

Review

Endocannabinoids as cardiovascular modulators

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Received 10 March 2000; received in revised form 19 May 2000; accepted 19 May 2000

Abstract

Cannabinoids, the bioactive constituents of the marijuana plant and their synthetic and endogenous analogs cause not only neurobehavioral, but also cardiovascular effects. The most important component of these effects is a profound decrease in blood pressure and heart rate. Although multiple lines of evidence indicate that the hypotensive and bradycardic effects of anandamide and other cannabinoids are mediated by peripherally located CB1 cannabinoid receptors, anandamide can also elicit vasodilation in certain vascular beds, which is independent of CB1 or CB2 receptors. Possible cellular mechanisms underlying these effects and the cellular sources of vasoactive anandamide are discussed. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cannabinoids; Marijuana; Cardiovascular effects

1. Background

The psychoactive properties of marijuana have been known to man for thousands of years, but it is only during the last few decades that the biological basis of the effects of marijuana and its bioactive ingredients, collectively called can-

nabinoids, has begun to unfold. The following are major milestones on this road to understanding.

1. The correct chemical structure of Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive ingredient of the marijuana plant, is established (Gaoni and Mechoulam, 1964). This opens the road to structure–activity studies using synthetic THC analogs, although synthetic cannabinoids have been prepared and studied as early as 1942 (Adams, 1942).
2. The saturable, high affinity binding of radiolabeled synthetic cannabinoids to stereoselective sites in the brain suggests the existence of specific cellular receptors for cannabinoids (Devane et al., 1988).

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3. Cannabinoid signaling through inhibition of adenylate cyclase and cAMP production is documented (Howlett and Fleming, 1984).
 4. Two cannabinoid receptors are identified by molecular cloning: CB1 receptors expressed primarily in the brain but also in some peripheral tissues (Matsuda et al., 1990), and CB2 receptors identified so far only in immune cells (Munro et al., 1993). These two receptors have fairly similar affinities for various cannabinoid agonists (Matsuda, 1997), although there are exceptions (Showalter et al., 1996).
 5. Selective antagonists for CB1 receptors (Rinaldi-Carmona et al., 1994) and CB2 receptors (Rinaldi-Carmona et al., 1998) are introduced. These drugs have provided powerful tools for identifying the putative physiological roles of cannabinoid receptors, although interpretation of their effects is complicated by their inverse agonist properties (Shire et al., 1999). A radioiodinated CB1 antagonist (Gatley et al., 1997) offers the possibility of detecting CB1 receptors when expressed at very low cellular densities (Liu et al., 2000).
 6. Selective agonists displaying two orders of magnitude or greater selectivity for CB1 or CB2 receptors have also been introduced. These include methanandamide (Pertwee, 1999) or arachidonyl-2-chloroethylamide (Hillard et al., 1999) for CB1 receptors, and JWH-133 (Pertwee, 1999; Huffman et al., 1999) or HU-308 (Mechoulam et al., 1995, 1998) for CB2 receptors.
 7. Knockout mice deficient in CB1 receptors (Ledent et al., 1999; Zimmer et al., 1999) and CB2 receptors (Buckley et al., 2000) are introduced, as another tool for deciphering the physiological functions of cannabinoid receptors.
 8. Endogenous ligands for cannabinoid receptors, named endocannabinoids, are discovered. The precedent of the presence of stereoselective opiate receptors in the mammalian brain (Pert and Snyder, 1973) leading to the discovery of endogenous opioid peptides (Hughes et al., 1973) was not lost on the cannabinoid field. Although there has been suggestive evidence for the possible existence of peptide-like substances released from nerves in the brain that bind to the cannabinoid receptor (Evans et al., 1992, 1994), Mechoulam and coworkers took a different approach. Based on the lipid solubility of plant-derived cannabinoids, they postulated a lipid-like endocannabinoid, which was ultimately isolated from porcine brain and identified as arachidonyl ethanolamide (anandamide, Devane et al., 1992). This was followed by the discovery of another endocannabinoid, 2-arachidonoyl glycerol (2-AG), present both in the brain (Sugiura et al., 1995), and in the gut (Mechoulam et al., 1995). Both these substances bind to cannabinoid receptors and mimic many of the actions of plant-derived and synthetic cannabinoids (Vogel et al., 1993; Felder et al., 1993).
 9. Finally, specific pathways for the biosynthesis (Bisogno et al., 1997a,b), enzymatic degradation (Deutsch and Chin, 1993) and facilitated uptake (Beltramo et al., 1997) of endocannabinoids are uncovered. There is evidence for the presence of both substances in neurons in the brain and for their stimulation-induced release (Di Marzo et al., 1994; Stella et al., 1997; Bisogno et al., 1997a,b), which implicates them in neurotransmission.
- Early research on plant-derived cannabinoids, primarily of THC, has indicated that, in addition to their neurobehavioral actions, these compounds are both potent and efficacious in eliciting cardiovascular effects. In man, the acute effect of smoking marijuana usually manifests as an increase in heart rate without a significant change in blood pressure (Kanakakis et al., 1976). However, prolonged use of cannabis in man as well as both acute and prolonged administration of THC to experimental animals reproducibly elicits a long lasting decrease in blood pressure and heart rate (Rosenkrantz, 1974; Benowitz and Jones, 1974). Because of the well-known effects of cannabinoids on central nervous system function, early studies of their cardiovascular actions concentrated on the ability of these compounds to inhibit sympathetic tone as the underlying mechanism of their action. Indeed, cross-perfusion experiments in dogs have provided some evidence for a centrally mediated sympatho-inhibitory effect of THC, al-

though additional peripheral sites of action could not be ruled out (Vollmer et al., 1974). Already at this early stage, the potential use of these compounds as antihypertensive agents has been considered, provided that their neurobehavioral and cardiovascular effects are separable. That this may be possible to achieve was first suggested by a 1977 publication of the biological effects of abnormal cannabidiol, a synthetic analog of the neurobehaviorally inactive, plant-derived cannabinoid, cannabidiol (Adams et al., 1977). However, more than 20 years have elapsed before this promising observation was followed up and extended (see below).

2. Cardiovascular effects of cannabinoids mediated by CB1 receptors

The discovery in 1992 of anandamide as the first endocannabinoid has logically raised the question whether it possesses cardiovascular activity similar to THC. Upon its intravenous bolus injection into urethane-anesthetized rats, anandamide was found to elicit a triphasic blood pressure response and bradycardia (Varga et al., 1995), similar to that reported earlier for THC (Siqueira et al., 1979). The first phase consists of a precipitous drop in heart rate and blood pressure that last a few seconds. These effects are vagally mediated, as they are absent in animals after bilateral transection of the cervical vagus nerve, or after pretreatment with methylatropine (Varga et al., 1995). This vagal component is followed by a brief pressor response, which persists in the presence of alpha-adrenergic blockade and also in animals in which sympathetic tone is abolished by pithing, and is thus not sympathetically mediated (Varga et al., 1995). Unlike the third, hypotensive, phase in the effect of anandamide (see below), this pressor component is not blocked by CB1 receptor antagonists and it persists in CB1 knockout mice (Járai et al., 1999), indicating the lack of involvement of CB1 receptors. Recent observations using the radiolabeled microsphere technique in rats suggest that this pressor component may be due to vasoconstriction in certain vascular beds, such as the spleen (Wagner et al., unpub-

lished observations). The third, and most prominent, component in the effect of anandamide is hypotension associated with moderate bradycardia that last 5–10 min. Interestingly, this third phase is absent in conscious normotensive rats (Stein et al., 1996; Lake et al., 1997b), but is present and more prolonged in conscious, spontaneously hypertensive rats (Lake et al., 1997a,b). Since sympathetic tone is known to be low in conscious, undisturbed normotensive rats (Caruba et al., 1987), these observations are compatible with a sympatho-inhibitory mechanism underlying anandamide-induced hypotension and bradycardia, as further discussed below. The finding that *R*-methanandamide, a metabolically stable analog of anandamide (Abadji et al., 1994), causes similar but more prolonged hypotension and bradycardia (Kunos et al., 2000), eliminates the possibility that the effects of anandamide are mediated indirectly by a metabolite. Three lines of evidence implicate CB1 receptors in anandamide-induced hypotension and bradycardia. First, these effects are effectively inhibited by the selective CB1 receptor antagonist SR141716A, at doses similar to those required to block other CB1 receptor-mediated effects (Varga et al., 1995; Lake et al., 1997b). Second, the rank order of the hypotensive and bradycardic potency of a series of cannabinoid analogs, including anandamide, is identical to the rank order of potency of the same substances for eliciting analgesia in rats or for binding to the rat brain CB1 receptor (Lake et al., 1997a). The third, and most important, evidence is the complete absence of anandamide-induced hypotension and bradycardia in CB1 receptor-knockout mice, while these effects are present in the wild-type littermates of these animals (Ledent et al., 1999; Járai et al., 1999).

2.1. Sympathoinhibitory mechanisms

The suggestion that a decrease in sympathetic tone may be the mechanism underlying the decline of blood pressure and heart rate following anandamide (see above) is compatible with early findings with THC, where experimental manipulations to decrease or abolish sympathetic tone resulted in a parallel decrease in the depressor

response to THC (Vollmer et al., 1974). Likewise, anandamide was unable to further reduce blood pressure in anesthetized rats after cervical transection of the spinal cord, or treatment with phentolamine, even though direct vasodilation with sodium nitroprusside was still possible (Varga et al., 1995). The plausible hypothesis that the reduction in sympathetic tone is central in origin was, however, contradicted by the finding that in urethane-anesthetized rats *i.v.* administration of anandamide failed to inhibit the activity of sympathetic premotor neurons of the 'vasomotor center' located in the rostral ventrolateral medulla (RVLM), or to decrease the firing rate of postganglionic sympathetic nerves (Varga et al., 1996). In baroreceptor denervated rats, *i.v.* administered anandamide attenuated the pressor response to electrical stimulation of the RVLM, but not to *i.v.* injections of phenylephrine (Varga et al., 1996). Together, these findings suggest that anandamide may indeed reduce sympathetic tone, but not through a central site of action, but rather by acting at a presynaptic site on peripheral sympathetic nerve terminals to inhibit norepinephrine release (Varga et al., 1996).

There is both *ex vivo* and *in vivo* evidence to support this possibility. In rat isolated atria and vas deferens, anandamide and THC concentration-dependently inhibit electrical stimulation-induced, but not tyramine-induced, norepinephrine release, and these effects are competitively antagonized by SR141716A. Furthermore, CB1 receptor mRNA could be detected and identified by RT-PCR and direct sequencing in the rat superior cervical ganglion, which contains cell bodies of postganglionic sympathetic nerves (Ishac et al., 1996). A functional correlate of these findings is the earlier observation that various cannabinoids inhibit the twitch response of isolated vasa deferentia to sympathetic nerve stimulation but not to exogenous norepinephrine (Pertwee et al., 1994). In recent *in vivo* studies using pithed rats (Malinowska et al., 1997) and rabbits (Niederhoffer and Szabo, 1999), certain synthetic cannabinoids were able to attenuate, in an SR141716A-sensitive manner, the increase in blood pressure and plasma norepinephrine spillover caused by electrical stimulation of preganglionic sympathetic

nerves. Furthermore, WIN 55,212-2, a potent hypotensive cannabinoid (Lake et al., 1997a), failed to lower blood pressure in pithed rabbits in which blood pressure was restored to normal levels by vasopressin infusion (Niederhoffer and Szabo, 1999). These observations also implicate a presynaptic site of cannabinoid action.

2.2. Direct vasodilation by cannabinoids

Nevertheless, the above observations do not exclude the possibility of a direct vasodilator action contributing to cannabinoid-induced hypotension. Indeed, in rats pretreated with 6-hydroxy-dopamine and then infused with vasopressin, the hypotensive cannabinoid HU-210 was still able to lower blood pressure (Vidrio et al., 1996). The finding that potent synthetic cannabinoids can lower blood pressure significantly below the level achieved by sympathetic blockade (Lake et al., 1997a) implies that direct vasodilation must contribute to the hypotension, whereas presynaptic sympatho-inhibition may be the primary mechanism of the long lasting bradycardia.

Whereas anandamide is a partial agonist of CB1 receptors, which also applies to its hypotensive effect (Lake et al., 1997a), 2-arachidonoyl glycerol (2-AG) appears to be a full agonist in certain test systems (Sugiura et al., 1999). In anesthetized rats, 2-AG causes a transient decrease in blood pressure (Varga et al., 1998; Mechoulam et al., 1998) that is partially inhibited by SR141716A and is associated with tachycardia rather than bradycardia (Varga et al., 1998). In anesthetized mice, 2-AG causes similar effects which, paradoxically, are resistant to blockade by SR141716A and persist in mice deficient in CB1 receptors (Járai et al., 2000). The finding that indomethacin, which inhibits the hypotensive effect of arachidonic acid, also inhibits 2-AG-induced hypotension suggested that the effect of 2-AG may be due to a metabolite. This was more directly confirmed by mass spectrometric evidence that 2-AG exposed to mouse blood is rapidly degraded with the parallel appearance of arachidonic acid (Járai et al., 2000), most likely through the action of a monoacylglycerol lipase (Goparaju et al., 1999; Di Marzo et al., 1999). Indeed, when

a metabolically stable analog of 2-AG was used in anesthetized mice, it produced hypotension and bradycardia, which were fully antagonized by SR141716A, and were absent in CB1 receptor knockout mice (Járai et al., 2000). Thus, 2-AG may play the role of an endogenous ligand of CB1 receptors mediating hypotension, as long as it is protected from rapid degradation.

Although the absence of anandamide-induced hypotension in CB1 receptor knockout mice attests to the obligatory involvement of these receptors (Ledent et al., 1999; Járai et al., 1999), their location and the underlying mechanisms are unclear. In preliminary experiments using the radiolabeled microsphere technique in anesthetized rats we found that anandamide and *R*-methanandamide decrease vascular resistance primarily in the coronaries and in the cerebral vascular bed, and these effects can be inhibited by SR141716A. These findings suggest that CB1 receptor-mediated hypotension is due, at least in part, to direct vasodilation in certain vascular beds. Indeed, CB1 receptors have been identified in cat cerebrovascular smooth muscle cells, where their activation was shown to elicit vasorelaxation linked to inhibition of L-type calcium channels (Gebremedhin et al., 1999). On the other hand, in rat carotid artery, activation of CB1 receptors by *R*-methanandamide does not lead to vasodilation, but instead inhibits forskolin-induced vasodilation and increase in cAMP accumulation (Holland et al., 1999). CB1 receptors have also been identified in vascular endothelial cells (Sugiura et al., 1998; Liu et al., 2000), where they can activate tyrosine kinase cascades (Liu et al., 2000). Whether activation of endothelial CB1 receptors can result in vasodilation is unclear. In endothelial cells from the rat renal artery, stimulation of CB1 receptor results in increased NO production (Deutsch et al., 1997), which could suggest that in some vascular beds anandamide may be able to elicit NO-dependent vasodilation. Such an effect, however, is unlikely to contribute to the hypotensive response to anandamide, because this *in vivo* response is unaffected by inhibition of endothelial NO synthase (Kunos et al., 2000). CB1 receptors located presynaptically on sympathetic nerve terminals may also contribute to cannabinoid-

induced hypotension and bradycardia (see above).

3. Anandamide-induced vasodilation via a non-CB1/non-CB2 mechanism

Although in the absence of CB1 receptors anandamide fails to elicit hypotension or bradycardia, recent evidence indicates that anandamide can cause localized vasodilation in the rat mesenteric vasculature by mechanisms that do not involve CB1 receptors. Micromolar concentrations of anandamide can bind to the capsaicin-sensitive vanilloid receptor (VR1), and at nanomolar concentrations anandamide can release calcitonin gene related peptide (CGRP), which can be prevented by the VR1 antagonist, capsazepine. Capsazepine as well as an antagonist of CGRP receptors were found to inhibit the endothelium-independent vasorelaxant effect of anandamide in isolated mesenteric arterial preparations, which led to the hypothesis that anandamide causes vasodilation by direct activation of VR1 receptors on sensory nerve terminals and the subsequent release of CGRP (Zygmunt et al., 1999). However, 1 μ M SR141716A, which inhibits the endothelium-dependent component of the mesenteric vasodilator effect of anandamide, does not influence vasodilation by capsaicin in the same preparation, and the endothelium-dependent, SR141716A-sensitive vasodilator effect of abnormal cannabidiol is not affected by capsazepine (Járai et al., 1999). Thus, an involvement of VR1 receptors in the effect of anandamide must be limited to its endothelium-independent, SR141716A-resistant component (Wagner et al., 1999; Chaytor et al., 1999).

Even though the endothelium-dependent component of the mesenteric vasodilator effect of anandamide and its metabolically stable analog, *R*-methanandamide, are susceptible to inhibition by SR141716A (Wagner et al., 1999; Chaytor et al., 1999; Járai et al., 1999), they do not involve activation of CB1 receptors. This was first suggested by the finding that THC, 2-AG and synthetic cannabinoids with high affinity for CB1 receptors do not elicit vasodilation in the iso-

lated, buffer perfused rat mesenteric vascular bed (Wagner et al., 1999). Furthermore, the vasodilator effect of anandamide and *R*-methanandamide persist in mice deficient in CB1 receptors (Járai et al., 1999). These findings pointed towards the existence of a unique endothelial site of action of anandamide distinct from CB1 or VR1 receptors. The existence of such a site was further supported by findings with a neurobehaviorally inactive, synthetic cannabinoid analog, called abnormal cannabidiol (abn-cbd; Adams et al., 1977). Abn-cbd does not bind to CB1 receptors, yet it elicits SR141716A-sensitive hypotension and mesenteric vasodilation in rats and in wild-type as well as CB1 receptor-knockout mice, which is fully endothelium-dependent (Járai et al., 1999). In the same study, it was also shown that the effect of abn-cbd is unaffected by pretreatment with an NO synthase inhibitor plus indomethacin, but can be blocked by the same combination of calcium-activated potassium channel toxins (apamin plus charybdotoxin) that was found to inhibit EDHF-induced vasodilation (Zygmunt and Högestätt, 1996; Edwards et al., 1998). This suggested that the vasodilation triggered through this endothelial site is NO- and prostanoid-independent and may be mediated through the release of EDHF (Járai et al., 1999). This is not to be confused with the alternative and intriguing hypothesis that EDHF itself may be an endocannabinoid acting on SR141716A-sensitive receptors in vascular smooth muscle (Randall and Kendall, 1998), as discussed in another recent review (Kunos et al., 2000). Anandamide is unlikely to be EDHF, given the fact that its endothelium-independent vasodilator action is not susceptible to block by SR141716A (see above).

The possibility that the endothelial site described above may be a receptor was suggested by a unique structure–activity relationship: the vasodilator potency of abn-cbd was substantially increased by shortening its alkyl side-chain, whereas cannabidiol, the parent compound of abn-cbd, turned out to be a selective antagonist of the cardiovascular effects of abn-cbd (Járai et al., 1999). It is noteworthy that anandamide was recently reported to elicit SR141716A-sensitive calcium transients in cultured vascular endothelial

cells (Mombouli et al., 1999; Fimiani et al., 1999), as this may serve as the trigger for the opening of calcium-activated potassium channels thought to be involved in the release of EDHF (Edwards et al., 1998). In contrast to the possible role of EDHF in mediating vasodilation triggered through this ‘novel’ endothelial receptor, activation of CB1 receptors in rabbit mesenteric arteries was recently reported to inhibit rather than stimulate EDHF release (Fleming et al., 1999).

The possibility that the non-CB1 endothelial site of action of anandamide may be the CB2 receptor is discounted by the persistence of anandamide and abn-cbd-induced mesenteric vasodilation in mice deficient in both CB1 and CB2 receptors (Járai et al., 1999). Thus, the hypotensive response observed with a novel, highly selective CB2 agonist must have a different mechanism (Mechoulam et al., 1995, 1998). The non-CB1/CB2 endothelial site is also distinct from the high affinity anandamide transporter, because AM404, a selective antagonist of the anandamide transporter (Beltramo et al., 1997), fails to inhibit the mesenteric vasodilator response to abn-cbd (Kunos et al., 2000). This distinction is important in the view of the recent findings that in the rabbit isolated mesenteric artery, the endothelium-dependent component of the effects of anandamide and *R*-methanandamide are antagonized by gap junction inhibitors, by a high concentration of SR141716A (10 μ M), which also inhibits gap junctional dye transfer, or by AM404, which does not inhibit gap junctional dye transfer (Chaytor et al., 1999). In this study, EDHF-induced vasodilation was also antagonized by gap junction inhibitors. These results led the authors to postulate that anandamide is taken up by endothelial cells via the high affinity anandamide transporter, which is then followed by the intracellular release and diffusion of an EDHF to adjacent smooth muscle cells through gap junctions. Together, these findings suggest that anandamide can act both via a membrane receptor site insensitive to AM404 (for which abn-cbd is a selective agonist) and through its high affinity transporter, which does not recognize abn-cbd. The possible role of gap junctions in the transfer of EDHF generated via the endothelial ‘receptor’ for abn-cbd remains to be tested.

4. Possible cellular sources of vasoactive anandamide

The presence of multiple vascular sites at which anandamide can elicit vasodilation raises the question whether endogenous anandamide is active at these sites and, if yes, what is its cellular origin. The lack of an *in vivo* pressor response to treatment with a CB1 antagonist (Lake et al., 1997a,b; Wagner et al., 1997) or with the transport inhibitor AM404 (Calignano et al., 1997) suggests the absence of a cannabinoid-mediated vasodilator 'tone' under normal conditions. However, such tonic activation appears to contribute to the hypotension of hemorrhagic shock (Wagner et al., 1997) or shock induced by gram-negative bacterial endotoxin (lipopolysaccharide or LPS, Varga et al., 1998). A source of anandamide and 2-AG under these conditions is activated circulating macrophages and platelets (Wagner et al., 1997; Varga et al., 1998), which are known to adhere to the vascular endothelium (McCuskey et al., 1996), and thus may 'deliver' these lipid mediators to their cellular site of action. These findings were not unexpected in the view of the ability of various leukocyte cell lines, including macrophages, to synthesize, take up and degrade anandamide (Di Marzo et al., 1996; Bisogno et al., 1997a,b).

Another potential source of endocannabinoids with cardiovascular activity is the vascular endothelium, in which the presence of anandamide (Deutsch et al., 1997) and 2-AG (Mechoulam et al., 1998; Sugiura et al., 1998) has been documented. There is also indirect evidence that the endothelium may be a source of vasodilator endocannabinoids in animals exposed to bacterial endotoxin (Wagner et al., 1999). Whether perivascular sensory nerves (Bukoski et al., 1997) or sympathetic nerve terminals contain and are capable of releasing endocannabinoids also deserves scrutiny, as are the multiple sites of the cardiovascular actions of these novel mediators.

Acknowledgements

The authors' work was supported by NIH grants HL59257 and HL49938 to GK. ZJ and

JAW were supported by fellowships from Sanofi Recherche and the Deutsche Forschungsgemeinschaft, respectively.

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