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Cannabinoid-Induced Hypotension and Bradycardia in Rats Is Mediated by CB_1-Like Cannabinoid Receptors[1]

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ABSTRACT

Previous studies indicate that the CB_1 cannabinoid receptor antagonist, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl (SR141716A), inhibits the anandamide- and Δ^9-tetrahydrocannabinol- (THC) induced hypotension and bradycardia in anesthetized rats with a potency similar to that observed for SR141716A antagonism of THC-induced neurobehavioral effects. To further test the role of CB_1 receptors in the cardiovascular effects of cannabinoids, we examined two additional criteria for receptor-specific interactions: the rank order of potency of agonists and stereoselectivity. A series of cannabinoid analogs including the enantiomeric pair (-)-11-OH-Δ^9-THC dimethylheptyl (+)-11-OH-Δ^9-THC dimethylheptyl were evaluated for their effects on arterial blood pressure and heart rate in urethane anesthetized rats. Six analogs elicited pronounced and long lasting hypotension and bradycardia that were blocked by 3 mg/kg of SR141716A. The rank order of potency was (-)-11-OH-Δ^9-THC dimethylheptyl $\geq$ (-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol $>$ (-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol $>$ THC $>$ anandamide $\geq$ (-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol, which correlated well with CB_1 receptor affinity or analgesic potency ($r = 0.96$-0.99). There was no hypotension or bradycardia after palmitoylethanolamine or (+)-11-OH-Δ^9-THC dimethylheptyl. An initial pressor response was also observed with THC and anandamide, which was not antagonized by SR141716A. We conclude that the similar rank orders of potency, stereoselectivity and sensitivity to blockade by SR141716A indicate the involvement of CB_1-like receptors in the hypotensive and bradycardic actions of cannabinoids, whereas the mechanism of the pressor effect of THC and anandamide remains unclear.

In anesthetized animals, the major psychoactive constituent of *Cannabis sativa*, THC, elicits a transient pressor response followed by hypotension and bradycardia (Dewey *et al*., 1970; Graham and Li, 1973; Estrada *et al.*, 1987). In rats with genetic or surgically induced hypertension, THC significantly lowers mean arterial blood pressure to normotensive levels (Stark and Dews, 1980; Birmingham, 1973; Nahas et *al*., 1973), and it has also been shown to prevent immobilization stress-induced hypertension (Williams and Ng, 1973).

Cannabinoid receptors have been identified in the rat by radioligand binding and autoradiography (Devane *et al.*, 1988; Herkenham *et al.*, 1990). Subsequently, two cannabinoid receptors have been cloned: the CB_1 receptor located in the brain (Matsuda *et al*., 1990; Gerard *et al*., 1991), and in some peripheral organs (Shire *et al*., 1995; Ishac *et al*., 1996), and the CB_2 receptor identified in macrophages (Munro *et al*., 1993; Galiegue *et al*., 1995). Additionally, a splice variant of the CB_1 receptor, the CB_{1A} receptor has also been described (Shire *et al*., 1995).

In 1992, an endogenous cannabinoid receptor ligand, anandamide (arachidonyl-2-ethanolamide), was extracted and purified from porcine brain (Devane *et al*., 1992). As with THC, anandamide binds to cannabinoid receptors (Vogel *et al*., 1993; Felder *et al*., 1993), inhibits adenylate cyclase via an inhibitory G-protein (Vogel *et al*., 1993), and inhibits voltage-gated N-type calcium channels (Felder *et al*., 1993). In neurobehavioral assays, anandamide has been shown to mimic THC in terms of inducing catalepsy, hypomotility, hypothermia and analgesia (Fride *et al*., 1993; Smith, *et al*., 1994). We previously demonstrated that in anesthetized rats, anand-

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ABBREVIATIONS: THC, Δ^9-tetrahydrocannabinol; CP-55,940, (-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol; HU-210, (-)-11-OH-Δ^9-THC dimethylheptyl; HU-211, (+)-11-OH-Δ^9-THC dimethylheptyl; WIN-55212-2, O-502, {(R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-(1-naphthalenyl) methanone} JWH-015, (-)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol;; O-502, 4′-(R)-OH-diadduct-Δ^9-THC; O-522, 4′-(S)-OH-diadduct-Δ^9-THC; SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl; ABN-CBD, abnormal-cannabidiol; MAP, mean arterial blood pressure; HR, heart rate; bpm, beats per minute; ED_{50}, agonist dose producing half-maximal effect, AD_{50}, antagonist dose causing 50% inhibition of agonist response

amide causes a pressor response followed by hypotension and mild bradycardia (Varga *et al.*, 1995), and similar effects have been observed in both conscious and anesthetized, spontaneously hypertensive rats (Lake *et al.*, 1997). The hypotensive and bradycardic responses to anandamide and THC are inhibited by the selective CB_1 receptor antagonist, SR141716A (Rinaldi-Carmona *et al.*, 1994), which suggests the involvement of CB_1 receptors (Varga *et al.*, 1995). Activation of these receptors was found to inhibit sympathetic tone via a presynaptic mechanism at peripheral sympathetic nerve terminals (Varga *et al.*, 1996; Ishac *et al.*, 1996). CB_1 receptors in the brain that mediate the neurobehavioral effects of cannabinoids have been characterized not only by their susceptibility to inhibition by SR141716A (Rinaldi Carmona *et al.*, 1994; Compton *et al.*, 1996), but also by their selective interaction with cannabinoid enantiomers (Little *et al.*, 1989). Furthermore, the rank order of potency of various cannabinoid analogs for eliciting neurobehavioral effects correlates well with their binding affinities for the brain cannabinoid receptor (Compton *et al.*, 1992a, 1993). To further test whether the CB_1 receptors mediating neurobehavioral and cardiovascular effects are pharmacologically similar, we analyzed the cardiovascular effects of a series of cannabinoid analogs, including enantiomeric pairs, in urethane-anesthetized rats. The results indicate that the pronounced hypotension and bradycardia induced by structurally different cannabinoids are mediated by receptors that are pharmacologically similar to brain CB_1 receptors mediating neurobehavioral effects. In contrast, the mechanism of the brief pressor response elicited by some cannabinoids remains unclear.

Methods

Animals. Adult male Sprague-Dawley rats weighing 280 to 400 g were obtained from Harlan (Indianapolis, IN) and were housed in suspension cages with food and water *ad libitum*. The animals were maintained at 24 to 26°C under a 14:10 hr light/dark cycle and were allowed to acclimate for at least 1 wk before surgery.

Surgical preparation. Anesthesia was induced with diethyl ether and a femoral vein was cannulated for i.v. drug administration. Ether anesthesia was then discontinued and urethane was administered (0.7 g/kg, i.v. + 0.3 g/kg, s.c.). Urethane administered according to this protocol does not depress basal blood pressure and does not interfere with cardiovascular regulatory mechanisms (Maggi and Meli, 1986). The femoral artery was cannulated and the catheter connected to a pressure transducer (Abbott, North Chicago, IL) for continuous monitoring of BP with a physiograph (Astromed, Cortland, NY). HR was monitored via a tachograph preamplifier driven by the pressure wave. The trachea was cannulated with PE-160 tubing to maintain an open airway. Body temperature was maintained at 37 to 38°C throughout the experiments by using a water circulating heating pad (Gaymar Industries, Orchard Park, NY) and rectal thermometer.

Experimental protocols. After a 30-min stabilization period, the animals received either vehicle or SR14716A (3 mg/kg, i.v.). Twenty min later, a single dose of an agonist was administered, and the changes in BP and HR were monitored for 60 min. As agonist effects on BP and HR were long lasting for most of the drugs and doses tested, each animal was tested with a single dose of an agonist, after either vehicle or SR141716A.

Drugs. The chemical structure of the cannabinoid agonists used is illustrated in Figure 1. Anandamide (arachidonyl-2-ethanolamide) and 4′-(R)- and 4′-(S)-OH-diadduct-Δ^9-THC (O-502 and O-522, respectively) were synthesized by Dr. Raj Razdan (Organix Inc., Woburn, MA). HU-210 and HU-211 were synthesized by Dr. Raphael Mechoulam (Hebrew University, Jerusalem, Israel). CP-55940 and SR141716A were provided by Dr. John Lowe at Pfizer Central Research. JWH-015 was synthesized by Dr. John Huffman (Clemson University). Δ^9-THC was obtained from the National Institute on Drug Abuse. WIN-55212-2 was purchased from Research Biochemicals International (Natick, MA). Palmitoylethanolamine was purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). All drugs were dissolved in 1:1:18 or 1:1:8 (emulphor-ethanol-saline). Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ) is currently available as Alkmulphor. The dosing volume was 0.5 to 1 ml/kg i.v., followed by a catheter line flush of 0.2 ml saline. Injection of the same volume of vehicle had no effect on blood pressure or heart rate. All drugs were injected i.v. as bolus doses over a 10- to 15-sec period.

Data analysis. MAP was calculated as 1/3(systolic-diastolic BP) + diastolic BP. Basal MAP and HR for all groups was 102 ± 4.8 mmHg and 325 ± 5.4 bpm, respectively (n = 96). Time-dependent, agonist-induced changes in MAP and HR in the absence or presence of SR141716A were compared using analysis of variance followed by Tukey's *post hoc* test. The ED_{50} for each agonist was calculated using ALLFIT, a nonlinear sigmoidal curve-fitting program (DeLean *et al.*, 1977). For anandamide and THC, which produce both a pressor and a subsequent depressor response, ED_{50}s for both components were calculated, using the peak response minus predrug baseline value in both cases. The ED_{50} for the depressor response to THC was calculated based on the descending portion of the biphasic curve. Correlation analysis and generation of the Pearson product-moment coefficient was performed using the StatView statistical package (Brainpower, Inc., Agoura Hills, CA). Data are presented as mean ± S.E. of the mean.

Results

THC (0.02-10 mg/kg, fig. 2A) elicited an initial brief pressor response followed by prolonged and marked hypotension and bradycardia. Dose-response relationships for these latter effects were biphasic with maximal hypotension (-62 ± 9 mmHg) observed at 2 mg/kg and maximal bradycardia (-140 ± 24 bpm) observed at 8 mg/kg of THC, beyond which doses the hypotensive and bradycardic effects were less pronounced (fig. 3). ED_{50} values were 0.27 ± 0.09 mg/kg for the hypotension and 0.62 ± 0.10 mg/kg for the bradycardic effect. Maximal decreases in BP and HR took 15 to 25 min to develop after the lower doses and 4 min after the 2 mg/kg dose, and each lasted more than 60 min. Pretreatment with SR141716A (3 mg/kg) blocked the hypotension and bradycardia elicited by the 4.0 mg/kg dose (fig. 2A). The initial pressor response (ED_{50} = 2.47 ± 0.85 mg/kg, data not shown) was observed at doses of THC ≥ 1 mg/kg, and was not antagonized by SR141716A (fig. 2A). No animal died following any dose of THC.

The stereoselectivity of cannabinoid-induced hypotension and bradycardia was evaluated using the enantiomers HU-210 and HU-211. At a dose of 0.01 mg/kg, HU-210 caused pronounced and long lasting hypotension and bradycardia without an initial pressor response (fig. 2B). In dose-response studies, HU-210 appeared to be more potent in causing hypotension than in eliciting bradycardia, the ED_{50}s for these being 0.0020 ± 0.0004 and 0.09 ± 0.01 mg/kg, respectively (fig. 3). Like THC, the effects of HU-210 were long lasting (fig. 2B), and the maximal decrease in MAP and HR exceeded those of THC (fig. 3). Pretreatment with SR141716A (3 mg/kg) blocked the hypotension and bradycardia elicited by the 0.01 mg/kg dose of HU-210 (fig. 2B). One in four animals died following the 0.3 mg/kg dose of HU-210, but none after lower

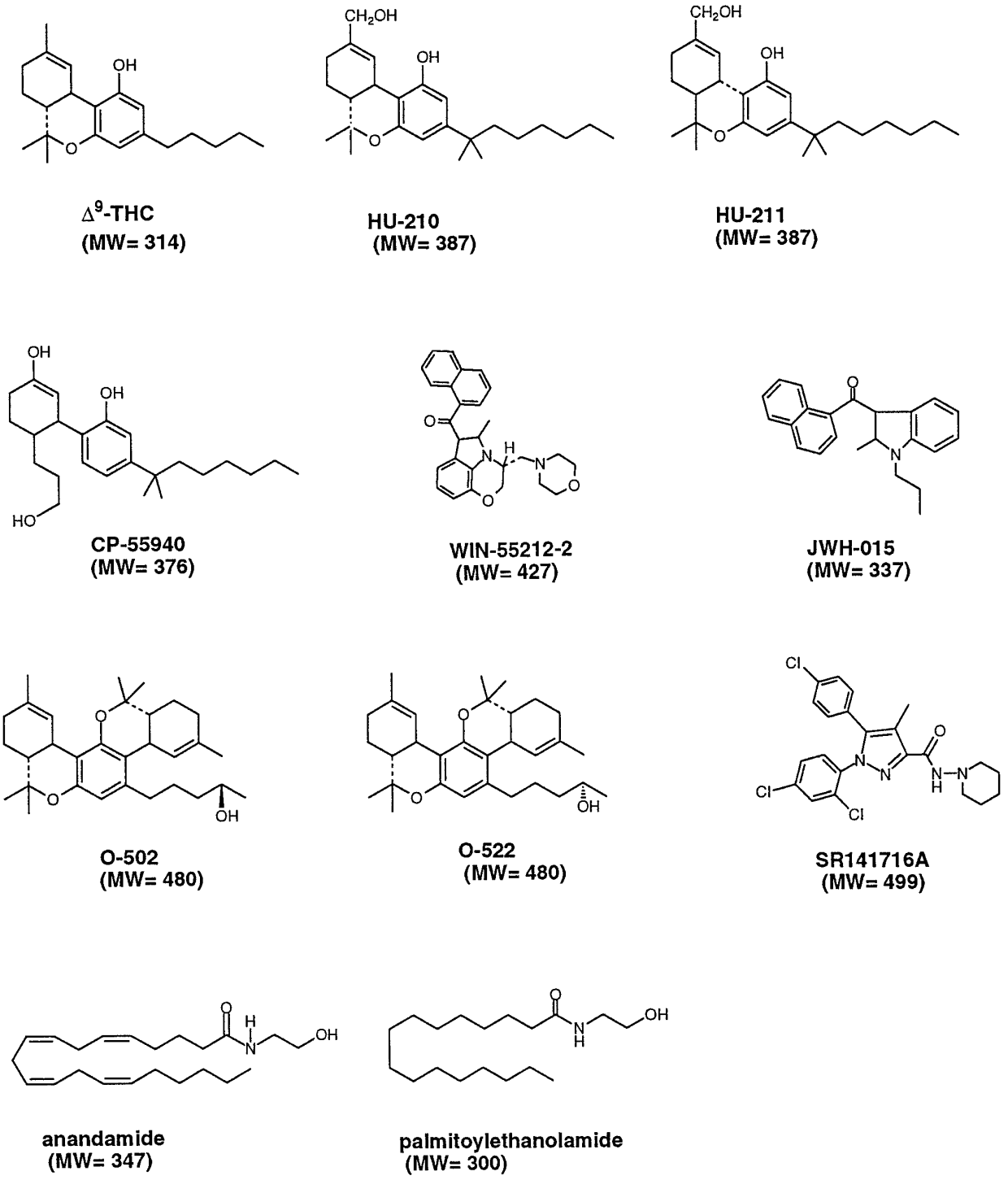

Fig. 1. Structure of cannabinoid agonists tested. Classical cannabinoids (Δ^9-THC, HU-210 and enantiomer HU-211), nonclassical compounds (CP-55940, WIN-55212-2, JWH-015, O-502 and stereoisomer O-522) and endogenous ethanolamide conjugated fatty acids (anandamide and palmitoylethanolamide).

doses. In contrast, the behaviorally inactive isomer HU-211 caused no changes in BP at doses of 0.1 and 1 mg/kg but, consistent with published observations (Mechoulam *et al.*, 1992), caused a slowly developing increase in HR (fig. 2B).

At a dose of 0.3 mg/kg, CP-55940 elicited pronounced hypotension and bradycardia without an initial pressor effect (fig. 4A), which was similar to the effects of HU-210. The ED_{50} was 0.011 ± 0.002 mg/kg for the hypotension and 0.11 ± 0.05 mg/kg for the bradycardia. Maximal hypotension (-83 ± 3 mmHg) and bradycardia (-218 ± 10 bpm) developed within 4 to 10 min. Pretreatment with SR141716A (3 mg/kg) blocked both the hypotension and the bradycardia elicited by 0.3 mg/kg CP-55,940 (fig. 4A). At a dose of 1 mg/kg, WIN-55212-2 also elicited long lasting hypotension and bradycardia, which developed within 1 to 5 min (fig. 4B). Similarly, WIN-55212-2 caused no initial pressor effect at any of the

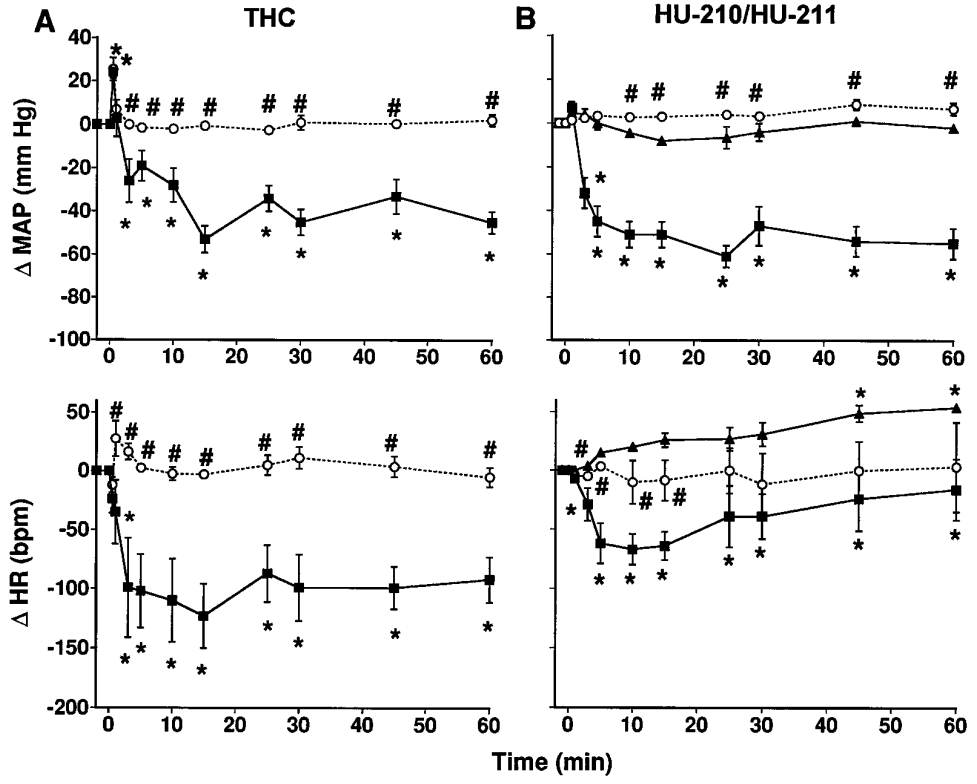

Fig. 2. Effects of classical cannabinoids on MAP and HR in anesthetized rats. A, Effects of THC (4 mg/kg, i.v.) after pretreatment with vehicle (■) or SR141716A (3 mg/kg, i.v., □). B, Effects of HU-210 (0.01 mg/kg i.v.) after vehicle (■) or SR141716A (3 mg/kg, i.v., □) or HU-211 (1 mg/kg i.v.) after vehicle (▲). Data are presented as mean ± S.E. (n = 5-9; *P < .05 from baseline, #P < .05 between groups).

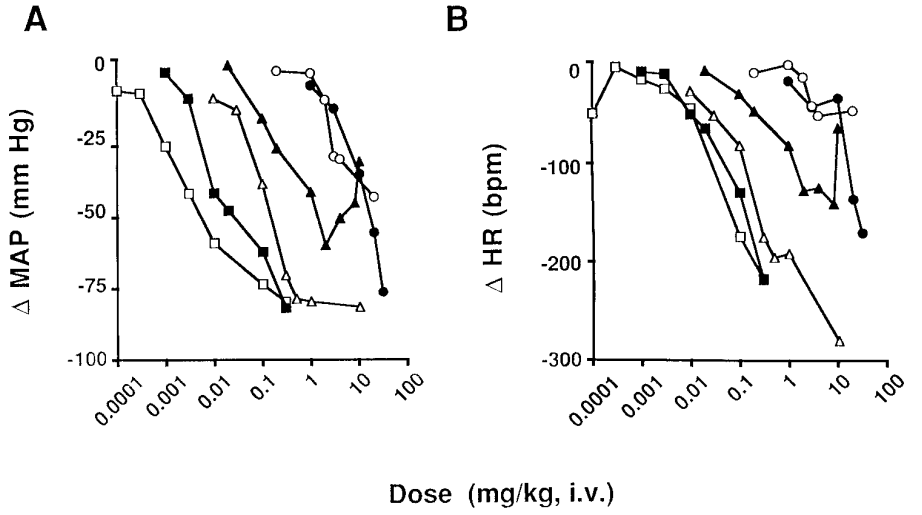

Fig. 3. Dose-dependent hypotension (A) and bradycardia (B) elicited by HU-210 (□), CP-55940 (■), WIN-55212-2 (△), Δ^9-THC (▲), anandamide (○) and JWH-015 (●). Each dose represents the mean of three to six experiments. The average S.E. was less than 10% of the mean.

doses tested and had an ED_{50} of 0.10 ± 0.02 mg/kg for hypotension and 0.29 ± 0.13 mg/kg for bradycardia (fig. 3). Pretreatment with SR141716A (3 mg/kg) blocked the hypotension, but only partially antagonized the bradycardia elicited by the 0.3 mg/kg dose of WIN-55212-2 (fig. 4B). One in three animals died after the highest dose tested (10 mg/kg), but none after lower doses. The time-response curve for both CP-55,940 and WIN-55212-2 was clearly biphasic, the reasons for which are not clear. The third compound in this class, JWH-015, was less potent than the first two, having an ED_{50} of 10.1 ± 1.6 mg/kg for hypotension and 16.4 ± 2.2 mg/kg for bradycardia (fig. 3). Pretreatment with SR141716A (3 mg/kg) blocked the long lasting hypotension and bradycardia elicited by 10 mg/kg of JWH-015 (fig. 4C). One in four animals died after the 20- and 30-mg/kg doses. As with CP-55,940 and WIN-55212-2, no initial pressor response was observed at any dose.

The stereoisomers O-502 and O-522 did not elicit any change in BP or HR at doses of 0.5 or 5 mg/kg. At a dose of 30 mg/kg, a brief (<4 min) pressor response was observed after either O-502 (+30 ± 6 mmHg) or O-522 (+41 ± 4 mm Hg). Both agents also caused moderate, but long lasting tachycardia (O-502: +50 ± 4 bpm; O-522: +45 ± 8 bpm) at the 30-mg/kg dose. Neither of these two cannabinoid isomers binds to the CB_1 receptor or elicits neurobehavioral effects in mice (table 1).

As described earlier (Varga *et al.*, 1995), anandamide elicited an initial transient bradycardia followed by a brief pres-

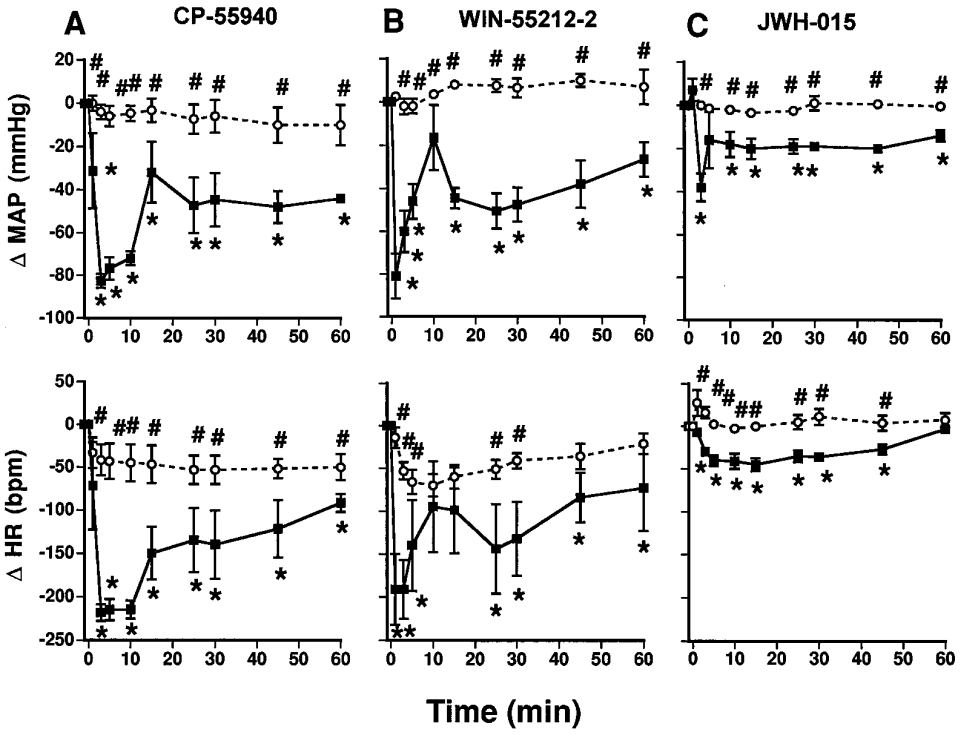

Fig. 4. Effects of nonclassical cannabinoids on MAP and HR in anesthetized rats. A, Effects of CP-55940 (0.3 mg/kg, i.v.) after pretreatment with vehicle (■) or SR141716A (3 mg/kg, i.v., □). B, Effects of WIN-55212-2 (1 mg/kg, i.v.) after vehicle (■) or SR141716A (3 mg/kg, i.v., □). C, Effects of JWH-015 (10 mg/kg, i.v.) after vehicle (■) or SR141716A (3 mg/kg, i.v., □). Points represent means ± S.E. from four experiments for each treatment group; *P < .05 from baseline and #P < .05 between groups.

sor and a more prolonged depressor response that lasted less than 15 min. During this latter hypotensive phase there was also moderate bradycardia (fig. 5). Dose-response studies yielded an ED_{50} of 2.5 ± 0.3 mg/kg for the hypotension with a peak change of -46 ± 3 mmHg, and an ED_{50} of 2.5 ± 0.2 mg/kg for the bradycardia with a peak of -43 ± 15 bpm (fig. 3). Pretreatment with SR141716A (3 mg/kg) inhibited the hypotension and parallel bradycardia elicited by the 4.0-mg/kg dose (fig. 5). The initial transient vagal bradycardia/hypotension and the subsequent brief pressor response that preceded the hypotension were not blocked by SR141716A (fig. 5). No animals died following up to 30 mg/kg of anandamide. In contrast, palmitoylethanolamine did not influence BP at doses of 1 to 20 mg/kg and caused a moderate delayed tachycardia at the 4 and 20 mg/kg doses.

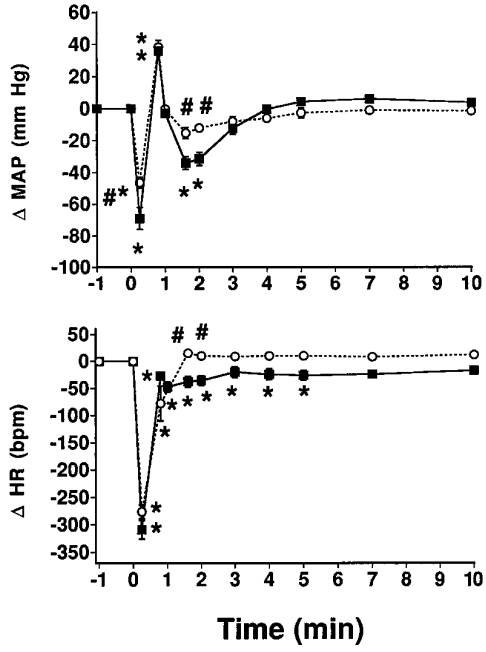

Fig. 5. Effects of anandamide (4 mg/kg i.v.) on MAP and HR in anesthetized rats pretreated with vehicle (■) or SR141716A (3 mg/kg, i.v., □). Points represent mean ± SE (n = 5-9); *P < .05 from baseline and #P < .05 between groups.

As illustrated in figure 3, the rank order of potency of the six analogs that elicited hypotension and bradycardia was the same for these two effects: HU-210 ≥ CP-55940 > WIN-55212-2 > THC > anandamide > JWH-015. In earlier studies, similar rank orders of potency have been determined for CB_1 receptor binding in rat brain membranes and for various behavioral effects in mice (table 1). Therefore, we determined the correlation between rat brain CB_1 receptor binding affinity (K_i) and the hypotensive and bradycardic ED_{50} values of the above six analogs. A significant positive correlation was observed between hypotension (r = 0.97) and bradycardia (r = 0.96) to the binding affinity (fig. 6A). A similar, significant positive correlation could be established between antinociceptive potency in mice and either the hypotensive (r = 0.99) or the bradycardic effects (r = 0.96) in rats (fig. 6B). Table 1 includes the absolute potencies of the various analogs tested for eliciting hypotension and bradycardia as determined in our study, and their potencies in eliciting hypothermia, antinociception, ring-immobility and hypoactivity, as well as their receptor binding affinities.

Discussion

It has long been recognized that the major psychoactive constituent of marijuana, THC, can cause pronounced and long lasting hypotension and bradycardia (Vollmer *et al*., 1974; Siqueira *et al*., 1979). More recently, analogous effects have been described for the endogenous cannabinoid ligand, anandamide (Varga *et al*., 1995; 1996). We have reported that these effects are inhibited by the CB_1 receptor antago-

TABLE 1

Summary of cardiovascular effects, receptor binding affinity in rat brain preparations and neurobehavioral effects in mice for selected cannabinoid compounds; compounds listed in descending rank order of potency (top to bottom)

Compound	Pressor	Hypotension		Bradycardia		binding K_i (nM)		Hypothermia	Antinociception	Ring Immobility	Hypoactivity
	ED_{50} (mg/kg, i.v.)	ED_{50} (mg/kg, i.v.)	E_{max} (mm Hg)	ED_{50} (mg/kg, i.v.)	E_{max} (bpm)	CB1	CB2	ED_{50} (mg/kg, i.v.)	ED_{50} (mg/kg, i.v.)	ED_{50} (mg/kg, i.v.)	ED_{50} (mg/kg, i.v.)
HU-210	NA	0.002	−82	0.09	−217	0.73[a]	0.22[b]	0.02[c]	0.009[c]	0.02[c]	0.004[c]
CP-55940	NA	0.01	−83	0.12	−218	0.58[b]	0.69[b]	0.3[d]	0.09[d]	0.4[d]	0.04[d]
WIN-55212-2	NA	0.1	−84	0.29	−267	1.82[b]	0.28[b]	12[e]	0.4[e]	1.1[e]	0.1[e]
Δ^9-THC	2.47	0.27	−62	0.62	−140	40.7[a]	36.4[b]	1.4[f]	1.4[f]	1.5[f]	1.0[f]
Anandamide	1.57	2.54	−46	2.54	−43	101[g]	371[b]	26.5[g]	6.2[g]	19.1[g]	17.9[g]
JWH-015	NA	10.1	−78	14.3	−170	336[h]	13.8[b]	ND	23.9[f]	ND	ND
HU-211	NA	NA	NA	NA	NA	1,990[a]	>10,000[b]	>30[c]	>30[c]	>30[c]	>30[c]
Palmitoylethanolamine	NA	>20	NA	NA	NA	>1,000[f]	>10,000[b]	ND	ND	ND	ND
O-522	>30	>30	NA	>30	NA	>10,000[f]	ND	>60[f]	>30[f]	>60[f]	>30[f]
O-502	>30	>30	NA	>30	NA	>10,000[f]	ND	>60[f]	>30[f]	>60[f]	>30[f]

NA, Not applicable; ND, not done.

[a] Compton *et al.*, 1993.
[b] Showalter *et al.*, 1996.
[c] Little *et al.*, 1989.
[d] Little *et al.*, 1988.
[e] Compton *et al.*, 1992b.
[f] Compton *et al.*, 1992a.
[g] Smith *et al.*, 1994.
[h] Kuster *et al.*, 1993.
[i] Compton *et al.*, unpublished data.
[j] Devane *et al.*, 1992.

nist SR141716A (Varga *et al.*, 1995) and that the inhibitory potency of SR141716A against the hypotensive response to THC or anandamide (Lake *et al.*, 1997) was similar to its inhibitory potency against the neurobehavioral effects of THC (Rinaldi-Carmona *et al.*, 1994). This has suggested that the cannabinoid-induced hypotension and bradycardia are mediated by CB_1-like receptors. In our study, we sought to further test this hypothesis by analyzing the cardiovascular effects of a series of cannabinoid analogs including two pairs of enantiomers in urethane-anesthetized rats. The analogs selected for testing belong to three classes: classical cannabinoids (THC, HU-210 and HU-211), nonclassical cannabinoids (CP-55940, WIN-55212-2, JWH-015, O-502 and O-522), and endogenous ethanolamine-conjugated fatty acids (anandamide and palmitoylethanolamine). This should minimize the possibility that their effects may be related to a common structural feature unrelated to their interaction with cannabinoid receptors. Six of the 10 analogs tested elicited hypotension and bradycardia, and they include compounds from all three classes. With the possible exception of anandamide, the hypotension and bradycardia were very pronounced and of long duration, with a clearly established rank order of potency. The involvement of specific receptors in these effects is indicated by their marked stereoselectivity. The behaviorally active analog HU-210 (Little *et al.*, 1989) displayed the same high potency for hypotension (ED_{50}: 2 μg/kg) as for eliciting neurobehavioral effects (ED_{50}s of 2-4 μg/kg, see table 1), although it was somewhat less potent in eliciting bradycardia. A significantly lower potency of HU-210 to elicit hypotension ($ED_{50} \approx 50$ μg/kg) was noted in Wistar rats, which may be related to the use of pentobarbital as anesthetic (Vidrio *et al.*, 1996). In contrast, the behaviorally inactive enantiomer, HU-211, did not elicit hypotension at doses up to 1 mg/kg, indicating a minimum of 500-fold stereoselectivity. At the 1-mg/kg dose, HU-211 elicited a moderate tachycardic effect, which is similar to earlier findings (Mechoulam *et al.*, 1992), and thus is probably not mediated by cannabinoid receptors.

The ability of SR141716A to inhibit the hypotensive and bradycardic effects of all six analogs is in agreement with earlier observations with THC and anandamide (Varga *et al.*, 1995; Lake *et al.*, 1997). In a previous study, the AD_{50} of SR141716A for inhibiting THC- or anandamide-induced hypotension and bradycardia in anesthetized rats was found to be in the range of 0.1 to 0.3 mg/kg iv. (Lake *et al.*, 1997). Our finding that a dose of 3 mg/kg SR141716A completely blocked the effects of most analogs is compatible with the involvement of CB_1 receptors in these effects. This possibility is further supported by the significant positive correlation between the potencies of the six analogs in eliciting hypotension and bradycardia and the binding affinity of these compounds or their analgesic potency in mice (fig. 6, table 1). However, despite this strong correlation there are also some subtle differences. First, it is evident that the absolute potencies of the six analogs tested for causing hypotension are slightly but consistently higher than their potencies for eliciting neurobehavioral effects (table 1). Second, the four most potent hypotensive analogs were appreciably less potent in eliciting bradycardia than hypotension (table 1). Third, the hypotensive ED_{50}s of the three most potent analogs (HU-210 > CP-55940 > WIN-55212-2) vary over two orders of magnitude, whereas their binding K_is for CB_1 receptors are

roughly equal (table 1). Finally, a dose of SR141716A that completely blocked the hypotensive response to 1 mg/kg WIN-55212-2, only partially inhibited its bradycardic effect (fig. 4B). Although the existence of a significant receptor reserve for the hypotensive action or differences in receptor coupling mechanisms may explain some of these discrepancies, we cannot exclude the possibility that they may reflect the existence of different isoforms of CB_1 receptors. Such a possibility is also suggested by reports that certain cannabinoid analogs devoid of neurobehavioral effects can elicit hypotension in experimental animals (Adams *et al.*, 1976; Zaugg and Kyncl, 1983), which we were able to confirm and extend in the case of abnormal cannabidiol (J. L. Wiley, E. J. N. Ishac, R. K. Razdan, B. R. Martin, K. Varga and G. Kunos, unpublished results).

Apart from the possibility of CB_1 receptor heterogeneity, it is also likely that the effects of some of the analogs are complex and involve more than one mechanism via CB_1 receptors located in different tissues. The results of our previous studies suggest that the hypotensive action of anandamide is due to inhibition of sympathetic tone (Varga *et al.*, 1995), and a similar mechanism has been proposed earlier for THC (Vollmer *et al.*, 1974; Siqueira *et al.*, 1979). Additional evidence has eliminated the central nervous system, sympathetic ganglia or postsynaptic sites in the vasculature or heart as possible sites of the sympathoinhibitory action of anandamide, and has suggested a presynaptic mechanism at sympathetic nerve terminals (Varga *et al.*, 1996). Indeed, this possibility is strongly supported by the demonstration of a CB_1 receptor-mediated inhibition of exocytotic norepinephrine release induced by anandamide or THC in isolated cardiac tissue or vas deferens, and by the presence of CB_1 receptor mRNA in a sympathetic ganglion (Ishac *et al.*, 1996). However, in our experiments the three most potent hypotensive analogs, CP-55,940, HU-210 and WIN-55212-2 lowered MAP by more than 80 mmHg (see table 1), which far exceeds the decrease in MAP caused by removing sympathetic tone. In a previous study done in the same strain of rats under identical conditions, the hypotensive response to anandamide was no longer present after *alpha*-adrenergic blockade with 2 mg/kg phentolamine, which lowered MAP from the same resting level by 51 ± 3 mmHg (Varga *et al.*, 1995). Therefore, CB_1 receptors other than those located presynaptically on sympathetic nerve terminals must contribute to the hypotensive effect of the more potent and efficacious cannabinoids.

Anandamide has been shown to bind to CB_1 as well as CB_2 receptors in transfected cell lines (Showalter *et al.*, 1996), whereas a saturated analog, palmitoylethanolamine, has been found to bind to the rat CB_2 receptor (Facci *et al.*, 1995) but not to the CB_1 receptor (Devane *et al.*, 1992; Felder *et al.*, 1993) or to the human CB_2 receptor (Showalter *et al.*, 1996). Our finding that palmitoylethanolamine did not elicit any change in blood pressure and caused moderate tachycardia argues against the possible role of CB_2 receptors in the hypotensive and bradycardic effects. The lack of CB_2 receptor involvement in these cannabinoid-induced effects is also suggested by their potent inhibition by SR141716A, an antagonist with 60-fold lower affinity for CB_2 (K_i: 702 nM) than for CB_1 receptors (K_i: 12.3 nM, Showalter *et al.*, 1996). Conclusive evidence against CB_2 receptor involvement awaits the development of a selective and potent CB_2 receptor antagonist.

Of the 10 compounds evaluated, only four elicited a transient pressor response: anandamide, THC, O-502 and O-522. The anandamide- and THC-induced pressor response was not CB_1 receptor-mediated as it was not attenuated by SR141716A. The involvement of CB_2 receptors is also unlikely, because JWH and WIN-55212-2 are even more potent at CB_2 than at CB_1 receptors (Showalter *et al.*, 1996), yet they produced no pressor effect. O-502 and its stereoisomer O-522, which are both devoid of cannabinoid-like neurobehavioral effects and do not bind to cannabinoid receptors (table 1), did not alter MAP or HR at the lower doses tested and caused a moderate pressor response and tachycardia only at a dose of 30 mg/kg. This suggests that the pressor and tachycardic effects are not mediated by cannabinoid receptors and may result from a nonreceptor- mediated direct effect on vascular smooth muscle, or an indirect effect via release of a vasoconstrictor agent. Sympathetic activation can be excluded as the underlying mechanism, in view of the earlier finding that spinal cord transection or *alpha*-adrenergic receptor blockade does not attenuate the pressor response to THC (Siqueira *et al.*, 1979) or anandamide (Varga *et al.*, 1995).

In summary, we have found that several cannabinoids,

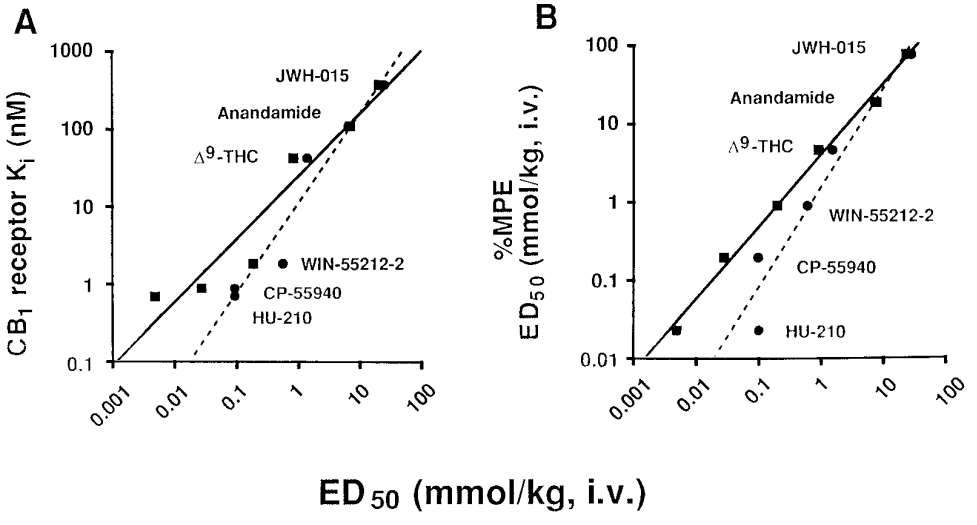

Fig. 6. A, Linear correlation between log K_i values for displacement of binding of ^{3}H-CP-55,940 to rat brain membranes and log ED_{50} values for maximal hypotension (■) or bradycardia (●), as shown on the abscissa. The correlation coefficient for hypotension was 0.97 ($P < .05$) and for bradycardia was 0.96 ($P < .05$). B, Linear correlation between log ED_{50} values for antinociception and log ED_{50}s for each compound for hypotension (■) or bradycardia (●). The correlation coefficient for hypotension was 0.99 ($P < .05$) and for bradycardia was 0.95 ($P < .05$).

including anandamide, elicit hypotension and bradycardia. These effects are dose-dependent, antagonized by SR141716A, enantioselective and display a similar same rank order of potency as observed for binding to CB_1 receptors in rat brain preparations and for eliciting antinociception in mice. All this indicates that the hypotension and bradycardia elicited by cannabinoids is mediated by a CB_1-like cannabinoid receptor. However, due to subtle differences in agonist potencies for the different effects of cannabinoids and in the inhibitory effects of SR141617A, the possible existence of CB_1 receptor isoforms cannot be excluded. Further studies are aimed at identifying cannabinoid analogs that cause hypotension but are devoid of neurobehavioral effects, and thus may prove of interest in the management of hypertension.

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