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 that are unaffected by the CB1 receptor antagonist SR141716A. The same dose of SR141716A (6 nmol/g IV) completely blocks the hypotensive effect and attenuates the bradycardic effect of anandamide. 2-AG elicits a similar hypotensive effect, resistant to blockade by either SR141716A or the CB2 antagonist SR144528, in both CB1−/− mice and their homozygous (CB1+/+) control littermates. In ICR mice, arachidonic acid (AA, 15 nmol/g IV) elicits hypotension and tachycardia, and indomethacin (14 nmol/g IV) inhibits the hypotensive effect of both AA and 2-AG. Synthetic 2-AG incubated with mouse blood is rapidly (<2 minutes) and completely degraded with the parallel appearance of AA, 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, Δ²-tetrahydrocannabinol (THC) causes prolonged hypotension and bradycardia. In 1992, arachidonoyl 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. Anandamide also elicits hypotension and bradycardia in anesthetized rats through a peripheral mechanism, as indicated by their susceptibility to blockade by SR141716A, a selective CB1 receptor antagonist, and by their absence in CB1 receptor knockout (CB1−/−) mice. In 1995, 2-arachidonoyl glycerol (2-AG), found in canine gut and rat brain, was identified as another endogenous cannabinoid. The concentration of 2-AG in the brain is several hundredfold higher than that of anandamide, and, unlike anandamide, which often acts as a partial agonist, 2-AG is a full agonist at CB1 receptors. This has led to the proposal that the CB1 receptor is a 2-AG receptor.

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, and anandamide has been identified in vascular endothelial cells. SR141716A also antagonizes hemorrhagic and endotoxin-induced hypotension, and circulating macrophages produce anandamide under such conditions, which suggests a paracrine mechanism in the control of vascular tone. Recently, 2-AG also has been identified in vascular endothelial...
found to increase in response to endotoxin treatment.22 Thus SR141716A.22 In the present study we analyzed the cardio-

animal care and usage committee.

preamplifier. The procedures used were approved by the institutional

derived from the pressure pulse with the use of a tachograph

was connected to a pressure transducer and physiograph. HR was

rinized saline for intravenous drug injections and monitoring blood

arterial cannula

as needed to maintain stable anesthesia. The jugular vein and the

anesthetized with 60 mg/kg IP sodium pentobarbital, supplemented

chow, and had access to drinking water ad libitum. Mice were

were kept under a 12:12 hours light/dark cycle, fed standard mouse

by the activation of SR141716A-sensitive receptors.

2-AG also may play a role in the local control of vascular tone

mediated hypotension and bradycardia.5 2-AG elicits tachycardia unaffected by

unlike anandamide, which causes CB1 receptor-mediated

is rapidly degraded in mouse blood, and the observed hypo-
tensive 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-

Methods

Cardiovascular Measurements

Ninety male ICR mice (25 to 30 g) from Harlan (Indianapolis, Ind)

were used. Male and female CB1−/− mice and their homozygous

littermates (64 animals) have been described.23 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 hepa-

rinized 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

derived from the pressure pulse with the use of a tachograph

preamplifier. The procedures used were approved by the institutional

animal care and usage committee.

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 concentra-
tion 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

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.26 The synthesis of an ether-linked analogue of

2-AG, 2-eicosatetraenylglycerol (2-AG-ether), has been detailed

elsewhere.17 The structure of all 3 compounds was confirmed by

nuclear magnetic resonance and electron impact mass spectrometry.

SR141716A5 (N-[piperidin-1-yl]-5-[4-chlorophenyl]-1-[2,4-
dichlorophenyl]-4-methyl-1H-pyrazol-3-carboxamide HCl) and the

CB2 receptor antagonist SR14452827 (N-(1S)-endo-1,3,3-trimethyl

cyclo[2.2.1]heptan-2-yl)-5-[(4-chloro-3-methylphenyl)-1-(4-
methyl-benzyl)-pyrazole-3-carboxamide) were gifts from Sanofi Re-

cherche (Montpellier, France). Anandamide was provided by Dr

Billy R. Martin.

Statistical Analyses

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.

Results

Bolus intravenous injection of 26.4 nmol (10 μg/g) 2-AG in
IRC mice caused hypotension and moderate tachycardia that
lasted ∼10 minutes. Unexpectedly, neither effect was influ-
enced by pretreatment with 6 nmol (3 μg/g) SR141716A (Figure 1A). The same dose of SR141716A nearly com-
pletely blocked the hypotension and attenuated the bradycar-
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<sup>−/−</sup> and CB1<sup>+/+</sup> 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<sup>−/−</sup> than in CB1<sup>+/+</sup> 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<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>−/−</sup> and CB1<sup>+/+</sup> 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 4B), 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

![Figure 2. Dose-dependence and lack of inhibition by SR141716A of the hypotensive effect of 2-AG and 1-AG in ICR mice. Columns and bars represent mean±SE (black: controls, n=6 to 15; shaded: after 6 nmol/g SR141716A, n=4 to 9). *Significant difference from baseline.](http://hyper.ahajournals.org/)

![Figure 3. Effects of 2-AG on mean BP (left) and HR (right) in CB1<sup>−/−</sup> (top) and CB1<sup>+/+</sup> mice (bottom). 2-AG (26.4 nmol/g) was injected intravenously before (C) and 10 minutes after the intravenous injection of 6 nmol/g SR141716A (●) or 10.4 nmol/g SR144528 (●). Points and bars represent mean±SE, n=6 to 9. *Significant difference from baseline.](http://hyper.ahajournals.org/)
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-AG 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-

Figure 4. Effect of indomethacin on 2-AG–induced (A) and AA-induced (B) changes in mean BP and HR in ICR mice. 2-AG (26.4 nmol/g) or AA (15 nmol/g) was injected intravenously 10 minutes after the intravenous injection of vehicle (○) or 14 nmol/g indomethacin (▲). AA was also tested after pretreatment with 6 nmol/g SR141716A (●). Points and bars represent mean±SE, n=5 to 6. *Significant difference (P<0.05) from baseline.

Figure 5. LC/MS analysis of 2-AG and anandamide incubated with mouse blood. Two selected ions were monitored simultaneously: m/z 379.4 for 2-AG ions and m/z 305.4 for AA ions in the left panels and m/z 348.4 for anandamide ions and m/z 305.4 for AA ions in the right panels. Samples used were extracts of 200 μL drug-free whole mouse blood (A); of 100 μg authentic 2-AG or anandamide dissolved in 200 μL buffer (B); and of 200 μL blood samples incubated with 100 μg 2-AG or anandamide for 1 minute (C) or 2 minutes (D).
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, 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 completely blocked by a dose of SR141716A previously shown to produce selective blockade of CB1 receptors. 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, where its levels are increased severalfold by the muscarinic agonist carbachol, 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, and 2-AG was found in platelets and macrophages, where its levels were increased 2- to 3-fold in response to bacterial lipopolysaccharide. 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

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, 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. 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, but both effects were completely blocked by a dose of SR141716A previously shown to produce selective blockade of CB1 receptors. 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. 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, where its levels are increased severalfold by the muscarinic agonist carbachol, and CB1 receptors are present in cerebrovascular smooth muscle. 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, and 2-AG was found in platelets and macrophages, where its levels were increased 2- to 3-fold in response to bacterial lipopolysaccharide. 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

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
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. 31 This “entourage effect” has been attributed, at least in part, to the protection of 2-AG against enzymatic breakdown. 31 Clearly, further studies are needed to explore the role of endogenous 2-AG as a cardiovascular regulator.

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References

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