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Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB_1 receptors

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Abstract

Activation of peripheral cannabinoid CB_1 receptors elicits hypotension. Using the radioactive microsphere technique, we examined the effects of cannabinoids on systemic hemodynamics in anesthetized rats. The potent cannabinoid CB_1 receptor agonist HU-210 ({−}-11-OH-Δ^9 tetrahydrocannabinol dimethylheptyl, 10 μg/kg i.v.) reduced mean blood pressure by 57 ± 5 mm Hg by decreasing cardiac index from 37 ± 1 to 23 ± 2 ml/min/100 g ($P < 0.05$) without significantly affecting systemic vascular resistance index. HU-210 elicited a similar decrease in blood pressure following ganglionic blockade and vasopressin infusion. The endogenous cannabinoid anandamide (arachidonyl ethanolamide, 4 mg/kg i.v.) decreased blood pressure by 40 ± 7 mm Hg by reducing systemic vascular resistance index from 3.3 ± 0.1 to 2.3 ± 0.1 mm Hg min/ml/100 g ($P < 0.05$), leaving cardiac index and stroke volume index unchanged. HU-210, anandamide, and its metabolically stable analog, *R*-methanandamide, lowered vascular resistance primarily in the coronaries and the brain. These vasodilator effects remained unchanged when autoregulation was prevented by maintaining blood pressure through volume replacement, but were prevented by pretreatment with the cannabinoid CB_1 receptor antagonist SR141716A (*N*-{piperidin-1-yl}-5-{4-chlorophenyl}-1-{2,4-dichlorophenyl}-4-methyl-1*H*-pyrazole-3-carboxamide HCl; 3 mg/kg i.v.). Only anandamide and *R*-methanandamide were vasodilators in the mesentery. We conclude that cannabinoids elicit profound coronary and cerebral vasodilation in vivo by direct activation of vascular cannabinoid CB_1 receptors, rather than via autoregulation, a decrease in sympathetic tone or, in the case of anandamide, the action of a non-cannabinoid metabolite. Differences between the hemodynamic profile of various cannabinoids may reflect quantitative differences in cannabinoid CB_1 receptor expression in different tissues and/or the involvement of as-yet-unidentified receptors.

Keywords: Anandamide; Cannabinoid CB_1 receptor; HU-210; *R*-methanandamide; Microsphere

1. Introduction

The psychoactive properties of cannabinoids, constituents of the marijuana plant, have long been recognized, and similar effects can be elicited by the endogenous ligand arachidonyl ethanolamide (anandamide) (Hillard, 2000). Recent research has revealed that cannabinoids elicit not only neurobehavioral but also cardiovascular effects (Kunos et al., 2000). The biological effects of cannabinoids are mediated by specific receptors, of which two subtypes have been identified, cannabinoid CB_1 receptors present in the brain but also in some peripheral tissues (Matsuda et al., 1990) and cannabinoid CB_2 receptors expressed by immune cells (Munro et al., 1993). Through the use of a selective cannabinoid CB_1 receptor antagonist, SR141716A (*N*-{piperidin-1-yl}-5-{4-chlorophenyl}-1-{2,4-dichlorophenyl}-4-methyl-1*H*-pyrazole-3-carboxamide HCl; Rinaldi-Carmona et al., 1994), the hypotensive and bradycardic effects of cannabinoids in rodents could be attributed to activation of peripheral cannabinoid CB_1 receptors (Varga et al., 1995; Járai et al., 1999). This conclusion has been confirmed by the absence of such effects in cannabinoid CB_1 receptor-knockout mice (Járai et al., 1999; Ledent et al., 1999).

Recent findings implicate endogenous cannabinoids in cardiovascular regulation. Circulating macrophages and platelets activated by hemorrhage or by bacterial endotoxin synthesize anandamide and another endogenous cannabi-

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noid, 2-arachidonoyl glycerol (2-AG), and these endocannabinoids contribute to the hypotension of hemorrhagic and septic shock via the activation of peripheral cannabinoid CB_1 receptors (Wagner et al., 1997; Varga et al., 1998). Vascular endothelial cells also contain anandamide (Deutsch et al., 1997) and 2-AG (Mechoulam et al., 1998; Sugiura et al., 1998), and functional cannabinoid CB_1 receptors are present on cerebrovascular smooth muscle cells (Gebremedhin et al., 1999). It has been proposed that the endothelium-derived hyperpolarizing factor (EDHF) released by various vasodilators may be an endocannabinoid (Randall et al., 1996), although this has been contested (Plane et al., 1997; Pratt et al., 1998).

There is evidence that various cannabinoids produce strong vasodilation in vitro (Chataigneau et al., 1998; Deutsch et al., 1997; Ellis et al., 1995; Wagner et al., 1999), but the underlying mechanisms are unclear (Pratt et al., 1998). However, there is a lack of information on the in vivo effects of cannabinoids on blood flow to various organs, and on the hemodynamic changes underlying their hypotensive action. In a study of the cardiovascular effects of a series of cannabinoids in anesthetized rats, the synthetic cannabinoid HU-210 ({−}-11-OH-Δ^9-tetrahydrocannabinol dimethylheptyl) was found to be the most potent analog in producing hypotension and bradycardia with ED_{50}'s (doses producing half-maximal effects) of 2 and 90 μg/kg, respectively (Lake et al., 1997a). Moreover, at a maximally effective i.v. dose, HU-210 lowered blood pressure by 75–80 mm Hg for up to 2 h, whereas a maximal hypotensive dose of anandamide lowered blood pressure transiently (∼15 min) by no more than 45 mm Hg (Lake et al., 1997a). The way this different degree of blood pressure reduction is achieved, e.g. reduction of cardiac output or of systemic vascular resistance, is unknown. Our aim was to analyze and compare the regional and systemic hemodynamic changes in response to anandamide, its metabolically stable analog *R*-methanandamide (Abadji et al., 1994), and HU-210, and the sensitivity of these changes to inhibition by SR141716A in urethane-anesthetized rats. The results indicate that cannabinoids differently decrease cardiac output and total peripheral resistance, and that all three cannabinoids are strong vasodilators in the heart and the brain via the activation of vascular cannabinoid CB_1 receptors. Furthermore, anandamide and *R*-methanandamide cause mesenteric vasodilation that does not appear to involve activation of cannabinoid CB_1 receptors.

2. Methods

2.1. Drugs, chemicals

The source of the cannabinoids used has been provided elsewhere (Lake et al., 1997a; Járai et al., 1999). Hexamethonium, arginine vasopressin, and urethane were from Sigma (St. Louis, MO). ^{46}Scandium and ^{57}Cobalt-labeled microspheres (diameter 15.5 ± 0.1 μm) were from NEN Dupont (Boston, MA).

2.2. Animals, surgery

A total of 80 male Sprague–Dawley rats (300–380 g) were used, according to procedures approved by the Institutional Animal Care and Use Committee. The rats were anesthetized with urethane, 0.7 g/kg i.v. + 0.3 g/kg i.p., and heparinized, 500 I.U./kg i.v. The trachea was cannulated and body temperature was kept at 37 °C by using a heating pad. The femoral vein was cannulated for injection of drugs. In some animals, a cannula in the contralateral femoral vein was used to infuse heparinized donor blood to counteract cannabinoid-induced hypotension. A catheter in the right femoral artery was connected to a pressure transducer and physiograph for monitoring of BP and heart rate (HR). Reference blood samples were obtained via a catheter in the abdominal aorta. Microspheres were injected through a catheter implanted into the left ventricle via the right carotid artery.

2.3. Microsphere injection

Preparation of the microspheres for injection was as described (Stanek et al., 1983; Levine et al., 1984). About 30,000 microspheres (∼0.6 μCi) in 1 ml saline + 0.05% Tween-80 were infused into the left ventricle over a 30-s period, followed by a 0.5-ml saline flush. Aortic reference samples (1.5 ml) were withdrawn at 1 ml/min, starting immediately prior to microsphere infusion. ^{46}Sc- or ^{57}Co-labeled microspheres were used in random order to determine baseline and one post-intervention value in the same animal. The second microsphere injection was done at the peak of the hypotensive response, i.e. 3, 15 or 30 min following anandamide, *R*-methanandamide, or HU-210, respectively. Experiments were terminated by an overdose of the anesthetic, the organs listed in Table 2 were removed and weighed, and trapped radioactivity was counted in a gamma spectrometer (LKB) set at the photopeak of each isotope. The total injected radioactivity and the radioactivity in the arterial reference sample were also determined.

2.4. Determination of hemodynamic parameters

The uniform distribution of the microspheres was verified by the < 10% difference in radioactivity entrapped in the two kidneys. Cardiac Index (ml/min/100 g), organ blood flow (ml/min/g wet weight), systemic vascular resistance index (mm Hg. min/ml/100 g), organ vascular resistance (mm Hg.min/ml/g tissue) and stroke volume index (μl/100 g) were calculated as described (Gulati et al., 1996).

2.5. Statistical analyses

To compare the effect of cannabinoid agonists with baseline or post-antagonist values in different groups of animals, we used one way analysis of variance (ANOVA) followed by an adjusted *t*-test for multiple comparisons with P values corrected by the Bonferroni method. Only if the ANOVA showed significant difference did we use a paired, two-tailed *t*-test to compare the percentage change in a parameter when both baseline and post-intervention values were determined in the same animal. P values of < 0.05 were considered significant.

3. Results

3.1. Basal cardiovascular parameters

Fig. 1 contains the basal cardiovascular parameters in urethane-anesthetized controls and rats treated with 3 mg/kg SR141716A i.v. 30 min prior to the measurements. Of note, basal mean blood pressure and heart rate were not different from values we reported in conscious, chronically cannulated rats (Lake et al., 1997b). The well-documented hypotension following intraperitoneal administration of urethane can be avoided by the treatment protocol described by Maggi and Meli (1986) and employed here. SR141716A caused a small reduction in basal blood pressure ($P < 0.05$), which was only observed with somewhat higher doses in previous studies (Lake et al., 1997b). This decrease in blood pressure reflects a small, statistically insignificant drop in systemic vascular resistance index, whereas cardiac index remained unchanged. The basal control values for these two parameters and for stroke volume index are within reference values reported in the literature for urethane-anesthetized rats, as measured by the radiolabeled microsphere technique (Gulati et al., 1996).

3.2. Effects of HU-210

The dose of HU-210 used (10 μg/kg i.v.) is five times its hypotensive ED_{50} (2 μg/kg i.v.)(Lake et al., 1997a), and was selected to provide a substantial decrease in blood pressure. As illustrated in Fig. 1, HU-210 reduced mean blood pressure by 57 ± 5 mm Hg ($n = 5$, $P < 0.001$), whereas the modest reduction in heart rate was not significant. This is as expected from earlier findings that HU-210 is about 40 times less potent in eliciting bradycardia than hypotension (Lake et al., 1997a). In agreement with earlier findings, HU-210 failed to alter either blood pressure or heart rate in animals pretreated with 3 mg/kg SR141716A. HU-210 also caused significant reductions in cardiac index and stroke volume index, both being inhibited by SR141716A, whereas systemic vascular resistance was not significantly altered (Fig. 1). As documented in Table 1, HU-210 caused major reductions in vascular resistance in the coronaries ($-46 \pm 6\%$ change from baseline, $P < 0.01$) and the cerebral vasculature ($-61 \pm 3\%$, $P < 0.001$), and a more modest reduction in renal vascular resistance ($-23 \pm 8\%$, $P < 0.05$). These effects were absent in SR141716A-pretreated animals. In the remaining organs,

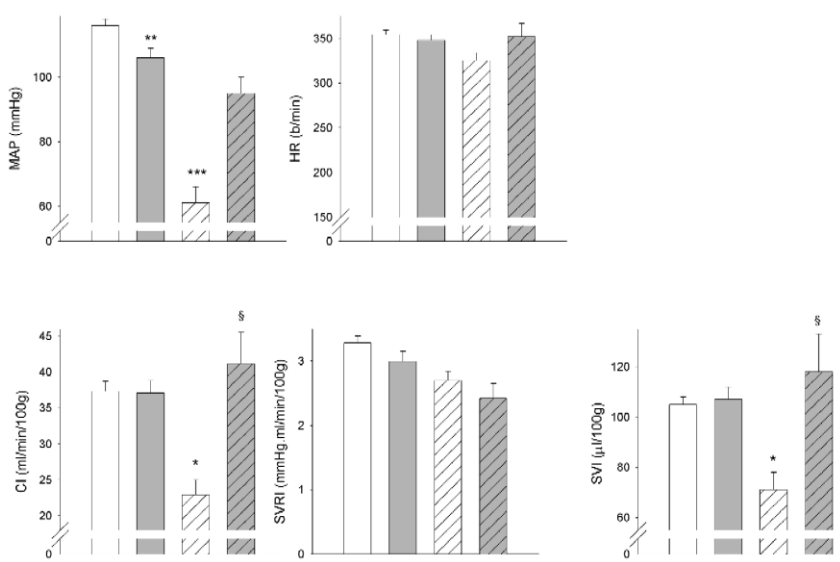

Fig. 1. The effects of HU-210 on systemic hemodynamics. Mean arterial pressure (MAP), heart rate (HR), cardiac index (CI), systemic vascular resistance (SVRI) and stroke volume index (SVI) were measured in urethane-anesthetized rats 15 min after the i.v. injection of vehicle (control, open bars, $n = 44$) or 6 μmol/kg SR141716A (shaded bars, $n = 24$), or 30 min after the i.v. injection of HU-210 (26 nmol/kg) in vehicle- (hatched bars, $n = 5$) or SR141716A-pretreated rats (shaded-hatched bars, $n = 6$). * indicate significant difference from drug-free control values ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). § indicates significant difference ($P < 0.05$) from corresponding values in the absence of SR141716A.

Table 1
Vascular resistance (mm Hg min/ml/g tissue) in different organs

Organ	Control (44)	SR (24)	HU-210 (5)	SR + HU-210 (6)	AN (5)	SR + AN (5)	M-AN (5)	SR + M-AN (4)
Kidney	33.6 ± 1.2	35.0 ± 1.8	23.4 ± 2.0 *	26.9 ± 2.7	30.8 ± 2.2	36.2 ± 4.2	29.1 ± 1.0 *	46.0 ± 3.0 * §
Heart	35.1 ± 2.5	28.3 ± 1.9	14.2 ± 3.6 *	23.5 ± 4.0§	14.2 ± 3 *	20.6 ± 4.6	15.4 ± 0.1 *	29.1 ± 4.8§
Brain	131 ± 8	131 ± 10	44.0 ± 4.8 * *	87.4 ± 9.1§	63 ± 15 * *	128 ± 17§	45.8 ± 4.5 *	119 ± 21§
Muscle	672 ± 68	472 ± 107	324 ± 72	510 ± 209	172 ± 27 *	162 ± 19 *	678 ± 88	285 ± 69 * §
Liver	375 ± 31	245 ± 26 *	281 ± 65	179 ± 22	246 ± 55	218 ± 31	218 ± 30 *	182 ± 44 *
Spleen	148 ± 20	254 ± 33	199 ± 60	182 ± 37	199 ± 38	633 ± 76 * §	128 ± 19	337 ± 59§
Stomach	212 ± 16	342 ± 37 *	204 ± 24	279 ± 57	151 ± 10	372 ± 55§	183 ± 20	392 ± 38 * §
Small intestine	116 ± 8	131 ± 10	71.5 ± 13.2	115 ± 18	119 ± 25	116 ± 11	56.7 ± 8.6 *	156 ± 50§
Large intestine	124 ± 9	147 ± 13	152 ± 32	159 ± 41	130 ± 25	203 ± 25	76.5 ± 7.5 *	202 ± 55§

Values are mean ± SE, number of experiments are in parenthesis. Significant difference (* $P < 0.05$; * * $P < 0.01$) from corresponding control value or (§, $P < 0.05$) from the response to the same agonist in the absence of SR141716A. SR: SR141716A (3 mg/kg i.v.); AN: anandamide (4 mg/kg i.v.); M-AN: *R*-methanandamide (5 mg/kg i.v.).

HU-210 did not influence vascular resistance. Only in the coronary and cerebral vasculatures was the decline in resistance large enough to result in an actual increase in blood flow, in spite of the marked reduction in blood pressure. The increase in flow was statistically significant in the brain (from 0.89 ± 0.18 to 1.42 ± 0.12 ml/min/g, or by +43 ± 9%, $P < 0.05$).

Earlier studies suggested that cannabinoids reduce blood pressure by decreasing sympathetic tone (Lake et al., 1997b; Malinowska et al., 1997; Niederhoffer and Szabo, 1999), and/or by direct vasodilation (Vidrio et al., 1996; Lake et al., 1997a). In order to distinguish between these possibilities, we treated three rats with the ganglionic blocker hexamethonium (20 μg/kg i.v.) to eliminate sympathetic tone, and then restored basal blood pressure and vascular tone by the continuous infusion of arginine vasopressin (0.5 μg/kg/min i.v.). Hexamethonium reduced blood pressure from 123 ± 5 to 74 ± 9 mm Hg ($P < 0.01$) and did not affect heart rate (293 ± 9 vs. 310 ± 22 beats/min), and the subsequent infusion of arginine vasopressin increased blood pressure to 124 ± 8 mm Hg and heart rate remained unchanged at 308 ± 22 beats/min. Although these last values were similar to baseline values in controls, cardiac index was significantly reduced and systemic vascular resistance significantly increased by the combination of hexamethonium and vasopressin (compare values in Fig. 1 and Table 2). As indicated by the data in Table 2, i.v. injection of HU-210 in the hexamethonium + vasopressin-treated rats reduced blood pressure by 58 ± 7 mm Hg, which was similar in magnitude to the response observed in control animals. However, the HU-210-induced decrease in cardiac index did not reach statistical significance under these conditions, and the small decrease in systemic vascular resistance was also not significant.

3.3. Effects of anandamide and its metabolically stable analog, R-methanandamide

Intravenous injection of 4 mg/kg anandamide (about twice its ED_{50}, Lake et al., 1997a) significantly ($P < 0.001$) reduced mean blood pressure by 40 ± 9 mm Hg, with no significant change in heart rate (Fig. 2). The greatest hypotension occurred at 3 min post-injection and blood pressure returned to baseline within 15 min. In contrast to the changes seen with HU-210, anandamide did not affect cardiac index or stroke volume index, but caused a significant ($P < 0.05$) reduction in systemic vascular resistance index (Fig. 2). *R*-methanandamide (5 mg/kg i.v.) also decreased blood pressure (by 36 ± 4 mm Hg, $P < 0.01$), but the effect reached a nadir at 15 min and was longer lasting than the effect of anandamide. *R*-methanandamide failed to alter blood pressure in the presence of SR141716A (Fig. 2).

Anandamide caused major reductions in vascular resistance in the brain (−51 ± 10%, $P < 0.01$), heart (−61 ± 9%, $P < 0.01$), and skeletal muscle (−79 ± 3%, $P < 0.001$) (Table 1). The effects in brain and heart were absent or significantly ($P < 0.001$) reduced after SR141716A pretreatment (brain: 0 ± 8%; heart: −20 ± 5%). *R*-methanandamide also caused SR141716A-sensitive reductions in vascular resistance in the brain (−57 ± 6% in the absence and −19 ± 14% in the presence of SR141716A, $P < 0.05$) and the heart (−44 ± 2% vs. −7 ± 12%, $P < 0.01$), but not in muscle. These differences were also evident in the pooled data shown in Table 1.

Table 2
The effect of HU-210 on hemodynamic parameters in the presence of ganglionic blockade and vasopressin

	Control	HU-210
MAP	124 ± 8	66 ± 3 *
HR	308 ± 22	300 ± 26
CI	19.5 ± 2.11	16.1 ± 4.05
SVRI	6.57 ± 1.1	4.96 ± 1.72
SVI	65 ± 11	54 ± 14

Values are means ± SE in three animals pretreated with hexamethonium (20 μg/kg iv.) and receiving vasopressin infusion (0.5 μg/kg/min iv.). MAP: mean arterial pressure, HR: heart rate, CI: cardiac index, SVRI: systemic vascular resistance index, SVI: stroke volume index.

* Significant difference ($P < 0.01$) from corresponding control value.

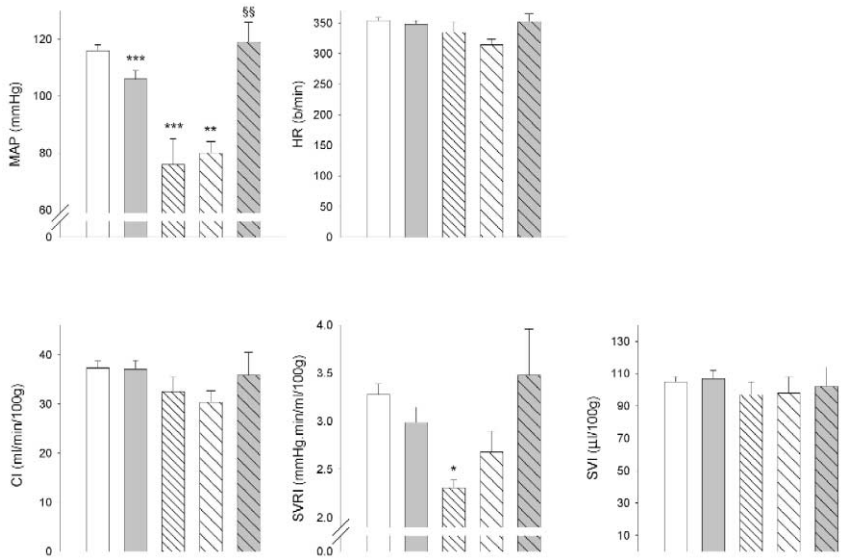

Fig. 2. The effects of anandamide and *R*-methanandamide on systemic hemodynamics. The same parameters as in Fig. 1 were measured under control conditions (open bars, $n = 44$), 15 min after the i.v. injection of 3 mg/kg SR141716A (shaded bars, $n = 24$), 3 min after the i.v. injection of 4 mg/kg anandamide (densely hatched bars, $n = 5$), or 15 min after the i.v. injection of 4 mg/kg *R*-methanandamide in control (lightly hatched bars, $n = 5$) or SR141716A-pretreated rats (shaded, lightly hatched bars, $n = 4$). * indicate significant difference from drug-free control values ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). §§ indicates significant difference ($P < 0.01$) from corresponding values in the absence of SR141716A.

In a previous study in the rat isolated, buffer-perfused mesenteric arterial preparation, anandamide and *R*-methanandamide elicited slowly developing vasodilation (maximum at 15–20 min) that lasted up to 1 h (Wagner et al., 1999; Járai et al., 1999). Accordingly, there was no significant change in mesenteric vascular resistance 3 min following the administration of anandamide (Table 1). However, in five animals in which the microspheres were injected 12 min after anandamide, at a time point when blood pressure was back to baseline, vascular resistance in the small intestine decreased from 116 ± 8 to 79 ± 2 mm Hg min/ml/g ($P < 0.05$). *R*-methanandamide caused major mesenteric vasodilation in both the small and large intestines, and both effects were completely blocked in the presence of SR141716A, as tested 15 min following the injection of *R*-methanandamide (see Table 1). It is also of interest that in the spleen, anandamide (but not HU-210) caused a significant increase in resistance in the presence of SR141716A (Table 1).

3.4. Coronary and cerebral vasodilation by HU-210 and R-methanandamide are not due to autoregulation

Since both HU-210 and *R*-methanandamide cause profound and long lasting hypotension, the major vasodilation observed in the coronary and cerebral vascular beds may result from autoregulation rather than the activation of cannabinoid CB_1 receptors in these blood vessels. To distinguish between these possibilities, in a group of animals the injection of either of these two cannabinoids was followed by the i.v. infusion of 10–15 ml of heparinized blood from normal donor rats, in order to restore blood pressure to near control levels by the time of the second microsphere injection. Under these conditions, HU-210 still elicited a significant decrease in cardiac index with no decrease in systemic vascular resistance index, whereas

Table 3
The effects of HU-210 (10 µg/kg i.v.) and *R*-methanandamide (5 mg/kg i.v.) on hemodynamic parameters (A) and blood flow (ml/min/g) and vascular resistance (mm Hg min/ml/g) in selected organs (B) after blood pressure restoration by volume replacement

(A)

	Control	HU-210	Control	*R*-methanandamide
MAP	119 ± 11	107 ± 8	122 ± 6	117 ± 8
HR	311 ± 4	285 ± 23	333 ± 11	304 ± 12
CI	24.6 ± 3.19	15.9 ± 3.9 *	32.9 ± 4.33	44.9 ± 5.9
SVRI	5.05 ± 0.67	9.15 ± 3.33	4.00 ± 0.53	2.82 ± 0.42 *
SVI	79 ± 9	54 ± 12 *	98 ± 11	147 ± 16 *

(B)

Organ	Control	HU-210	Control	*R*-methanandamide
Heart flow	2.94 ± 0.36	4.97 ± 0.58 *	2.11 ± 0.48	5.62 ± 0.88 *
Resistance	42.7 ± 3	24.3 ± 2.9 * *	71.2 ± 15	24.8 ± 6.7 *
Brain flow	0.76 ± 0.07	1.7 ± 0.18 * *	0.69 ± 0.11	1.93 ± 0.36 *
Resistance	161 ± 5	70 ± 8 * *	202 ± 30	73.4 ± 15.5 *
Spleen flow	0.86 ± 0.21	0.81 ± 0.27	1.00 ± 0.17	0.33 ± 0.04 *
Resistance	205 ± 71	259 ± 109	139 ± 27	373 ± 51 * *

Each of the drugs was tested in five animals, values are means ± SE. To restore MAP following cannabinoid injection, animals received 10–15 ml donor blood i.v. (see text). Asterisks indicate significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$) from corresponding control value. Abbreviations and units as in Table 2.

R-methanandamide caused a decrease in systemic vascular resistance index and an increase in stroke volume index (Table 3A). Furthermore, both cannabinoids caused statistically significant increases in brain and coronary blood flows and corresponding decreases in vascular resistance. Interestingly, *R*-methanandamide also caused a major decrease in blood flow and increase in resistance in the spleen in these animals (Table 3B).

4. Discussion

The profound and long lasting hypotension elicited via activation of peripheral cannabinoid CB_1 receptors is unsurpassed by hypotension triggered through any other mechanism (Lake et al., 1997a). Known ligands for these receptors include not only plant-derived and synthetic cannabinoids, but also endogenous substances such as anandamide (Devane et al., 1992). Both anandamide and the synthetic agonist HU-210 elicit hypotension, and although anandamide is less efficacious and its effect is much shorter lasting than that of HU-210, the effects of both compounds are inhibited by the selective cannabinoid CB_1 antagonist SR141716A (Lake et al., 1997a), which suggests a common underlying mechanism. However, the present findings indicate that the hypotensive effect of HU-210 is predominantly due to a reduction in cardiac output, whereas the similar effect of anandamide can be attributed to a decrease in total peripheral resistance. This difference is unlikely to be due to the greater hypotension elicited by HU-210 than anandamide. In a recent study in rats in which cardiac output was measured by the thermodilution technique, the exclusive role of decreased vascular resistance in the hypotensive action of anandamide was documented for both 4 and 10 mg/kg doses of anandamide, the latter dose causing a 50 mm Hg decrease in blood pressure, which is comparable to the effect of 10 μg/kg HU-210 in the present study (Garcia et al., 2001).

The reduction in cardiac output by HU-210 is completely blocked by SR141716A. In doses similar to that used in the present study, SR141716A is a selective antagonist of cannabinoid CB_1 receptors (Rinaldi-Carmona et al., 1994), which implicates cannabinoid CB_1 receptors in the effect of HU-210 on cardiac output, but does not indicate their location. Although there is no published report on the cardiovascular effects of centrally administered HU-210, neither anandamide (Varga et al., 1995) nor WIN 55,212-2 ({−}-3-[2-hydroxy-4-{1,1-dimethylheptyl} phenyl]-4-[3-hydroxy-propyl]cyclohexan-1-ol; Malinowska et al., 1997), another potent cannabinoid CB_1 agonist (Showalter et al., 1996), produce hypotension upon intracisternal or intracerebroventricular administration, which discounts the possibility of a central site of action. Both of these ligands inhibit the pressor response to preganglionic sympathetic nerve stimulation in decerebrate (Malinowska et al., 1997) or barodenervated rats (Varga et al., 1996), and WIN 55,212-2 was found to reduce blood pressure and norepinephrine spillover in an SR141716A-sensitive manner in pithed rabbits (Niederhoffer and Szabo, 1999). Furthermore, there is evidence for presynaptic cannabinoid CB_1 receptors located on cardiac sympathetic nerve terminals, stimulation of which inhibits norepinephrine release (Ishac et al., 1996). Together, these findings support the notion that the decrease in cardiac output by HU-210 may be due to presynaptic inhibition of sympathetic tone to the heart, resulting in a decrease in stroke volume and heart rate. This is also supported by the finding that once sympathetic tone had been eliminated by ganglionic blockade, HU-210 no longer caused a significant decrease in cardiac output or heart rate (Table 2). The alternative possibility that HU-210 reduces cardiac index by increasing vagal tone is unlikely, as presynaptic cannabinoid CB_1 receptors identified on cholinergic nerve terminals are also inhibitory (Coutts and Pertwee, 1997). Although a cannabinoid CB_1 receptor-mediated direct myocardial depressor effect cannot be ruled out, cannabinoid CB_1 receptor mRNA was undetectable in rat cardiac tissue (Ishac et al., 1996). The situation is different in humans whose myocardium has detectable levels of cannabinoid CB_1 receptor mRNA (Galiegue et al., 1995) and in whom marijuana smoking causes SR141716A-sensitive tachycardia (Huestis et al., 2001).

The question arises as to why was there no decrease in cardiac output in response to anandamide. It is possible that the level of expression of cannabinoid CB_1 receptors is much lower on sympathetic nerve terminals than in vascular tissue. If this is the case, a partial agonist such as anandamide is expected to be much less efficacious at sympathetic neuronal than at vascular cannabinoid CB_1 receptors, whereas a potent full agonist such as HU-210 could be equally effective at both.

Both anandamide and HU-210 elicited marked cerebral and coronary vasodilation, inhibited by SR141716A. There is strong evidence for the presence of cannabinoid CB_1 receptors in cerebrovascular smooth muscle cells that mediate vasodilation (Gebremedhin et al., 1999), and microvascular endothelial cells in the cerebral vasculature also contain functional cannabinoid CB_1 receptors (Chen et al., 2000). Such receptors may be involved in the observed reduction in cerebrovascular resistance in response to HU-210 and anandamide, as well as in the reported increase in cerebral blood flow in marijuana smokers (Mathew et al., 1992). In a recent study using bovine isolated coronary arteries, the dilator effect of anandamide was unaffected by SR141716A, but could be blocked by an inhibitor of anandamide degradation, suggesting the role of a non-cannabinoid metabolite (Pratt et al., 1998). This latter mechanism is unlikely to account for the present findings, where the metabolically stable analog *R*-methanandamide produced similar coronary and cerebral vasodilation as anandamide, and in both cases the effects were antagonized by SR141716A. It is possible that

cannabinoid CB_1 receptors absent from larger coronary vessels are present in resistance arterioles. An analogous situation regarding presynaptic cannabinoid CB_1 receptors has been reported in rats, where functional cannabinoid CB_1 receptors were found to be present in neurons innervating resistance vessels, but not in those innervating larger conduit arteries (Malinowska et al., 1997).

Cannabinoid CB_1 receptor mRNA has also been detected in the vascular endothelium (Deutsch et al., 1997; Sugiura et al., 1998; Liu et al., 2000), and vascular cannabinoid CB_1 receptors present in smooth muscle and/or endothelium are likely to mediate the vasodilator effect of cannabinoids in certain vascular beds, most prominently in the coronary and cerebral circulation. The alternative possibility that the vasodilation observed in these organs was an autoregulatory response to the profound hypotension caused by the cannabinoids can be ruled out. When blood pressure was maintained through volume replacement following the administration of cannabinoids, the degree of coronary and cerebral vasodilation remained unchanged compared to that seen in the absence of volume replacement.

Earlier findings in anesthetized rats indicate that anandamide and THC, but not synthetic cannabinoids such as HU-210, elicit a transient pressor response before lowering blood pressure, and this pressor component is unaffected either by sympathetic blockade or SR141716A (Varga et al., 1995; Lake et al., 1997a). The present finding that anandamide, but not HU-210, causes SR141716A-resistant vasoconstriction in the spleen (Table 1) suggests that vasoconstriction in certain vascular beds, such as the spleen, may be the underlying mechanism.

We recently reported that anandamide and *R*-methanandamide caused prolonged vasodilation in the rat isolated, buffer-perfused mesenteric vascular bed, which could be inhibited by SR141716A. However, HU-210, WIN 55,212-2 and THC were ineffective or caused vasoconstriction, which suggested that receptors other than cannabinoid CB_1 receptors may be involved (Wagner et al., 1999). This possibility was further confirmed by the persistence of anandamide-induced mesenteric vasodilation in mice deficient in cannabinoid CB_1 receptors or both cannabinoid CB_1 and CB_2 receptors and the ability of certain non-psychoactive cannabinoids to elicit mesenteric vasodilation (Járai et al., 1999). The findings in the present study are compatible with this interpretation: anandamide and *R*-methanandamide both elicited slowly developing, SR141716A-sensitive mesenteric vasodilation, whereas HU-210 had no such effect. The SR141716A-sensitive, mesenteric vasodilator effect of anandamide was also recently documented in a study in which mesenteric blood flow was monitored by Doppler sonography (Garcia et al., 2001). This difference in the effect of anandamide and HU-210 on the mesenteric circulation may also be responsible, at least in part, for the difference in their effect on systemic vascular resistance in spite of similar reductions in cerebrovascular and coronary resistance. Whether the mesenteric vascular effects of anandamide and *R*-methanandamide under the present in vivo conditions are mediated by endothelial 'anandamide receptors' (Járai et al., 1999), or by vanilloid VR_1 receptors and the subsequent release of calcitonin gene-related peptide from sensory nerves, as proposed in recent studies using isolated blood vessels (Zygmunt et al., 1999) or the isolated, perfused mesenteric bed (Ralevic et al., 2000), remains to be determined.

In summary, we have documented the hemodynamic profile of cannabinoid ligands. The presence of multiple mechanisms involved in profound and organ-specific vasodilation could serve as the basis for further studies into the possible cardiovascular regulatory functions of endocannabinoids, and for the development of novel therapeutic agents targeting these receptors.

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