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REVIEW

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Cardiovascular actions of cannabinoids and their generation during shock

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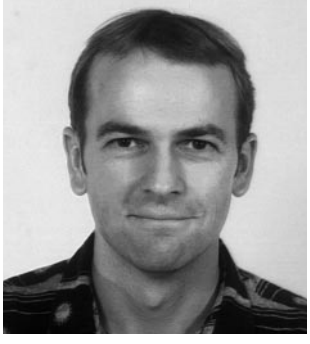
Abstract Marijuana is a widely abused recreational drug well known for its psychoactive properties. Cannabinoids, the active ingredients of marijuana, elicit their neurobehavioral effects by interacting with the CB_1 cannabinoid receptor subtype, expressed primarily in the brain but also present in some peripheral tissues. A second receptor subtype, the CB_2 receptor, is expressed on cells of the immune system and is thought to be responsible for the immunosuppressant effects of cannabinoids. Recently, endogenous lipidlike substances have been identified, including arachidonyl ethanolamide (anandamide) and 2-arachidonyl glyceride, that bind to cannabinoid receptors and mimic many of the neurobehavioral effects of plant-derived cannabinoids. Both plant-derived cannabinoids and the endogenous ligands have been shown to elicit hypotension and bradycardia via activation of peripherally located CB_1 receptors. Possible underlying mechanisms include presynaptic CB_1 receptor mediated inhibition of norepinephrine release from peripheral sympathetic nerve terminals, and/or direct vasodilation via activation of vascular cannabinoid receptors. The latter may also be the target of endocannabinoids of vascular endothelial origin. Recent studies indicate that a peripheral endogenous cannabinoid system in circulating macrophages and platelets is activated in hemorrhagic and septic shock and may contribute to the hypotension associated with these conditions via activation of vascular cannabinoid receptors. The potential role of this mechanism in human shock conditions is under investigation.

Key words Cannabinoid · Hypotension · Blood pressure · Anandamide · 2-Arachidonyl-glycerol

Abbreviations *2-AG* 2-Arachidonyl monoglyceride · *EDHF* Endothelium-derived hyperpolarizing factor · *GC/MS* Gas chromatography–mass spectrometry · *HU-210* (–)-11-OH-Δ^9-Tetrahydrocannabinol dimethylheptyl · *L-NAME* N^G-Nitro-L-arginine methylester · *LPS* Lipopolysaccharide · *SR141716 A* *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl · *THC* Δ^9-Tetrahydrocannabinol · *WIN 55212-2* (R)-(+)-[2,3-Dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl]-(1-naphthalenyl) methanone



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Introduction

Next to alcohol, marijuana is the most widely used recreational drug in Western countries. The major psychoactive constituent of the marijuana plant, cannabis sativa, is Δ^9-tetrahydrocannabinol (THC; Fig. 1). The characteristic neurobehavioral effects of THC in humans include analgesia, euphoria, and acoustic hallucinations, whereas in rodents the classical tetrad of hypothermia, antinociception, catalepsia, and hypomotility is considered diagnostic for cannabinoid action [1]. Although the main focus of contemporary cannabinoid research has been on these neurobehavioral effects and the underlying cellular/molecular mechanisms, it has long been known that cannabinoids also have immunomodulatory [2] and cardiovascular actions [3–9]. This review concentrates on the cardiovascular effects of cannabinoids and on the putative role of endocannabinoids as a novel class of cardiovascular regulators. Other aspects of cannabinoid research are briefly summarized, and the reader is referred to several excellent in depth reviews, as cited in the text.

Cannabinoids have been catapulted onto the center stage of biomedical research by several key discoveries during the past decade. First, specific cannabinoid receptor binding sites have been identified in the brain and in some peripheral tissues, with properties characteristic of the superfamily of G protein coupled receptors [10]. Subsequently, two such receptors have been cloned: the CB_1 receptor, originally discovered in brain [11, 12] but also present in some peripheral tissues [13, 14], and the CB_2 receptor, first identified in macrophages in the marginal zone of spleen [15] and also found in the tonsils, thymus, mast cells, and some blood cells [16]. The human CB_1 receptor has a molecular weight of 52.8 kDa and contains 472 amino acids (Table 1; for review see [16]). In a recent study a cannabinoid receptor with about 50% sequence identity with the human CB_1 receptor was discovered in leech, indicating that this signaling system has been conserved during evolution [17]. The amino acid sequence homology between the human CB_1 and CB_2 receptors is only about 44%, and the CB_2 receptor is also smaller than the CB_1 receptor (360 amino acids, 40.2 kDa), due to a shorter third intracellular loop and C-terminal tail. The gene locus designations for the CB_1 and CB_2 receptors are CNR1 (human, on chromosome 6 [14, 18]; mouse, on chromosome 4 [19]) and CNR2 (mouse, on chromosome 4 [20]). The messenger RNA for a splice variant of the CB_1 receptor – termed CB_{1A} – has also been identified [21], although there is no evidence as yet whether this message is translated. In Chinese hamster ovary cells stably transfected with either CB_1 or CB_{1A} receptor mRNA, cannabinoids were found to inhibit forskolin-stimulated cAMP accumulation, although the effects elicited via CB_{1A} receptors were smaller than those triggered by CB_1 receptors [22].

The second important development was the discovery of endogenous lipidlike substances that bind to cannabinoid receptors, mimic most of the effects of cannabinoids, and may be involved in intercellular signaling. The first such ligand, arachidonyl ethanolamide, was isolated from porcine brain in 1992 [23] (see Fig. 1). The term "anandamide" was coined from the Sanskrit *ananda*, meaning "bliss" or "happy," and the chemical designation "amide." The name "anandamide" initially included two related molecules with similar binding affinities for cannabinoid receptors: *N*-docosatetraenoyl ethanolamide and *N*-homo-γ–linolenoyl ethanolamide. Because most of the follow-up work so far has concentrated on the originally isolated arachidonyl-ethanolamide, the name "anandamide" is used to designate this substance (Fig. 1).

As with the plant-derived THC, anandamide binds to cannabinoid receptors [24, 25], inhibits adenylate cyclase via an inhibitory G protein [24], and inhibits voltage-gated N-type calcium channels [25]. Anandamide and other cannabinoids have also been found to modulate the voltage-dependent potassium A current [26] and to activate inwardly rectifying potassium channels via CB_1 receptors coupled to a pertussis toxin sensitive G protein [27]. Cannabinoid receptor mediated cellular responses also include the pertussis toxin-sensitive activation of mitogen-activated protein kinases [28], the activation of a neuronal form of focal adhesion kinase [29], and the mobilization of arachidonic acid [30], blocked by the CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl) -4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR141716 A) [31]. Although anandamide also binds to transfected CB_2 receptors, it does not activate them [32, 33].

More recently the demonstration of stimulation-induced, calcium-dependent release of anandamide from neurons in the brain has strongly suggested that anandamide has a neurotransmitter or neuromodulatory function [34, 35]. This has been further supported by findings that neurons can both synthesize and degrade anandamide. It was first thought that the biosynthesis of anandamide occurs via enzymatic condensation of arachidonic acid and ethanolamine [36, 37]. Subsequent findings indicate, however, that anandamide is most likely generated from a precursor phospholipid, *N*-arachidonyl phosphatidyl ethanolamide, by phospholipase D-catalyzed cleavage that yields anandamide and phosphatidic acid. *N*-Arachidonyl phosphatidyl ethanolamide, in turn, may be generated by the action of an *N*-acyltransferase which catalyzes the transfer of arachidonic acid from the sn-1 position of phospholipids to the amine group of phosphatidylethanolamine (reviewed in [38, 39]).

The enzyme anandamide amidohydrolase, which is capable of catalyzing the synthesis of anandamide at exceptionally high substrate concentrations [40, 41], is mainly responsible for the biodegradation of anandamide and other unsaturated *N*-acylethanolamides, resulting in the release of free arachidonic acid [36, 42, 43]. Inhibitors of this enzyme, including the serine protease inhibitor phenylmethyl sulfonylfluoride, have been shown to increase the apparent binding affinity of anandamide at cannabinoid receptors [44, 45] and to potentiate its physiological actions [46, 47]. Recently, several potent and practically irreversible inhibitors of anandamide amidohydrolase have been described [48–52]. Although these provide powerful tools for studying

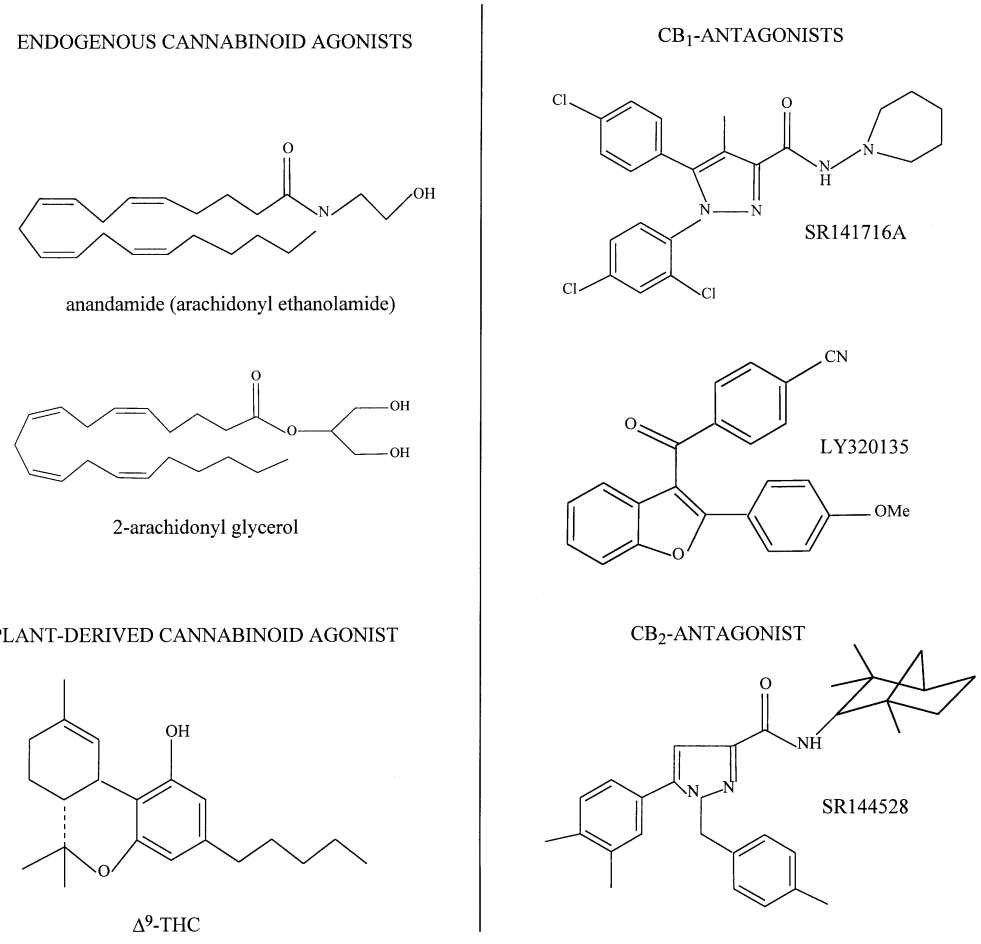

Fig. 1 Structures of the endogenous cannabinoid ligands anandamide (arachidonyl ethanolamide) and 2-arachidonyl glycerol, the major active constituent of marijuana, Δ^9-tetrahydrocannabinol (*THC*), the selective CB_1 receptor antagonists SR141716 A and LY320135, and the selective CB_2 receptor antagonist SR144528

Table 1 Gene localization and molecular size of cannabinoid receptors cloned to date

Receptor	Species cloned	Chromosomal location	Amino acid sequence	Gene Bank accession	Reference
CB1	Human	6q14–15	472	X54937	[14, 18]
	Rat	–	473	X55812	[11]
	Mouse	4	473	U17985	[19, 120]
CB1 A	Human	–	411	X81120	[21]
	Rat	–	411	X81121	[21]
	Mouse	–	–	–	
CB2	Human	–	360	X74328	[15]
	Rat	–	–	–	
	Mouse	4	347	–	[20, 121]

the mechanisms of anandamide inactivation, lack of selectivity is a drawback. Several of these compounds have been found to bind to CB_1 receptors with an affinity similar to or greater than their affinity for the enzyme [48–50], or to potently inhibit phospholipase A_2 [51–52]. Anandamide is also present in some nonneuronal tissues, such as a macrophage cell line [53] and circulating rat macrophages [54, 55], which have been shown to be able to both synthesize [53–55] and degrade anandamide [53] (also reviewed in [39]). Enzymatic degradation may not be the only, or not even the most important, mechanism for terminating the biological action of anandamide. A high-affinity uptake mechanism for anandamide has been identified in neurons [56] and macrophages [57], and blocking this uptake by selective inhibitors has been shown to potentiate the vasodepressor effect of anandamide [58]. Furthermore, anandamide may be a substrate for a lipoxygenase, and the product, 12-hydroxyanandamide, has been found to be a functional cannabinoid agonist at the brain cannabinoid receptor [59, 60].

Another endogenous cannabinoid ligand, 2-arachidonyl glyceride (2-AG) has been isolated from canine gut [61]. As with anandamide, 2-AG binds to CB_1 cannabinoid receptors and mimics some of the biochemical and neurobehavioral effects of cannabinoids [61, 62]. In binding assays 2-AG has been found to have a K_d of 472 nM [61] or 2.4 μM [62] at the transfected or native CB_1 receptor, respectively, and it was 2–24 times less potent than anandamide. Unlike anandamide, 2-AG not only binds to but also activates CB_2 receptors [63], for review see [64]. Recent findings indicate that 2-AG is present in the brain at much higher concentrations than anandamide, and its stimulation-induced release can trigger the CB_1 receptor-mediated inhibition of long-term potentiation [65]. 2-AG can be synthesized as well as enzymatically degraded by neuroblastoma cells [66] or normal rat brain tissue [62], which further supports its neuromodulatory function. Unlike anandamide, whose generation is catalyzed by phospholipase D, 2-AG has been proposed to arise from inositol phospholipid precursors via cleavage by phospholipase C, yielding diacylglycerol, which is acted on by diacylglycerol lipase to give rise to 2-AG [65]. Alternatively, 2-AG may be generated from sn-1-lyso-2-arachidonyl phospholipids by phospholipase C [66]. Unlike the situation with anandamide, there appears to be no facilitated cellular uptake system for 2-AG [67]. However, the same amidohydrolase enzyme may be involved in the degradation of both anandamide and 2-AG [68].

Two additional endogenous lipids have been shown to bind to cannabinoid receptors: mead ethanolamide, having anandamide-like biochemical activity and binding profile [69], and the putative CB_2 receptor selective agonist, palmitoyl ethanolamide [33].

Finally, the advent of potent and highly selective antagonists first for the CB_1 receptor [70, 71] and, more recently, for the CB_2 receptor [72] (see Fig. 1), has provided critically important tools for uncovering the physiopathological role(s) of endogenous cannabinoids. The first CB_1 selective antagonist, SR141716 A [70], is about 50 times more potent at CB_1 (K_d: 12.3 nM [70]) than at CB_2 receptors (K_d: 702 nM [70, 73]), and at similar submicromolar concentrations it has been found not to bind to 30 different neurotransmitter receptors and 6 ion channels tested [70]. Interestingly, SR141716 A is reported to have properties of an inverse agonist in Chinese hamster ovary cells transfected with CB_1 receptors: it inhibits the high constitutive mitogen-activated protein kinase activity of these cells, and its binding to CB_1 receptors is enhanced by guanyl nucleotides [74]. Therefore, when this antagonist is found to unmask the "tonic" activity of an endogenous cannabinoid, the alternative explanation of inverse agonism must be considered. Another, more recently introduced CB_1 receptor antagonist, LY320135 (see Fig. 1), has been reported to have similar CB_1/CB_2 selectivity but about 20 times lower absolute potency than SR141716 A [71]. The first potent and selective CB_2 antagonist, SR144528, is reported to have subnanomolar affinity (K_i: 0.6 nM) for both the rat spleen and the cloned human CB_2 receptor, whereas its affinity for the rat brain and cloned human CB_1 receptor is 700 times lower (K_i: 400 nM)[72].

Cardiovascular effects of plant-derived and synthetic cannabinoids

Since the early 1970s several studies have documented the hypotensive effect of THC in both humans and laboratory animals [3–7]. In humans, blood pressure remains unaffected after an acute dose of THC, which elicits dose-related tachycardia [8]. However, chronic oral ingestion of THC has been shown to induce bradycardia and supine hypotension [9]. Total peripheral resistance is decreased even after acute administration of THC, when the lack of decrease in blood pressure is probably due to increased cardiac output secondary to the tachycardia [75]. In rats and most of the other mammalian species tested, acute administration of THC elicits prolonged hypotension and bradycardia, preceded by a brief pressor effect [76]. Several studies have demonstrated that the hypotensive/bradycardic reponse to THC can be reduced or abolished after surgical or pharmacological elimination of sympathetic tone. Such observations have been interpreted to suggest that these effects of THC are due to a centrally mediated inhibition of sympathetic tone [3]. In contrast, the initial pressor response to THC is not affected by such interventions, suggesting that it is due to peripheral, nonsympathetically mediated vasoconstriction [77, 78]. Early studies describe tolerance to the cardiovascular effects of cannabinoids; chronic intraperitoneal application of THC over 14 days resulted in the disappearance of the hypotensive and bradycardic effect [5].

A recent study examined the effects of a series of six cannabinoid analogs on arterial blood pressure and heart rate in urethane-anesthetized rats [78]. All analogs tested elicited pronounced and long-lasting hypotension and bradycardia, which could be blocked by pretreatment with the CB_1 receptor antagonist SR141716 A. The hypotensive and bradycardic potencies of these analogs displayed a highly significant, positive correlation with their analgesic potency (r=0.99) as well as their binding affinity to the brain cannabinoid receptor (r=0.97). In all four test systems, the following rank order of potencies was evident: (–)-11-OH-Δ^9-tetrahydrocannabinol dimethylheptyl (HU-210) ≥CP-55,940>WIN-55212-2>THC>anandamide (endogenous ligand)≥JWH-015 (for chemical structures see [78]). The similar rank orders of agonist potencies and the similar susceptibility to inhibition by SR141716 A in the various paradigms provide strong evidence that these diverse effects are mediated by the same receptor subtype, i.e., the CB_1 receptor. Stereoselectivity, another indication of specific receptor mechanisms, was also observed. HU-211, the inactive stereoisomer of the potent cannabinoid agonist HU-210, failed to influence blood pressure or heart rate at doses up to three orders of magnitude higher than the maximal hypotensive dose of HU-210 [78]. Interestingly, HU-211 was recently reported to protect animals from endotoxic shock by a mechanism unrelated to cannabinoid receptors, and probably involving suppression of tumor necrosis factor α production [79].

Of the six agonists tested, only THC and anandamide elicited an initial pressor response, which could not be antagonized by SR141716 A, suggesting that it is not mediated by CB_1 receptors [78]. As for the subsequent hypotension and bradycardia, both THC and anandamide acted as partial agonists. Their peak effects amounted to less than 50% of the profound decrease in blood pressure (by up to 80 mmHg) and heart rate (by up to 200 beats/min) elicited by maximally effective doses of the four other ligands. The extreme hypotension elicited by the latter compounds clearly exceeds the hypotension triggered by complete removal of sympathetic tone, which suggests that a direct vascular effect is responsible for some or even most of the hypotension observed. A similar possiblity is supported by the finding that the potent synthetic cannabinoid HU-210 retains its hypotensive action after chemical sympathectomy using 6-OH-dopamine [80].

The strong hypotensive properties of various cannabinoid analogs raises the possibility that such compounds might serve as the basis for the development of antihypertensive agents acting via a novel mechanism of action. For such compounds to be acceptable for human therapy, the cardiovascular and neurobehavioral activities must be separable. In a study of azacannabinoids, the hydroxyacetyl derivatives were found to be far less potent in eliciting centrally mediated hypomotility than the potent synthetic cannabinoid dimethylheptylpyran, whereas their hypotensive activity remained unchanged [81]. Furthermore, "abnormal cannabidiol"; an analog of the behaviorally inactive cannabidiol, is similarly inactive in triggering certain cannabinoidlike behavioral effects in dogs but, unlike its parent compound, is reasonably potent in eliciting hypotension [82]. Our group was able to confirm and extend these findings in rats and mice, and also to show that the hypotensive action of abnormal cannabidiol can be attenuated by SR141716 A (Lake et al., submitted for publication).

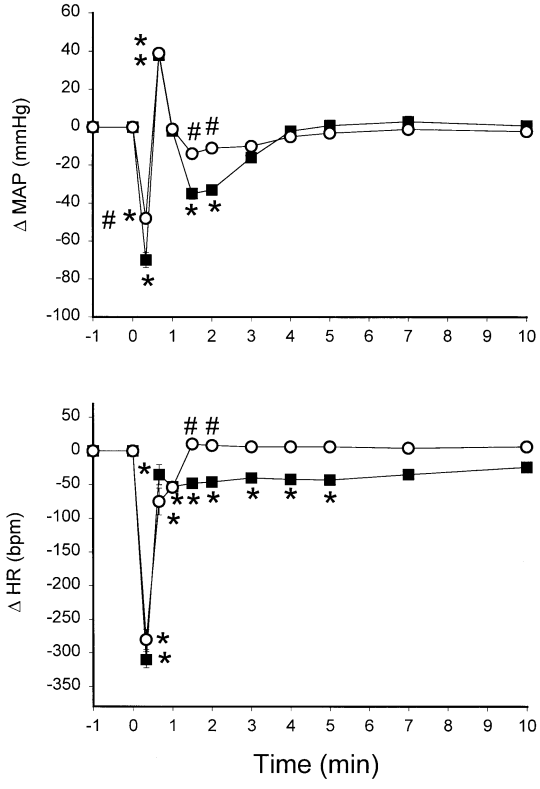

Fig. 2 Effects of anandamide (4 mg/kg i.v.) on mean arterial pressure (*MAP*) and heart rate (*HR*) in anesthetized normotensive Sprague-Dawley rats pretreated with vehicle (*filled squares*) or SR141716 A (3 mg/kg i.v.; *open circles*). *Points*, mean±SE (n=5–9); *P<0.05 from baseline; $^{\#}P$<0.05 between groups. (Reproduced by permission from [78]

Cardiovascular effects of endogenous cannabinoids

Anandamide mimics THC in neurobehavioral assays [83, 84] and elicits similar cardiovascular effects. In urethane-anesthetized rats anandamide elicits a triphasic response (see Fig. 2): a transient vagal bradycardia and hypotension (phase 1) are followed by a brief pressor response (phase 2) and then a more prolonged depressor response that lasts 3–5 min (phase 3) [85]. Both the amplitude and the duration of the phase 3 hypotension and bradycardia are significantly increased by pretreatment of the animals with phenylmethyl sulfonylfluoride (60 mg/kg i.p.), and the hypotension elicited by methanandamide, a metabolically stable analog of anandamide [86], has been found to be much longer lasting (20–25 min) than the hypotension caused by anandamide (J.A. Wagner et al. unpublished observations). These findings suggest that rapid metabolism of anandamide is responsible, at least in part, for its relatively short duration of action. Although THC usually elicits only the brief pressor and subsequent prolonged depressor response, one study reported the same triphasic response as observed for anandamide [87]. The initial transient bradycardia and hypotension in response to anandamide are vagally mediated, as they can be abolished by methylatropine or by cervical vagotomy. As observed earlier with THC, the phase 3 hypotensive response to anandamide is greatly reduced after α-adrenergic blockade or transection of the cervical spinal cord, whereas the brief pressor response, if anything, is enhanced after such interventions. This suggests that the depressor response is due to inhibition of sympathetic tone, whereas the pressor response is independent of the sympathetic nervous system [85].

Using the selective CB_1 antagonist SR141716 A, it was possible to determine the role of CB_1 receptors in the cardiovascular effects of anandamide. As tested in urethane-anesthetized rats, SR141716 A dose-dependently inhibited the phase 3 hypotensive response to anandamide, whereas the initial vagal bradycardia and the brief pressor response were unaffected [78, 85] (see Fig. 2). The ID_{50} of SR141716 A is similar for inhibiting the hypotensive response to anandamide (0.29 mg/kg) or THC (0.08 mg/kg) [88], and both values are similar to the ID_{50} of SR141716 A for inhibiting the neurobehavioral effects of cannabinoids [70]. At doses up to 3 mg/kg, SR141716 A does not affect basal blood pressure or heart rate, whereas at higher

doses a moderate decrease in blood pressure is usually observed. These findings suggest that the prolonged hypotension, but not the initial vagal activation and the subsequent brief pressor response, are mediated by CB_1 receptors, which are not tonically active. Recently we were able to obtain definitive evidence for the role of CB_1 receptors in the hypotensive and bradycardic actions of anandamide and other cannabinoids, using CB_1 receptor knockout mice developed at the National Institutes of Mental Health by Drs. Andreas Zimmer and Tom Bonner. In preliminary, unpublished experiments we found that in pentobarbital-anesthetized CB_1 receptor knockout (–/–) mice anandamide, THC, HU-210, and (R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl]-(1-naphthalenyl) methanone (WIN 55212-2) failed to elicit hypotension and bradycardia, which fully developed in the control (+/+) littermates treated with the same drugs. Interestingly, anandamide and THC caused a slight increase in blood pressure and a moderate but sustained increase in heart rate in the CB_1 receptor knockout mice.

Although a central site of the hypotensive action of anandamide appeared plausible, subsequent findings ruled out such a mechanism. First, intracerebroventricular administration of anandamide fails to reduce blood pressure in anesthetized rats. Second, the activity of sympathetic premotor (barosensitive) neurons in the rostral ventrolateral medulla is not reduced during the hypotensive response to systemically administered anandamide [89]. Third, in barodenervated rats anandamide fails to attenuate the increase in postganglionic sympathetic nerve activity triggered by electrical stimulation of the rostral ventrolateral medulla, yet it blunts the simultaneous pressor response [89]. The latter observation also excludes a possible ganglionic blocking action and suggests that anandamide inhibits norepinephrine release from postganglionic sympathetic nerve terminals, although it does not exclude a possible direct vasodilator effect.

Since anesthesia might increase sympathetic outflow [90], two other studies examined the effects of anandamide in conscious, chronically cannulated rats: in normotensive Sprague-Dawley rats anandamide elicited the transient, vagally mediated bradycardia and the subsequent brief pressor response, but the phase 3 hypotension seen in anesthetized rats was absent [88, 91]. Spontaneously hypertensive rats are known to have higher basal sympathetic outflow than Sprague-Dawley rats. Spontaneously hypertensive rats, either conscious or under anesthesia, displayed the full triphasic blood pressure response to anandamide, including the prolonged hypotensive response [88]. Interestingly, the phase 3 hypotensive response to anandamide lasted much longer in spontaneously hypertensive (>45 min) than in anesthetized Sprague-Dawley rats (3–5 min). These observations are compatible with the possibility that preexisting sympathetic tone determines the hypotensive response to anandamide [88].

Two recent studies have confirmed the existence of sympatho-inhibitory, presynaptic CB_1 receptors [92, 93]. In the first study using rat isolated atria and vasa deferentia preloaded with [^{3}H]norepinephrine, anandamide and THC concentration-dependently inhibited nerve-stimulation induced [^{3}H]norepinephrine release, and these effects were competitively antagonized by SR141716 A [92]. The nonexocytotic release of [^{3}H]norepinephrine by tyramine was unaffected by either anandamide or THC. The same study demonstrated the presence of CB_1 receptor mRNA in various rat tissues using reverse transcription polymerase chain reaction with appropriate primers. Criteria for identifying cannabinoid receptor mRNA included the appearance of an amplicon of the expected size, Southern hybridization of the amplicon with a CB_1 receptor cDNA, and verification of the structure of the amplicon by dideoxy chain termination sequencing. Using these criteria, CB_1 receptor mRNA could be identified in the superior cervical ganglion, which contains cell bodies of postganglionic sympathetic neurons [92]. The presence of CB_1 receptor mRNA in a sympathetic ganglion suggests that the receptor protein is located presynaptically on postganglionic sympathetic nerve terminals. Anandamide may activate such receptors in the heart and vasculature, contributing to its hypotensive and bradycardic properties. The functional role of presynaptic cannabinoid receptors has subsequently been documented in a whole animal model. In anesthetized, pithed rats the pressor response to electrical stimulation of thoracolumbar preganglionic sympathetic neurons was dose-dependently inhibited by systemically administered cannabinoids, and the inhibition could be prevented by SR141716 A [93]. Interestingly, the results of a recent study indicate that in the mouse vas deferens the ability of cannabinoids to inhibit norepinephrine release may be mediated by CB_2 receptors [94]. Presynaptic cannabinoid receptors may not be limited to peripheral sympathetic nerve terminals. Presynaptic cannabinoid receptors have been also identified in the brain [95], and cannabinoids have been found to suppress acetylcholine release in the guinea pig myenteric plexus [96] as well as at the neuromuscular junction [97].

Additional direct effects of cannabinoids on the peripheral vasculature, which contains functional CB_1 receptors [98], is likely [99–101]. In an isolated, buffer-perfused mesenteric arterial bed preparation, SR141716 A is reported to inhibit the nitric oxide and prostanoid-independent component of the vasodilator response to carbachol or to the calcium ionophore A23187 [100], effects commonly attributed to a putative endothelium-derived hyperpolarizing factor (EDHF) [102]. Furthermore, either SR141716 A, high potassium, or the cytochrome P-450 inhibitors clotrimazole and proadifen (SKF525 A) inhibited the vasodilator effect of anandamide in this preparation [100, 101]. However, the conclusion of these studies that anandamide is identical to EDHF [100, 101] has been contested. Another group using the same in vitro model [103] found that the vasodilator response to anandamide cannot be antagonized by SR141716 A, and anandamide- and EDHF-induced vascular smooth muscle cell hyperpolarizations were attributed to activation of different types of potassium channels [103, 104]. SR141716 A similarly failed to reduce the vasodilatory effect of anandamide in a perfused bovine coronary artery preparation, whereas the effect was

abolished in the presence of an amidohydrolase inhibitor that prevented the hydrolysis of anandamide [105]. This led to the suggestion that anandamide produces endothelium-dependent vasorelaxation as a result of its catabolism to arachidonic acid, which is then metabolized to yield vasodilatory eicosanoids such as prostacyclin or the epoxyeicosatrienoic acids [105]. In contrast, in a study by Randall and Kendall [106] anandamide caused coronary vasorelaxation which could be abolished by SR141716 A. In the porcine coronary artery, anandamide was found neither to hyperpolarize nor to relax smooth muscle cells [104]. The ability of endothelial cells to generate anandamide has recently been shown in rat cultured renal endothelial cells [107]. Together with the finding of CB_1 receptor dependent vasodilation after exogenous anandamide in juxtamedullary afferent arterioles [107], these latter findings suggest an endocannabinoid signaling system in the kidney, exerting vasorelaxation.

The question whether EDHF is an endocannabinoid remains controversial. It is clear, however, that anandamide is able to relax [100, 101, 103–109] and to hyperpolarize [103, 104, 109] smooth muscle cells in endothelium-intact arteries. Whether these effects are CB_1 receptor-mediated [100, 106–109] or are independent of CB_1 receptor activation [103–105] is uncertain and may vary from tissue to tissue. The resistance of anandamide effects to inhibition by SR141716 A in some of these studies, also observed in a classical neurobehavioral paradigm in mice [110], could suggest that the effects are due to a metabolite of anandamide which does not act via cannabinoid receptors. Even when the vasodilator effect of anandamide is sensitive to inhibition by SR141716 A in certain vascular beds, the receptors involved may be distinct from CB_1 receptors. This is strongly suggested by the lack of a vasodilator response of such preparations to the synthetic cannabinoids HU-210 [103, 104] or WIN 55,212–2 [103], which bind with high affinity to CB_1 receptors and produce dramatic decreases in blood pressure (blocked by SR141716 A) in intact, anesthetized rats [78]. Indeed, in a recent preliminary study using the isolated, buffer-perfused rat mesenteric artery preparation we found that anandamide and methanandamide were potent vasodilators, whereas HU-210, WIN 55212–2, THC, and 2-AG had no dilator effect. SR141716 A inhibited the effect of anandamide in intact, but not in endothelium-denuded, preparations. These findings could suggest that anandamide-induced mesenteric vasodilation in the rat is mediated by an SR141716A-sensitive receptor distinct from CB_1 receptors and located on endothelial cells, whereas a direct vasodilator effect of anandamide on vascular smooth muscle is mediated by an SR141716A-insensitive mechanism [111].

Additionally, the generation and release of 2-AG from human vascular endothelial cells after stimulation with either thrombin or the calcium ionophore A23187 has been recently reported [112]. At the same time, we examined the cardiovascular effects of 2-AG in urethane-anesthetized rats [55]. As with anandamide, 2-AG (1 and 3 mg/kg i.v.) elicited hypotension that could be attenuated by SR141716 A. Unlike anandamide, 2-AG also elicited moderate tachycardia that was unaffected by SR141716 A pretreatment [55].

Cannabinoid receptor activation during shock

The profound and long-lasting hypotension that can be triggered via the activation of peripherally located CB_1 receptors suggests that activation of such receptors by endogenous ligands is involved in pathological states associated with extreme hypotension, such as the various forms of cardiovascular shock. The selective CB_1 receptor antagonist SR141716 A does not alter basal blood pressure or heart rate in doses up to 3 mg/kg i.v., which suggests that "hypotensive" CB_1 receptors are not tonically active. However, in anesthetized rats subjected to controlled hemorrhage (stepwise removal of approximately 50% of the total blood volume until mean blood pressure stabilized at 40 mmHg), the intravenous injection of 3 mg/kg SR141716 A caused an acute increase in blood pressure, which remained elevated for 20–30 min [54] (see Fig. 3). The pressor effect was dose dependent (Fig. 3, inset) and peripherally mediated because injection of 300 µg/kg SR141716 A into the cisterna magna or the 4th cerebral ventricle failed to influence blood pressure. Furthermore, the mere lowering of blood pressure did not activate the endogenous cannabinoid system: SR141716 A caused no pressor effect after blood pressure had been reduced to approx. 50 mmHg by phentolamine (2 mg/kg i.v.) [54], which also discounts the possibility that the pressor response observed in shock is due an inverse agonist action of SR141716 A. These findings therefore suggest that shock induces the release of endogenous cannabinoids acting at peripheral CB_1-like receptors to lower blood pressure.

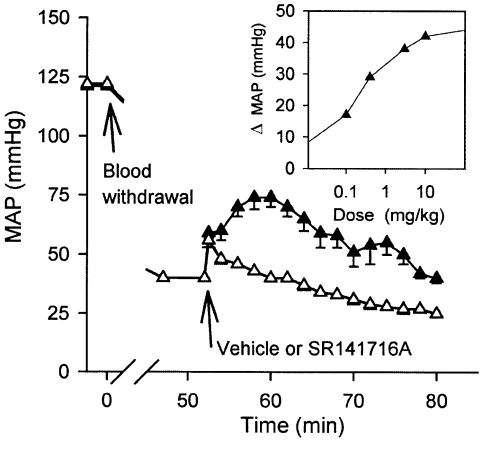

Fig. 3 The effects of SR141716 A (3 mg/kg i.v. in 0.2 ml, *n*=7; *filled triangles*) or vehicle (0.2 ml, *n*=7; *open triangles*) on blood pressure in rats subjected to hemorrhagic shock. *Inset*, dose-dependence of the pressor effect of SR141716 A in shock, each point representing the mean of three to seven experiments. The ED_{50} value for the pressor effect of SR141716 A was calculated by using the ALLFIT program. *Vertical bars*, SEM. (Reproduced by permission from [54])

On the assumption that the released cannabinoids become blood-borne, various components of peripheral blood from animals in shock were tested for hypotensive activity in normal recipient rats. Macrophages and platelets, but not other blood cells or plasma, isolated from hemorrhaged rats elicited CB_1 receptor mediated hypotension in normotensive recipients, whereas similar cells from normal controls had no such effects. Macrophages from hemorrhaged rats or control rat macrophages stimulated in vitro with phospholipase D or the calcium ionophore ionomycin were found to incorporate arachidonic acid or ethanolamine into a product that coeluted with anandamide on reverse-phase HPLC [54]. Finally, macrophages stimulated in vitro with ionomycin plus phospholipase D produced anandamide, as identified by gas chromatography– mass spectrometry (GC/MS) [54].

Several observations discount the role of the NO system in the CB_1 receptor mediated hypotension in hemorrhagic shock. Pretreatment of recipient rats with the irreversible NO synthase inhibitor N^G-nitro-L-arginine methylester (L-NAME) did not prevent the hypotensive response to "shock" blood, nor was the transfer of hypotensive activity by macrophages blocked by pretreatment of donor rats with L-NAME. Furthermore, the rate of nitrite and nitrate production was similar in control and "shock" macrophages within the first 3 h following their isolation. Finally, the hypotensive response to intravenous administration of the NO donor sodium nitroprusside was unaffected by SR141716 A [54].

One may wonder about the survival value of a vasodilator mechanism activated under conditions when blood pressure is already reduced due to blood loss. In this regard it was somewhat unexpected that pretreatment of rats with SR141716 A before subjecting them to hemorrhage drastically would worsen rather than prolong their survival [54]. This could suggest that the cannabinoid-induced vasodilation is beneficial, as it may serve to maintain adequate tissue oxygenation in the face of excessive reflex sympathetic vasoconstriction.

The intriguing implication of these observations is that macrophage-derived anandamide may activate peripheral CB_1 receptors contributing to hemorrhagic hypotension. These findings also raise two related questions. First, is a similar mechanism involved in other forms of shock, and, second, in the absence of detectable anandamide in "shocked" platelets [54], what is the mechanism by which these cells elicit CB_1 receptor mediated hypotension?

Lipopolysaccharide (LPS) present in the outer membrane of gram-negative bacteria is an important, although not exclusive, pathogenic factor in septic or endotoxic shock. LPS has also been implicated in the pathogenesis of hemorrhagic shock [113], which suggests a certain commonality in the underlying cellular mechanisms. LPS administered to experimental animals initiates cytokine activation associated with hemodynamic consequences [114], which provides a widely used model for studying the mechanisms involved in endotoxic shock. Current management of human septic shock in intensive care units includes volume replacement, vasopressor drugs, antibiotics, monoclonal antibodies against endotoxin, and hemodialysis. Despite the use of such measures the mortality rate still ranges between 25% and 75% [115]. Hence, further research to evaluate the physiopathological events leading to circulatory failure, organ hypoperfusion, and concomitant dysfunction resulting in death is warranted.

Intravenous injection of 15 mg/kg *Escherichia coli* LPS into anesthetized rats consistently elicits gradually developing, prolonged hypotension associated with tachycardia [55]. The same pattern of response is observed in control rats injected with macrophages plus platelets isolated from 3 ml blood from a LPS-treated donor rat or injection of control rat macrophages (contaminated with platelets) or pure platelets preincubated in vitro with 200 μg/ml LPS. In all four cases the hypotension but not the tachycardia is prevented by pretreatment of the recipient rat with 3 mg/kg SR141716 A. To test whether the hypotension evoked by LPS-activated macrophages or platelets is dependent on preexisting sympathetic tone, rats were pretreated with phentolamine (2 mg/kg i.v.) to eliminate α-adrenergic vasoconstrictor tone and then continuously infused with vasopressin (2 μg/kg/min i.v.) to restore basal blood pressure to control levels. The hypotensive and tachycardic effects of subsequently injected, LPS-activated macrophages or platelets did not differ from that in controls, which indicates that the hypotension is independent of sympathetic tone and is most likely due to a direct vascular effect. The additional observation that pretreatment of recipient rats with L-NAME does not alter the hypotensive response to LPS-treated macrophages/platelets suggests that, as in hemorrhagic shock, endothelial NO does not significantly contribute to this vasodilator effect [55].

Control and LPS-treated macrophages and platelets were analyzed for the presence of the endogenous cannabinoids anandamide and 2-AG, by using reverse-phase HPLC followed by GC/MS. Such analyses revealed that platelet-depleted macrophage preparations treated in vitro with LPS contain substantial amounts of anandamide, whereas anandamide is undetectable in control macrophages and in pure platelet preparations treated with LPS (Fig. 4). These findings were very similar to those obtained earlier in the hemorrhagic shock model and confirmed the absence of anandamide in platelets. Control platelets, however, were found to contain 2-AG, and their in vitro exposure to LPS (200 μg/ml) markedly increased the cellular levels of 2-AG. The additional finding that in anesthetized rats 2-AG elicits hypotension, which can be prevented by SR141716 A pretreatment [55], suggests that 2-AG contributes to the CB_1 receptor-mediated hypotensive effect of "shock" platelets.

Taken together these findings indicate that during certain shock conditions, platelets, and macrophages produce at least two different endogenous cannabinoids. Anandamide and 2-AG may be paracrine mediators of hypotension during shock, acting via CB_1 receptors localized in the peripheral vasculature. However, the cellular localization of such receptors needs to be defined. Although blood-borne mediators may act on vascular smooth muscle cells directly, the vascular endothelium may also be a target of mac-

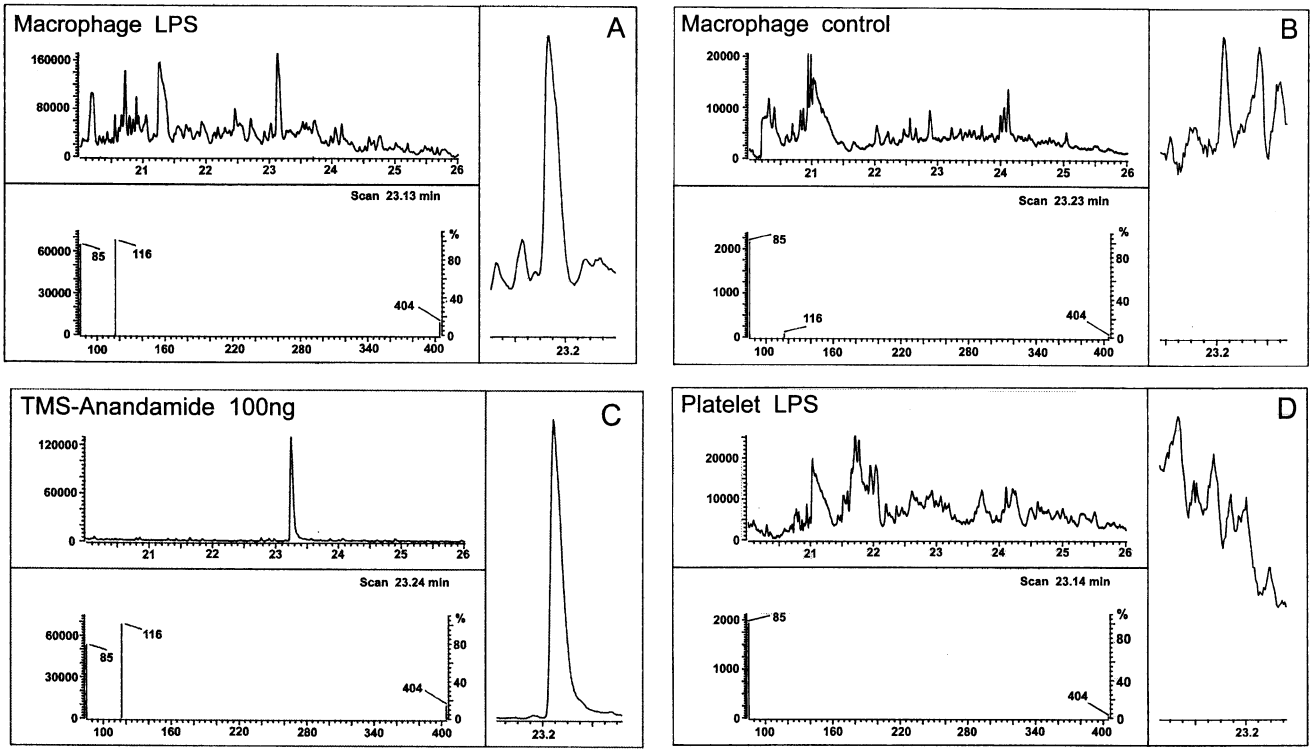

Fig. 4A–D GC/MS analysis of anandamide in rat macrophages and platelets. Macrophages (**A**, **B**) and platelets (**D**) isolated from 100 ml normal rat blood were incubated in vitro with 200 µg/ml LPS (**A**, **D**) or vehicle (**B**) for 90 min. Ethyl acetate extracts of cells plus medium were prepurified by reverse-phase HPLC, and the anandamide fractions were subjected to GC/MS analysis. **C** GC/MS analysis of 100 ng authentic anandamide. *Upper left panels* (**A**–**D**), combined currents obtained by selected ion monitoring on m/z 85, m/z 116, and m/z 404; *lower left panels*, ratio of the monitored ions at the apex of the anandamide peak; *right panels*, expanded view of the anandamide region of the ion chromatogram. The amount of anandamide in the GC elution peak in LPS-stimulated macrophages (**A**, 130 ng) was estimated from a calibration curve generated with 50, 100, and 150 ng anandamide. The limit of sensitivity for anandamide is 1 ng in this GC/MS system. (Reproduced by permission from [55])

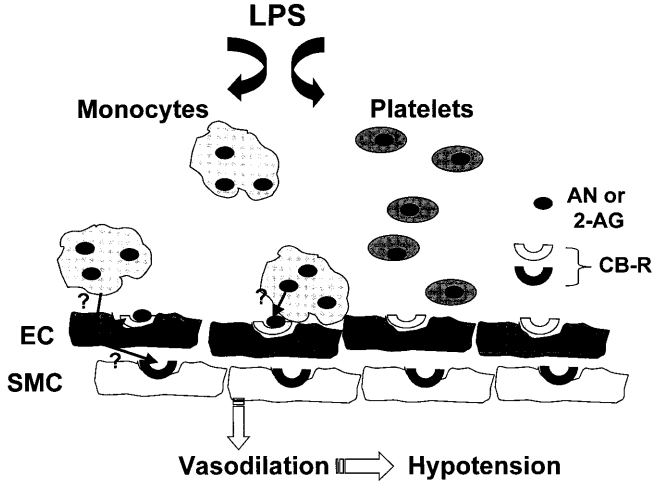

Fig. 5 Schematic illustration of the proposed role of macrophage-, platelet-, and endothelium-derived endogenous cannabinoids and vascular cannabinoid receptors in the hypotension of septic shock

rophage- and platelet-derived endocannabinoids. The presence of CB_1 receptors on endothelial cells is suggested by the detection of CB_1 receptor mRNA by reverse transcription-polymerase chain reaction in cultured endothelial cells from human umbilical vein [112]. Furthermore, endotoxin-induced activation of vascular endothelial cells has been found to be dependent on the presence of macrophages [116]. Activated macrophages not only adhere to the vascular endothelium [117] but may develop junctional communication with endothelial cells via the expression of the gap junction protein connexin-43 [118, 119]. All this suggests that the vascular endothelium is also involved in cannabinoid-mediated vasodilation in shock conditions. It could be an additional source of cannabinoids, the target of macrophage- and platelet-derived cannabinoids, or both. Preliminary evidence suggesting the existence of SR141716A-sensitive “anandamide receptors” located on vascular endothelial cells has been briefly discussed above. Results of the same preliminary study [111] also suggest that endothelial cells are a source of endocannabinoids released by LPS. Using a buffer-perfused mesenteric vascular bed preparation isolated from control rats, we found that the inclusion of 0.5–5.0 µM SR141716 A in the perfusion buffer does not influence perfusion pressure. However, in preparations obtained from rats that have been pretreated in vivo with 15 mg/kg LPS the same concentrations of SR141716 A cause a sustained, 10- to 15-mmHg increase in perfusion pressure, which can be abolished by endothelial denudation prior to the infusion with SR141716 A [111]. Thus, LPS-induced mesenteric vasodilation may involve the release of an endocannabinoid from the endothelium. Since macrophages are known to be involved in LPS-induced endothelial activa-

tion [116], this provides an alternative explanation of our finding that LPS-activated macrophages elicit SR141716A-sensitive hypotension. However, it remains unclear whether the mediator responsible for such release is also an endocannabinoid or another macrophage-derived substance.

The scheme depicted in Fig. 5 summarizes the proposed sources of endocannabinoids (macrophages, platelets, vascular endothelial cells) and their vascular targets (endothelial and vascular smooth muscle cells) in LPS-induced hypotension.

Research prospects and clinical implications

In view of the widely suspected role of endogenous cannabinoids as central modulators of neurobehavioral functions, it is somewhat ironic that the first evidence of a physiological function for this novel class of mediators is related to their cardiovascular effects. Endogenous cannabinoids have significant effects on cardiovascular variables, and may be involved as vasodilator mediators in the peripheral regulation of vascular tone. Recent evidence indicates that activation of vascular cannabinoid receptors contributes to the hypotension associated with hemorrhagic and endotoxic shock, and that macrophage- and platelet-derived cannabinoids are likely contributors. The vascular endothelium has also been implicated as a possible source and/or target of vasoactive cannabinoids. The possible involvement of cannabinoids and their receptors in cardiovascular shock states in humans is currently under investigation. Such studies could lead to the development of a novel pharmacological approach for the management of extreme hypotension associated with certain pathological conditions.

Certain synthetic cannabinoids cause profound and long-lasting hypotension via activation of vascular CB_1 receptors. Studies using the isolated rat mesenteric vascular bed preparation suggest that additional receptors with unique sensitivity to anandamide are also involved in vasodilation in certain vascular beds. Attempts to separate hemodynamic activity from undesired psychoactive properties of cannabinoid analogs may lead to the development of a new class of antihypertensive agents.

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