Endocannabinoids Acting at Cannabinoid-1 Receptors Regulate Cardiovascular Function in Hypertension

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Background—Endocannabinoids are novel lipid mediators with hypotensive and cardiodepressor activity. Here, we examined the possible role of the endocannabinergic system in cardiovascular regulation in hypertension.

Methods and Results—In spontaneously hypertensive rats (SHR), cannabinoid-1 receptor (CB1) antagonists increase blood pressure and left ventricular contractile performance. Conversely, preventing the degradation of the endocannabinoid anandamide by an inhibitor of fatty acid amidohydrolase reduces blood pressure, cardiac contractility, and vascular resistance to levels in normotensive rats, and these effects are prevented by CB1 antagonists. Similar changes are observed in 2 additional models of hypertension, whereas in normotensive control rats, the same parameters remain unaffected by any of these treatments. CB1 agonists lower blood pressure much more in SHR than in normotensive Wistar-Kyoto rats, and the expression of CB1 is increased in heart and aortic endothelium of SHR compared with Wistar-Kyoto rats.

Conclusions—We conclude that endocannabinoids tonically suppress cardiac contractility in hypertension and that enhancing the CB1-mediated cardiodepressor and vasodilator effects of endogenous anandamide by blocking its hydrolysis can normalize blood pressure. Targeting the endocannabinoid system offers novel therapeutic strategies in the treatment of hypertension. (Circulation. 2004;110:1996-2002.)

Key Words: hypertension ■ blood pressure ■ contractility ■ endocannabinoids ■ pharmacology

Hypertension is a major health problem and, when untreated, predisposes to cardiovascular morbidity and premature death. Effective treatment of hypertension reduces complications and improves life expectancy; however, side effects of available antihypertensive medications and differences in their efficacy in preventing end-organ damage justify the search for antihypertensive agents that act through novel mechanisms of action.

Δ⁹-Tetrahydrocannabinol, the psychoactive ingredient of marijuana, lowers blood pressure and heart rate in experimental animals, and hypotension has been reported after chronic marijuana use in humans. The endogenous cannabinoid ligands arachidonoyl ethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG) also lower blood pressure and heart rate in rodents. Cannabinoids interact with G protein-coupled receptors to produce their effects. To date, 2 such receptors have been identified: cannabinoid-1 receptors (CB1), expressed at high levels in the brain and also present in peripheral tissues, including the heart and the vasculature, and CB2, expressed by immune and hematopoietic cells. Cannabinoids fail to lower blood pressure after selective blockade of CB1, or in CB1-knockout mice, which implicates CB1 in this effect.

Treatment of normotensive rats and mice with CB1 antagonists alone does not affect blood pressure, and baseline blood pressure is similar in CB1-knockout mice and their wild-type littermates, which indicates that CB1 receptors are not tonically active. This is also suggested by the lack of hypotension after inhibition of anandamide transport, in agreement with the relatively modest hypotensive effect of anandamide in normotensive animals. However, an increase in the hypotensive efficacy of cannabinoids has been noted in spontaneously hypertensive rats (SHR). Therefore, we examined whether endogenous cannabinoids may have a cardiovascular regulatory function in hypertension. Three different models of experimental hypertension were used to reduce the possibility of detecting changes limited to a given model rather than to hypertension itself. The results document tonic activation of cardiac and vascular CB1 in hypertension that limits increases in blood pressure and cardiac contractility. They also indicate that upregulation of CB1 is responsible for this tone and that increasing it by...
inhibiting the inactivation of endogenous anandamide can normalize blood pressure and cardiac contractile performance in hypertension.

Methods

Materials
Anandamide, the CB1 receptor antagonist AM251, and the anandamide transport inhibitors AM404 and OMDM-2 were from Tocris; the fatty acid amidohydrolase inhibitor URB597 was from Cayman Chemicals; and the CB1 antagonist SR141716 and the CB2 antagonist SR144528 were from the National Institute on Drug Abuse drug supply program. The synthetic CB agonist HU-210 was a gift from R. Mechoulam (Hebrew University, Jerusalem, Israel). Drugs were mixed in corn oil and sonicated for 5 minutes at 4°C. The mixture was added to 4 parts of Pluronic F68 (Sigma-Aldrich) solution (40 mg/mL) dissolved in water and sonicated to obtain a stable suspension for bolus intravenous injections.

Animals
Rats were obtained from Harlan (Indianapolis, Ind). Male, 8- to 10-month-old SHR, age-matched male Wistar Kyoto rats (WKY), and 8- to 10-week-old male Sprague-Dawley rats were maintained on standard rat chow and water ad libitum. Dahl salt-sensitive and salt-resistant rats (male, Rapp strain, 6 weeks old) were maintained for 4 weeks on rat chow containing either 0.12% or 8% NaCl. Systolic blood pressure monitored daily by the tail-cuff technique was 120±11 mm Hg (salt-sensitive, 0.12% NaCl), 180±14 mm Hg (salt-sensitive, 8% NaCl), and 118±9 mm Hg (salt-resistant, 8% NaCl). Hypertension was induced in Sprague-Dawley rats by chronic infusion of angiotensin II (60 ng/min) via an osmotic minipump, as described previously. Rats were used 10 to 12 days after implantation.

Figure 1. Hemodynamic effects of CB1 antagonist SR141716 (3 mg/kg), FAAH antagonist URB597 (10 mg/kg), and anandamide (AEA; 10 mg/kg) in WKY (■) and SHR (○). Drugs were injected at 0 minutes. Values are mean±SEM. *P<0.05 of corresponding baseline values (n=4 to 10 for each condition). HR indicates heart rate.
Hemodynamic Measurements

Rats were anesthetized with pentobarbital sodium (60 mg/kg IP) and tracheotomized to facilitate breathing. Animals were placed on controlled heating pads, and core temperature, measured via a rectal probe, was maintained at 37 °C. A microtip pressure-volume catheter (SPR-838; Millar Instruments) was inserted into the right carotid artery and advanced into the left ventricle (LV) under pressure control as described previously.

Immunohistochemistry

Affinity purified CB1, polyclonal antibody was raised in rabbit against the first 100 amino acids of human CB1. Formalin-fixed, paraffin-embedded aortic sections deparfaffinized and rehydrated in PBS were preincubated in blocking buffer (1% BSA, 0.6% Triton-X-100 in PBS, pH 7.4) for 1 hour, then incubated with the primary antibody at 24 °C for 24 hours. Sections were washed, and endogenous peroxidase activity was suppressed with 3% H2O2 in PBS for 10 minutes. Sections were washed 3 times and placed on a computer. Heart rate, maximal LV systolic pressure, MAP, and maximal slope of systolic pressure increment (+dP/dt) were computed with a cardiac pressure-volume analysis program (PVAN2.9; Millar). Cardiac output calculated and corrected according to in vitro and in vivo volume calibrations with PVAN2.9 was normalized to body weight (cardiac index [CI]). Total peripheral resistance index (TPRI) was calculated by the equation TPRI = MAP/CI. In 3 experiments, drugs were microinjected into the fourth cerebral ventricle as described previously.

Western Blotting

Frozen myocardial tissue from SHR and WKY was minced and homogenized in ice-cold lysis buffer (1% BSA, 0.6% Triton-X-100 in PBS, pH 7.4) for 1 hour, then incubated with the primary antibody at 24 °C for 1 hour and then at 4 °C for 24 hours. Sections were washed, and endogenous peroxidase activity was suppressed with 3% H2O2 in PBS for 10 minutes. Sections were washed 3 times in PBS and incubated in biotinylated secondary antibody (goat-anti-rabbit IgG 1:1000; Vector) and in ABC reagents according to the manufacturer’s instructions. Color was developed in 3,3’-diaminobenzidine and staining visualized by light microscopy and quantified by densitometry. Specificity of the reaction was controlled by preabsorption of the antibodies with 1 to 10 µg/mL of the immunizing peptide.

Measurement of Endocannabinoid Levels

Tissue levels of anandamide and 2-AG were quantified by liquid chromatography/in-line mass spectrometry, as described in detail elsewhere. Values are expressed as femtomoles or picomoles per milligram of wet tissue.

Statistical Analyses

Time-dependent variables were analyzed by ANOVA followed by the Dunnett post hoc test. In other cases, the Student t test was used, as appropriate. Values with P<0.05 were considered statistically significant.
induced hypertension (Figure 3b). In salt-sensitive Dahl rats, SR141716 had no effect on mean blood pressure in normotensive animals kept on a low-salt diet but elicited a pressor response in animals made hypertensive by an 8% NaCl-containing diet. Salt-resistant Dahl rats kept on the same 8% NaCl diet also decreased by treatment with the anandamide transport inhibitors AM404 (10 mg/kg, 0.1 pmol/mL), and the change in pressure/volume effects of anandamide were similar to those of URB597 (Figure 2g through 2i), and anandamide-induced hypotension was potentiated in rats with angiotensin II–induced hypertension (Figure 3c). The effects of the synthetic CB agonist HU210 were similarly potentiated, with its hypotensive EC$_{50}$ reduced from 5.9 to 1.5 μg/kg and its maximal hypotensive effect increased from $-39 \pm 14$ to $-108 \pm 11$ mm Hg in WKY (n=6) versus SHR (n=6, $P<0.01$), respectively.

Because anandamide is a known ligand for vanilloid receptors (VR1), we tested the ability of the VR1 antagonist capsazepine to inhibit the hypotensive response to URB597 or anandamide in SHR. As illustrated in Figure 5, the hypotensive response to either agent was not affected by capsazepine pretreatment.

**Upregulation of Cardiac and Vascular Endothelial CB$_1$ in SHR**

The expression of CB$_1$ in the myocardium and aorta of SHR and WKY was analyzed by immunohistochemistry and Western blotting with a CB$_1$ antibody. CB$_1$ expression was significantly greater in both cardiac tissue and the aortic endothelium of SHR than in WKY (Figure 6).

**Endocannabinoid Levels and FAAH Expression in WKY and SHR**

In myocardium, FAAH expression was unexpectedly increased and anandamide levels correspondingly decreased in SHR compared with WKY (Figure 7), with no difference in 2-AG levels. However, URB597 treatment caused a greater increase in myocardial anandamide levels in SHR than in WKY (Figure 7). Interestingly, a similar increase was observed when myocardial anandamide levels in SHR myocardium were determined under control conditions ($10.8 \pm 0.4$ fmol/mg, n=3) and 10 minutes after the intravenous injection of 10 mg/kg anandamide, ie, at the time of the peak hypotensive response ($14.9 \pm 1.3$ fmol/mg, n=4, $P<0.05$). Plasma levels of anandamide were not different at the same time points ($1.43 \pm 0.05$ versus $1.53 \pm 0.1$ pmol/mL), and the URB597-induced increase in plasma anandamide ($1.78 \pm 0.20$ versus $2.44 \pm 0.20$ pmol/mL, or 1.3-fold) was also smaller than the increase observed in the myocardium (2.2-fold; Figure 7b).
Discussion

The present findings provide evidence for a novel cardiovascular regulatory mechanism mediated by endocannabinoids acting at CB1, stimulation of which lowers blood pressure through reductions in both cardiac contractility and vascular resistance. In various forms of hypertension, this endocannabinoid system becomes tonically active, probably owing to an upregulation of cardiac and vascular endothelial CB1. Furthermore, increased activation of CB1 through blocking of the metabolic degradation or intracellular uptake of the endocannabinoid anandamide normalizes blood pressure, offering a novel approach for the pharmacotherapy of hypertension.

CB1 antagonists did not affect blood pressure in normotensive rats, and inhibitors of FAAH or anandamide transport were similarly ineffective, which indicates that the cannabinoid system is inactive under normotensive conditions. In contrast, CB1 antagonists elicited sustained further increases in blood pressure in rats with 3 different forms of hypertension, which suggests that the elevated basal blood pressure itself is responsible for the tonic activation of CB1. Interestingly, CB1 antagonists increased cardiac contractile performance without significantly affecting peripheral vascular resistance, which suggests that the primary target of endocannabinoids in hypertension is the heart. The lack of change in heart rate further suggests that they decrease contractility directly rather than through inhibition of sympathetic tone. Correspondingly, inhibition of FAAH by URB597 decreased cardiac contractility without affecting heart rate. However, URB597 reduced both cardiac contractility and peripheral vascular resistance, and these effects are remarkably similar to those of exogenous anandamide, except that anandamide also caused bradycardia. This reduction in vascular resistance suggests that CB1 effects on vascular tone require higher levels of endocannabinoids than does suppression of cardiac contractile performance, which could result from differences in the relative concentration of endogenous ligand and receptor in the 2 tissues. URB597 preferentially increases anandamide rather than 2-AG levels in the brain, and the same was shown here for the heart. Thus, anandamide or a related fatty acid amide, rather than 2-AG, may be responsible for the activation of CB1 in hypertension.

CB1 receptors in the vasculature and in the myocardium, they mediate negative inotropy, and both of these sites may be implicated in the hypotensive effect of anandamide. Additionally, presynaptic CB1 receptors on sympathetic nerve terminals inhibit norepinephrine release and may mediate anandamide-induced bradycardia. The lack of bradycardia after URB597 may be related to low tissue levels of anandamide, FAAH, or both at cardiac sympathetic nerve terminals. Central administration of SR141716 at a dose that blocks cannabinoid-induced behavioral effects caused no hemodynamic changes in SHR, which rules out a central site of action.

Anandamide can elicit vasodilation through an additional G/iG o -coupled receptor distinct from CB1 or CB2, which is inhibited by SR141716 but not by other CB1 antagonists such as AM251 and SR141716 were equally effective in eliciting pressor and cardiostimulatory responses and in blocking the depressor effects of URB597 in hypertensive rats. This indicates that their likely target is CB1 rather than the non-CB1/non-CB2...
The vanilloid VR1 receptor can also mediate anandamide-induced vasodilation; however, it is not involved in the effects described here, because SR141716, which does not affect VR1-mediated vasodilation by capsaicin, completely blocked the effect of URB597 (Figure 2f), and the VR1 antagonist capsazepine failed to influence the hypotensive response to URB597 or anandamide (Figure 5). Furthermore, we found no difference in the hypotensive response to anandamide in VR1−/− mice and their controls, and SR141716 completely blocked the effect in both.

Responses to CB1 agonists and URB597 were greatly potentiated in hypertensive rats, which suggests increased target-organ sensitivity rather than increased endocannabinoid levels as the underlying mechanism. Indeed, an increase in CB1 expression was evident in both the myocardium and the aortic endothelium of SHR versus WKY, although receptor expression in the aorta may not reflect that in resistance vessels. Additional changes in the coupling of CB1 to signaling pathways, such as may result from an upregulation of inhibitory G proteins, are also possible. Tissue levels of endocannabinoids were unchanged or even reduced in SHR compared with WKY, although the greater rise in myocardial anandamide after blockade of FAAH in SHR may also contribute to their increased cardiovascular response to the FAAH antagonist.

The increased reactivity of CB1 in hypertension can be exploited therapeutically. Direct activation of CB1 by plant-derived or synthetic agonists is unacceptable because of psychotropic effects. However, by blocking the inactivation of endocannabinoids, a more selective action may be achieved due to the distinct tissue distribution of FAAH. Indeed, in a recent study, URB597 elicited CB1-mediated anxiolytic and analgesic responses without causing other cannabinoid-like effects, such as hypothermia and catalepsy. Although a higher dose of URB597 was required to reduce blood pressure in SHR, the effect was completely blocked by CB1 antagonists, and URB597 was ineffective in normotensive rats. This suggests that the action of URB597 is fully accounted for by endocannabinoid-mediated stimulation of CB1.

The elevated systolic performance of the hypertensive ventricle has been attributed to a hypertrophic response that compensates for the increased wall stress. Blocking endocannabinoid inactivation reduces both the elevated arterial pressure and the increased LV contractile performance without affecting the same parameters in normotensive animals. This offers an innovative approach to the pharmacotherapy of hypertension by simultaneously targeting and normalizing the inappropriately increased LV contractile performance and vascular tone.

References


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_Circulation_. 2004;110:1996-2002; originally published online September 27, 2004; doi: 10.1161/01.CIR.0000143230.23252.D2
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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