Cardiovascular Effects of 2-Arachidonoyl Glycerol in Anesthetized Mice


Abstract—Cannabinoids, including the endogenous ligand anandamide, elicit pronounced hypotension and bradycardia through the activation of CB1 cannabinoid receptors. A second endogenous cannabinoid, 2-arachidonoyl glycerol (2-AG), has been proposed to be the natural ligand of CB1 receptors. In the present study, we examined the effects of 2-AG on mean arterial pressure and heart rate in anesthetized mice and assessed the role of CB1 receptors through the use of selective cannabinoid receptor antagonists and CB1 receptor knockout (CB1–/–) mice. In control ICR mice, intravenous injections of 2-AG or its isomer 1-AG elicit dose-dependent hypotension and moderate tachycardia, whereas anandamide is stable under the same conditions. A metabolically stable ether analogue of 2-AG causes prolonged hypotension and bradycardia in ICR mice, and both effects are completely blocked by SR141716A, whereas anandamide is stable under the same conditions. A metabolically stable ether analogue of 2-AG causes prolonged hypotension and bradycardia in ICR mice, and both effects are completely blocked by SR141716A, whereas the same dose of 2-AG-ether does not influence blood pressure and heart rate in CB1–/– mice. These findings are interpreted to indicate that exogenous 2-AG is rapidly degraded in mouse blood, probably by a lipase, which masks its ability to interact with CB1 receptors. Although the observed cardiovascular effects of 2-AG probably are produced by an arachidonate metabolite through a noncannabinoid mechanism, the CB1 receptor–mediated cardiovascular effects of a stable analogue of 2-AG leaves open the possibility that endogenous 2-AG may elicit cardiovascular effects through CB1 receptors. (Hypertension. 2000;35:679-684.)

Key Words: receptors • mice • hypotension • cannabinoid

It has long been known that cannabinoids have powerful cardiovascular effects. In anesthetized rats, Δ9- tetrahydrocannabinol (THC) causes prolonged hypotension and bradycardia.1 In 1992, arachidonyl ethanolamide (anandamide) extracted from porcine brain was identified as an endogenous cannabinoid based on its ability to bind to the brain cannabinoid receptor and to mimic the neurobehavioral effects of THC.2 Anandamide also elicits hypotension and bradycardia in anesthetized rats through a peripheral mechanism.3–5 These effects are mediated by CB1 cannabinoid receptors,6 as indicated by their susceptibility to blockade by SR141716A,7 a selective CB1 receptor antagonist,7 and by their absence in CB1 receptor knockout (CB1–/–) mice.8 In 1995, 2-arachidonoyl glycerol (2-AG), found in canine gut9 and rat brain,10 was identified as another endogenous cannabinoid. The concentration of 2-AG in the brain is several hundredfold higher than that of anandamide,10,11 and, unlike anandamide, which often acts as a partial agonist,12–15 2-AG is a full agonist at CB1 receptors.11,16,17 This has led to the proposal that the CB1 receptor is a 2-AG receptor.17

Endogenous cannabinoids also have been implicated in the local control of vascular tone. In certain vascular preparations, SR141716A inhibits the vasodilation induced by the endothelium-derived hyperpolarizing factor,18,19 and anandamide has been identified in vascular endothelial cells.20 SR141716A also antagonizes hemorrhagic21 and endotoxin-induced hypotension,22 and circulating macrophages produce anandamide under such conditions, which suggests a paracrine mechanism in the control of vascular tone.21,22 Recently, 2-AG also has been identified in vascular endothelial

Received June 30, 1999; first decision August 26, 1999; revision accepted September 27, 1999.
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We and others have reported that intravenous injection of 2-AG in anesthetized rats causes dose-dependent hypotension. However, this effect was antagonized by SR141716A less effectively than anandamide-induced hypotension, and, unlike anandamide, which causes CB1 receptor-mediated bradycardia, 2-AG elicits tachycardia unaffected by SR141716A. In the present study we analyzed the cardiovascular effects of 2-AG in anesthetized mice and assessed the role of CB1 receptors through the use of selective antagonists and CB1 knockout mice. The results indicate that 2-AG is rapidly degraded in mouse blood, and the observed hypotensive effect does not involve CB1 receptors but probably is caused by an arachidonic acid (AA) metabolite. In contrast, a metabolically stable analogue of 2-AG causes CB1 receptor-mediated hypotension and bradycardia.

Methods

Cardiovascular Measurements

Ninety male ICR mice (25 to 30 g) from Harlan (Indianapolis, Ind) were used. Male and female CB1−/− littermates (64 animals) have been described. Animals were kept under a 12:12 hours light/dark cycle, fed standard mouse chow, and had access to drinking water ad libitum. Mice were anesthetized with 60 mg/kg IP sodium pentobarbital, supplemented as needed to maintain stable anesthesia. The jugular vein and the carotid artery were cannulated with PE-10 tubing filled with heparinized saline for intravenous drug injections and monitoring blood pressure (BP) and heart rate (HR), respectively. The arterial cannula was connected to a pressure transducer and physiograph. HR was monitored (alternating at 0.2 seconds), as specified in the legend of Figure 1A. The same dose of SR141716A nearly completely blocked the hypotension and attenuated the bradycardia. The dose- or time-dependent effects of drugs on BP and HR and on neurobehavioral parameters were analyzed by ANOVA followed by Tukey’s post hoc test. Differences from corresponding baseline values with a value of P < 0.05 were considered statistically significant.

Analysis of 2-AG and Anandamide by Liquid Chromatography/Mass Spectrometry

For assessing the stability of 2-AG and anandamide in mouse blood, 200-μL aliquots of heparinized blood were withdrawn from ICR mice and incubated with 100 μg of synthetic 2-AG (final concentration 1.32 mmol/L) or 100 μg of anandamide (1.44 mmol/L) for 1 to 5 minutes at 37°C. At the end of the incubation, the whole blood was extracted 3 times with 500 μL of cold diethyl ether + 20 μL 1.0N HCl; the organic phase was dried under a stream of nitrogen and resuspended in 100 μL methanol. The sample was fractionated by reversed-phase high-performance liquid chromatography on an ODS column (Supelcosil, 5 μm, 4.6 mm×15 cm), with the use of a mobile phase of methanol/water/acetic acid (85:15:0.03 vol/vol/vol) at a flow rate of 1 mL/min on a Waters 2690 system. This was followed in line by mass spectroscopic analysis on a Micromass Quattro II mass spectrometer equipped with an atmospheric pressure chemical ionization source. Two selected ions were simultaneously monitored (alternating at 0.2 seconds), as specified in the legend of Figure 5.

Drugs

2-AG and 1- (or 3-)arachidonoyl glycerol (1-AG) were synthesized as described earlier. The synthesis of an ether-linked analogue of 2-AG, 2-eicosatetraenylglycerol (2-AG-ether), has been detailed by the activation of SR141716A-sensitive receptors. SR141716A also may play a role in the local control of vascular tone mediated hypotension and bradycardia, unlike anandamide, which causes CB1 receptor-mediated hypotension, and, therefore, does not involve CB1 receptors but probably is caused by an arachidonic acid (AA) metabolite. In contrast, a metabolically stable analogue of 2-AG causes CB1 receptor-mediated hypotension and bradycardia.
dia elicited by an equihypotensive dose (11.5 nmol [4 μg/g]) of anandamide (Figure 1B). Basal BP in the absence or presence of SR141716A was 74±3 versus 76±5 mm Hg, and HR was 365±13 versus 414±16 bpm. The dose dependence of the hypotensive response to 2-AG is illustrated in Figure 2A. At none of the doses tested was any inhibition by SR141716A observed. 1-AG, which has been described to have similar potency as 2-AG in producing cannabinoid effects,11 also caused dose-dependent hypotension, which was also not blocked by SR141716A pretreatment (Figure 2B). In fact, SR141716A potentiated the effect of 1-AG, which was significant at the 2.6 nmol/g dose. Because the lack of inhibition by SR141716A called into question the role of CB1 receptors in the effects of 2-AG, we tested these effects in CB1−/− and CB1+/+ mice (Figure 3). In both types of animals, 26.4 nmol/g of 2-AG caused hypotension and tachycardia similar to the effects seen in ICR mice, although the hypotension was somewhat less in CB1−/− than in CB1+/+ animals. Nevertheless, the effect of SR141716A (6 nmol/g IV) on the hypotensive effect of 2-AG was similar in the 2 groups: no inhibition was observed. In CB1+/+ mice, basal BP in the absence or presence of SR141716A was 81±6 versus 84±9 mm Hg and basal HR was 278±19 versus 416±10 bpm. In CB1−/− mice, the corresponding values were 75±4 versus 75±1 mm Hg and 331±20 versus 413±28 bpm. Although the tachycardic effect of 2-AG was reduced by SR141716A in both CB1−/− and CB1+/+ mice, this may have been related to the marked increase in basal HR caused by SR141716A in both groups. Pretreatment of CB1 receptor knockout mice with the CB2 receptor antagonist SR144528 (10.5 nmol [5 μg/g IV, Figure 3), which did not affect basal BP or HR, also failed to affect the responses to 2-AG. In contrast to SR141716A, indomethacin (14 nmol [5 μg/g IV) pretreatment of ICR mice significantly inhibited the hypotensive response to 2-AG, whereas the moderate tachycardia was unaffected (Figure 4A). AA (15 nmol [5 μg/g IV) also elicited hypotension and moderate tachycardia, and the hypotension but not the tachycardia was inhibited by 14 nmol/g indomethacin (Figure 4B). On the other hand, SR141716A pretreatment (6 nmol/g IV) failed to influence the effects of AA (Figure 4A), and, conversely, indomethacin (14 nmol/g IV) did not influence the hypotensive response to 11.5 nmol/g anandamide (18±2 vs 19±3 mm Hg, n=5, before and after indomethacin, respectively). Because this suggested that 2-AG may break down to release AA, which could be further metabolized into a hypotensive cyclooxygenase product(s), we tested the stability of 2-AG in mouse blood. Authentic 2-AG or anandamide (100 μg each) was added to 0.2-mL aliquots of heparinized mouse blood at 37°C, the blood samples were extracted after
various time intervals with diethyl ether, and the extracts were analyzed by liquid chromatography/mass spectrometry. As illustrated in the left panels of Figure 5, 2-AG disappeared from the blood within 2 minutes, with the parallel appearance of AA. In contrast, most of the anandamide added could be recovered unmetabolized after similar incubations (Figure 5, right panels) and even after a 5-minute incubation (not shown). These findings confirm the extreme instability of 2-AG in mouse blood, whereas anandamide appears to be more stable under similar conditions.

Because of its instability, 2-AG may not reach its site of action in the vasculature in amounts sufficient to activate CB1 receptors. Therefore we analyzed the cardiovascular effects of a metabolically stable ether analogue of 2-AG17,24 in ICR mice and in CB1−/− mice. As seen in Figure 6A, intravenous injection into ICR mice of 56 nmol (20 μg)/g 2-AG–ether elicited prolonged hypotension and bradycardia, and both effects were completely blocked by pretreatment with 6 nmol/g SR141716A. Similar effects were observed in CB1+/+ mice (data not shown), whereas 2-AG-ether failed to elicit any change in BP and caused a modest increase in HR in CB1−/− mice (Figure 6B). This further confirms the role of CB1 receptors in the effects of 2-AG-ether.

Discussion

The results presented in this article illustrate an unexpected difficulty in documenting the role of cannabinoid receptors in a biological effect of 2-AG on its intravenous administration to mice. Cannabinoids, including anandamide, evoke hypotension and bradycardia by activating CB1 receptors.3,5 Al-
though 2-AG also elicits hypotension, it increases rather than decreases HR, which is unusual in view of its documented ability to interact with CB1 receptors. The inability of SR141716A to inhibit the hypotensive effect of 2-AG and the persistence of such an effect in CB1−/− mice clearly indicate the lack of CB1 receptor involvement. 2-AG induces similar tachycardia in CB1−/− and CB1+/+ mice, which discounts the involvement of CB1 receptors in this effect as well. Although the tachycardic effect of 2-AG was reduced by SR141716A in both groups of mice, the apparent inhibition probably was related to the substantial increase in basal HR after SR141716A treatment. SR141716A does not cause tachycardia in rats,9 and the presence of the tachycardic effect in both CB1−/− and CB1+/+ mice rules out the involvement of CB1 receptors. The mechanism of SR141716A-induced tachycardia in mice thus remains unclear.

We present 3 lines of evidence to indicate that these paradoxical findings are due to the rapid degradation of 2-AG in mouse blood and the subsequent generation of a hypotensive arachidonate metabolite whose actions are not mediated by cannabinoid receptors. First, the effects of 2-AG could be mimicked by AA, and the effects of both 2-AG and AA were antagonized by indomethacin. Because indomethacin did not influence the hypotensive response to anandamide, its effect cannot be attributed to a nonspecific interaction with CB1 receptors but is more likely to be caused by inhibition of the generation of an as-yet-unidentified hypotensive cyclooxygenase product.

The second evidence is the rapid disappearance of 2-AG from mouse blood. The parallel appearance of AA suggests that 2-AG is rapidly hydrolyzed to yield AA and glycerol. Because the breakdown is essentially complete in 2 minutes, well before the peak of the hypotensive response or a significant increase in HR develops, it is very unlikely that 2-AG would be responsible for these effects. The finding that anandamide is much more stable under the same conditions is consistent with its CB1-receptor–mediated hypotensive and bradycardic effects. The differential stability of these two endocannabinoids also suggests that the rapid breakdown of 2-AG is not catalyzed primarily by an amidohydrolase, which can degrade both anandamide and 2-AG,28 but rather by a lipase such as sn-2 monoacylglycerol lipase, which may be involved in the degradation of 2-AG but not of anandamide.29,30 Further studies may be warranted to analyze the activity of this enzyme in blood.

The third line of evidence is the effects of a metabolically stable ether analogue of 2-AG. Not only did this compound produce hypotension as well as bradycardia, as expected from an agonist of CB1 receptors,5 but both effects were completely blocked by a dose of SR141716A previously shown to produce selective blockade of CB1 receptors.7 Furthermore, both effects of 2-AG-ether were absent in CB1−/− mice. In a recent study, 2-AG-ether was 2 orders of magnitude less potent and also less efficacious than 2-AG in inducing CB1 receptor–mediated calcium transients in NG108 to 15 cells.17 If a similar relation holds for the cardiovascular effects of these two compounds, 2-AG should be a highly potent hypotensive and bradycardic agent if it were protected from degradation before reaching CB1 receptors. The present findings indicate that this does not happen even with high intravenous doses of 2-AG. However, endogenous 2-AG produced and released at sites close to the receptors may escape metabolic degradation. For example, 2-AG was identified in vascular endothelial cells,23 where its levels are increased severalfold by the muscarinic agonist carbachol,24 and CB1 receptors are present in cerebrovascular smooth muscle.15 If 2-AG is produced in and released from the endothelium, it may reach CB1 receptors on smooth muscle without being degraded. CB1 receptors also have been identified in vascular endothelium,23 and 2-AG was found in platelets22 and macrophages,10 where its levels were increased 2- to 3-fold in response to bacterial lipopolysaccharide.22,30 Because lipopolysaccharide increases the adhesion of platelets to the endothelium, platelet-derived 2-AG could act as a “juxtacrine” regulator of vascular tone, where its proximity to an endothelial site of action could protect it from...
degradation. 2-AG is highly lipid soluble, and it may remain associated with the cell membrane after its release, which could further protect it against rapid degradation by blood-borne lipases. In various tissues, 2-AG is accompanied by certain 2-acyl glycerol esters, which have no cannabinoid activity of their own but can potentiate the binding of 2-AG to CB1 receptors. This “entourage effect” has been attributed, at least in part, to the protection of 2-AG against enzymatic breakdown. Clearly, further studies are needed to explore the role of endogenous 2-AG as a cardiovascular regulator.

Acknowledgments

This work was supported by National Institutes of Health grant HL-59257 (to G.K.). Fellowship support (to Z.J.) was from Sanofi Recherche (Montpellier, France) and from the Deutsche Forschungsgemeinschaft (to J.A.W.). We thank D. Troy Bridgen for assistance with the LC/MS analyses.

References


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Hypertension. 2000;35:679-684
doi: 10.1161/01.HYP.35.2.679

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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