe et al., 2001, 2002, 2003) underscore pharmacological differences between (R)-methanandamide and Δ^9-THC revealed by their interactions with SR-141716.

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