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Cannabinoid antagonist SR-141716 inhibits endotoxic hypotension by a cardiac mechanism not involving CB1 or CB2 receptors

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Abstract

Endocannabinoids and CB1 receptors have been implicated in endotoxin (LPS)-induced hypotension: LPS stimulates the synthesis of anandamide in macrophages, and the CB1 antagonist SR-141716 inhibits the hypotension induced by treatment of rats with LPS or LPS-treated macrophages. Recent evidence indicates the existence of cannabinoid receptors distinct from CB1 or CB2 that are inhibited by SR-141716 but not by other CB1 antagonists such as AM251. In pentobarbital-anesthetized rats, intravenous injection of 10 mg/kg LPS elicited hypotension associated with profound decreases in cardiac contractility, moderate tachycardia, and an increase in lower body vascular resistance. Pretreatment with 3 mg/kg SR-141716 prevented the hypotension and decrease in cardiac contractility, slightly attenuated the increase in peripheral resistance, and had no effect on the tachycardia caused by LPS, whereas pretreatment with 3 mg/kg AM251 did not affect any of these responses. SR-141716 also elicited an acute reversal of the hypotension and decreased contractility when administered after the response to LPS had fully developed. The LPS-induced hypotension and its inhibition by SR-141716 were similar in pentobarbital-anesthetized wild-type, CB1^{-/-}, and CB1^{-/-}/CB2^{-/-} mice. We conclude that SR-141716 inhibits the acute hemodynamic effects of LPS by interacting with a cardiac receptor distinct from CB1 or CB2 that mediates negative inotropy and may be activated by anandamide or a related endocannabinoid released during endotoxemia.

Keywords

endotoxin; cannabinoids; negative inotropy

Activation of Cannabinoid receptors by plant-derived and endogenous cannabinoids elicits well-documented cardiovascular effects (17,25). In humans, acute ingestion of Δ^9 -tetrahydrocannabinol, the main psychoactive ingredient in marijuana, usually elicits tachycardia (24). However, prolonged use in humans and acute as well as chronic administration in most animal models cause long-lasting hypotension and bradycardia (4,26), and similar depressor effects have been also reported in response to the acute administration of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) (22,45). The introduction in 1994 of the first selective cannabinoid-1 receptor (CB1) antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR-141716; Ref. 40), allowed

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these responses to be characterized as CB1 mediated (45), a conclusion later borne out by the absence of cannabinoid-induced hypotension and bradycardia in mice with genetic ablation of CB1 (23,27). Although the overwhelming majority of CB1 in mammals are located in the brain (16), the cardiovascular depressor effects of cannabinoids appear to involve CB1 expressed in peripheral tissues, including blood vessels (13,17,29), the heart (5), and sympathetic nerve terminals (20,30). The possible involvement in cardiovascular depressor responses of CB2 (15), another cannabinoid receptor primarily expressed by immune cells (33), is less well documented.

Activation of CB1 by certain synthetic cannabinoids can cause profound and prolonged hypotension (26), which has raised the possible involvement of the CB1/endocannabinoid system in pathological states associated with hypotension, such as various forms of shock. Indeed, the CB1 antagonist SR-141716 has been reported to inhibit or reverse the hypotension associated with hemorrhagic (48), endotoxemic (46), and cardiogenic (47) shock and the hypotension that accompanies advanced liver cirrhosis (2,41). There is also evidence that in these conditions macrophage- and platelet-derived endocannabinoids, including anandamide and 2-AG, are responsible for the activation of SR-141716-sensitive receptors (2,41,46–48, 50).

Recent studies indicate that anandamide can elicit vasodilation through a number of mechanisms in addition to the possible activation of vascular CB1 (17,25), including the activation of vanilloid TRPV1 receptors on sensory nerve terminals (51). Of particular interest is a novel endothelial site of action that, similar to CB1, is G_i/G_o coupled and inhibited by SR-141716 but does not interact with other CB1 or CB2 agonists or antagonists (18,23,32, 34). A similar situation may exist in the heart, where the negative inotropic effects of cannabinoids including anandamide can be mediated both by CB1 receptors (5) and by an SR-141716-sensitive mechanism that does not involve CB1 receptors (8). Shock-related hypotension, such as the hypotension induced by bacterial endotoxin (LPS), may involve both vasodilation and decreased cardiac contractility, and the relative roles of endocannabinoids and their receptors in these two mechanisms have not been determined. Furthermore, cannabinoid receptors have been implicated in endotoxin-induced hypotension based on the ability of SR-141716 to prevent this effect, and the relative role of CB1 versus SR-141716-sensitive receptors distinct from CB1 or CB2 needs to be explored. Here we report that LPS elicits similar, SR-141716-sensitive hypotension in anesthetized wild-type mice and in mice deficient in CB1 or in both CB1 and CB2. In anesthetized rats, the acute (<2 h) hypotensive phase following LPS injection is primarily due to a decrease in cardiac contractility, and both the hypotension and the decreased contractility are prevented by SR-141716 but are unaffected by another CB1 antagonist, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251; Ref. 12).

Materials and Methods

Reagents

SR-141716 was provided by the National Institute on Drug Abuse drug supply service (Research Triangle Park, NC); AM251 was from Tocris Cookson (Baldwin, MO). SR-141716 and AM251 were dissolved in a vehicle of corn oil-water (1:4) emulsified by the addition of Pluronic F-68 (40 mg/ml). The concentration of the antagonists in this vehicle was 10 mg/ml, and vehicle or drug was injected in a volume of 0.3 ml/kg body wt. LPS (*Escherichia coli*, 0127:B8) and Pluronic F-68 were from Sigma (St. Louis, MO).

Animals

Male Sprague-Dawley rats weighing 300–350 g were obtained from Harlan (Indianapolis, IN). $CB1^{-/-}$ mice, their homozygous controls ($CB1^{+/+}$), and $CB1^{-/-}/CB2^{-/-}$ double knockouts were developed and backcrossed to a C57BL/6J background by Andreas Zimmer (Univ. of Bonn, Bonn, Germany) and Nancy E. Buckley (California State Polytechnic Univ., Pomona, CA), as described previously (23). $CB1^{-/-}$ mice were bred from heterozygote breeding pairs and genotyped with a PCR-based assay and DNA extracted from tail snips obtained at the time of weaning. $CB1^{-/-}/CB2^{-/-}$ double-knockout mice were bred from breeding pairs that were homozygote knockouts for both receptor genes.

Hemodynamic measurements

Rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized to facilitate breathing. The animals were placed on heating pads, and core temperature measured via a rectal probe was maintained at 37°C. To measure left ventricular (LV) systolic pressure (LVSP) and the maximal slope of systolic pressure increment ($+dP/dt$), a microtip catheter (SPR-524; Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the LV under pressure control as described previously (36–38). A P50 tube was also inserted into the right femoral artery and vein for measurement of arterial blood pressure and intravenous injections, respectively. In some experiments, a saline-filled cannula connected to a pressure transducer was inserted into the right jugular vein for monitoring central venous pressure. Aortic blood flow was measured with transit time technology by placing a flow probe (2.5SB733, Transonic Systems, Ithaca, NY) on the abdominal aorta below the renal arteries after midline laparotomy. Blood flow in the abdominal aorta was used to calculate hindquarter vascular resistance, representing predominantly skeletal muscle, skin, and bone. After stabilization for 20 min, the signals were continuously recorded with a Powerlab/4SP analog-to-digital converter at 1 kHz (AD Instruments, Mountain View, CA), stored, and displayed on a personal computer (36–38). Heart rate, maximal LVSP, mean arterial pressure (MAP), and $+dP/dt$ were calculated as previously described (36–38). Peripheral resistance index (PRI) was calculated as $MAP \cdot \text{mean aortic blood flow}^{-1} \cdot 100 \text{ g body wt}^{-1}$, and changes are expressed as % of control.

Mice were anesthetized with pentobarbital sodium (50 mg/kg ip). Polyethylene cannulas (P10) were inserted into the carotid artery and jugular vein for the measurement of MAP and for drug injections, respectively.

All animal procedures were in accordance with the guidelines of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee.

Data analyses

Values are expressed as means $\pm$ SE. Time-dependent variables were analyzed by ANOVA followed by Bonferroni's post hoc test. Differences were deemed statistically significant when $P < 0.05$.

Results

Hemodynamic effects of LPS in anesthetized rats are inhibited by SR-141716

In pentobarbital-anesthetized rats, intravenous injection of 10 mg/kg LPS caused an acute hypotensive response that lasted up to 1 h. As shown in Fig. 1, the hypotension was associated with moderate tachycardia and a major decrease in LV contractility, as indicated by dramatic decreases in $+dP/dt$ and LVSP. Aortic blood flow, an indicator of cardiac output, also decreased, and PRI increased. Pretreatment of the rats with either vehicle or 3 mg/kg SR-141716 10 min before injection of LPS had no significant hemodynamic effect by itself,

as also illustrated by the 0 min values in Fig. 1, which were similar in the vehicle- and SR-141716-treated groups. However, SR-141716 pretreatment nearly completely prevented the hypotension without influencing the tachycardic response to LPS and also completely prevented the LPS-induced decrease in cardiac contractility but only slightly delayed the decrease in aortic blood flow and the increase in PRI.

SR-141716 was also able to reverse the effects of LPS when administered after the acute hemodynamic effects of LPS had developed. As illustrated in Fig. 2, in five rats intravenous injection of 3 mg/kg SR-141716 15 min after LPS treatment induced an immediate and rapid increase in arterial pressure that significantly exceeded the gradual increase in pressure in five other LPS-treated animals injected with vehicle only. SR-141716 also caused a rapid increase in LVSP and $+dP/dt$ that not only reached but then exceeded pre-LPS baseline levels.

Differential effects of SR-141716 and AM251 on hemodynamic response to LPS

To explore the role of CB1 in the acute hypotensive response to LPS, rats were pretreated with a 3 mg/kg intravenous dose of the CB1 antagonists SR-141716 or AM251 10 min before the similar administration of 10 mg/kg LPS. This dose of SR-141716 was shown to completely block the hypotensive response of anesthetized rats to a near-maximal dose (10 μ g/kg iv) of the potent synthetic CB1 agonist HU-210 (26), and in separate experiments 3 mg/kg AM251 caused a similar, complete blockade of the hypotensive effect of 10 μ g/kg HU-210 (not shown). As illustrated in Fig. 3, there was a dramatic difference between the ability of the two antagonists to antagonize the effect of LPS. Unlike SR-141716, which nearly completely prevented the hypotensive response to LPS (see also Figs. 1 and 2), AM251 had no effect at all: LPS caused as great a hypotensive response in the absence as in the presence of AM251.

LPS-induced hypotension in wild-type and CB receptor-deficient mice

The inability of AM251 to inhibit the hypotensive response to LPS suggested that the SR-141716-sensitive receptors involved are not CB1. To further test this, the acute hypotensive response to LPS was tested in pentobarbital-anesthetized mice deficient in CB1 (CB1^{-/-}) or both CB1 and CB2 (CB1^{-/-}/CB2^{-/-}) and in their wild-type littermates. As illustrated in Fig. 4, intravenous injection of 100 μ g/kg LPS caused similar hypotension in the three groups, and in all three groups the hypotensive response to LPS could be prevented by pretreatment with 3 mg/kg SR-141716.

Discussion

We previously reported (46) that the CB1 antagonist SR-141716 inhibits acute hypotension and decreases mortality in response to LPS treatment in rats. The present findings confirm the ability of SR-141716 to inhibit LPS-induced hypotension, yet they indicate that this effect and the underlying hemodynamic changes are not mediated by CB1. AM251 is a selective CB1 antagonist at least equipotent with SR-141716 (12), and at the in vivo dose used here it was reported to block the hemodynamic effects of potent CB1 agonists in rats (11). Thus its complete inability to influence the LPS response (Fig. 3) is not compatible with CB1 involvement. Furthermore, LPS-induced hypotension in mice and its sensitivity to inhibition by SR-141716 were not influenced by the genetic ablation of CB1 or both CB1 and CB2 (Fig. 4).

Several recent studies point to the existence of additional cannabinoid receptors distinct from CB1 or CB2. At least two of these, a putative G_i/G_o-coupled endothelial receptor mediating vasodilation in certain vascular beds (18,23,32,34) and a receptor postulated to be present on glutamatergic terminals in the hippocampus (14), have been shown to be uniquely sensitive to inhibition by SR-141716 but not by other CB1 antagonists such as AM251, although their

differential sensitivity to the agonist WIN-55,212-2 (14,32,34) suggests that they are distinct molecular entities. LPS is a potent stimulant of anandamide synthesis in macrophages (28, 46), and LPS-treated macrophages were found to elicit SR-141716-sensitive hypotension when injected into normal control rats (28,46). Because anandamide can interact with the SR-141716-sensitive non-CB1/non-CB2 receptors described above (23,32,34), one might postulate that such a receptor, rather than CB1, may mediate the acute hemodynamic effects of LPS.

Macrophages isolated from mice deficient in fatty acid amidohydrolase, the enzyme responsible for anandamide metabolism, and stimulated in vitro with LPS have higher anandamide levels and elicit a greater decrease in blood pressure in recipient rats than similarly treated cells isolated from wild-type littermates (28). Although these findings suggest that macrophage-derived anandamide or a related fatty acid amide may mediate the acute hemodynamic effect of LPS, other mechanisms, such as a reported LPS-induced increase in target organ sensitivity to endocannabinoids (35), may also play a role.

The present findings also indicate, however, that the hypotensive effect of LPS is due to decreased cardiac contractility, which leads to a decrease in stroke volume and cardiac output rather than vasodilation, and the decrease in contractility is so profound that arterial pressure decreases despite a parallel increase in peripheral resistance. This hemodynamic pattern of a primary decrease in cardiac contractility is similar to that reported in several recent studies in both anesthetized (19,31,36,39) and conscious (42) rats, although an LPS-induced vasodilation in certain vascular beds, such as the renal vasculature (11) and the heart and the brain (44), would not be detected in the present experiments because of the positioning of the aortic flow probe. The primary cardiodepressor effect of LPS suggests that SR-141716 must have a myocardial site of action as well. Indeed, SR-141716 not only prevents the marked decrease in $+dP/dt$ and LVSP of subsequently administered LPS but elicits an immediate reversal of the decline in these parameters to levels above control values when it is administered after the hemodynamic response to LPS has fully developed. Similarly, cardiac contractility increased above control values when LPS was administered after pretreatment with SR-141716 (Fig. 1). This is an interesting phenomenon in which increased sympathetic nervous system drive may play a role: LPS has been reported to increase sympathetic tone in rats, as indicated by a rise in plasma catecholamines (21). Thus the net effect of LPS on cardiac contractility may be determined by the balance between endocannabinoid-mediated negative and sympathetically mediated positive inotropy, although the involvement of additional mechanisms cannot be excluded.

In contrast, SR-141716 only slightly delays but does not significantly inhibit the LPS-induced decrease in aortic blood flow and increase in peripheral resistance. Although these findings do not exclude the possibility that SR-141716 may antagonize LPS-induced vasodilation in vascular beds where they do occur, it appears that the primary site of action of SR-141716 in this experimental model is cardiac, not vascular. An SR-141716-sensitive, AM251-insensitive negative inotropic response to anandamide has been described in the rat Langendorff heart preparation (8), and a similar receptor site may be involved in the in vivo effects described here. The molecular identity of this site and its possible relation to the G_i/G_o -coupled endothelial receptor for anandamide remain to be determined.

Interestingly, our recent observations (3) indicate that in various forms of hypertension a compensatory hypotensive endocannabinergic tone is activated, which can be reversed equally effectively by SR-141716 and AM251 and is associated with upregulation of vascular and cardiac CB1. Thus endocannabinoids are hypotensive regulators whose tonic effects may be mediated by different types of receptors in different pathological conditions.

Previous studies implicated TNF- α (Refs. 10 and 43) and platelet-activating factor (PAF; Ref. 1) in the cardiodepressor effects of endotoxin. SR-141716 does not inhibit PAF or TNF- α receptors (unpublished observations). However, TNF- α and/or PAF may act via the release of an endocannabinoid such as anandamide, which would then interact with an SR-141716-sensitive receptor. Indeed, we recently showed (28) that a component of the hypotensive response of rats to PAF can be inhibited by SR-141716. Further studies are needed to identify the SR-141716-sensitive myocardial site apparently involved in the acute hemodynamic effects of LPS.

CB1 receptors in the central nervous system have been implicated in the control of appetite (7) and the rewarding effects of nicotine (6) and alcohol (49). In light of such findings, the antagonist SR-141716, recently named rimonabant, is being developed for the treatment of obesity, nicotine dependence, and alcoholism. The present findings indicate that SR-141716 has additional peripheral sites of action that are not shared by other CB1 antagonists but may also be of therapeutic importance.

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Fig. 1.

Hemodynamic effects of LPS in anesthetized rats in the absence and presence of the CB1 antagonist SR-141716. LPS (10 mg/kg iv) was injected at 0 min, 10 min after the injection of vehicle (●) or SR-141716 (3 mg/kg iv; ○). Mean arterial pressure (MAP; A), heart rate (HR; B), left ventricular systolic pressure (LVSP; C), maximal slope of systolic pressure increment (+dP/dt; D), mean aortic blood flow (MAF; E), and change (Δ) in peripheral resistance index (PRI; F) were monitored or computed as described in materials and methods. Values are means ± SE from experiments in 4–5 separate animals. Significant difference ($P < 0.05$): *from corresponding baseline value in the vehicle + LPS group; #between corresponding values in the 2 treatment groups.

Fig. 2.

SR-141716 reverses the hemodynamic effects of LPS. SR-141716 (3 mg/kg) or vehicle was injected intravenously 15 min after the intravenous injection of 10 mg/kg LPS, as indicated by arrows. Values are means $\pm$ SE from 5 rats treated with SR-141716 (○) or 5 other animals treated with vehicle (●). MAP (A), LVSP (B), and $+dP/dt$ (C) were continuously monitored and calculated at the indicated time points.

Fig. 3.

LPS-induced hypotension is inhibited by SR-141716 but not by AM251. *A*: representative tracings of the effects of LPS on MAP in a rat pretreated with vehicle (*left*), SR-141716 (3 mg/kg iv; *center*), and AM251 (3 mg/kg iv; *right*). *B*: mean $\pm$ SE Δ MAP from similar experiments from 4–5 animals. *Significant difference from values in vehicle + LPS-treated rats ($P < 0.05$).

Fig. 4. Effect of LPS on MAP in wild-type (A), CB1^{-/-} (B), and CB1^{-/-}/CB2^{-/-} (C) mice pretreated with vehicle (●) or SR-141716 (3 mg/kg iv; ○). The numbers of animals in the 3 groups were 5 (A), 5 (B), and 4 (C). *Significant difference from corresponding value in the vehicle + LPS group.