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Effects of the CB1R agonist WIN-55,212-2 and the CB1R antagonists SR-141716 and AM-1387: Open-field examination in rats

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Abstract

This study examined the open-field (O-F) effects in rats of the cannabinoid 1 receptor (CB1R) agonist WIN-55,212-2 (WIN; 1 to 5.6 mg/kg) and its interaction with the CB1R antagonist/inverse agonist SR-141716 (1 to 5.6 mg/kg). Additionally, separate studies examined the O-F effects of SR-141716 (1 to 10 mg/kg) and a newly synthesized CB1R selective antagonist/inverse agonist AM-1387 (3 and 10 mg/kg) when these ligands were administered alone. Both antagonists are characterized *in vitro* by decreased of GTPγS binding and increased cAMP accumulation (inverse agonism). WIN dose dependently reduced ambulation (horizontal activity) and rearing (vertical activity); SR-141716 completely (WIN 3 mg/kg) or partially (WIN 5.6 mg/kg) normalized these behaviors. WIN alone resulted in circling and in an increased latency to leave the start area of the O-F, effects blocked by all doses of SR-141716. Both the increased scratching and grooming, associated with SR-141716 administration, were attenuated but not abolished by WIN. SR-141716 alone tended to reduce ambulation (significant at 10 mg/kg) and rearing (non-significant), had no effect on latency, and increased scratching and grooming (both frequency and duration), at doses of 3 mg/kg and up. At the doses examined, AM-1387 had no effect on ambulation, rearing, latency but significantly increased scratching (10 mg/kg); there was also a trend for increased grooming (both frequency and duration). The O-F profile of WIN suggests more similarity with the effects of THC rather than methanandamide (and presumably also anandamide). Intrinsic activity (scratching and grooming) by SR-141716 was re-affirmed and seemed to be associated with administration of AM-1387 as well. AM-1387 was less potent than SR-141716.

Keywords: WIN-55,212-2; SR-141716; AM-1387; Open-field; Rats

1. Introduction

Cannabis has a long history of use and abuse. The discovery of a cannabinoid receptor (CB1R) in the brain and of an endogenous receptor ligand named anandamide (arachidonoyl ethanolamine) as well as other arachidonoyl amides and later also 2-arachidonoylglycerol (2-AG), provides the opportunity to examine the pharmacological and physiological mechanisms underlying the psychoactive effects of cannabis largely responsible for its abuse. The endocannabinoid system also includes a second cannabinoid receptor (CB2R) with predominant peripheral localization but recently also discovered in brain (Van Sickle et al., 2005); enzymes which catalyze the hydrolytic degradation of endocannabinoids; and oxidative enzymes. There may also be an anandamide transporter involved in the action of endocannabinoids (Pertwee, 2006; Thakur et al., 2005).

An interesting aspect of cannabinoid research is the considerable structural diversity of the known classes of cannabinoid ligands. These include but are not limited to: (1) classical (tricyclic, e.g., delta-9-tetrahydrocannabinol, Δ^9-THC) and non-classical (bicyclic, e.g., CP-55,940) cannabinoids (acting as agonists on either CB1R or CB2R); (2) aminoalkylindoles (AAI's) acting as CB1R (e.g., WIN-55,212-2) or CB2R agonists

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(e.g., AM-1241) or alternatively as CB2R antagonists (AM-630); (3) anandamide analogs as CB1R selective agonists, anandamide transport (putative) inhibitors or anandamide amidase inhibitors; (4) 2-AG analogs as CB1R agonists; (5) pyrazole analogs acting as CB1R (e.g., SR-141716; AM-251) or CB2R (e.g., SR-144528) antagonists/inverse agonists (Thakur et al., 2005); (6) diarylether sulfonate mixed CB1R/CB2R agonists (De Vry and Jentzsch, 2004; De Vry et al., 2004; Mauler et al., 2002).

The cannabinoid receptors are members of the G-protein superfamily. It has become increasingly clear that the variety of ligands shown to be able to activate these receptors do not do so in an identical manner (e.g., Bonhaus et al., 1998; Mukhopadhyay and Howlett, 2005; Picone et al., 2005; Shoemaker et al., 2005). The realization that G associated receptor proteins can be activated through multiple binding motifs allow for the expression of several related but diverse biological effects (Howlett et al., 2004). One of the most commonly used CB1R ligands for probing the cannabinoid system in pre-clinical pharmacology is the aminoalkylindole (AAI) WIN-55,212-2 (WIN). WIN is considered a highly potent, full acting CB1R agonist. However, this mixed CB1R/CB2R ligand (as well as anandamide) can produce, for example, antinociception by activating G proteins not directly involving the CB1 receptor (summarized in Howlett et al., 2004). Such findings have fueled speculations about additional cannabinoid receptor subtypes (Wiley and Martin, 2002; see also Monory et al., 2002).

One impetus for the current investigation was our finding that WIN did not readily substitute for Δ^9-THC (THC), nor was WIN generalization readily antagonized by the CB1R selective antagonist SR-141716 in rats discriminating between 3 mg/kg THC and vehicle (Järbe et al., unpublished; see also Compton et al., 1992; Pério et al., 1996). Admittedly, indices of differences in the discriminative stimulus effects between WIN and THC are, however, not consistent across studies (e.g., Wiley et al., 1998). In our hands, two other AAI ligands (CB1R selective) generalized completely to the THC cue and their THC-like discriminative stimulus effects efficiently blocked by SR-141716 (Järbe et al., unpublished). Additionally, although the stable chiral anandamide analog methanandamide (Abadji et al., 1994) will generalize to THC under certain conditions, its interaction with SR-141716 appears different compared to the interaction between this antagonist and THC. For example, surmountable antagonism is not easily obtained with SR-141716 and methanandamide but readily apparent with THC and SR-141716 (Järbe et al., 2001). This may be due to differential rate depressant effects (Baskfield et al., 2004; Järbe et al., 2001, 2003b). Likewise, open-field behaviors (Järbe et al., 2002, 2003a) as well as other unconditioned effects (Adams et al., 1998) in rodents are differentially affected by SR-141716 and anandamide/methanandamide on the one hand compared to THC on the other hand. As noted above, postulating that different cannabinoid ligands can activate the CB1R through multiple binding motifs and/or multiple mechanisms may explain such variations in biological effects (see also Thomas et al., 2005).

SR-141716 was the first selective CB1R antagonist developed (Rinaldi-Carmona et al., 1994). *In vitro* biochemical assays showed that higher concentrations of SR-141716 decreased GTPγS binding and increased cAMP production suggesting that this ligand has inverse agonist properties (Howlett et al., 2004; Pertwee, 2005). In addition to its ability to antagonize the effects of CB1R agonists both *in vitro* and *in vivo,* SR-141716 produces its own behavioral effects. For example, increased locomotion has been described in mice (Bass et al., 2002; Compton et al., 1996; see also Cosenza et al., 2000) as well as an increased incidence of scratching (Darmani and Pandya, 2000; Janoyan et al., 2002). However, occurrence of scratching may be dependent on the mouse strain used (Lichtman et al., 2001). Likewise, increased levels of scratching as well as grooming have been observed for rats (Navarro et al., 1997; Järbe et al., 2002; Rubino et al., 1998, 2000; Vickers et al., 2003). These effects were attenuated but not completely blocked by THC (Järbe et al., 2002).

In view of conflicting data for rats concerning ambulation (horizontal activity) and rearing (vertical activity) between our open-field study (Järbe et al., 2002) and the data by Costa and Colleoni (1999) we decided to re-examine the effects of SR-141716 on open-field behaviors of rats. Costa and Colleoni (1999) reported that 3 mg/kg SR-141716 singly markedly increased ambulation and rearing in rats whereas we observed either no change or a decrease of these behaviors compared to controls (Järbe et al., 2002). As an expansion of the current work, we also examined a newly synthesized CB1R antagonist/inverse agonist AM-1387 as determined by *in vitro* biochemical assays (decreased GTPγS binding and increased cAMP production; McLaughlin et al., 2006). Yet, Bass et al. (2002) had concluded that inverse agonism at CB1R, as determined by decreased GTPγS binding or for that matter CB1R activation alone, were not sufficient to explain the locomotor stimulatory effects of SR-141716 and some of its analogs in mice.

2. Materials and methods

2.1. Animals

A total of 230 adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY) being between 2.5 and 3 months old upon arrival to the Temple quarters were used. The animals were quarantined for 1 week and thereafter the animals were handled each weekday for 2 weeks prior to when the test phase began. 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" (National Institutes of Health, 1996) were followed.

2.2. Treatments

In tests with WIN-55,212-2 and SR-141716, rats were given two i.p. injections (2 ml/kg each) on either side of the peritoneal midline 30 min prior to testing in the open-field arena; this corresponds to our previous open-field examination with THC and SR-141716 (Järbe et al., 2002). Groups of rats ($n=10$) were given either WIN and vehicle or SR-141716, or vehicle and vehicle (non-drug controls). The doses of WIN were 1, 3 or 5.6 mg/kg, and the doses of SR-141716 were 1, 3, and 5.6 mg/kg in the interaction studies. When examining SR-141716 singly,

we also included a dose of 10 mg/kg. AM-1387 was also injected i.p. (1 ml/kg) 30 min pre-session and examined at two doses, viz., 3 and 10 mg/kg but the vehicle was different from the above studies (see Section 2.5). The limited amounts available of AM-1387 precluded examining a wider dose range. The potential clinical interest of AM-1387 lies in its rather short duration of action compared to SR-141716 and the related CB1R antagonist AM-251 as determined by schedule maintained responding for food in rats; responding was equally suppressed at 10 and 120 min after administration of AM-1387 (4 mg/kg) but no longer evident when examined 8 h after i.p. injection (McLaughlin et al., 2006).

Treatments (i.e., various combinations of drugs and dosages) were staggered such that about one-third of the rats in each condition completed the open-field test before the second round commenced, which was followed by a third, and final round. This precaution was aimed at counterbalancing for the possible influence of length of stay in the vivarium prior to testing. Open-field sessions occurred during the lighted portion (1 to 4 PM) of the light/dark cycle during weekdays to minimize the influence of diurnal variations. 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

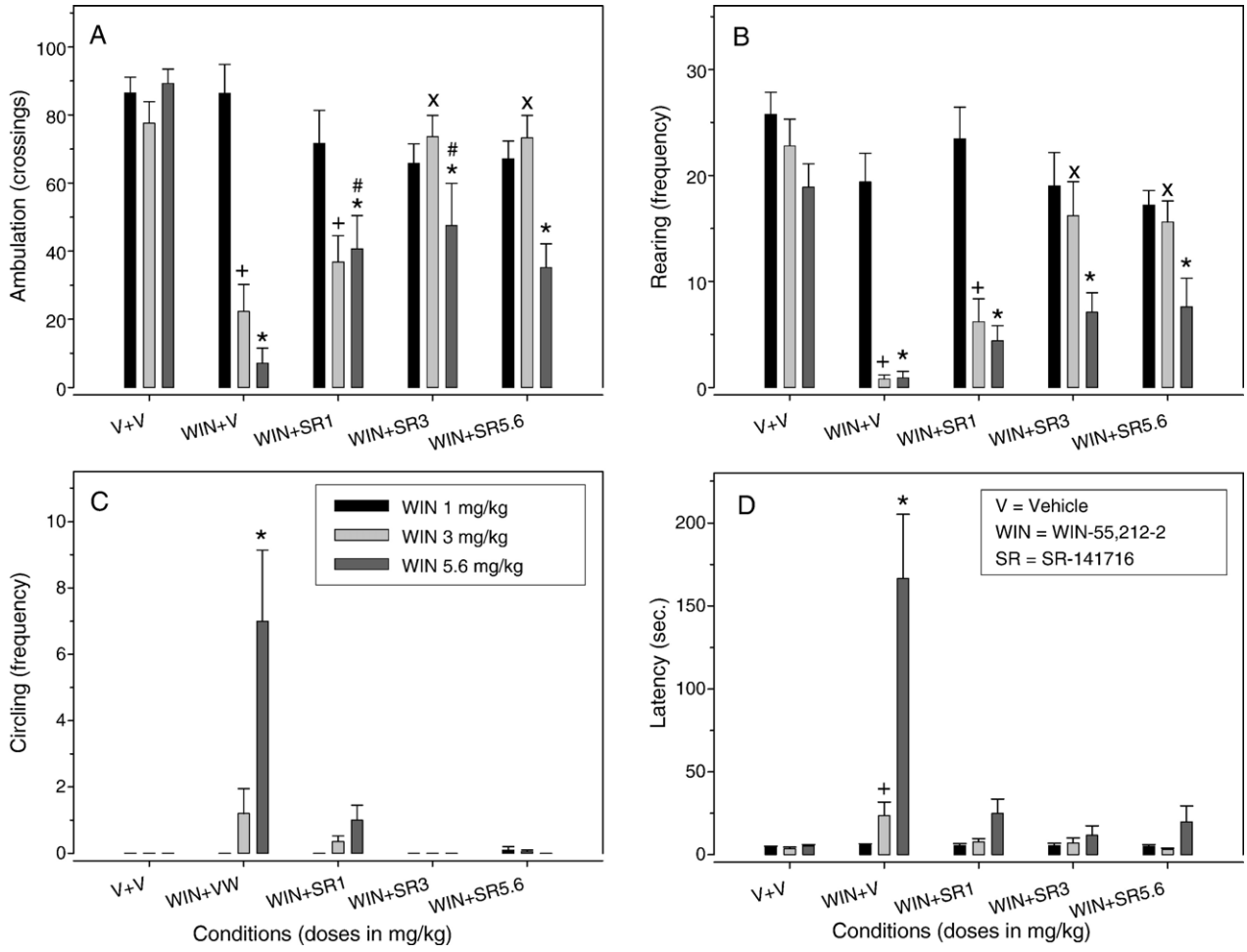

Fig. 1. The effects of WIN 55,212-2 (WIN, 1, 3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3, and 5.6 mg/kg) on ambulation (A), rearing (B), circling (C) and latency (D) in different groups of Sprague–Dawley rats ($n=10$, except for WIN 1 mg/kg plus SR-141716 5.6 mg/kg and the corresponding control group, i.e., the left bar above V+V, where $n=9$ because of lost recordings). WIN and SR-141716 injections were given i.p. 30 min prior to session onset; controls received two vehicle (2 ml/kg each) injections (V+V). The left bar above "V+V" constitutes the control condition pertaining to the interaction study involving 1 mg/kg WIN, the middle bar pertains to 3 mg/kg WIN, and the right hand bar refers to the examination involving 5.6 mg/kg WIN. The data points represent the means (+S.E.M.) during a 5-min observation period in an open-field arena. Ambulation (A): i) WIN 1 mg/kg [F (4, 43)=2.17; $p>0.05$]. ii) WIN 3 mg/kg [F (4, 45)=13.20; $p<0.001$] — +) WIN+V and WIN+SR1 ≠ V+V (middle bar); ×) WIN+SR3 and WIN+SR5.6 ≠ WIN+V and WIN+SR1. iii) WIN 5.6 mg/kg [F (4, 45)=.15; $p<0.001$] — *) WIN+V, WIN+SR1, WIN+SR3, and WIN+SR5.6 ≠ V+V (right bar); #) WIN+SR1 and WIN+SR3 ≠ WIN+V. Rearing (B): i) WIN 1 mg/kg [F (4, 43)=1.86; $p>0.05$]. ii) WIN 3 mg/kg [F (4, 45)=14.99; $p<0.001$] — +) WIN+V and WIN+SR1 ≠ V+V (middle bar); ×) WIN+SR3 and WIN+SR5.6 ≠ WIN+V and WIN+SR1. iii) WIN 5.6 mg/kg [F (4, 45)=12.80; $p<0.001$] — *) WIN+V, WIN+SR1, WIN+SR3, and WIN+SR5.6 ≠ V+V (right bar). Circling (C): i) WIN 1 mg/kg [F (4, 43)=0.95; $p>0.05$]. ii) WIN 3 mg/kg [F (4, 45)=2.24; $p>0.05$]. iii) WIN 5.6 mg/kg [F (4, 45)=9.73; $p<0.001$] — *) V+V (right bar), WIN+SR1, WIN+SR3, and WIN+SR5.6 ≠ WIN+V. Latency (D): i) WIN 1 mg/kg [F (4, 43)=0.30; $p>0.05$]. ii) WIN 3 mg/kg [F (4, 45)=4.25; $p=0.005$] — +) V+V (middle bar), WIN+SR3 and WIN+SR5.6 ≠ WIN+V. iii) WIN 5.6 mg/kg [F (4, 45)=13.66; $p<0.001$] — *) V+V (right bar), WIN+SR1, WIN+SR3, and WIN+SR5.6 ≠ WIN+V.

center of the field. The floor was covered with a piece of acrylic, which was cleaned between sessions. A video camera was mounted 1.5 m above the floor of the open-field arena, such that the whole arena was viewable on camera. Lighting was provided by overhead florescent lights and two clip-on incandescent lamps with 40-W bulbs about 2 m above the box floor.

Animals were transported to the experimental room about 3 h before any session started. Thirty minutes after injection, the session began by placing the rat in the center circle and sessions ended after 5 min. The entire 5-min session was recorded on videotape and scored later. Scoring was not conducted blind to treatments.

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 second 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 recorded whether circling (or turning behavior) consistently was directed to the left or right and also if 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), i.e., "A scratching episode produced by a particular hind limb consisted of 1 or more repetitive scratches with less than 2 s in between. If the interval between consecutive scratches by a particular hind limb was greater than 2 s, 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".

2.5. Drugs

WIN 55,212-2 (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate) was purchased from Tocris Cookson, Inc., Ellisville, MI. SR-141716, as base [(*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] was kindly provided by the National Institute on Drug Abuse (NIDA), Bethesda, MD. The drugs were dissolved in a solution of propylene glycol (PG) and Tween-80 (T-80), and stored at −20 °C. Shortly before

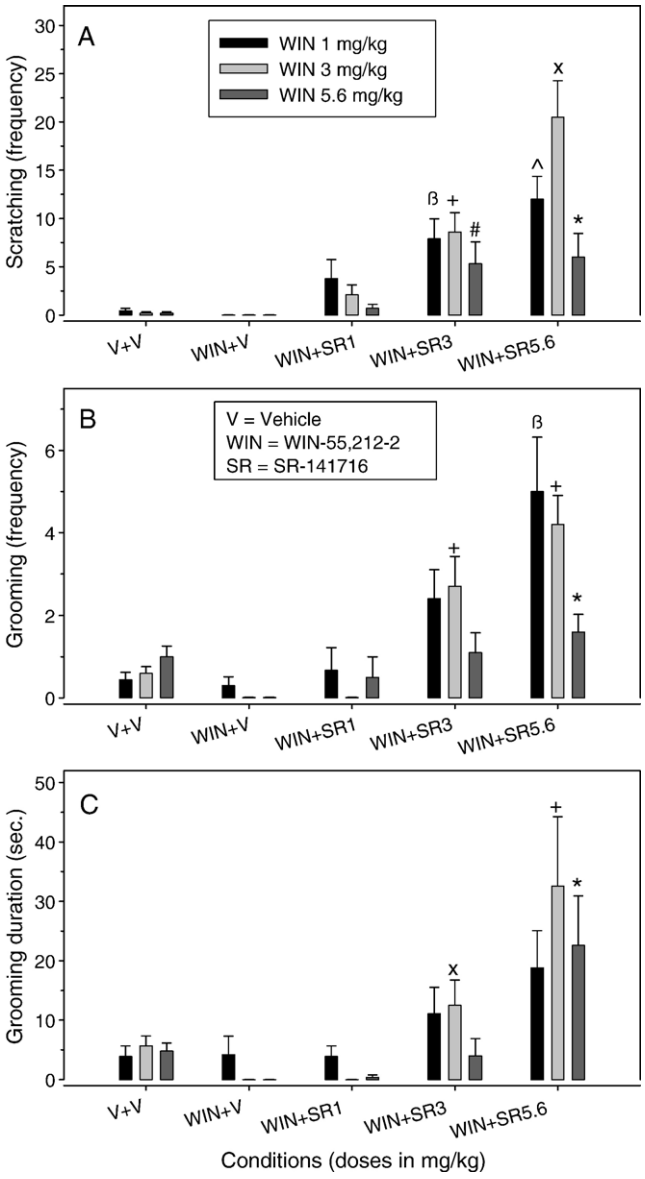

Fig. 2. The effects of WIN 55,212-2 (WIN, 1, 3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3, and 5.6 mg/kg) on scratching (A), grooming frequency (B), and grooming duration (C) in different groups of Sprague–Dawley rats ($n=10$, except for WIN 1 mg/kg plus SR-141716 5.6 mg/kg and the corresponding control group, i.e., the left bar above V+V, where $n=9$ because of lost recordings). WIN and SR-141716 injections were given i.p. 30 min prior to session onset; controls received two vehicle (2 ml/kg each) injections (V+V). The left bar above "V+V" constitutes the control condition pertaining to the interaction study involving 1 mg/kg WIN, the middle bar pertains to 3 mg/kg WIN, and the right hand bar refers to the examination involving 5.6 mg/kg WIN. The data points represent the means (±S.E.M.) during a 5-min observation period in an open-field arena. Scratching (A): i) WIN 1 mg/kg [F (4, 43)=9.34 $p<0.001$] — β) V+V (left bar) and WIN+V≠WIN+SR3; ^) V+V, WIN+V, and WIN+SR1≠WIN+SR5.6. ii) WIN 3 mg/kg [F (4, 45)=19.63; $p<0.001$] — +) V+V (middle bar) and WIN+V≠WIN+SR3; ×) V+V (middle bar), WIN+V, WIN+SR1, and WIN+SR3≠WIN+SR5.6. iii) WIN 5.6 mg/kg [F (4, 45)=5.03; $p=0.002$] — *) V+V (right bar) and WIN+V≠WIN+SR5.6; #) WIN+V≠WIN+SR3. Grooming (frequency) (B): i) WIN 1 mg/kg [F (4, 43)=7.36 $p<0.001$] — β) V+V (left bar), WIN+V, and WIN+SR1≠WIN+SR5.6. ii) WIN 3 mg/kg [F (4, 45)=17.15; $p<0.001$] — +) V+V (middle bar), WIN+V, and WIN+SR1≠WIN+SR3; ×) V+V (middle bar), WIN+V, and WIN+SR1≠WIN+SR5.6. iii) WIN 5.6 mg/kg [F (4, 45)=4.73; $p=0.003$]—*)WIN+V≠WIN+SR5.6.Grooming(duration)(C):i)WIN1mg/kg[F (4, 43)=1.19; $p>0.05$]. ii) WIN 3 mg/kg [F (4, 45)=11.80; $p<0.001$] — +) V+V (middle bar), WIN+V, and WIN+SR1≠WIN+SR5.6; ×) WIN+V and WIN+SR1≠WIN+SR3. iii) WIN 5.6 mg/kg [F(4, 45)=5.43; $p=0.001$] — *) V+V (right bar), WIN+V, WIN+SR1, and WIN+SR3≠WIN+SR5.6.

being used, the solute was diluted with normal (0.9%) saline after the solute had been sonicated for 20–30 min. The final concentrations of the vehicle were propylene glycol (5%)/Tween-80 [3% (1 and 3 mg/kg), 4% (5.6 mg/kg) or 5% (10 mg/kg)] and saline. The increase in the amount of T-80 occurred at the expense of saline. All doses were administered i.p. in a volume of 2 ml/kg. AM-1387 [′1-(2,4-dichlorophenyl)-4-(hydroxymethyl)-*N*-(piperidinyl)-5-arylphenyl-1*H*-pyrazole-3-carboxamide] was synthesized at the Center for Drug Discovery, Northeastern University, and dissolved in DMSO because of its lower potency compared to SR-141716 and given i.p. (1 ml/kg).

2.6. Statistics

Completely randomized one-way analyses of variance (ANOVA) were calculated using SigmaStat (v. 3.10; Systat Software, Inc., Point Richmond, CA; www@systat.com). When ANOVA was significant, *post-hoc* analyses were carried out with the Holm–Sidak all pair-wise comparisons method with alpha, two-tailed set at 0.05 (i.e., for the collection of comparisons).

3. Results

3.1. WIN-55,212-2 alone and in combination with SR-141716

Fig. 1 shows the effects of WIN 55,212-2 (WIN, 1, 3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3, and 5.6 mg/kg) on ambulation (A), rearing (B), circling (C) and latency (D). Detailed statistics are given in the Fig. 1 legend.

3.1.1. Ambulation (A)

WIN dose-dependently suppressed this horizontal exploratory behavior (see data above label WIN+V). This suppression was antagonized by SR-141716 (see WIN+SR). Doses of 3 and 5.6 mg/kg SR-141716 brought the activity counts in WIN 55,212-2 (3 mg/kg) treated rats (middle light grey bars) to levels comparable to that of the controls (V+V, middle light grey bar). In the high-dose WIN (5.6 mg/kg) condition, however, none of the antagonist doses did so. Thus, even though two (1 and 3 mg/kg) of the three antagonist doses together with WIN significantly increased ambulation compared to that observed for WIN singly (5.6 mg/kg), the activity level was still significantly below control levels (right grey bar

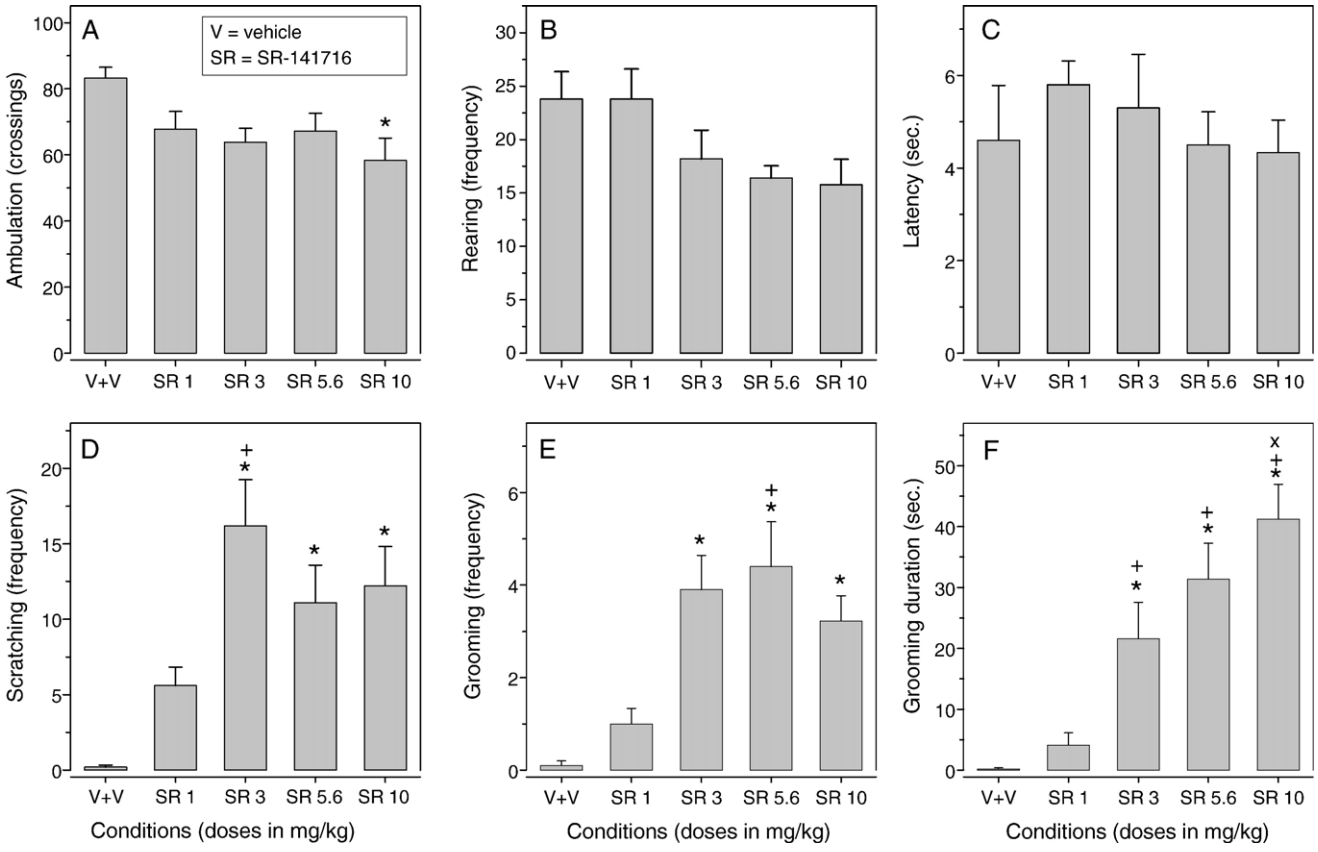

Fig. 3. The effects of SR-141716 (0, 1, 3, 5.6 and 10 mg/kg) on ambulation (A), rearing (B), latency (C), scratching (D), grooming frequency (E), and grooming duration (F) in different groups of Sprague–Dawley rats ($n=10$, except for SR-141716 10 mg/kg where $n=9$ because of lost recordings). SR-141716 plus vehicle injections were given i.p. 30 min prior to session onset; controls received two vehicle (2 ml/kg each) injections (V+V). The data points represent the means (±S.E.M.) during a 5-min observation period in an open-field arena. Ambulation (A): [F (4, 44)=3.30; $p=0.019$] — *) SR10≠V+V. Rearing (B): [F (4, 44)=2.70; $p=0.043$]. Latency (C): [F (4, 44)=0.47; $p>0.05$]. Scratching (D): [F (4, 44)=8.41; $p<0.001$] — *) SR3, SR5.6, and SR10≠V+V; +) SR3≠SR1. Grooming bouts (E): [F (4, 44)=9.32; $p<0.001$ — *) SR3, SR5.6, and SR10≠V+V; +) SR3 and SR5.6≠SR1. Grooming duration (F): [F (4, 44)=14.42; $p<0.001$] — *) SR3, SR5.6, and SR10≠V+V; +) SR3, SR5.6 and SR10≠SR1; ×) SR10≠SR3.

in graph A above label V+V) for all of the latter drug combinations.

3.1.2. Rearing (B)

WIN dose-dependently suppressed this vertical exploratory behavior (see data above label WIN+V). This suppression was antagonized by SR-141716. Doses of 3 and 5.6 mg/kg SR-141716 brought the activity counts in WIN (3 mg/kg) treated rats (middle light grey bars) to levels comparable to that of the controls (V+V, middle light grey bar). In the high-dose WIN (5.6 mg/kg) condition, however, none of the antagonist doses did so. Thus, the rearing level was still significantly below control levels (right grey bar in graph B above label V+V) even though there was a tendency for increased rearing with the addition of the antagonist to WIN (see WIN+SR) compared to that observed for WIN singly (5.6 mg/kg, i.e., the right bar above WIN+V).

3.1.3. Circling (C)

This behavior was significantly increased in the high-dose WIN condition (5.6 mg/kg, i.e., the right bar above WIN+V) compared to all other groups and efficiently antagonized by all the three SR-141716 doses (1 to 5.6 mg/kg) examined (see WIN+SR, right grey bars in graph C).

3.1.4. Latency (D)

Latency to leave the middle circle of the open-field arena was significantly increased in the high-dose WIN condition (5.6 mg/kg, see right bar above WIN+V) and efficiently antagonized by all the three SR-141716 doses examined (1 to 5.6 mg/kg; see right dark grey bars in graph D above WIN+SR). Latency was also increased significantly after 3 mg/kg WIN (see middle bar above WIN+V) compared to the controls (middle bar above V+ V), and this latency increase was significantly attenuated by all SR-141716 doses (middle light grey bars in graph D).

Fig. 2 shows the effects of WIN 55,212-2 (WIN, 1, 3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3, and 5.6 mg/kg) on scratching (A), grooming frequency (B), and grooming duration (C). Detailed statistics are given in the Fig. 2 legend.

3.1.5. Scratching (A)

SR-141716 (in combination with WIN) produced dose-dependent increases in the amount of scratching compared to vehicle (V+V) and WIN singly (WIN+V), significantly so with 3 and 5.6 mg/kg SR-141716 (see bars above labels WIN+SR3 and WIN+SR5.6).

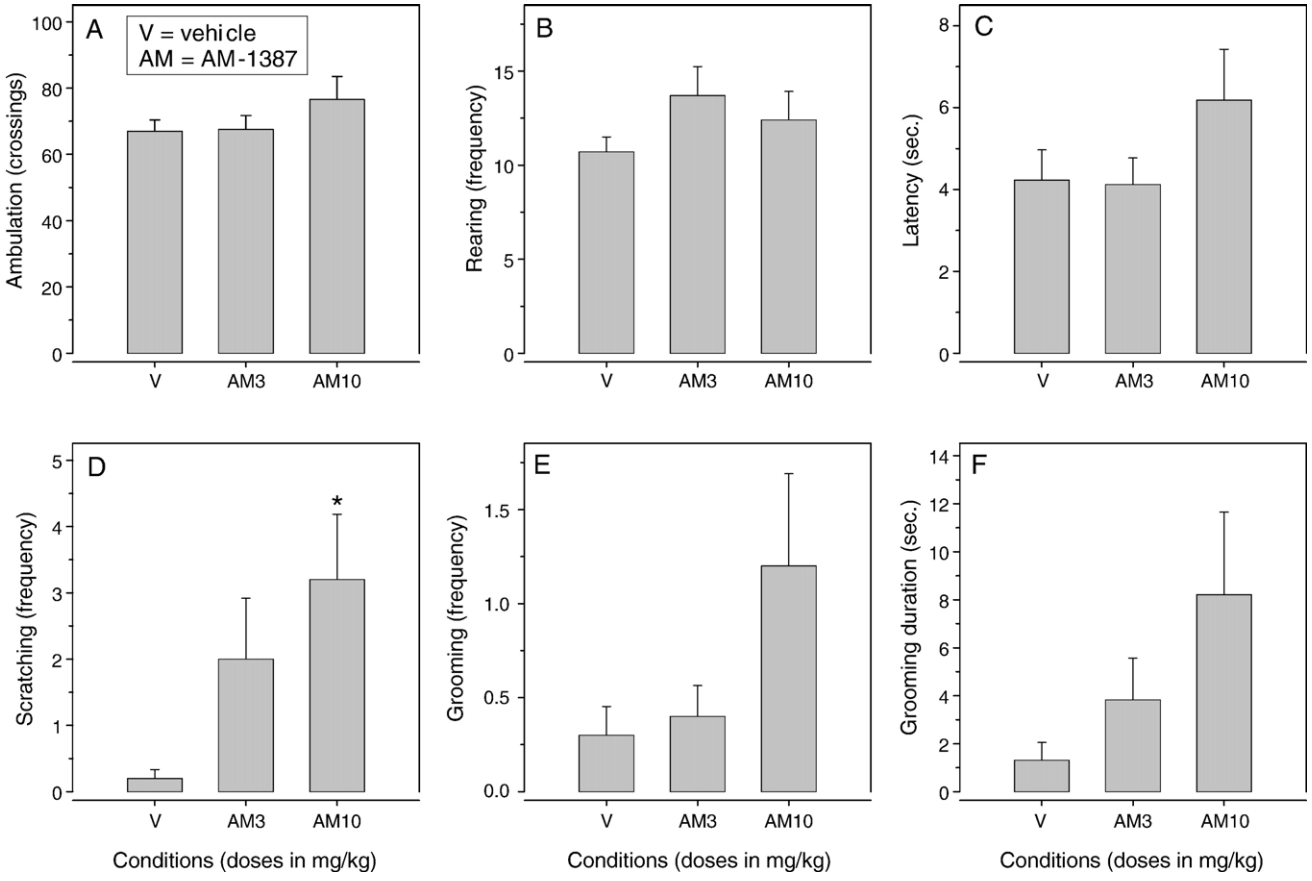

Fig. 4. The effects of AM-1387 (0, 3, and 10 mg/kg) on ambulation (A), rearing (B), latency (C), scratching (D), grooming frequency (E), and grooming duration (F) in different groups of Sprague–Dawley rats ($n=10$). AM-1387 (AM) and vehicle (V) injections were given i.p. 30 min prior to session onset. The data points represent the means (±S.E.M.) during a 5-min observation period in an open-field arena. Scratching (D): [$F(2, 27)=3.73$; $p=0.037$] — *) AM10 ≠ V+V. All of the other ANOVA's were non-significant.

3.1.6. Grooming frequency (B)

Grooming increased significantly with 5.6 mg/kg SR-141716 (plus WIN 1 mg/kg) compared to vehicle (V+V, left dark bar) and WIN singly (1 mg/kg, i.e., left dark bar above label WIN+V) as well as when compared to the combination of 1 mg/kg WIN and SR-141716 (left dark bar above label WIN+SR1). The same pattern occurred when examining 5.6 mg/kg SR-141716 (together with WIN 3 mg/kg) as illustrated by the light grey middle bars. Also a lower dose SR-141716 (3 mg/kg plus WIN 3 mg/kg) resulted in a grooming frequency significantly above the levels of the controls (V+V), WIN (3 mg/kg) alone (WIN+V), and when this dose of WIN was combined with 1 mg/kg SR-141716 (WIN+SR1). Grooming frequency after the combination of 5.6 mg/kg SR-141716 and 5.6 mg/kg WIN (WIN+SR5.6, dark bar) was significantly elevated compared to WIN (5.6 mg/kg) alone (WIN+V).

3.1.7. Grooming duration (C)

Addition of SR-141716 to WIN tended to increase also the grooming duration. Thus, addition of 3 and 5.6 mg/kg SR-141716 to 3 mg/kg WIN increased grooming duration above that seen with 3 mg/kg of WIN alone (WIN+V), and this dose of WIN together with 1 mg/kg SR-141716 (WIN+SR1); and in the case of WIN plus 5.6 mg/kg SR-141716, also above the control values (V+V, middle light grey bar). The combination of 5.6 mg/kg WIN plus 5.6 mg/kg SR-141716 (right bar above label WIN+SR5.6) increased grooming duration significantly compared to all the other comparison conditions, i.e., controls (V+V, right bar), 5.6 mg/kg WIN alone (WIN+V), WIN (5.6 mg/kg) plus 1 mg/kg SR-141716 (WIN+SR1), and WIN (5.6 mg/kg) plus 3 mg/kg SR-141716 (WIN+SR3).

3.2. SR-141716 alone

Fig. 3 shows the effects of SR-141716 (0, 1, 3, 5.6 and 10 mg/kg) singly on ambulation (A), rearing (B), latency (C), scratching (D), grooming frequency (E), and grooming duration (F). Detailed statistics are given in the Fig. 3 legend.

There was one instance of reduced ambulatory (horizontal) activity (A), *viz.,* locomotion was significantly lower in the 10 mg/kg SR-141716 (SR10) treated animals compared to the controls (V+V). There was a tendency ($p=0.043$) for a reduced rearing level (B). No pair-wise comparisons, however, were significant regarding rearing. The latency to leave the circle (C) was non-significant; circling behavior was absefsnt (not shown).

SR-141716 doses of 3 mg/kg and up significantly increased the frequency of scratching (D) and grooming (E), as well as the total time spent grooming (F) compared to the vehicle (V+V) treated animals.

3.3. AM-1387 alone

Fig. 4 shows the effects of AM-1387 (0, 3, and 10 mg/kg) singly on ambulation (A), rearing (B), latency (C), scratching (D), grooming frequency (E), and grooming duration (F); circling was absent (not shown). The only statistically significant change occurred in the category scratching (D); 10 mg/kg AM-1387 (AM10) produced elevated scores of scratching compared to the controls (V). Statistics are given in the Fig. 4 legend.

4. Discussion

The current open-field studies were undertaken to determine if the CB1R agonist WIN would produce a different behavioral profile relative to THC given previously observed differences in the discriminative stimulus effects of these two CB1R ligands (Introduction; see also Patel and Hillard, 2006; Robinson et al., 2003; Thomas et al., 2005). As part of this objective we also re-examined the open-field profile of SR-141716 alone — this allowed for a replication of our previous findings (Järbe et al., 2002) that SR-141716 does not necessarily "induce in rats a behavioral pattern opposite to that of CB1 receptor agonists" (Costa and Colleoni, 1999). The latter investigators reported marked increases in ambulation and rearing in rats examined after a single dose of 3 mg/kg SR-141716. A third objective was to explore *in vivo* a newly synthesized CB1R ligand (AM-1387) with antagonist/inverse agonist properties as determined *in vitro*. Of particular interest for the latter comparison were the categories scratching and grooming (both frequency and duration). These behavioral categories have consistently emerged after SR-141716 administration in our previous open-field examinations; see also Vickers et al. (2003) and Rubino et al. (1998).

Like THC, WIN (3 and 5.6 mg/kg) depressed ambulation and rearing. This WIN induced suppression was antagonized by SR-141716 (see also Darmani, 2001a). As with THC (Järbe et al., 2002), the suppression observed after the highest WIN dose (5.6 mg/kg) was not completely restored to vehicle levels irrespective of SR-141716 dose (1 to 5.6 mg/kg) particularly regarding rearing. That complete restoration of behaviors suppressed by high doses of CB1R agonists may be difficult to achieve has been noted also by other investigators (McMahon et al., 2005 and references cited therein; see also Darmani, 2001a). The lowest dose (1 mg/kg) of WIN currently examined did not significantly change either ambulation or rearing. A lower dose (0.6 mg/kg) of WIN increased ambulation and possibly also rearing in rats (Drews et al., 2005). That also other CB1R agonists can exert behaviorally stimulatory activity under certain conditions has been discussed by e.g. Järbe et al. (2002) (see also Sanudo-Peña et al., 2000).

However, of more interest here is that the addition of SR-141716 (1, 3 and 5.6 mg/kg) did not significantly change the ambulation and rearing levels following 1 mg/kg WIN. This contrasts with combinations of methanandamide and SR-141716 which did not normalize these behaviors. Rather, increasing doses of SR-141716 together with the "behaviorally" non-active dose of 10 mg/kg as well as the active dose of 18 mg/kg methanandamide tended to suppress these behaviors even below the levels noted for these agonist doses alone (Järbe et al., 2003a). In this regard it is interesting to note that Breivogel et al. (2001) and Monory et al. (2002) have suggested the existence of an as yet unidentified binding site in brains of mice activated by anandamide (and thus presumably also by methanandamide) and WIN but not by several

other CB1R agonists. However, the distribution of the new putative binding site in brain differed between the two studies (using differently derived CB1R knockouts).

Significant levels of circling occurred after administration of 5.6 mg/kg WIN (see Fig. 1C). Circling is a motor disturbance occurring after administration of higher doses of various CB1R agonists including THC's (Δ^8-THC and Δ^9-THC), cannabinol, AM-411, HU-210 (Sjödén et al., 1973; Järbe and Hiltunen, 1987; Järbe et al., 2002, 2004; Ferrari et al., 1999) as well as WIN (current study) but not methanandamide (Järbe et al., 1998, 2003a). Circling (as well as the increased latency to leave the middle start area of the open-field arena) after WIN administration was readily antagonized by SR-141716, which is consistent with previous findings with the above mentioned CB1R agonists. Additionally, no consistent patterns in the direction (i.e., rotating left or right) of the circling behavior was evident (see also Järbe et al., 2002).

WIN efficiently blocked scratching in mice (Darmani and Pandya, 2000; Janoyan et al., 2002). Although some attenuation of this response may be inferred (compare Figs. 2A and 3D), WIN did not abolish SR-141716 produced scratching in the current study. This was the general consensus also from our previous open-field studies involving SR-141716 and THC, methanandamide or AM-411 (Järbe et al., 2002, 2003a, 2004). Scratching levels among the controls (V+V) were low and have been so throughout all of the above open-field studies; this may be an age dependent phenomenon in rats (Berendsen and Broekkamp, 1991a). Administration of the above CB1R agonists, including WIN (current study), has also been associated with very low levels of scratching. Darmani and colleagues have suggested that the SR-141716 induced scratching in mice primarily is the result of serotonin (5-HT) activation downstream. Several different pathways were implicated but notably the selective 5-$HT_{2A/C}$ antagonist SR-463449B potently blocked SR-141716 induced scratching (Darmani and Pandya, 2000). Darmani (2001b) also showed that the selective 5-$HT_{2A/C}$ racemic agonist DOI [(±)-1-(2,5-dimethoxy-4iodophenyl)-2-aminopropane] induced scratching in mice can be blocked in a dose dependent manner by various CB1R agonists including WIN. However, this may be a species specific response as Berendsen and Broekkamp (1991b) reported that racemic DOI did not produce hind-limb scratching in rats. Rather DOI tended to reduce 5-methoxytryptamine (5-MeOT) induced scratching. In a direct comparison, other investigators (Lay et al., 2000) have noted differences between the two species in their pharmacological (*in vitro*) response to CB1R agonists.

Grooming among controls has been more variable across our studies. Grooming levels after CB1R agonist administration have been consistently low as they were also in the current study. Additions of SR-141716 to the CB1R agonists (including THC, Järbe et al., 2002) have tended to increase grooming as was the case also in this study focusing on WIN. As with scratching, attenuation of the SR-141716 induced grooming by WIN was observed (compare Fig. 2B and C with Fig. 3E and F). Yet, similarly to e.g., THC, complete blockade was not achieved. Generally, grooming duration seems to be the more sensitive measure compared to frequency of grooming. Thus, with increasing doses of CB1R agonists the time spent grooming after SR-141716 administration is reduced more than the number of grooming instances. Interestingly, grooming frequency (duration was not recorded, nor was scratching) increased significantly in rats after termination of sub-chronic THC treatment (19 consecutive injections) in the open-field (Sjödén et al., 1973). Such a rebound ("withdrawal") phenomenon has also been described more recently for rats treated with the CB1R agonist HU-210 (Giuliani et al., 2000). This behavioral pattern also seems part of the constellation of effects elicited in rats after SR-141716 induced abrupt withdrawal of continuous THC or WIN pretreatment (Aceto et al., 1996, 2001; Rubino et al., 1998). However, the intrinsic activity of SR-141716 complicates the interpretation of antagonist precipitated THC or WIN withdrawal in rodents.

Administration of SR-141716 singly (summarized in Fig. 3) tended to reduce ambulation (and possibly also rearing) but not the latency to leave the middle circle. This is in agreement with our previous open-field study; in both studies circling was not present (current study; Järbe et al., 2002). The increased incidence of scratching and grooming (both the frequency and duration) is also in accord with our previously described data for SR-141716 alone in rats (Järbe et al., 2002; see also Navarro et al., 1997; Rubino et al., 1998, 2000; Vickers et al., 2003).

The newly synthesized AM-1387 (McLaughlin et al., 2006) seemed to share the open-field profile with SR-141716 as AM-1387 significantly increased scratching and also tended to increase both the frequency and duration grooming behavior. However, the magnitude of the effect appeared less than that observed after SR-141716 administration. Although data are lacking, it may be that AM-1387 does not easily penetrate the blood–brain barrier or that AM-1387 is a CB1R inverse agonist displaying fewer effects attributable to inverse agonism compared to SR-141716. Whatever the reason for the potency difference, the behavioral outcome after AM-1387 administration provides an additional instance to suggest that CB1R inverse agonism is associated with increased levels of scratching and grooming in rats. However, more CB1R inverse agonists need to be studied to broaden the generality of such a concept. Alternatively, the intrinsic activity of the CB1R antagonists may be due to a change in endocannabinoid tone resulting from the CB1R blockade.

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