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Differences in the relative potency of SR 141716A and AM 251 as antagonists of various in vivo effects of cannabinoid agonists in C57BL/6J mice

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Abstract

Although the cannabinoid CB_1 antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (SR 141716A) blocks many of the in vivo effects of cannabinoids, the antagonist activity of SR 141716A is limited under some conditions. The general aims of this study were to: 1) examine whether the limited antagonist activity of SR 141716A generalizes to the cannabinoid CB_1 antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (AM 251); and 2) examine mechanisms by which cannabinoids produce hypothermia, catalepsy, and hypoactivity in C57BL/6J mice. SR 141716A and AM 251 were administered alone and in combination with the cannabinoid agonists Δ^9-tetrahydrocannabinol (Δ^9-THC) and *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl] pyrrolol-[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone (WIN 55212−2). Δ^9-THC and WIN 55212−2 produced catalepsy, hypothermia, and hypoactivity with similar potency; WIN 55212−2 produced greater hypothermia than Δ^9-THC, otherwise differences in maximal effect were not detected in the other assays. When administered alone, the antagonists did not produce catalepsy or alter body temperature and they decreased locomotor activity. SR 1417167A and AM 251 blocked catalepsy and hypothermia, and partially attenuated hypoactivity, produced by Δ^9-THC and WIN 55212−2. While the antagonists were equipotent in blocking agonist-induced hypothermia, SR 141716A was 6-fold more potent than AM 251 in blocking agonist-induced catalepsy. The results demonstrate that SR 141716A and AM 251 have strikingly similar behavioral activity, i.e., they block some and not other in vivo effects of cannabinoid agonists, and further demonstrate differences in the maximum effect of cannabinoid agonists that might be related to differences in agonist efficacy. While the results strongly suggest that cannabinoid CB_1 receptors mediate the hypothermic and cataleptic effects of cannabinoids, differences in the relative potency of antagonists suggest that mechanisms responsible for these effects are not identical.

Keywords

AM 251; cannabinoid; Δ^9-tetrahydrocannabinol; SR 141716A; WIN 55212−2

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1. Introduction

Many cannabinoid agonists (e.g., Δ^9-tetrahydrocannabinol; Δ^9-THC) bind non-selectively to at least two cannabinoid receptor subtypes (cannabinoid CB_1 and CB_2); however, one of those subtypes (cannabinoid CB_1 receptors) appears to be responsible for the behavioral effects of cannabinoids. Studies with the cannabinoid CB_1 receptor-selective antagonist SR 141716A (rimonabant) support this view insofar as the hypothermic, cataleptic, antinociceptive, and discriminative stimulus effects of Δ^9-THC are antagonized by SR 141716A (Wiley et al., 1995b;Compton et al., 1996;McMahon et al., 2005;Beardsley and Thomas, 2005 for review; McMahon, 2006). Although an effective antagonist of several in vivo effects of cannabinoid agonists, SR 141716A does not consistently block cannabinoid-induced decreases in locomotor activity or operant responding; instead, SR 141716A tends to mimic the effects of agonists under these conditions (Järbe et al., 2002;2003;De Vry and Jentzsch, 2004;McMahon et al., 2005; however, see Winsauer et al., 1999).

The extent to which limitations in the antagonist activity of SR 141716A generalize to other cannabinoid CB_1 antagonists is not clear. If such limitations are related to inhibition of endogenous tone or to inverse agonist activity at cannabinoid CB_1 receptors (Bouaboula et al., 1997), then other cannabinoid CB_1 receptor inverse agonists should be similarly limited in their cannabinoid antagonist activity. One goal of this study was to compare SR 141716A to another cannabinoid CB_1-receptor inverse agonist (AM 251; Lan et al., 1999) in procedures (i.e., measures of catalepsy, body temperature, and activity; Martin et al., 1991) sensitive not only to cannabinoid antagonism but also to the direct effects of cannabinoid CB_1 antagonists (Compton et al., 1996). To examine whether limitations in antagonism are specific to a particular cannabinoid agonist (i.e., Δ^9-THC), studies were conducted with another cannabinoid agonist (WIN 55212−2) that was reported to mimic the effects of Δ^9-THC in these assays, e.g., both compounds produced catalepsy and decreased body temperature and activity (Compton et al., 1992). A second goal was to compare the mechanisms by which cannabinoids produce some of their effects in vivo. If the same mechanism is responsible for cannabinoid-induced hypothermia, catalepsy, and hypoactivity, then a cannabinoid antagonist should exert similar antagonism of these effects. C57BL/6J mice were chosen for study because this mouse strain has been used to generate CB_1 receptor knockouts (Ledent et al., 1999;Zimmer et al., 1999).

2. Methods

2.1. Subjects

Male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) weighing 17−25 g were housed 4 per cage on a 12-/12-h light/dark cycle, had free access to food (rodent sterilizable diet; Harlan Teklad, Madison, WI) and water, and were experimentally naive before testing. Mice were allowed at least 7 days to habituate to the experimental room prior to testing, and testing was conducted during the light period. Mice were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

2.2. Procedure

Mice received one drug treatment and were assayed at different times during an experimental session. Mice were housed separately between time points and, at each time point, activity, catalepsy and body temperature were measured consecutively as follows. Activity was measured for 5 min in four 30- × 15- × 15-cm customized acrylic boxes (Instrumentation

Services, The University of Texas Health Science Center at San Antonio) that were separately enclosed in commercially-available, sound-attenuating chambers (model ENV-022M, MED Associates, St. Albans, VT). The floor consisted of a parallel grid of 2.3-mm stainless-steel rods mounted 6.4 mm apart, or of perforated 16-gauge stainless steel with 6.4-mm round holes (9.5-mm staggered centers); floor types were counterbalanced between animals. Four infrared light beams were spaced 6 cm apart and located 2 cm above the floor of each box. Occlusions of the infrared light beams were counted using commercially-available computer software (Multi-Varimex version 1.00, Columbus Instruments, Columbus, OH). Catalepsy was measured by placing the front paws over a 1-cm-diameter, horizontal, stainless-steel bar supported 4 cm above the floor by two 8- × 8-cm pieces of Plexiglas (Instrumentation Services, The University of Texas Health Science Center at San Antonio). The time that both paws remained on the bar was measured up to 30 s. Finally, a probe was inserted 2 cm into the rectum and temperature was recorded with a commercially-available thermometer (model BAT-7001H, Physitemp Instruments, Inc., Clifton, NJ).

To characterize the onset and duration of action of Δ^9-THC (1−100 mg/kg) and WIN 55212−2 (1−32 mg/kg), effects were measured at 10, 30, 60, 120, 240, 480 and 1440 min after administration; a baseline was obtained 10 min before drug administration. The maximum effect was obtained 60 min after Δ^9-THC and 30 min after WIN 55212−2 (see Results). Antagonism tests were conducted with doses (10 and 32 mg/kg, respectively) of WIN 55212−2 and Δ^9-THC producing comparable effects in the activity, catalepsy, and temperature assay. Some antagonism tests also were conducted with a larger dose (32 mg/kg) of WIN 55212−2. The interval for antagonist pretreatment was chosen from preliminary studies and, to maintain a constant interval (90 min) between time of antagonist administration and time of peak agonist effect, antagonists were administered 30 min before Δ^9-THC and 60 min before WIN 55212−2. Effects were measured 10 min before administration of the agonists, and 60 and 30 min after administration Δ^9-THC and WIN 55212−2, respectively. To examine the effects of SR 141716A and AM 251 alone, they were studied at doses of 0.32−32 mg/kg in combination with vehicle using the same temporal parameters. The order in which drugs and doses were tested was non-systematic.

2.3. Drugs

N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (SR 141716A) and Δ^9-tetrahydrocannabinol (Δ^9-THC; The Research Technology Branch, National Institute on Drug Abuse, Rockville, MD); *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolol-[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate salt (WIN 55212−2; Sigma, St. Louis, MO); and *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (AM 251; Tocris, Ellisville, MO) were dissolved in a 1:1:18 mixture of absolute ethanol, Emulphor-620 (Rhone-Poulenc Inc., Princeton, NJ) and physiologic saline. Doses were expressed as the weights of the forms listed above. All drugs were administered i.p. and drug concentrations were adjusted so that the volume was 0.3−0.5 ml for agonist or vehicle and 0.5−0.7 ml for antagonist or vehicle.

2.4. Data analyses

Body temperature (°C) was expressed as a difference from baseline measured 10 min before agonist administration, catalepsy was expressed as seconds that both front paws remained on the horizontal bar (maximum 30 s), and activity was expressed as the number of beam breaks counted in 5 min. Because baseline locomotor activity decreased over time, the time course of hypoactivity induced by Δ^9-THC and WIN 55212−2 was expressed as a percentage of vehicle-control activity at corresponding time points.

Agonist and antagonist dose-effect curves were generated from data obtained at time of peak effect (i.e., 60 and 30 min following Δ^9-THC and WIN 55212−2, respectively; 90 min following administration of SR 141716A and AM 251). Agonist dose-effect data were analyzed by expressing each dose as a percentage of the maximum effect (defined by data obtained with 32 mg/kg). For antagonism of catalepsy and hypothermia induced by Δ^9-THC (32 mg/kg) and WIN 55212−2 (10 mg/kg), results were expressed as a percentage of maximum antagonism (i.e., percentage of the difference between the effects obtained with the dose of the agonist and with vehicle control). Agonist and antagonist potencies were estimated by: 1) expressing the data as a percentage of the maximum effect and 2) analyzing the dose-effect curves by simultaneously fitting straight lines to the individual dose-response data, separately for hypothermia, catalepsy, and hypoactivity, by means of GraphPad Prism version 4.03 for Windows (San Diego, CA), using the following equation: effect = slope * log(dose) + intercept. Straight lines were fitted to the linear portion of dose-effect curves, defined by doses producing 25−75% of the maximum effect, including not more than one dose producing less than 25% of the maximum effect, and not more than one dose producing more than 75% of the maximum effect.

If the slopes of the dose-effect curves were not significantly different (as determined by an F-ratio test), then a common, best-fitting slope was used for further analyses (for detailed examples of this approach, see Kenakin, 1997). Parallel line analyses of data from individual subjects was used to calculate doses corresponding to the 50% level of the effect (ED_{50} values for agonists ad ID_{50} values for antagonists), potency ratios, and their 95% confidence limits (Tallarida, 2000). In one case (i.e., antagonist of WIN 55212−2-induced catalepsy by AM 251), extrapolation was used to calculate the ID_{50} value. The potencies were considered significantly different when the 95% confidence limits of their potency ratio did not include 1. Differences in maximum effect were analyzed with a Student *t*-test for independent samples or one-way ANOVA using the non-transformed data.

Antagonists decreased activity and antagonism of agonist-induced hypoactivity was incomplete (see Results). Therefore, an antagonist was considered to have significantly altered locomotor activity or to have significantly blocked agonist-induced hypoactivity when the slope of the dose-effect curve was significantly different from 0; all doses were included in these analyses. The same analysis was used to examine the effects of the antagonists alone in the body temperature and catalepsy assays. Time course data were averaged from 4 mice and all other data were averaged from 6−9 mice.

3. Results

3.1. Catalepsy, hypothermia, and hypoactivity induced by Δ-^{9}-THC and WIN 55212−2

Fig. 1 shows the time course for Δ^9-THC (1−100 mg/kg; left) and WIN 55212−2 (1−32 mg/kg; right) when studied 10−1440 min after administration. Δ^9-THC and WIN 55212−2 dose-dependently decreased temperature, produced catalepsy, and decreased activity (Fig. 1). The effects of Δ^9-THC were maximal at doses of 32 and 100 mg/kg; the time course varied somewhat for each measure, with hypothermia and hypoactivity generally occurring at 30−240 min, and catalepsy lasting somewhat longer (30−480 min). The effects of Δ^9-THC were no longer evident at 1440 min after administration. The effects of WIN 55212−2 were maximal at doses of 10 and 32 mg/kg; WIN 55212−2 generally had a more rapid onset of action (10 min) than Δ^9-THC, and its duration of action was similar to that of Δ^9-THC.

3.2. Effects of SR 1417167A and AM 251 on cannabinoid-induced hypothermia

Based on the time course data, the effects of Δ^9-THC and WIN 55212−2 were examined at 60 and 30 min, respectively, after administration. A test for parallel lines revealed that the agonist

dose-effect curves for hypothermia did not significantly deviate from parallelism; the potencies (Fig. 2, top and bottom left, closed circles connected to single diamond; values and corresponding dashed lines represent ED_{50} values in mg/kg; Table 1) were not significantly different. Δ^9-THC produced significantly less hypothermia than WIN 55212−2 ($P < 0.01$); for example, body temperature was decreased by 4.8 and 7.5 °C, respectively, at a dose of 32 mg/kg for each. Hypothermia produced by the smaller dose (10 mg/kg) of WIN 55212−2 was the same as hypothermia produced by 32 mg/kg of Δ^9-THC ($P > 0.20$); therefore, antagonism studies were conducted with these equally effective doses of agonist. In contrast to the agonists, SR 141716A and AM 251 (up to 32 mg/kg) did not alter body temperature (Fig. 2, top rightmost panels, open circles).

Expressing the data as a percentage of the maximum effect, the dose-effect curves obtained with each of the antagonist-agonist combinations did not deviate significantly from parallelism (Fig. 2, rightmost panels, diamonds); the magnitude of antagonism of hypothermia was not different for these drug combinations ($P > 0.20$). For antagonism of Δ^9-THC (32 mg/kg)-induced hypothermia, AM 251 and SR 141716A did not differ in potency (ID_{50} values were 0.71 and 0.65 mg/kg; Table 1). For antagonism of WIN 55212−2 (10 mg/kg)-induced hypothermia, AM 251 and SR 141716A also did not differ in potency (ID_{50} values were 0.45 and 0.25 mg/kg; Table 1). When comparing the potency of SR 141716A as an antagonist of hypothermia produced by Δ^9-THC (32 mg/kg) and WIN 55212−2 (10 mg/kg), there was a significant difference in potency, i.e., the 95% confidence limits (1.2−5.8) of the potency ratio (2.6) did not include 1. However, AM 251 did not exhibit this difference in potency as an antagonist of the hypothermic response produced by Δ^9-THC (32 mg/kg) and WIN 55212−2 (10 mg/kg).

The highest magnitude of hypothermia obtained with 32 mg/kg of WIN 55212−2 was dose-dependently antagonized by AM 251 and SR 141716A (data not shown); ID_{50} values (95% confidence limits) were 1.2 (0.73−2.0) mg/kg and 0.83 (0.49−1.4) mg/kg, respectively. Thus, antagonism of this highest hypothermic response required doses of AM 251 and SR 141716A that were larger than doses required to antagonize the smaller hypothermic response produced by WIN 55212−2 (10 mg/kg) and Δ^9-THC (32 mg/kg).

3.3. Effects of SR 141716A and AM 251 on cannabinoid-induced catalepsy

The Δ^9-THC and WIN 55212−2 dose-effect curves could be fitted with a common slope, and the potencies (Fig. 3, top and bottom left, closed circles connected to single diamond; Table 2) were not significantly different. The magnitude of catalepsy produced by WIN 55212−2 (10 mg/kg) and Δ^9-THC (32 mg/kg) was not significantly different ($P > 0.20$). SR 141716A and AM 251 (up to 32 mg/kg) did not produce catalepsy (Fig. 3, top rightmost panels, open circles).

A test of parallel lines for each of the 4 antagonist-agonist combinations revealed that the antagonist dose-effect curves did not deviate from parallelism (Fig. 3, rightmost panels, diamonds); the magnitude of antagonism was not different for these drug combinations ($P > 020$). In contrast to antagonism of hypothermia, there was a marked difference between antagonists in their potency for blocking agonist-induced catalepsy, i.e., AM 251 was 6.1- and 5.4-fold less potent than SR 141716A as an antagonist of catalepsy produced by Δ^9-THC and WIN 55212−2, respectively (Fig. 3; Table 2). There also was a significant difference in the potency of SR 141716A to antagonize catalepsy as a function of agonist, i.e., the potency of SR 141716A in the presence of Δ^9-THC (32 mg/kg) was 3.6-fold less than in the presence of WIN 55212−2 (10 mg/kg) (Table 2). However, AM 251 did not exhibit this difference in potency as an antagonist of catalepsy produced by Δ^9-THC and WIN 55212−2 (Table 2).

3.4. Hypoactivity induced by Δ^9-THC, WIN 55212−2, SR 141716A, and AM 251.

The potencies of Δ^9-THC and WIN 55212−2 for producing hypoactivity were not significantly different, i.e., the ED_{50} values (95% confidence limits) were 6.5 (3.4−12) and 2.8 (1.4−5.5) mg/kg, respectively, and the 95% confidence limits (1.0−5.6) of their potency ratio (2.3) was not significantly different from 1 (Fig. 4, top and bottom left). The magnitude of hypoactivity induced by WIN 55212−2 (10 mg/kg) and Δ^9-THC (32 mg/kg) was not significantly different ($P > 0.20$). When administered alone, SR 141716A and AM 251 decreased activity (Fig. 4, top rightmost panels, open circles), as evidenced by a significant dose-effect relationship (i.e., slopes were significantly different from 0) for SR 141716A. When linear regression was conducted with all doses (0.32−32 mg/kg) of AM 251, the slope of the dose-effect relationship was not significantly different from 0 ($P = 0.06$). However, including not more than two ineffective doses of AM 251 in the linear regression showed the slope to be significantly different from 0 ($P < 0.05$).

There was no significant difference in slope for each of the 4 antagonist-agonist combinations (i.e., the lines did not deviate from parallelism). SR 141716A and not AM 251 significantly attenuated hypoactivity induced by Δ^9-THC (32 mg/kg); maximum antagonism by SR 141716A was 45% (Fig. 4). Both SR 141716A and AM 251 significantly attenuated hypoactivity induced by WIN 55212−2 (10 mg/kg); maximum antagonism was 63% and 60%, respectively (Fig. 4). There was a tendency for SR 141716A to be a more potent antagonist of WIN 55212−2-induced hypoactivity than Δ^9-THC-induced hypoactivity ($P = 0.05$).

4. Discussion

The cannabinoid CB_1 receptor-selective antagonists SR 141716A and AM 251 blocked the cataleptic and hypothermic effects of Δ^9-THC and WIN 55212−2 without producing catalepsy or modifying body temperature on their own in C57BL/6J mice. While the antagonists were equipotent in blocking agonist-induced hypothermia, their potency was 6-fold different for blocking agonist-induced catalepsy. The antagonists decreased activity and only partially reversed Δ^9-THC- and WIN 55212−2-induced hypoactivity. Collectively, these results show AM 251 and SR 141716A to have similar effects in vivo; however, differences in their relative potency as antagonists suggest that mechanisms underlying the hypothermic and cataleptic effects of cannabinoids are not identical.

The cannabinoid agonists Δ^9-THC and WIN 55212−2 have similar behavioral effects under a variety of conditions (Fan et al., 1994;Wiley et al., 1995a;McMahon, 2006), and the current study extends these findings to include catalepsy, hypothermia, and hypoactivity in C57BL/6J mice. The agonists were equipotent to each other within assays, and their individual potencies were similar across assays. WIN 55212−2 produced greater hypothermia than Δ^9-THC, perhaps reflecting in vitro data showing WIN 55212−2 to have higher efficacy (i.e., greater activation of G-proteins) at cannabinoid CB_1 receptors than Δ^9-THC (Breivogel and Childers, 2000). While simultaneous production of hypothermia, catalepsy and hypoactivity is consistent with cannabinoid agonism (Martin et al., 1991), non-cannabinoids also can produce this profile of activity (Wiley and Martin, 2003). Cannabinoid CB_1 receptor-selective antagonists can therefore be exploited to further examine the mechanism by which drugs produce hypothermia, catalepsy, and hypoactivity.

The cannabinoid CB_1-receptor selective antagonists SR 141716A and AM 251 dose-dependently attenuated the hypothermic and cataleptic effects of Δ^9-THC and WIN 55212−2; therefore, the cannabinoid agonists appear to act at cannabinoid CB_1 receptors to produce hypothermia and catalepsy. The mechanisms responsible for hypothermia and catalepsy, however, appeared to differ inasmuch as the antagonists differed in their relative potency for blocking these effects. SR 141716A and AM 251 had similar potency for blocking the

hypothermic effects of the cannabinoids, consistent with their similar potency as antagonists of the discriminative stimulus effects of cannabinoid agonists in other species (Järbe et al., 2006;McMahon, 2006). In contrast, AM 251 was 6-fold less potent than SR 141716A as an antagonist of the cataleptic effects of the cannabinoid agonists. Rather than establishing the potency of an antagonist by determining shifts in agonist dose-effect curves, the current study generated antagonist dose-effect curves in the presence of a fixed dose of agonist (i.e., inhibition curves). Characterizing antagonists with inhibition curves can be limited inasmuch as a comparison among antagonists requires their potency to be established with equi-effective doses of agonist; moreover, this approach makes it difficult to differentiate between competitive versus non-competitive antagonism (Kenakin, 2006). These limitations notwithstanding, differences in the relative potency of SR 141716A and AM 251 suggest that, in addition to a common mechanism of action at cannabinoid CB_1 receptors, there is another mechanism that differentially contributes to the hypothermic and cataleptic effects of cannabinoid agonists in C57BL/6J mice.

SR 141716A was shown in previous studies to be limited in its capacity to antagonize the effects of cannabinoids on rates of operant responding and locomotor activity (Järbe et al., 2002;2003;De Vry and Jentzsch, 2004;McMahon et al., 2005), and the current study shows similar limitations in C57BL/6J mice insofar as SR 141716A did not fully attenuate cannabinoid-induced hypoactivity. AM 251 also did not fully attenuate agonist-induced hypoactivity and this incomplete antagonism was not limited to a single cannabinoid agonist (i.e. similar limitations were obtained with both Δ^9-THC and WIN 55212−2). While these results might reflect a role for non-cannabinoid CB_1 receptors in the effects of cannabinoids on locomotor activity, incomplete antagonism of cannabinoid-induced hypoactivity might be due to the direct effects of cannabinoid antagonists which, in turn, could be due to inverse agonism at cannabinoid receptors (e.g., Bouaboula et al., 2002). The hypoactivity produced by SR 141716A and AM 251 might be inconsistent with inverse agonism insofar as the bi-directional effects of cannabinoid agonists and inverse agonists on receptor signaling in vitro yields similar bi-directionality in vivo (i.e., hypoactivity and hyperactivity, respectively). However, even when cannabinoid antagonists produce hyperactivity, this activity is not clearly related to inverse agonism (Bass et al., 2002), and other mechanisms (i.e., inhibition of endogenous cannabinoids) could be responsible for the direct behavioral effects of SR 141716A and AM 251.

In summary, SR 141716A and AM 251 had strikingly similar behavioral activity in C57BL/6J mice, blocking some (hypothermia and catalepsy) and not other effects (hypoactivity) of cannabinoid agonists. The limited antagonism of cannabinoid-induced hypoactivity was related to the direct effects of the antagonists on locomotor activity, and factors (i.e., inhibition of endogenous cannabinoid tone) responsible for these direct effects have not yet been identified. The current study showed that body temperature, catalepsy, and locomotor activity were differentially sensitive to cannabinoid CB_1 receptor activity. While locomotor activity was sensitive to the direct effects of cannabinoid antagonists, this behavioral measure was relatively insensitive to antagonism of exogenous administration of cannabinoid agonists. On the other hand, body temperature was especially sensitive to cannabinoid CB_1 receptor activity, providing not only a measure of antagonism of exogenous cannabinoids but also a measure of cannabinoid CB_1 agonism that appeared to vary as a function of the extent to which agonists activate G-protein signaling at cannabinoid CB_1 receptors (i.e., agonist efficacy).

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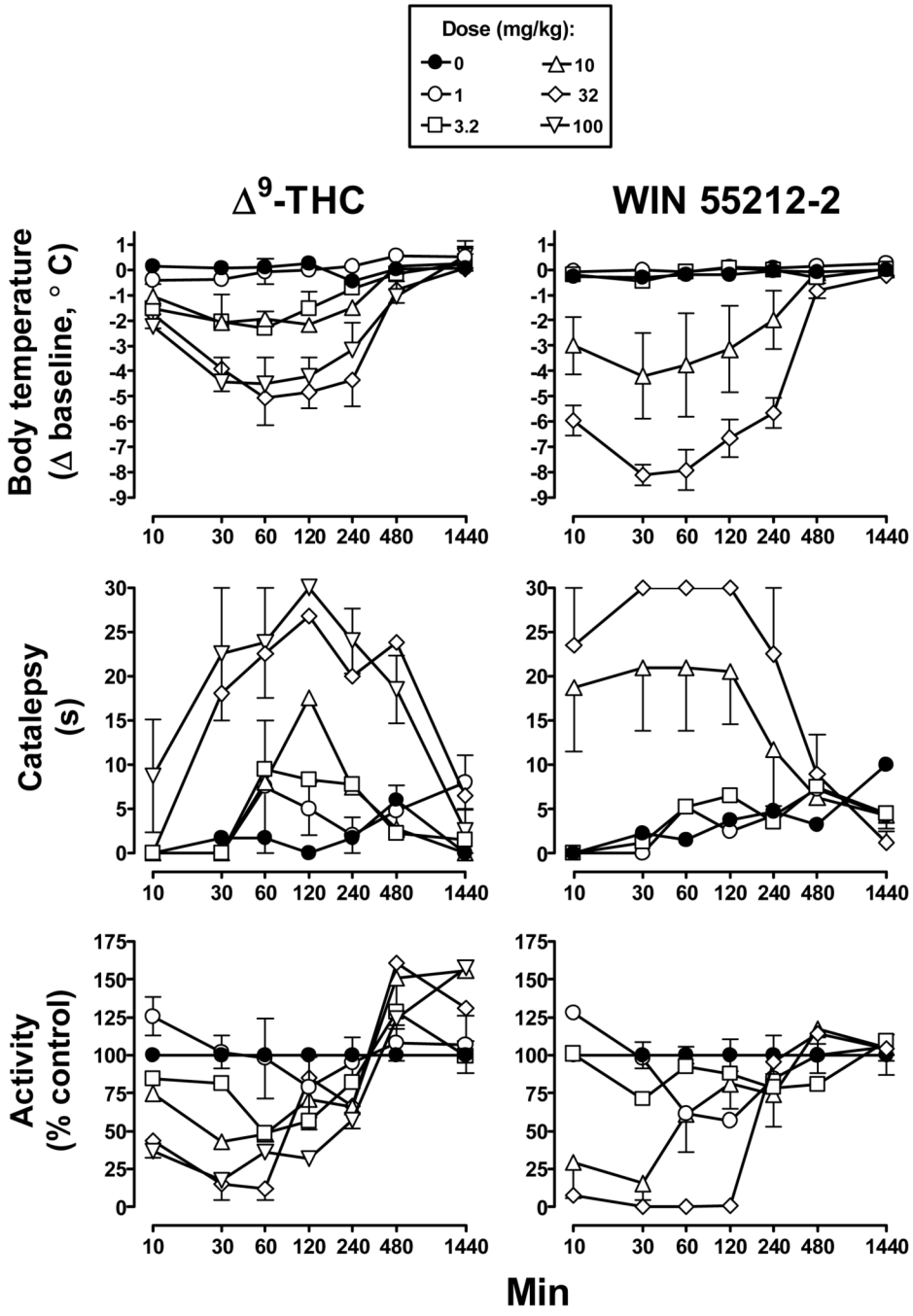

Fig 1.

Time course of hypothermia, catalepsy, and hypoactivity induced by different doses of Δ^9-THC (left) and WIN 55212−2 (right). Abscissae: min expressed on log scale. Ordinates: mean (± S.E.M.) change in body temperature as ° C (top), catalepsy in s (middle), and activity counts (bottom).

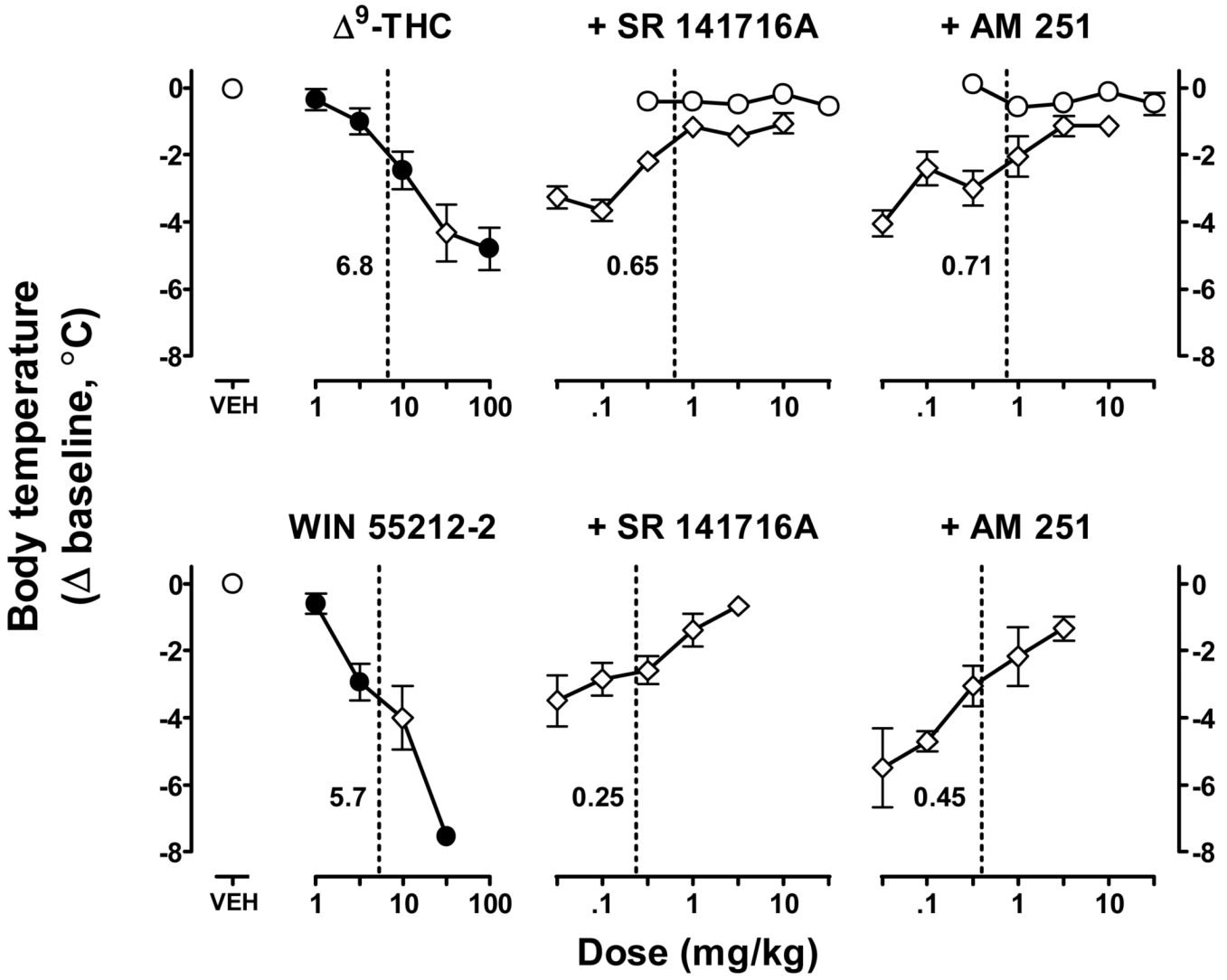

Fig 2.

Hypothermic effects of Δ^9-THC (top) and WIN 55212−2 (bottom), and antagonism by SR 141716A and AM 251. Abscissae: dose in mg/kg body weight. Ordinate: mean (± S.E.M.) change in body temperature as ° C. Leftmost panels, effects of vehicle (VEH), alone or in combination with Δ^9-THC and WIN 55212−2 (closed circles). Diamonds, effects of Δ^9-THC (32 mg/kg) and WIN 55212−2 (10 mg/kg), alone or in combination with SR 141716A and AM 251. Open circles in rightmost panels, effects of antagonist alone. Values and dashed lines, ED_{50} values (agonists) and ID_{50} values (antagonists) in mg/kg.

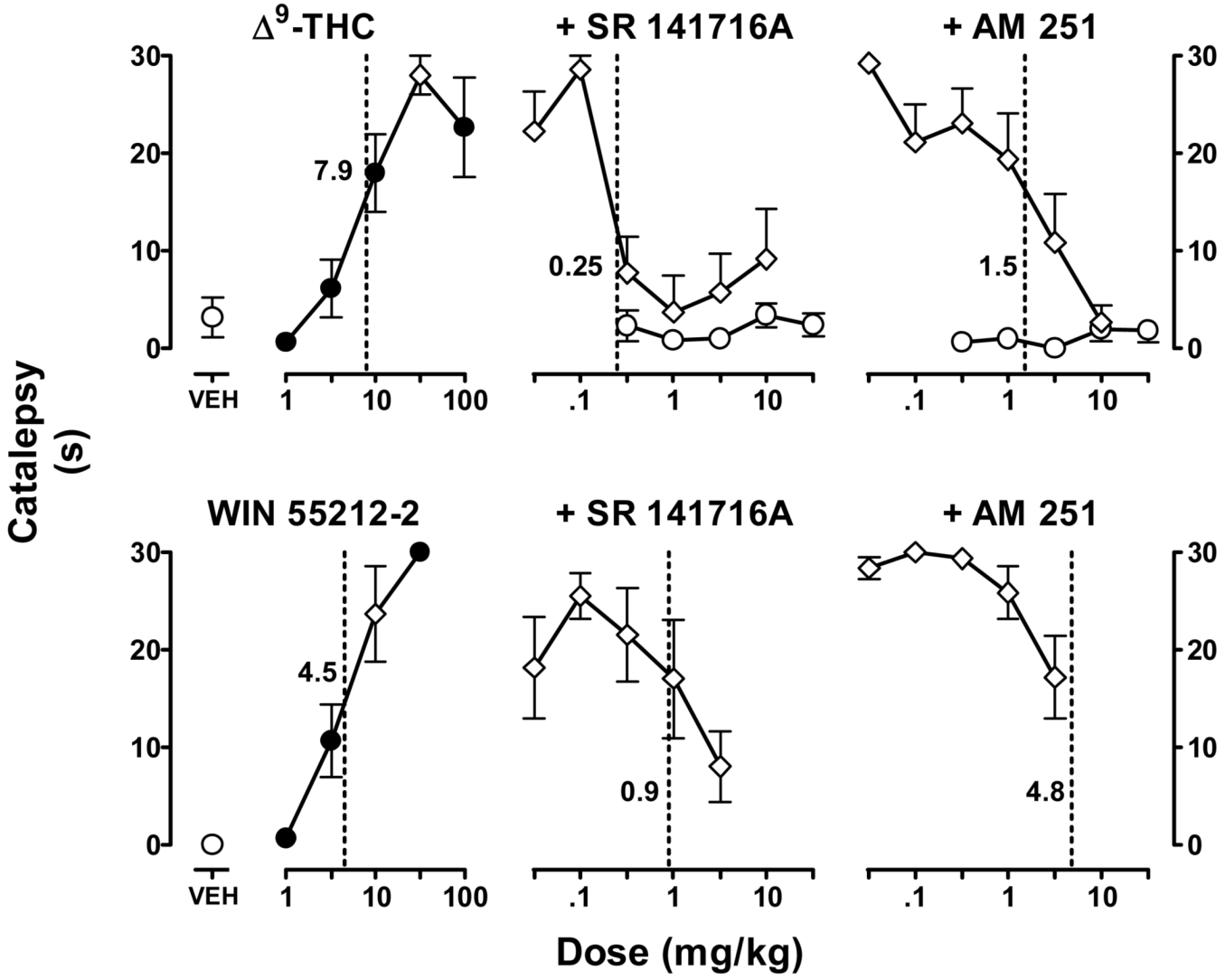

Fig 3.

Cataleptic effects of Δ^9-THC (top) and WIN 55212−2 (bottom), and antagonism by SR 141716A and AM 251. Ordinate: mean (± S.E.M.) catalepsy in s. See Fig. 2 legend for other details.

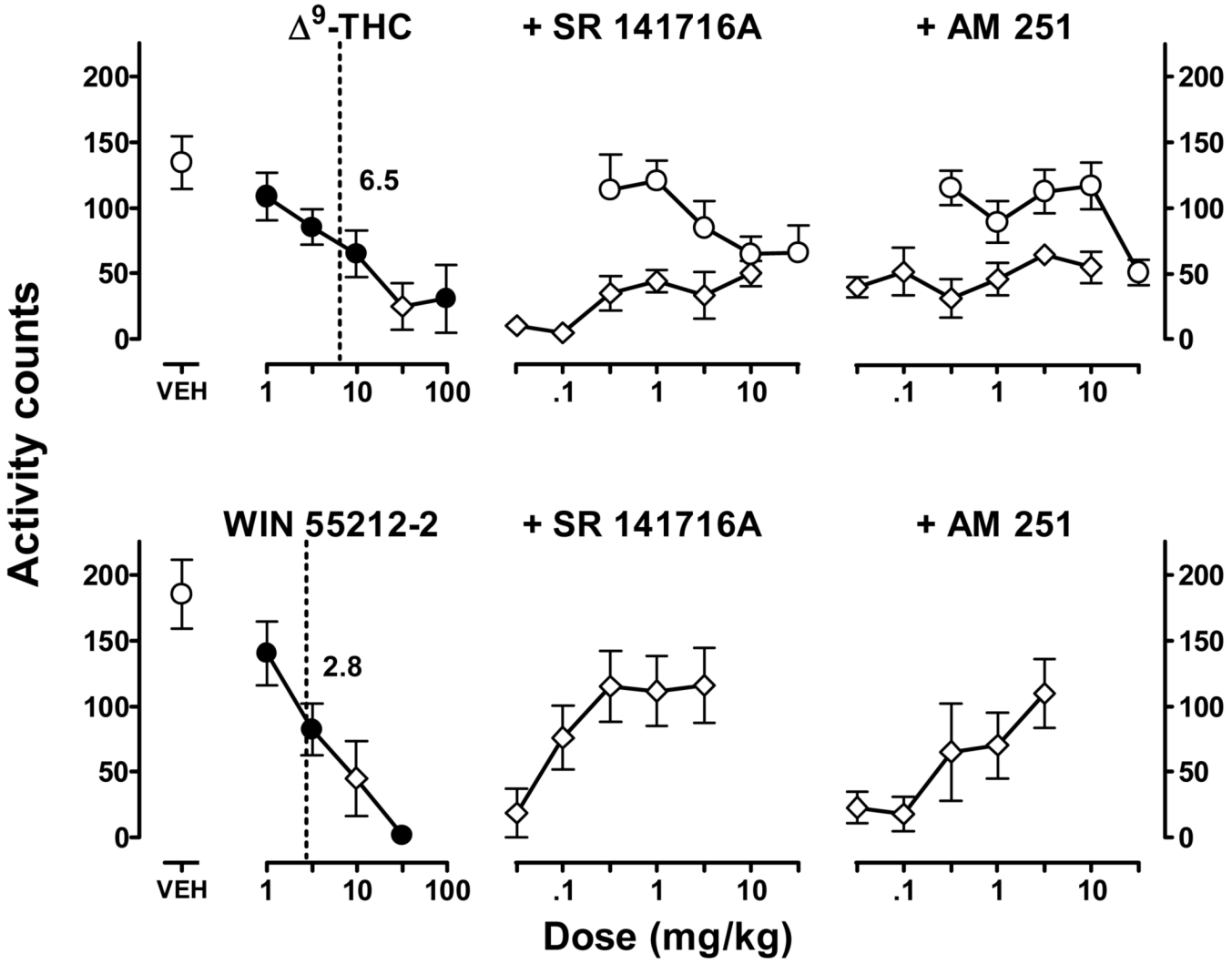

Fig 4.

Hypoactivity induced by Δ9-THC (top) and WIN 55212−2 (bottom), alone and in combination with SR 141716A and AM 251. Ordinate: mean (± S.E.M.) activity counts. See Fig. 2 legend for other details.

Table 1

ED_{50} values in mg/kg for agonist-induced hypothermia, ID_{50} values in mg/kg for antagonism of agonist-induced hypothermia, and corresponding potency ratios and 95% confidence limits (95% CL). Potency ratios are expressed as a ratio of the least potent divided by the most potent.

Hypothermia	***ED_{50} (95% CL)***	***Potency Ratio (95% CL)***
Δ^9-THC	6.8 (3.5−13)	
WIN 55212−2	5.7 (4.2−7.6)	1.2 (0.7−2.2)
Δ^9-THC antagonism	***ID_{50} (95% CL)***	
AM 251	0.71 (0.37−1.4)	
SR 141716A	0.65 (0.40−1.1)	1.1 (0.5−2.6)
WIN 55212−2 antagonism		
AM 251	0.45 (0.15−1.4)	
SR 141716A	0.25 (0.12−0.49)	1.8 (0.6−5.3)

Table 2

ED_{50} values in mg/kg for agonist-induced catalepsy, ID_{50} values in mg/kg for antagonism of agonist-induced catalepsy, and corresponding potency ratios and 95% confidence limits (95% CL). Potency ratios are expressed as a ratio of the least potent divided by the most potent.

Catalepsy	***ED_{50} (95% CL)***	***Potency Ratio (95% CL)***
Δ^9-THC	7.9 (5.3−12)	
WIN 55212−2	4.5 (2.8−7.1)	1.8 (1.0−3.1)
Δ^9-THC antagonism	***ID_{50} (95% CL)***	
AM 251	1.5 (0.80−2.8)	
SR 141716A	0.25 (0.13−0.47)	6.1 (2.5−15)[a]
WIN 55212−2 antagonism		
AM 251	4.8 (0.88−27)	
SR 141716A	0.90 (0.44−1.8)	5.4 (1.8−16)[a]

[a] significant difference in ID_{50}