

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 CB₁ cannabinoid receptor subtype, expressed primarily in the brain but also present in some peripheral tissues. A second receptor subtype, the CB₂ 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 CB₁ receptors. Possible underlying mechanisms include presynaptic CB₁ 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.



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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-1H-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 CB₁ receptor, originally discovered in brain [11, 12] but also present in some peripheral tissues [13, 14], and the CB₂ 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 CB₁ 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 CB₁ receptor was discovered in leech, indicating that this signaling system has been conserved during evolution [17]. The amino acid sequence homology between the human CB₁ and CB₂ receptors is only about 44%, and the CB₂ receptor is also smaller than the CB₁ receptor (360 amino acids, 40.2 kDa), due to a shorter third intracellular loop and C-terminal tail. The gene locus designations for the CB₁ and CB₂ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₂ 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 sulfonyl fluoride, 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 CB₁ receptor antagonists SR141716 A and LY320135, and the selective CB₂ receptor antagonist SR144528

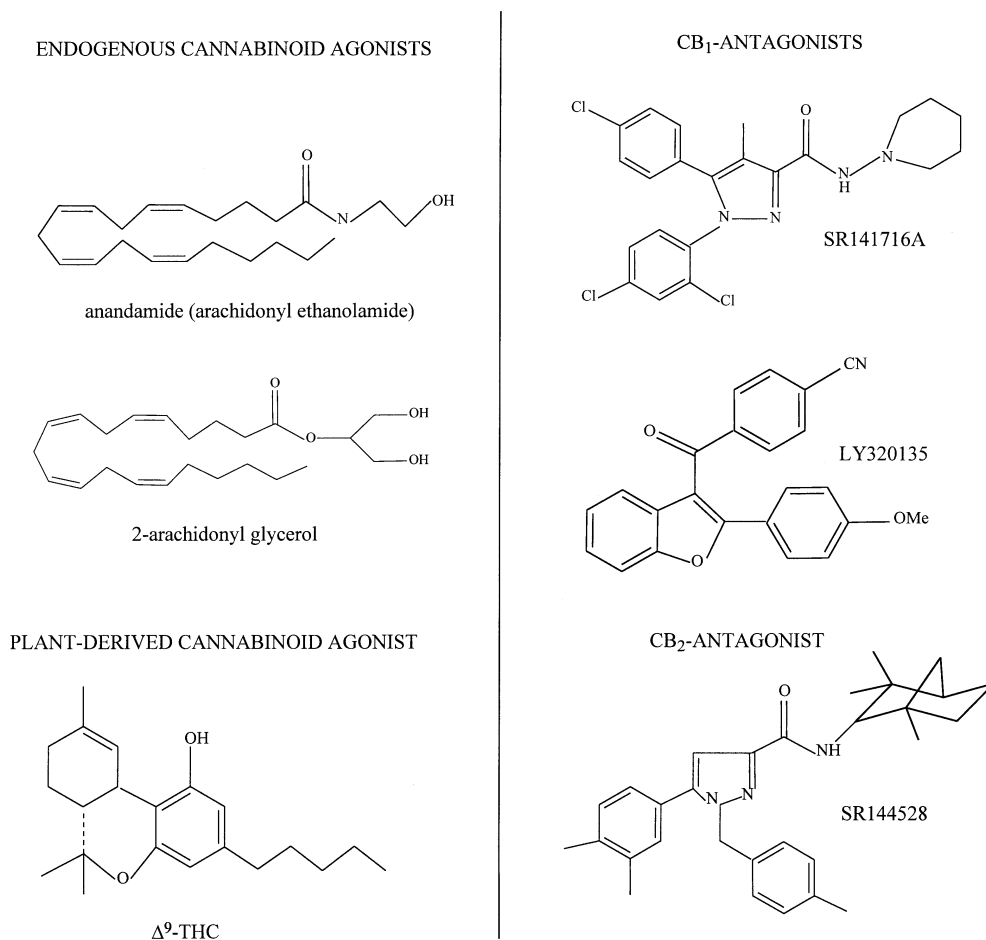


Table 1 Gene localization and molecular size of cannabinoid receptors cloned to date

| 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 CB₁ receptors with an affinity similar to or greater than their affinity for the enzyme [48–50], or to potently inhibit phospholipase A₂ [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-hydroxy-anandamide, 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 CB₁ 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 μ M [62] at the transfected or native CB₁ receptor, respectively, and it was 2–24 times less potent than anandamide. Unlike anandamide, 2-AG not only binds to but also activates CB₂ 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 CB₁ 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 CB₂ receptor selective agonist, palmitoyl ethanolamide [33].

Finally, the advent of potent and highly selective antagonists first for the CB₁ receptor [70, 71] and, more recently, for the CB₂ receptor [72] (see Fig. 1), has provided critically important tools for uncovering the physiopathological role(s) of endogenous cannabinoids. The first CB₁ selective antagonist, SR141716 A [70], is about 50 times more potent at CB₁ (K_i : 12.3 nM [70]) than at CB₂ receptors (K_i : 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 CB₁ receptors: it inhibits the high constitutive mitogen-activated protein kinase activity of these cells, and its binding to CB₁ 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 CB₁ receptor antagonist, LY320135 (see Fig. 1), has been reported to have similar CB₁/CB₂ selectivity but about 20 times lower absolute potency than SR141716 A [71]. The first potent and selective CB₂ antagonist, SR144528, is reported to have subnanomolar affinity (K_i : 0.6 nM) for both the rat spleen and the cloned human CB₂ receptor, whereas its affinity for the rat brain and cloned human CB₁ 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 response 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 CB₁ 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) \geq CP-55,940 $>$ WIN-55212-2 $>$ THC $>$ anandamide (endogenous ligand) \geq 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 CB₁ 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 CB₁ 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 possibility 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).

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,

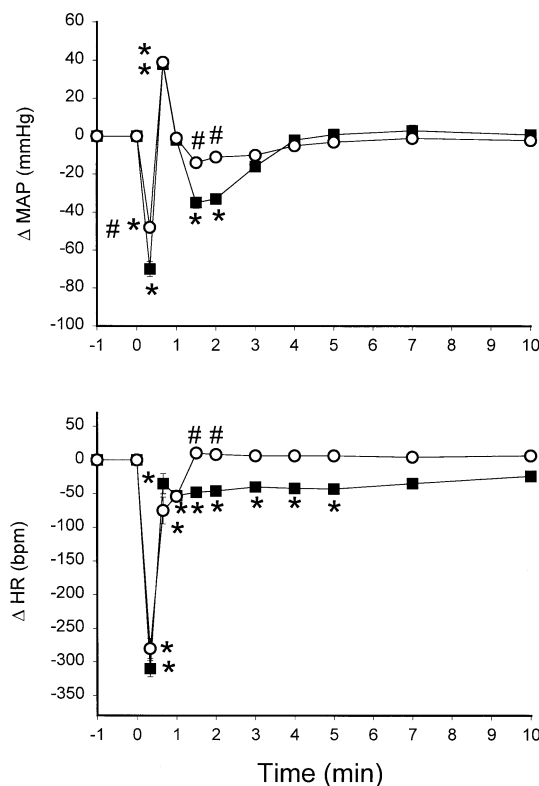


Fig. 2 Effects of anandamide (4 mg/kg i.v.) on mean arterial pressure (MAP) and heart rate (HR) in anesthetized normotensive Sprague-Dawley rats pretreated with vehicle (filled squares) or SR141716 A (3 mg/kg i.v.; open circles). Points, mean \pm SE ($n=5-9$); * $P<0.05$ from baseline; # $P<0.05$ between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

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 CB₁ antagonist SR141716 A, it was possible to determine the role of CB₁ 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 ID₅₀ 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 ID₅₀ 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 CB₁ receptors, which are not tonically active. Recently we were able to obtain definitive evidence for the role of CB₁ receptors in the hypotensive and bradycardic actions of anandamide and other cannabinoids, using CB₁ 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 CB₁ 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 CB₁ 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 CB₁ receptors [92, 93]. In the first study using rat isolated atria and vasa deferentia preloaded with [³H]norepinephrine, anandamide and THC

concentration-dependently inhibited nerve-stimulation induced [³H]norepinephrine release, and these effects were competitively antagonized by SR141716 A [92]. The nonexocytotic release of [³H]norepinephrine by tyramine was unaffected by either anandamide or THC. The same study demonstrated the presence of CB₁ 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 CB₁ receptor cDNA, and verification of the structure of the amplicon by dideoxy chain termination sequencing. Using these criteria, CB₁ receptor mRNA could be identified in the superior cervical ganglion, which contains cell bodies of postganglionic sympathetic neurons [92]. The presence of CB₁ 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 CB₂ 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 CB₁ 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 CB₁ 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 CB₁ receptor-mediated [100, 106–109] or are independent of CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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” CB₁ 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 CB₁-like receptors to lower blood pressure.

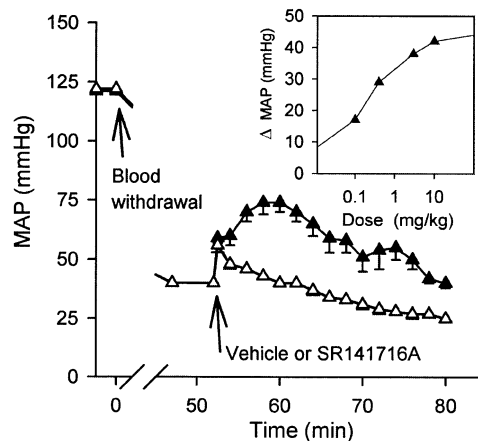


Fig. 3 The effects of SR141716 A (3 mg/kg i.v. in 0.2 ml, $n=7$; filled triangles) or vehicle (0.2 ml, $n=7$; open triangles) on blood pressure in rats subjected to hemorrhagic shock. *Inset*, dose-dependence of the pressor effect of SR141716 A in shock, each point representing the mean of three to seven experiments. The ED₅₀ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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-

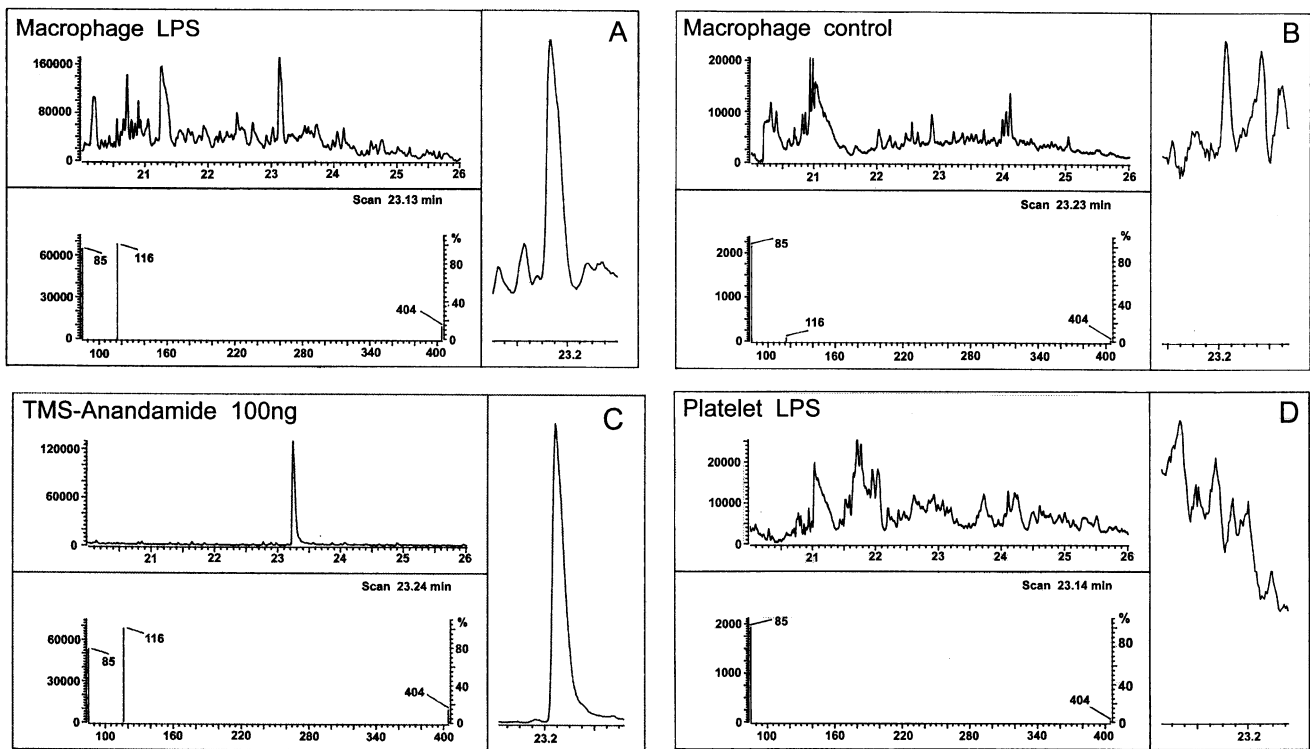


Fig. 4A–D GC/MS analysis of anandamide in rat macrophages and platelets. Macrophages (**A, B**) and platelets (**D**) isolated from 100 ml normal rat blood were incubated *in vitro* with 200 $\mu\text{g}/\text{ml}$ LPS (**A, D**) or vehicle (**B**) for 90 min. Ethyl acetate extracts of cells plus medium 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])

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-

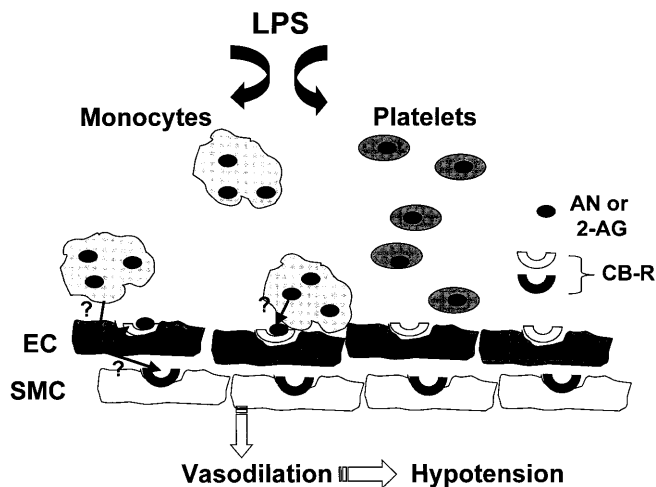


Fig. 5 Schematic illustration of the proposed role of macrophage-, platelet-, and endothelium-derived endogenous cannabinoids and vascular cannabinoid receptors in the hypotension of septic shock

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 CB₁ 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.

References

- Dewey WL (1986) Cannabinoid pharmacology. *Pharmacol Rev* 38:151–178
- Friedmann H, Klein TW, Newton C, Daaka Y (1995) Marijuana, receptors and immunomodulation. *Adv Exp Med Biol* 373:103–113
- Vollmer RR, Cavero I, Ertel RJ, Solomon TA, Buckley JP (1974) Role of the central nervous system in the hypotension and bradycardia induced by (–)- Δ^9 -trans-tetrahydrocannabinol. *J Pharm Pharmacol* 26:186–192
- Cavero I, Solomon T, Buckley JP, Jandhyala BS (1973) Studies on the hypotensive activity of (–)- Δ^9 -trans-tetrahydrocannabinol in anesthetized dogs. *Res Commun Chem Pathol Pharmacol* 6:527–540
- Graham JDP, Li DMF (1973) Cardiovascular and respiratory effects of cannabis in cat and rat. *Br J Pharmacol* 49:1–10
- Dewey WL, Harris LS, Howes JF, Kennedy JS, Granchelli FE, Pars HG, Razdan RK (1970) Pharmacology of some marijuana constituents and two heterocyclic analogs. *Nature* 226:1255–1257
- Hardman HF, Domino EF, Seevers MH (1971) General pharmacological actions of some synthetic tetrahydrocannabinol derivatives. *Pharmacol Rev* 23:295–315
- Kanakis C, Pouget JM, Rosen KM (1976) The effects of Δ^9 -THC (cannabis) on cardiac performance with or without beta blockade. *Circulation* 53:703–709
- Benowitz NL, Jones RT (1975) Cardiovascular effects of prolonged delta-9-tetrahydrocannabinol ingestion. *Clin Pharmacol Ther* 18:287–297
- Devane WA, Dysarz FA, Johnson LS, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34:605–613
- Matsuda LA, Lolait SJ, Brownstein BJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346:561–564
- Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 87:1932–1936
- Kaminski NE, Abood ME, Kessler FK, Martin BR, Schatz AR (1992) Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. *Mol Pharmacol* 42:736–742
- Gerard CM, Mollereau C, Vassart G, Parmentier M (1991) Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* 279:129–134
- Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61–65
- Matsuda LA (1997) Molecular aspects of cannabinoid receptors. *Crit Rev Neurobiol* 11:143–166
- Stefano GB, Salzet B, Salzet M (1997) Identification and characterization of the leech CNS cannabinoid receptor: coupling to nitric oxide release. *Brain Res* 753:219–224
- Caenazzo L, Hoehe MR, Hsieh W-T, Berretini WH, Bonner TI, Gershon ES (1991) HindIII identifies a two allele DNA polymorphism of the human cannabinoid receptor gene (CNR). *Nucleic Acids Res* 19:4798
- Stubbs L, Chittenden L, Chakrabarti A, Onaivi E (1996) The gene encoding the central cannabinoid receptor is located in proximal mouse chromosome 4. *Mamm Genome* 7:165–166
- Onaivi ES, Stubbs L, Chakrabarti A, Chittenden L, Hurst DP, Akinshola BE, Shire D, Reggio PH (1998) Murine cannabinoid receptor genetics. *FASEB J* 12:A194
- Shire D, Carrilon C, Kaghad M, Calandra B, Rinaldi-Carmona M, LeFur G, Ferrara P (1995) An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J Biol Chem* 270:3726–3731
- Rinaldi-Carmona M, Calandra B, Shire D, Bouaboula M, Oustric D, Barth F, Casellas P, Ferrara P, Le Fur G (1996) Characterization of two cloned human CB₁ cannabinoid receptor isoforms. *J Pharmacol Exp Ther* 278:871–878
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258:1946–1949
- Vogel Z, Barg J, Levy R, Saya D, Heldman E, Mechoulam R (1993) Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *J Neurochem* 61:352–355
- Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA (1993) Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned cannabinoid receptor and stimulates receptor mediated signal transduction. *Proc Natl Acad Sci USA* 90:7656–7660
- Deadwyler SA, Hampson RE, Bennett BA, Edwards TA, Mu J, Pacheco MA, Ward SJ, Childers SR (1993) Cannabinoids modu-

- late potassium currents in cultured hippocampal neurons. *Receptor Channels* 1:121–134
27. Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci* 15:6552–6561
 28. Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, LeFur G, Casellas P (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312:637–641
 29. Derkinderen P, Toutant P, Burgaya F, Le Bert M, Siciliano JC, de Francis V, Gelman M, Girault J-A (1996) Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* 273:1719–1722
 30. Burstein S, Budrow J, Debatis M, Hunter SA, Subramanian A (1994) Phospholipase participation in cannabinoid-induced release of free arachidonic acid. *Biochem Pharmacol* 48:1253–1264
 31. Shivachar AC, Martin BR, Ellis EF (1996) Anandamide- and Δ^9 -tetrahydrocannabinol-evoked arachidonic acid mobilization and blockade by SR141716 A. *Biochem Pharmacol* 51:669–676
 32. Bayewitch M, Avidor-Reiss T, Levy R, Barg J, Mechoulam R, Vogel Z (1995) The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling. *FEBS Lett* 375:143–147
 33. Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* 92:3376–3380
 34. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz J-C, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372:686–691
 35. Cadas H, Gaillet S, Beltramo M, Venance L, Piomelli D (1996) Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J Neurosci* 16:3934–3942
 36. Deutsch DG, Chin SA (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* 46:791–796
 37. Devane WA, Axelrod J (1994) Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid receptor, by brain membranes. *Proc Natl Acad Sci USA* 91:6698–6701
 38. Hillard CJ, Campbell, WB (1997) Biochemistry and pharmacology of arachidonylethanolamide, a putative endogenous cannabinoid. *J Lipid Res* 38:2383–2398
 39. Di Marzo V, Deutsch DG (1998) Biochemistry of the endogenous cannabinoid ligands. *Semin Neurosci* (in press)
 40. Ueda N, Kurahashi Y, Yamamoto S, Tokunaga T (1995) Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J Biol Chem* 270:23823–23827
 41. Arreaza G, Devane WA, Omeir RL, Sajjani G, Kunz J, Cravatt BF, Deutsch DG (1997) The cloned rat hydrolytic enzyme responsible for the breakdown of anandamide also catalyzes its formation via the condensation of arachidonic acid and ethanolamine. *Neurosci Lett* 234:59–62
 42. Schmid PC, Zuzarte-Augustin ML, Schmid HH (1985) Properties of rat liver N-acylethanolamine amidohydrolase. *J Biol Chem* 260:14145–14149
 43. De Petrocellis L, Melck D, Ueda N, Maurelli S, Kurahashi Y, Yamamoto S, Marino G, Di Marzo V (1997) Novel inhibitors of brain, neuronal and basophilic anandamide amidohydrolase. *Biochem Biophys Res Commun* 231:82–88
 44. Childers SR, Sexton T, Roy MB (1994) Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem Pharmacol* 47:711–715
 45. Hillard CJ, Edgemont WS, Campbell WB (1995) Characterization of ligand binding to the cannabinoid receptor of rat brain membranes using a novel method: application to anandamide. *J Neurochem* 64:677–683
 46. Pertwee RG, Fernando SR, Griffin G, Abadji V, Makriyannis A (1994) Effect of phenylmethylsulphonyl fluoride on the potency of anandamide as an inhibitor of electrically evoked contractions of two isolated tissue preparations. *Eur J Pharmacol* 272:73–78
 47. Adams IB, Ryan W, Singer M, Thomas BF, Compton DR, Razdan RK, Martin BR (1995) Evaluation of cannabinoid receptor binding and in vivo activities for anandamide analogs. *J Pharmacol Exp Ther* 273:1172–1181
 48. Koutek B, Prestwich GD, Howlett AC, Chin SA, Salehani D, Akhavan N, Deutsch DG (1994) Inhibitors of arachidonoyl ethanolamide hydrolysis. *J Biol Chem* 269:22937–22940
 49. Deutsch DG, Omeir R, Arreaza G, Salehani D, Prestwich GD, Huang Z, Howlett A (1997) Methyl arachidonoyl fluorophosphate: a potent irreversible inhibitor of anandamide amidase. *Biochem Pharmacol* 53:255–260
 50. Edgemont WS, Greenberg MJ, McGinly PJ, Muthian S, Campbell WB, Hillard CJ (1998) Synthesis and characterization of diazomethylarachidonoyl ketone: an irreversible inhibitor of N-arachidonylethanolamine amidohydrolase. *J Pharmacol Exp Ther* 286:184–190
 51. Beltramo M, di Tomaso E, Piomelli D (1997) Inhibition of anandamide hydrolysis in rat brain tissue by (E)-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one. *FEBS Lett* 403:263–267
 52. De Petrocellis L, Melck D, Ueda N, Maurelli S, Kurahashi Y, Yamamoto S, Marino G, Di Marzo V (1997) Novel inhibitors of brain, neuronal, and basophilic anandamide amidohydrolase. *Biochem Biophys Res Commun* 231:82–88
 53. Di Marzo V, De Petrocellis L, Sepe N, Buono A (1996) Biosynthesis of anandamide and related acylethanolamides in mouse J774 macrophages and N18 neuroblastoma cells. *Biochem J* 316:977–984
 54. Wagner JA, Varga K, Ellis EF, Rzigalinski BA, Martin BR, Kunos G (1997) Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. *Nature* 390:518–521
 55. Varga K, Wagner JA, Bridgen DT, Kunos G (1998) Platelet- and macrophage-derived cannabinoids are involved in endotoxin-induced hypotension. *FASEB J* 12:1035–1044
 56. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277:1094–1097
 57. Bisogno T, Maurelli S, Melck D, De Petrocellis L, Di Marzo V (1997) Biosynthesis, uptake, and degradation of anandamide and palmitoylethanolamide in leukocytes. *J Biol Chem* 272:3315–3323
 58. Calignano A, La Rana G, Beltramo M, Makriyannis A, Piomelli D (1997) Potentiation of anandamide hypotension by the transport inhibitor, AM404. *Eur J Pharmacol* 337:R1–R2
 59. Hampson AJ, Hill WA, Zan-Phillips M, Makriyannis A, Leung E, Egle RM, Bornheim LM (1995) Anandamide hydroxylation by brain lipoxygenase: metabolite structures and potencies at the cannabinoid receptor. *Biochim Biophys Acta* 1259:173–179
 60. Ueda N, Yamamoto K, Yamamoto S, Tokunaga T, Shirakawa E, Shinkai H, Ogawa M, Sato T, Kudo I, Inoue K, et al (1995) Lipoxygenase-catalyzed oxygenation of arachidonoyl-ethanolamide, a cannabinoid receptor agonist. *Biochim Biophys Acta* 1254:127–134
 61. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminsky NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50:83–90
 62. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 215:89–97
 63. Lee M, Yang KH, Kaminski NE (1995) Effects of putative cannabinoid receptor ligands, anandamide and 2-arachidonoyl-glycerol, on immune function in B6C3F1 mouse splenocytes. *J Pharmacol Exp Ther* 275:529–536
 64. Mechoulam R, Ben Shabat S, Hanus L, Fride E, Vogel Z, Bayewitch M, Sulcova AE (1996) Endogenous cannabinoid ligands

- chemical and biological studies. *J Lipid Mediat Cell Signal* 14:45–49
65. Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388:773–778
 66. Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, Di Marzo V (1997) Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. *Biochem J* 322:671–677
 67. Di Marzo V, Bisogno T, Sugiura T, Melck D, De Petrocellis L (1998) The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. *Biochem J* 331:15–19
 68. Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S (1998) Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett* 422:69–73
 69. Priller J, Briley EM, Mansouri J, Devane WA, Mackie K, Felder CC (1995) Mead ethanolamide, a novel eicosanoid, is an agonist for the central (CB₁) and peripheral (CB₂) cannabinoid receptors. *Mol Pharmacol* 48:288–292
 70. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, Ferrara P, Soubrie P, Breliere JC, LeFur G (1994) SR141716 A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350:240–244
 71. Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, Cullinan GJ, Hunden DC, Johnson DW, Chaney MO, Koppel GA, Brownstein M (1998) LY320135, a novel cannabinoid CB₁ receptor antagonist, unmasks coupling of the CB₁ receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* 284:291–297
 72. Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere JC, Le Fur GL (1998) SR 144528, the first potent and selective antagonist of the CB₂ cannabinoid receptor. *J Pharmacol Exp Ther* 284:644–650
 73. Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB₂): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther* 278:989–999
 74. Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand JP, LeFur G, Casellas P (1997) A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J Biol Chem* 272:22330–22339
 75. Malit LA, Johnstone RE, Bourke DI, Kulp RA, Klein V, Smith TC (1975) Intravenous Δ^9 -Tetrahydrocannabinol: effects on ventilatory control and cardiovascular dynamics. *Anesthesiology* 42:666–673
 76. Rosenkrantz H (1983) Cannabis, marijuana and cannabinoid toxicological manifestations in man and animals. In: O'Brien K, Kalant H (eds) Cannabis and health hazards. Addiction Research Foundation, Toronto, pp 91–175
 77. Adams MD, Earnhardt JT, Dewey WL, Harris LS (1976) Vasoconstrictor actions of Δ^8 - and Δ^9 -tetrahydrocannabinol in the rat. *J Pharmacol Exp Ther* 196:649–656
 78. Lake KD, Compton DR, Varga K, Martin BR, Kunos G (1997) Cannabinoid-induced hypotension and bradycardia in rats is mediated by CB₁-like cannabinoid receptors. *J Pharmacol Exp Ther* 281:1030–1037
 79. Gallily R, Yamin A, Waksman Y, Ovadia H, Weidenfeld J, Bar-Joseph A, Biegon A, Mechoulam R, Shohami E (1997) Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production by dexamabinol (HU-211), a nonpsychotropic cannabinoid. *J Pharmacol Exp Ther* 283: 918–924
 80. Vidrio H, Sanchez-Salvatori MA, Medina M (1996) Cardiovascular effects of (–)-11-OH-delta 8-tetrahydrocannabinol-dimethylheptyl in rats. *J Cardiovasc Pharmacol* 28:332–336
 81. Zaugg HE, Kyncl J (1983) New antihypertensive cannabinoids. *J Med Chem* 26:214–217
 82. Adams MD, Earnhardt JT, Martin BR, Harris LS, Dewey WL, Razdan RK (1977) A cannabinoid with cardiovascular activity but no overt behavioral effects. *Experientia* 33:1204–1205
 83. Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther* 270:219–227
 84. Frider E, Mechoulam R (1993) Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur J Pharmacol* 231:313–314
 85. Varga K, Lake K, Martin BR, Kunos G (1995) Novel antagonist implicates the CB₁ cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 278:279–283
 86. Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makryannis A (1994) (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem* 37:1889–1893
 87. Siqueira SW, Lapa AJ, Ribeiro do Valle J (1979) The triple effect induced by delta 9-tetrahydrocannabinol on the rat blood pressure. *Eur J Pharmacol* 58:351–357
 88. Lake KD, Martin BR, Kunos G, Varga K (1997) Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension* 29:1204–1210
 89. Varga K, Lake KD, Huangfu D, Guyenet PG, Kunos G (1996) Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension* 28:682–686
 90. Carruba MO, Bondiolotti GP, Picotti GB, Catteruccia N, Da Prada M (1987) Effects of diethyl ether, halothane, ketamine and urethane on sympathetic activity in the rat. *Eur J Pharmacol* 134:15–24
 91. Stein EA, Fuller SA, Edgemont WS, Campbell WB (1996) Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. *Br J Pharmacol* 119:107–114
 92. Ishac E, Jiang L, Lake KD, Varga K, Abood M, Kunos G (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB₁ receptors on peripheral sympathetic nerves. *Br J Pharmacol* 118:2023–2028
 93. Malinowska B, Godlewski G, Bucher B, Schlicker E (1997) Cannabinoid CB₁ receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn Schmiedebergs Arch Pharmacol* 356:197–202
 94. Griffin G, Fernando SR, Ross RA, McKay NG, Ashford ML, Shire D, Huffman JW, Yu S, Lainton JA, Pertwee RG (1997) Evidence for the presence of CB₂-like cannabinoid receptors on peripheral nerve terminals. *Eur J Pharmacol* 339:53–61
 95. Herkenham M, Lynn AB, Johnson MR, Melvin LS, deCosta BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11:563–583
 96. Coutts AA, Pertwee RG (1997) Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *Br J Pharmacol* 121:1557–1566
 97. Kumbaraci NM, Nastuk WL (1979) Effects of Δ^9 -tetrahydrocannabinol on excitable membranes and neuromuscular transmission. *Mol Pharmacol* 17:344–349
 98. Gebremedhin G, Campbell WB, Hillard CJ, Harder DR (1996) Inhibitory effects of anandamide on Ca²⁺ currents in cat cerebral arteriolar smooth muscle cells. *FASEB J* 10:A302
 99. Ellis EF, Moore SS, Willoughby K (1995) Anandamide and Δ^9 -THC-dilation of cerebral arterioles is blocked by indomethacin. *Am J Physiol* 269:H1859–H1864
 100. Randall MD, Alexander SPH, Bennett T, Boyd EA, Fry JR, Gardiner SM, Kemp PA, McCulloch AI, Kendall DA (1996) An endogenous cannabinoid as an endothelium derived vasorelaxant. *Biochem Biophys Res Commun* 229:114–120
 101. Randall MD, McCulloch AI, Kendall DA (1997) Comparative pharmacology of endothelium-derived hyperpolarizing factor and anandamide in rat isolated mesentery. *Eur J Pharmacol* 333 (2–3):191–197

102. Mombouli JV, Vanhoutte PM (1997) Endothelium-derived hyperpolarizing factor(s): updating the unknown. *Trends Pharmacol Sci* 18:252–256
103. Plane F, Holland M, Waldron GJ, Garland CJ, Boyle JP (1997) Evidence that anandamide and EDHF act via different mechanisms in rat isolated mesenteric arteries. *Br J Pharmacol* 121:1509–1511
104. Chataigneau T, Feletou M, Thollon C, Villeneuve N, Vilaine JP, Duhault J, Vanhoutte PM (1998) Cannabinoid CB₁ receptor and endothelium-dependent hyperpolarization in guinea-pig carotid, rat mesenteric and porcine coronary arteries. *Br J Pharmacol* 123:968–974
105. Pratt PF, Hillard CJ, Edgemond WS, Campbell WB (1998) N-Arachidonylethanolamide relaxation of bovine coronary artery is not mediated by CB₁ cannabinoid receptor. *Am J Physiol* 274:H375–H381
106. Randall MD, Kendall DA (1997) Involvement of a cannabinoid in endothelium-derived hyperpolarizing factor-mediated coronary vasorelaxation. *Eur J Pharmacol* 335:205–209
107. Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, Moore LC (1997) Production and physiological actions of anandamide in the vasculature of the rat kidney. *J Clin Invest* 100:1538–1546
108. White R, Hiley CR (1997) A comparison of EDHF-mediated and anandamide-induced relaxations in the rat isolated mesenteric artery. *Br J Pharmacol* 122:1573–1584
109. Zygmunt PM, Hogestatt ED, Waldeck K, Edwards G, Kirkup AJ, Weston AH (1997) Studies on the effects of anandamide in rat hepatic artery. *Br J Pharmacol* 122:1679–1686
110. Adams IB, Compton DR, Martin BR (1998) Assessment of anandamide interaction with the cannabinoid brain receptor: SR141716 A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. *J Pharmacol Exp Ther* 284:1209–1217
111. Wagner JA, Varga K, Kunos G (1998) Endotoxin induces cannabinoid-receptor mediated, endothelium-dependent vasodilation in rat mesenteric arteries. *Hypertension* (in press)
112. Sugiura T, Kodaka T, Nakane S, Kishimoto S, Kondo S, Waku K (1998) Detection of an endogenous cannabimimetic molecule, 2-arachidonylglycerol, and cannabinoid CB₁ receptor mRNA in human vascular cells: is 2-arachidonylglycerol a possible vasomodulator? *Biochem Biophys Res Commun* 243:838–843
113. Yao Y-M, Bahrami S, Leichtfried G, Redl H, Schlag G (1995) Pathogenesis of hemorrhage-induced bacteria/endotoxin translocation in rats. *Ann Surg* 221:398–405
114. Ruetten H, Thiemermann C, Vane JR (1996) Effects of the endothelin receptor antagonist, SB 209670, on circulatory failure and organ injury in endotoxic shock in the anesthetized rat. *Br J Pharmacol* 118:198–204
115. Nathanson C, Hoffman WD, Suffredini AF, Eichacker PQ, Danner RL (1994) Selected treatment strategies for septic shock based on proposed mechanisms of pathogenesis. *Ann Intern Med* 120:771–783
116. Pugin J, Ulevitch RJ, Tobias PS (1993) A critical role for monocytes and CD14 in endotoxin-induced endothelial cell activation. *J Exp Med* 178:2193–2200
117. McCuskey RS, Urbaschek R, Urbaschek B (1996) The microcirculation during endotoxemia. *Cardiovasc Res* 32:752–763
118. Chandross KJ, Kessler JA, Cohen RI, Simburger E, Spray DC, Bieri P, Dermietzel R (1996) Altered connexin expression after peripheral nerve injury. *Mol Cell Neurosci* 7:501–518
119. Polacek D, Bech F, McKinsey JF, Davies PF (1997) Connexin43 gene expression in the rabbit arterial wall: effects of hypercholesteremia, balloon injury and their combination. *J Vasc Res* 34:19–30
120. Chakrabarti A, Onaivi ES, Chaudhuri G (1995) Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Sequence* 5:385–388
121. Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, Bonnin-Cabanne O, Le Fur G, Caput D, Ferrara P (1996) Molecular cloning, expression and function of the murine CB₂ peripheral cannabinoid receptor. *Biochim Biophys Acta* 1307:132–136