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Cardiovascular Pharmacology of Cannabinoids

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Abstract

Cannabinoids and their synthetic and endogenous analogs affect a broad range of physiological functions, including cardiovascular variables, the most important component of their effect being profound hypotension. The mechanisms of the cardiovascular effects of cannabinoids in vivo are complex and may involve modulation of autonomic outflow in both the central and peripheral nervous systems as well as direct effects on the myocardium and vasculature. Although several lines of evidence indicate that the cardiovascular depressive effects of cannabinoids are mediated by peripherally localized CB_1 receptors, recent studies provide strong support for the existence of as-yet-undefined endothelial and cardiac receptor(s) that mediate certain endocannabinoid-induced cardiovascular effects. The endogenous cannabinoid system has been recently implicated in the mechanism of hypotension associated with hemorrhagic, endotoxic, and cardiogenic shock, and advanced liver cirrhosis. Furthermore, cannabinoids have been considered as novel antihypertensive agents. A protective role of endocannabinoids in myocardial ischemia has also been documented. In this chapter, we summarize current information on the cardiovascular effects of cannabinoids and highlight the importance of these effects in a variety of pathophysiological conditions.

Keywords

Cannabinoid; Anandamide; CB_1 receptor; Blood pressure; Cardiac function; Vascular; Ischemia

1 Introduction

The biological effects of marijuana and its main psychoactive ingredient, Δ^9-tetrahydrocannabinol (THC), are mediated by specific, G protein-coupled receptors (GPCRs) (Howlett et al. 1990). To date, two cannabinoid (CB) receptors have been identified by molecular cloning: the CB_1 receptor, which is by far the most abundant of all neurotransmitter receptors in the brain (Matsuda et al. 1990), but is also present in various peripheral tissues including the heart and vasculature (Gebremedhin et al. 1999; Liu et al. 2000; Bonz et al. 2003), and the CB_2 receptor, expressed primarily by immune (Munro et al. 1993) and hematopoietic cells (Valk and Delwel 1998). The natural ligands of these receptors are endogenous, lipid-like substances called endocannabinoids, which include arachidonoyl ethanolamide or anandamide and 2-arachidonoylglycerol (2-AG), as the two most widely studied members of this group (reviewed by Mechoulam et al. 1998).

Cannabinoids and their synthetic and endogenous analogs are best known for their prominent psychoactive properties, but their cardiovascular effects were also recognized as early as the 1960s. The most important component of these effects is a profound decrease in arterial blood pressure, cardiac contractility, and heart rate (Lake et al. 1997a,b; Hillard 2000; Kunos et al. 2000, 2002; Randall et al. 2002; Ralevic et al. 2002; Hiley and Ford 2004). Although several

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lines of evidence indicate that the cardiovascular depressive effects of cannabinoids are mediated by peripherally localized CB_1 receptors, cannabinoids can also elicit vascular and cardiac effects, which are independent of CB_1 and CB_2 receptors, as discussed in detail later in this chapter.

Recent findings implicate the endogenous cannabinoid system in the pathomechanism of hypotension associated with various forms of shock, including hemorrhagic (Wagner et al. 1997), endotoxic (Varga et al. 1998; Wang et al. 2001; Liu et al. 2003; Bátkai et al. 2004a), and cardiogenic shock (Wagner et al. 2001a, 2003), as well as the hypotension associated with advanced liver cirrhosis (Bátkai et al. 2001; Ros et al. 2002). Furthermore, the possible use of cannabinoids as novel antihypertensive agents has been entertained (Birmingham 1973; Archer 1974; Varma et al. 1975; Crawford and Merritt 1979; Zaugg and Kynel 1983; Lake et al. 1997b; Bátkai et al. 2003 and 2004; Li et al. 2003). In addition, a protective role of endocannabinoids has been described in myocardial ischemia (reviewed in Hiley and Ford 2003, 2004).

The goal of this chapter is to summarize the cardiovascular effects of cannabinoids and to highlight the unique therapeutic potential of the pharmacological manipulation of the endocannabinergic system in a variety of pathological conditions.

2 Cardiovascular Effects of Cannabinoids In Vivo

The in vivo cardiovascular effects of cannabinoids are complex and may involve modulation of the autonomic outflow in both the central and peripheral nervous systems as well as direct effects on the myocardium and vasculature. However, their peripheral actions appear to play the dominant role, at least upon systemic administration at the doses used by most investigators. Moreover, the effects of endocannabinoids are complicated by their rapid metabolism, which may liberate other vasoactive substances and their precursors (reviewed in Mechoulam et al. 1998; Kunos et al. 2002; Randall et al. 2002; Ralevic et al. 2002).

In humans, the acute effect of smoking cannabis usually manifests as an increase in heart rate with no significant change in blood pressure (Kanakis et al. 1976). However, chronic use of cannabis in man, as well as both acute and prolonged administration of THC to experimental animals, elicit a long-lasting decrease in blood pressure and heart rate (Rosenkratz 1974; Benowitz and Jones 1975). 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. Indeed, cross-perfusion experiments in dogs have provided some evidence for a centrally mediated sympatho-inhibitory effect of THC, although 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 was considered (Archer 1974), in the hope that their neurobehavioral and cardiovascular effects would turn out to be 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 two decades have elapsed before this promising observation was followed up and extended (see below).

2.1 Role of CB_1 Receptors in the Cardiovascular Effects of Cannabinoids

The discovery of anandamide, the first endocannabinoid (Devane et al. 1992), has raised the obvious question whether it possesses cardiovascular activity similar to THC. Upon its intravenous bolus injection into anesthetized rats and mice, anandamide was found to elicit a triphasic blood pressure response and bradycardia (Varga et al. 1995; Pacher et al. 2004; Bátkai et al. 2004b; see Fig. 1) similar to that reported earlier for THC (Siqueira et al. 1979). The first

phase of the response consists of a precipitous drop in heart rate and blood pressure that lasts for a few seconds only. 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 α-adrenergic blockade and also in rats in which sympathetic tone is abolished by pithing, and is thus not sympathetically mediated (Varga et al. 1995). This pressor component is also unaffected by CB_1 receptor antagonists and it persists in CB_1 knockout mice (Járai et al. 1999; Pacher et al. 2004), indicating the lack of involvement of CB_1 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. 2001b). The third, and most prominent, phase in the effect of anandamide is hypotension associated with moderate bradycardia that last about 2–10 min. Interestingly, this third phase is absent in conscious normotensive rats (Stein et al. 1996; Lake et al. 1997a), but is present and more prolonged in conscious, spontaneously hypertensive rats (Lake et al. 1997b; Bátkai et al. 2004b). Since sympathetic tone is known to be low in conscious, undisturbed normotensive rats (Carruba et al. 1987), these observations appear to be 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 hypotensive and bradycardic effects of anandamide are mediated indirectly by a metabolite.

Several lines of evidence indicate that cannabinoid-induced hypotension is mediated by CB_1 receptors. First, the hypotension is effectively antagonized by the CB_1-selective antagonist SR141716 (Varga et al. 1995, 1996; Calignano et al. 1997). SR141716 can block hypotension induced by plant-derived and synthetic cannabinoids as well as anandamide (Lake et al. 1997a). However, when tested in anesthetized mice, the hypotensive effect of 2-AG was unexpectedly resistant to inhibition by SR141716, but could be antagonized by indomethacin, suggesting the involvement of a cyclo-oxygenase metabolite (Járai et al. 2000). Indeed, 2-AG was found to be rapidly (<30 s) degraded in mouse blood to generate arachidonic acid. Accordingly, when the metabolically stable analog 2-AG ether was tested, its hypotensive effect was antagonized by SR141716 and it was absent in CB_1-deficient mice indicating that, similar to anandamide, it is an effective agonist of hypotensive CB_1 receptors (Járai et al. 2000). 2-AG ether has been recently identified as an endogenous brain constituent (Hanus et al. 2001), thus it may also be involved in cardiovascular regulation.

The second line of evidence for CB_1 receptor involvement is the strong, positive correlation between the concentrations of various cannabinoid agonists producing half-maximal hypotensive and bradycardic responses (EC_{50}) and their affinity constants for binding to CB_1 receptors in the brain (Lake et al. 1997a). The strongest evidence, however, is the total absence of cannabinoid-induced hypotension and bradycardia in mice lacking the CB_1 receptor (Járai et al. 1999; Ledent et al. 1999). Interestingly, the isolated tachycardia in response to the acute intake of THC by human volunteers who are not chronic marijuana users is similarly inhibited by SR141716 (Huestis et al. 2001). In healthy young adults, the heart is under dominant vagal tone, and the tachycardic effect of THC is most likely due to inhibition of acetylcholine release from cardiac vagal efferents via presynaptic CB_1 receptors (Szabo et al. 2001).

Acute drug effects on blood pressure are the result of changes in peripheral vascular resistance, cardiac output, or both. Recent unpublished results in the authors' laboratory, using the pressure-volume conductance system (Pacher et al. 2003 and 2004; Fig. 2) in pentobarbital-anesthetized mice in vivo, indicate that the hypotensive effect of (–)-11-OH-Δ^9-THC dimethylheptyl (HU-210) results primarily from a decrease in cardiac contractility (Fig. 2). In

contrast, anandamide reduces both cardiac contractility (Fig. 1) and total peripheral resistance (TPR) (Pacher et al. 2004; Bátkai et al. 2004b), which is in agreement with the cardiodepressant effects of HU-210 and anandamide observed in isolated Langendorff rat hearts and in isolated, electrically stimulated human atrial appendages in vitro (Bonz et al. 2003; Ford et al. 2002; Nahas and Trouve 1985; see below). These cardiodepressor effects may underlie the ability of anandamide and HU-210 to decrease cardiac output observed in studies using the radiolabeled microsphere technique (Wagner et al. 2001b). Wagner et al. also demonstrated that both HU-210 and anandamide produce major vasodilation in the coronary and cerebral circulation, which could be antagonized by SR141716 (Wagner et al. 2001b). Collectively, these findings suggest that cannabinoids cause cardiodepressor effects as well as coronary and cerebral vasodilation via SR141716-sensitive CB_1 receptors.

Despite strong evidence for the exclusive role of CB_1 receptors in the hypotensive effect of cannabinoids, there is growing evidence over the last few years that anandamide-induced vasodilation in the mesenteric, and possibly some other vascular beds, is independent of CB_1 or CB_2 receptors (see below, Sect. 3). This apparent paradox may be resolved by the observation that in anesthetized rats, abnormal cannabidiol (abn-cbd), a non-psychoactive cannabinoid with vasodilator activity (Adams et al. 1977; Járai et al. 1999), can elicit a significant increase in mesenteric blood flow in vivo as measured by Doppler sonography, without having a significant effect on blood pressure (S. Bátkai, P. Pacher, G. Kunos, unpublished observations). It is possible that the vasodilation elicited through local release of endocannabinoids in certain vascular beds is compensated by sympathetic vasoconstriction in others, resulting in no net effect on blood pressure.

2.2 Role of Central Versus Peripheral Mechanisms in the Cardiovascular Effects of Cannabinoids

Early work with THC suggested that cannabinoids lower blood pressure through a centrally mediated sympatho-inhibitory mechanism (Vollmer et al. 1974). However, the hypotension elicited by anandamide in urethane-anesthetized rats is not associated with any change in the activity of sympathetic premotor neurons in the medullary vasomotor center or in the activity of sympathetic postganglionic nerves (Varga et al. 1996), which ruled out centrally mediated sympatho-inhibition or ganglionic blockade as possible underlying mechanisms, at least for anandamide. Intra-cerebroventricular administration in rabbits of the potent synthetic cannabinoid WIN55,212-2 was found to increase rather than decrease sympathetic tone, which also argues against a central mechanism for the hypotensive effect (Niederhoffer and Szabo 2000). Yet, the pressor response triggered by electrical stimulation of the vasomotor center was reversibly inhibited by anandamide, whereas the effect of exogenous phenylephrine was unaffected, suggesting a presynaptic-inhibitory effect of norepinephrine release from peripheral sympathetic nerve terminals (Varga et al. 1996). Indeed, stimulation of presynaptic CB_1 receptors inhibits norepinephrine release both in vitro (Ishac et al. 1996; Deutsch et al. 1997; Schlicker et al. 1997; Christopoulos et al. 2001; Vizi et al. 2001) and in vivo (Malinowska et al. 1997; Niederhoffer and Szabo 2000). However, when sympathetic tone is eliminated by ganglionic blockade and vascular tone is restored by vasopressin infusion, the hypotensive response to the potent synthetic cannabinoid HU-210 remains unchanged, although its bradycardic effect is lost (Wagner et al. 2001b). This suggests that cannabinoid-induced bradycardia may be due to inhibition of sympathetic tone to the heart, but the hypotensive response is due to direct vasodilation, as also indicated by its presence in rats following chemical sympathetic denervation (Vidrio et al. 1996).

3 Cardiovascular Effects of Cannabinoids In Vitro

3.1 Direct Vasorelaxant Effects of Cannabinoids

An early report on the effect of anandamide on cerebral blood flow indicated that the observed vasodilator response could be inhibited by indomethacin (Ellis et al. 1995). The obvious implication of this finding was that anandamide causes vasorelaxation indirectly through the generation of arachidonic acid and its subsequent metabolism by cyclooxygenase. Although THC also produced an indomethacin-sensitive response, subsequent studies could not document an effect of cyclooxygenase inhibition on anandamide-induced vasorelaxation in other blood vessels, including the mesenteric and coronary vasculature (Randall et al. 1996; Randall and Kendall 1997; Plane et al. 1996; White and Hiley 1997), ruling out increased prostanoid formation as a major mechanism for the vasodilatory effect.

The vasorelaxant effect of anandamide displays tissue and interspecies differences. Anandamide has been found to relax rat hepatic and guinea pig basilar arteries (Zygmunt et al. 1999), bovine coronary arteries (Pratt et al. 1998), but not rat carotid arteries (Holland et al. 1999) or the rat aorta (Darker et al. 1998). Anandamide has been reported to mediate vasodilation in kidney afferent arterioles through the endothelial release of nitric oxide (NO) (Deutsch et al. 1997). Anandamide was also found to release NO in a variety of human blood vessels as well as the right atrium (Bilfinger et al. 1998). In contrast, in other studies the anandamide-induced vasorelaxation was insensitive to inhibition of NO synthase (Randall et al. 1996; White and Hiley 1997; Járai et al. 1999). Both anandamide and the CB agonist HU-210 caused up-regulation of the expression and activity of the inducible NO synthase in human umbilical vein endothelial cells (HUVEC), which is unlikely to contribute to the acute vasodilatory effect, but may play an important role in terminating the action of endogenous anandamide by affecting its cellular uptake (Maccarrone et al. 2000).

The interest in the vasodilator action of endocannabinoids was further stimulated by a report in 1996 that the mesenteric vasodilation attributable to an endothelium-derived hyperpolarizing factor (EDHF) is sensitive to inhibition by SR141716 (Randall et al. 1996). The corollary of this finding was that EDHF might be an endocannabinoid released from the vascular endothelium and acting at SR141716-sensitive CB receptors on vascular smooth muscle cells, which it would hyperpolarize and relax (Randall et al. 1996). Interestingly, carbachol was found to induce 2-AG production by the rat aortic endothelium (Mechoulam et al. 1998b), which is compatible with 2-AG being an EDHF.

Inhibition of EDHF-induced vasorelaxation by SR141716 was confirmed in some (White and Hiley 1997) but not other (Chataigneau et al. 1998; Fulton and Quilley 1998; Niederhoffer and Szabo 1999a; Pratt et al. 1998) studies. A possible source of these discrepancies may be the different species and vascular preparations tested. Additionally, the finding that the vasodilator action of anandamide has both an endothelium-dependent and an endothelium-independent component, and only the former is sensitive to inhibition by SR141716 (Chaytor et al. 1999; Mukhopadhyay et al. 2002; Wagner et al. 1999), also argues against anandamide itself being EDHF, although it leaves open the possibility that anandamide or another endocannabinoid acting at an SR141716-sensitive receptor on vascular endothelial cells may *release* an EDHF (Járai et al. 1999). This latter possibility is compatible with findings that anandamide triggers calcium transients in cultured vascular endothelial cells (Fimiani et al. 1999; Mombouli et al. 1999), and that the anandamide-induced hyperpolarization of the rat hepatic artery is endothelium dependent (Zygmunt et al. 1997). Interestingly, the mesenteric vasodilation caused by the non-psychotropic cannabinoid abn-cbd is inhibited by the same combination of calcium-activated potassium channel toxins (apamin+charybdotoxin; Járai et al. 1999; Ho and Hiley, 2003) that were reported to inhibit EDHF-induced vasodilation (Randall and Kendall 1998), although no such inhibition was observed in rat hepatic arteries (Zygmunt et al. 1997),

and in some other preparations the effect of anandamide was inhibited by iberiotoxin, which blocks a different (large conductance) calcium-activated potassium channel (Ishioka et al. 1999; Begg et al. 2001; White et al. 2001). The findings with abn-cbd would suggest that cannabinoids might release EDHF via activation of an endothelial site distinct from CB_1 receptors (which recognizes abn-cbd, see below). Indeed, activation of bona fide CB_1 receptors may have an opposite effect, i.e., inhibition of the release of EDHF, as indicated by the findings of Fleming et al. (1999) in porcine coronary and rabbit carotid and mesenteric arteries.

Another mechanism by which anandamide elicits SR141716-sensitive, endothelium-dependent vasodilation may be through an intracellular site of action at gap junctions. Evidence for this mechanism is the ability of various gap junction inhibitors as well as the anandamide transport inhibitor AM404 to antagonize anandamide-induced mesenteric vasodilation and the ability of SR141716 to inhibit dye transfer through gap junctions (Chaytor et al. 1999). Together, these findings form the basis of the hypothesis that anandamide induces vasodilation at an intracellular site in endothelial cells where it would facilitate the gap junctional transfer of an EDHF to vascular smooth muscle (Chaytor et al. 1999). However, in other studies the vasodilator effect of anandamide was inhibited by some but not other gap junction inhibitors, and the ones that were inhibitory (18α-glycyrrhetinic acid, ouabain) also blocked Na^+, K^+-ATPases at the concentrations used, suggesting a mechanism of action unrelated to gap junction inhibition (Harris et al. 2002). In rabbit aortic rings, which are relaxed by anandamide in a partially endothelium-dependent manner, the anandamide response was unaffected by gap junction inhibitors (Mukhopadhyay et al. 2002), and similar findings were reported for rat isolated coronary arteries (White et al. 2001).

Finally, Zygmunt et al. (1999) described an unusual indirect pathway. They demonstrated that anandamide induces vasorelaxation in rat mesenteric and hepatic arteries, and in guinea pig basilary artery through the activation of TRPV1 receptors on sensory neurons, causing the release of the vasodilatory peptide calcitonin gene-related peptide (CGRP) (see also Sect. 4 below, “Role of Vanilloid TRPV1 Receptors in the Cardiovascular Effects of Cannabinoids”).

3.2 Novel Endothelial Endocannabinoid Receptor

The possible existence of cannabinoid receptors distinct from CB_1 or CB_2 was first suggested by findings that potent synthetic cannabinoids as well as THC do not elicit vasodilation in the same rat mesenteric vascular bed preparations in which anandamide and methanandamide have strong vasodilator activity (Wagner et al. 1999). In these experiments, the effects of anandamide and methanandamide could be inhibited by SR141716, but the concentration required was somewhat higher (1–10 μM) than required for inhibition of CB_1 receptors (Járai et al. 1999; Chaytor et al. 1999; Mukhopadhyay et al. 2002; Wagner et al. 2001; White et al. 2001). Also, the ability of SR141716 to inhibit anandamide-induced vasodilation was lost following endothelial denudation (Chaytor et al. 1999; Járai et al. 1999; Mukhopadhyay et al. 2002; Wagner et al. 2001). These findings led to the postulation of an endothelial site, somewhat sensitive to inhibition by SR141716 but distinct from CB_1 or CB_2 receptors, that contributes to anandamide-induced vasodilation in the mesenteric circulation (Járai et al. 1999) and, possibly, in other vascular beds, such as the rat coronary circulation (Ford et al. 2002). In this latter study, a nonCB_1/nonCB_2 mechanism mediating the negative inotropic effect of anandamide has been also identified.

More recently, a non-CB_1/non-CB_2 site was also postulated to exist on glutamatergic terminals in the mouse hippocampus, where its activation by cannabinoids inhibits glutamatergic transmission and excitatory postsynaptic potentials (EPSPs) (Hájos et al. 2001). Similar to the endothelial site, the site in the hippocampus is susceptible to inhibition by SR141716, but it can be activated by the synthetic cannabinoid WIN55,212-2 (Hájos et al. 2001). A similarly WIN55,212-2-sensitive, but SR141716-insensitive, non-CB_1/non-CB_2 site that can activate

guanosine triphosphate (GTP)γS labeling in brain membranes has been identified in CB_1 knockout mice (Breivogel et al. 2001) and in astrocytes, where its stimulation inhibits cyclic adenosine monophosphate (cAMP) production (Sagan et al. 1999). Since the endothelial site is insensitive to WIN55,212-2 in the rat mesentery (Wagner et al. 1999) or in the rabbit aorta (Mukhopadhyay et al. 2002), and abn-cbd does not inhibit glutamatergic EPSPs in rat hippocampus (M. Begg, D.M. Lovinger, G. Kunos, unpublished results), it is very likely distinct from the non-CB_1 site described in the rat CNS.

Abn-cbd is a synthetic analog of the behaviorally inactive plant-derived cannabinoid, cannabidiol. Several years ago, abn-cbd was reported to be inactive in two, rather non-specific, behavioral paradigms used to screen cannabinoids in mice, but to cause profound hypotension in dogs (Adams et al. 1977). This prompted us to speculate that abn-cbd may be a selective agonist of the putative vascular endothelial cannabinoid receptor. A detailed study of the pharmacology of abn-cbd supported this possibility (Járai et al. 1999). Abn-cbd does not bind to CB_1 receptors in the rat brain or to the human CB_2 receptor at concentrations up to 100 μM (Offertáler et al. 2003), and is inactive in the Martin behavioral tetrad in mice at doses up to 60 mg/kg (Járai et al. 1999). Yet, abn-cbd (20 mg/kg i.v.) causes SR141716-sensitive hypotension in both wild-type and CB_1 receptor knockout mice. Furthermore, abn-cbd causes endothelium-dependent vasodilation in the buffer-perfused mesenteric vascular bed isolated from rats or from wild-type as well as CB_1 knockout mice, and these effects are also inhibited by 1–5 μM of SR141716 (Járai et al. 1999). The parent compound of abn-cbd, cannabidiol (10 μM), has no vasodilator activity in the rat isolated, perfused mesenteric vascular bed preparation, but is able to inhibit the vasodilation caused by abn-cbd or anandamide (Járai et al. 1999). This suggested that abn-cbd is an agonist and cannabidiol is an antagonist of a novel endothelial cannabinoid receptor mediating vasodilation. Additional experiments indicated that the vasodilator response to abn-cbd is not affected by N^G-nitro-l-arginine methylester (L-NAME)+indomethacin, suggesting that endothelial NO and prostacyclin are not involved. However, a combination of apamin (100 nM) and charybdotoxin (100 nM), inhibitors of calcium-activated potassium channels, significantly attenuated the vasodilation caused by abn-cbd. As the same combination of potassium channel blockers inhibits EDHF-induced mesenteric vasodilation (Randall and Kendall 1998), these findings were compatible with the possible release of EDHF through activation of this novel endothelial site (Járai et al. 1999; also see above).

Capsazepine, an inhibitor of the vanilloid TRPV1 receptor, does not influence the mesenteric vasodilator response to abn-cbd at a concentration that blocks the vasodilator effect of capsaicin or the vasodilator response to anandamide in endothelial-intact preparations (Járai et al. 1999). These observations distinguish the endothelial cannabinoid receptor from TRPV1 receptors, but are compatible with the involvement of the latter in the endothelium-independent, SR141716-insensitive component of the effect of anandamide, as suggested earlier by Zygmunt et al. (1999).

Similar conclusions were reached by Howlett and coworkers, who investigated the vasodilator action of anandamide in isolated aortic rings from rabbits (Mukhopadhyay et al. 2002). In those experiments, the vasorelaxant effect of anandamide had a major (80%) endothelium-dependent and a minor endothelium-independent component, thus making it an attractive model for further exploration of the pharmacological properties of the endothelial site (Mukhopadhyay et al. 2002). The endothelium-dependent component was found to be SR141716-sensitive and also to involve pertussis toxin (PTX)-sensitive G proteins and NO production, whereas the endothelium-independent minor component appeared to be via a PTX-insensitive mechanism involving TRPV1 receptors, CGRP, and NO (Mukhopadhyay et al. 2002). These findings are in general good agreement with the earlier findings in the rat and suggest, in addition, that the non-CB_1 endothelial receptor is coupled to G_i/G_o.

The possibility that the endothelial cannabinoid receptor is a GPCR is supported by recent findings in rat-isolated mesenteric artery segments (representing small conduit vessels approximately 200 μm in diameter) set up in a wire myograph (Offertáler et al. 2003). The responses of these preparations were similar to those observed using the perfused mesenteric vascular bed, where resistance changes reflect the response of precapillary arterioles (20–30 μm in diameter), in that the vasorelaxant effect of abn-cbd was NO-independent (resistant to inhibition by L-NAME) but sensitive to inhibition by apamin plus charybdotoxin. The observed insensitivity to NO, also observed in the case of anandamide in the same preparation (Harris et al. 2002), is different from the situation in the rabbit aorta (see above) or in the rat renal artery (Deutsch et al. 1997), and may reflect species- and/or vascular region-specific differences. Importantly, the vasorelaxation by abn-cbd was not inhibited by the vanilloid TRPV1 receptor antagonist capsazepine, and the inhibitory effects of the toxin combination implicate calcium-activated potassium channels. However, in agreement with the observations of Mukhopadhyay et al. (2002) with anandamide, mesenteric vasorelaxation by abn-cbd could be inhibited by PTX in endothelium-intact but not in endothelium-denuded preparations, which is compatible with the endothelial cannabinoid receptor being a GPCR coupled to G_i/G_o (Offertáler et al. 2003). Ho and Hiley (2003) reported, however, that the mesenteric vasodilator effect of abn-cbd was unaffected by PTX, even though its other properties, including its endothelium dependence and susceptibility to inhibition by the compound O-1918 (see below) were similar to those reported by Offertáler et al.

An endothelial site of action of abn-cbd is further documented by its ability to activate p42/44 MAP kinase and Akt phosphorylation in cultured HUVEC (Offertáler et al. 2003). As in the earlier studies using the perfused mesenteric vascular bed, in the myograph preparations abn-cbd is a full agonist, i.e., it completely reverses phenylephrine-induced contractions with an EC_{50} of 2–3 μM. Unexpectedly, cannabidiol and SR141716, both of which antagonize abn-cbd-induced vasodilation in the resistance vessels of the mesenteric arterial bed preparation, act as full agonists in the small conduit vessels (EC_{50} of 0.82 μM for cannabidiol and 6.4 μM for SR141716). Thus, these latter compounds are most likely partial agonists rather than pure antagonists at the endothelial cannabinoid receptor. This prompted us to develop structurally modified analogs of cannabidiol to search for a pure antagonist. The compound O-1918 does not relax mesenteric arterial segments at concentrations up to 30 μM, but competitively inhibits the vasodilator response to abn-cbd without affecting vasodilation to carbachol or CGRP (Offertáler et al. 2003). The endothelial site of action of O-1918 is further supported by its ability to antagonize the activation of p42/44 mitogen-activated protein (MAP) kinase and Akt phosphorylation in HUVEC (Offertáler et al. 2003). The finding that O-1918 also inhibits the mesenteric vasorelaxant effect of anandamide strongly suggest that the same endothelial receptor is the site of action of anandamide. Similar to abn-cbd, O-1918 does not bind to CB_1 or CB_2 receptors. Recent studies indicate that O-1918 inhibits the mesenteric vasorelaxant effect of two putative novel endocannabinoids, *N*-arachidonoyl dopamine (O'Sullivan et al. 2004) and virodhamine (C.R. Hiley, personal communication). These findings are important because the relatively low potency of anandamide in eliciting mesenteric vasorelaxation could suggest that the primary endogenous ligand at these receptors is not anandamide.

Inhibition of the vasodilator effect of abn-cbd by charybdotoxin (Ho and Hiley 2003; Offertáler et al. 2003; see also above) has suggested the involvement of a calcium-activated K^+ channel in this effect. In a recent study using the whole cell patch-clamp technique, we have described a voltage-dependent outward current in HUVEC that is carried by K^+ ions and is blocked by charybdotoxin and iberiotoxin, suggesting the involvement of the large conductance calcium-activated potassium (BK_{Ca}) channel (Begg et al. 2003). Although abn-cbd did not elicit a current on its own, it caused a concentration-dependent increase in the voltage-induced K^+ current that was sensitive to PTX, suggesting the involvement of a G_i/G_o-coupled receptor. The increase in K^+ current by abn-cbd was unaffected by relevant concentrations of SR141716

or SR144528, which argues against the involvement of CB_1 and CB_2 receptors (Begg et al. 2003). This was further supported by the lack of effect on K^+ currents of HU-210, a potent CB_1/CB_2 receptor agonist, which is devoid of mesenteric vasodilator activity. On the other hand, the abn-cbd-induced increase in K^+ current was antagonized by O-1918, which produced no effect by itself (Begg et al. 2003). The finding that the iberiotoxin-sensitive current induced by the selective BK_{Ca} opener NS-1619 was unaffected by O-1918 indicates that blockade of the effect of abn-cbd by O-1918 occurs at a site proximal to the channel, most likely at the receptor (Begg et al. 2003). This compound as well as PTX similarly inhibited the endothelium-dependent vasorelaxing effect of abn-cbd in the rat isolated mesenteric artery (Offertáler et al. 2003). This raises the possibility that activation of BK_{Ca} channels is involved in the mesenteric vasodilation mediated by this novel endothelial receptor.

The endogenous cannabinoid anandamide also increased the K^+ current evoked by a single voltage step; however, its effect was only observed at high concentrations. The effect of anandamide was partially inhibited by O-1918 or PTX; thus, part of the effect of anandamide may not be mediated by the same pathway as abn-cbd (Begg et al. 2003). Anandamide acted as a full agonist in the rat isolated mesenteric artery preparation with an EC_{50} comparable to that of abn-cbd (Offertáler et al. 2003), suggesting that there may be subtle differences between the rat and human receptors. The low apparent efficacy of anandamide for the human endothelial receptor could also suggest the existence of an endogenous ligand(s) other than anandamide (see also above).

In HUVEC, intracellular cyclic guanosine monophosphate (cGMP) increased the voltage-induced outward current, comparable with the effect of abn-cbd. A similar increase in outward current was also produced by YC-1, an activator of soluble guanylyl cyclase. The increases evoked by abn-cbd and cGMP were inhibited by a protein kinase G inhibitor KT-5823, and the effect of abn-cbd, but not of cGMP, was also blocked by the guanylyl cyclase inhibitor 1*H*-(1,2,4)oxadiazolo[4,3-*a*]quinozalin-1-one (ODQ). Furthermore, cGMP continued to increase the K^+ current under Ca^{2+}-clamped conditions, indicating that its action is not due to modulation of $[Ca^{2+}]_i$. The effects of abn-cbd, cGMP, and YC-1 on K+ currents were not additive, suggesting that these compounds utilize a common intracellular pathway (Begg et al. 2003). Finally, abn-cbd was found to increase cellular cGMP levels, and this effect could be inhibited by O-1918 (Begg et al. 2003). Together, these data suggest that the novel, G_i/G_o-coupled receptor activated by abn-cbd is positively coupled to guanylyl cyclase to raise intracellular cGMP, which activates protein kinase G.

Recently, it has been proposed that TRPV4 Ca^{2+} entry channels in vascular endothelial cells contribute to the vasorelaxant effect of anandamide via its enzymatic degradation, yielding arachidonic acid and its subsequent P450 epoxygenase-dependent metabolism (Watanabe et al. 2003). TRPV4 channels are unlikely to be involved in the effects of abn-cbd on the outward current or on vascular tone, because these effects are sensitive to PTX, whereas TRPV4-mediated calcium entry is not. Furthermore, potentiation of the outward current by abn-cbd persisted in the presence of clamped intracellular calcium (Begg et al. 2003). Also, *R*-methanandamide, an anandamide analog resistant to enzymatic degradation and therefore unlikely to give rise to P450 metabolites, has a mesenteric vasodilator effect similar to that of anandamide (Wagner et al. 1999).

Extracellular calcium is known to have a potent vasodilator effect, particularly in the mesenteric circulation, where it is thought to contribute to the postprandial vasodilation associated with the intestinal absorption of nutrients. Bukoski and coworkers found that SR141716 can inhibit Ca^{2+}-induced mesenteric vasodilation through a sensory nerve-dependent mechanism, which led them to suggest that anandamide may be a sensory nerve-derived vasodilator mediator (Ishioka and Bukoski 1999). Interestingly, O-1918 also inhibits calcium-induced mesenteric

vasorelaxation, which is similar in wild-type and CB_1 receptor knockout mice (Bukoski et al. 2002), suggesting that the vasodilation by extracellular calcium is most likely mediated by the endothelial abn-cbd-sensitive receptor.

Collectively, the above-mentioned results indicate that the synthetic cannabinoid ligands abn-cbd and O-1918 act as a selective agonist and silent antagonist, respectively, of a novel vascular endothelial receptor distinct from CB_1 and CB_2 that mediates mesenteric vasodilation, and is coupled to a phosphoinositide (PI)3-kinase/Akt-dependent pathway through G_i/G_o.

3.3 Direct Cardiodepressant Effects of Cannabinoids

In contrast to the growing knowledge on the vascular effects of cannabinoids, little is known about cannabinoid-induced direct cardiac effects. The endocannabinoid anandamide (Felder et al. 1996), anandamide amidohydrolase (Bilfinger et al. 1998), and traces of the message for the CB_1 receptor (Galiegue et al. 1995) have all been detected in the human heart. In a more recent study, the existence of CB_1 receptors was confirmed in human atrial myocytes by immunoblotting and immunohistochemistry (Bonz et al. 2003). In the same study, it was demonstrated that anandamide, *R*-methanandamide, and HU-210 dose-dependently decrease contractile performance in isolated, electrically paced human atrial muscle. A selective and potent CB_1 antagonist, AM251 (Gatley et al. 1997), blocked the negative inotropic effect of all three drugs, and the involvement of CB_2 receptor activation, NO, or prostanoid release could all be excluded (Bonz et al. 2003). Consistently with these in vitro results, HU-210 decreases cardiac output in rats in vivo in a CB_1 receptor-dependent manner (Wagner et al. 2001b). Previous studies have also demonstrated that anandamide caused SR141716-sensitive coronary vasorelaxation in isolated perfused rat hearts (Randall and Kendall 1997; Fulton and Quilley 1998), implicating cannabinoid receptors. These effects of anandamide were not mimicked by arachidonic acid, indicating that the vasodilator effect was not mediated by arachidonic acid metabolites.

In isolated, perfused, rat Langendorff heart preparations, anandamide and *R*-methanandamide, but not palmitoylethanolamide or the selective CB_2 receptor agonist JWH015, significantly reduced both left ventricular developed and coronary perfusion pressures, indicating decreased myocardial contractile function and coronary vasodilation (Ford et al. 2002). Interestingly, anandamide-mediated vasodilatation and negative inotropy were both sensitive to inhibition by the CB_1 antagonist SR141716 and the CB_2 antagonist SR144528, but not to the TRPV1 antagonist capsazepine, which led the authors to propose a novel site distinct from classic CB_1 and CB_2 receptors (Ford et al. 2002).

In agreement with the observations on isolated cardiac preparations, our recent unpublished results using the Millar pressure-volume conductance system (see below and also Figs. 1 and 2) to directly measure cardiac performance in vivo strongly suggest the crucial importance of the cardiac component in the hemodynamic effects of cannabinoids. Taken together, the above-mentioned studies suggest that CB_1 receptors are present in cardiomyocytes, and cannabinoids may decrease cardiac contractility through both CB_1-dependent and CB_1-independent mechanisms.

4 Role of Vanilloid TRPV1 Receptors in the Cardiovascular Effects of Cannabinoids

Structural similarities between anandamide and vanilloid compounds such as capsaicin (Di Marzo et al. 1998) raised the possibility of an interplay between these two systems. Indeed, Zygmunt et al. (1999) demonstrated that in rat mesenteric arteries, the endothelium-independent vasodilator effect of anandamide is inhibited by the TRPV1 receptor antagonist

capsazepine or by a CGRP receptor antagonist. They further demonstrated that anandamide binds to the cloned TRPV1 receptor with micromolar affinity, and at nanomolar concentrations it releases immunoreactive CGRP from sensory nerve terminals located in the vascular adventitia (Zygmunt et al. 1999). A similar involvement of TRPV1 receptors in the mesenteric vasodilator action of methanandamide has also been suggested (Ralevic et al. 2000). These observations support the hypothesis that anandamide-induced vasodilation involves activation of TRPV1 receptors in sensory nerves and the subsequent release of the potent vasodilator peptide CGRP.

The above observations do not implicate the endothelium in the vasodilator response to anandamide. Other studies, which documented both endothelium-dependent and endothelium-independent components for the vasodilator effect of anandamide, confirmed the role of TRPV1 receptors but only for the endothelium-independent component (Járai et al. 1999; Mukhopadhyay et al. 2002). The endothelium-dependent vasodilator effect of anandamide in the rabbit aorta or the similar effect of abn-cbd in rat mesenteric arteries is unaffected by capsazepine (Mukhopadhyay et al. 2002; Járai et al. 1999; Offertáler et al. 2003; Ho and Hiley 2003). Interestingly, sensory nerve terminals also appear to have CB_1 receptors, stimulation of which by very low doses of anandamide or by the synthetic cannabinoid HU-210, neither of which results in activation of TRPV1 receptors, inhibits sensory neurotransmission (reviewed in Ralevic et al. 2002). Furthermore, a recent study by Zygmunt et al. (2002) indicates that THC and cannabinol, but not other psychotropic cannabinoids, can elicit CGRP release from periarterial sensory nerves by a mechanism that is independent of not only CB_1 and CB_2 receptors, but also of vanilloid TRPV1 receptors. Thus, the sensory nerve-dependent effects of cannabinoids are complex, as interactions with CB_1 and TRPV1 receptors appear to have opposite functional consequences, and there may be additional actions independent of both of these receptors. TRPV1 receptors are not involved in the dilation of isolated coronary arteries by anandamide either in the sheep, where the effect is endothelium dependent (Grainger and Boachie-Ansah 2001), or in the rat, where it is endothelium independent (White et al. 2001). Furthermore, in the rat mesenteric arterial bed, the role of sensory nerves and vanilloid receptors in the dilator effect of anandamide was found to be conditional on the presence of NO (Harris et al. 2002).

TRPV1-containing afferent nerve fibers are present on the epicardial surface of the heart and the activation of these receptors by epicardially injected capsaicin evokes a sympathoexcitatory response with a brief increase in blood pressure (Zahner et al. 2003). Capsaicin infusion also induces a moderate pressor effect in pigs (Kapoor et al. 2003). We have recently found (Pacher et al. 2004) that i.v. injection of 10 μg/kg capsaicin evokes only a brief pressor response in wild-type mice, while at the much higher dose of 100 μg/kg its effect has both a depressor and a pressor component. In contrast, capsaicin elicited no change in blood pressure in $TRPV1^{-/-}$ mice, suggesting that TRPV1 receptors mediate the cardiogenic sympathetic or Bezold-Jarisch reflex in mice, which is in agreement with recent reports in which the cardiovascular effects of capsaicin were inhibited by TRPV1 receptor antagonists (Smith and McQueen 2001; Zahner et al. 2003).

In the absence of anandamide-induced hypotension in CB_1 knockout mice, the physiological relevance of the interaction of anandamide with TRPV1 receptors has been questioned (Szolcsányi 2000). We previously reported that anandamide causes a triphasic blood pressure response where the predominant hypotensive effect is preceded by a transient, vagally mediated drop in heart rate and blood pressure followed by a brief pressor response (Varga et al. 1995, see also Sect. 2 and Fig. 1). This initial component is missing in $TRPV1^{-/-}$ mice (Pacher et al. 2004) and is unaffected by the CB_1 antagonist SR141716 in rats (Varga et al. 1995). Bolus intravenous injections of anandamide may reach high enough plasma concentrations for a few seconds to activate TRPV1 receptors, which may explain the above findings. Indeed, the phase

I transient hypotension and bradycardia do not appear when anandamide is injected slowly to limit its peak plasma concentration (Z. Jarai, J.A. Wagner, G. Kunos, unpublished observations). In contrast, the prolonged hypotensive phase of the anandamide response is characterized by decreased cardiac contractility and total peripheral resistance (TPR), which are similar in $TRPV1^{+/+}$ and $TRPV1^{-/-}$ mice and were completely antagonized by SR141716, implicating CB_1 receptors (Pacher et al. 2004). In agreement with this observation, the sustained hypotensive and bradycardic effects of cannabinoids are totally absent in mice lacking the CB_1 receptor (Ledent et al. 1999; Járai et al. 1999). Thus, TRPV1 receptors are not involved in the sustained cardiovascular response to anandamide, but may become transiently activated in response to pharmacological concentrations achieved after bolus i.v. injections. These findings are in agreement with a recent report by Malinowska et al (2001) who found that in rats the transient vagal activation to a bolus injection of anandamide was partially blocked by the TRPV1 antagonists capsazepine or ruthenium red, whereas the CB_1-mediated prolonged hypotension remained unaffected.

Collectively, the above-mentioned studies show that the sustained hypotensive effect of anandamide involves a marked cardiodepressor component in addition to a decrease in TPR, and these effects are mediated by CB_1 but not TRPV1 receptors. The role of TRPV1 receptors is limited to the transient activation of the Bezold-Jarisch reflex by very high plasma concentrations of anandamide.

5 Pathophysiological Role of the Endocannabinergic System in Cardiovascular Disorders

Recent studies indicate that the endogenous cannabinergic system plays an important role in cardiovascular regulation under various pathophysiological conditions, and pharmacological manipulation of this system may offer novel therapeutic approaches in a variety of cardiovascular disorders.

5.1 Role of the Endocannabinergic System in Hemorrhagic, Endotoxic, and Cardiogenic Shock and Liver Cirrhosis

The profound and long-lasting, yet reversible, hypotension elicited by potent synthetic cannabinoids (Lake et al. 1997a) suggested that endocannabinoids may be involved in pathological conditions associated with extreme hypotension such as various forms of shock. Observations over the last decade have provided evidence for a key role of endocannabinoids in the hypotension associated with hemorrhagic (Wagner et al. 1997), endotoxic (Varga et al. 1998; Liu et al. 2003; Bátkai et al. 2004a; Wang et al. 2001; Godlewski et al. 2004), and cardiogenic shock (Wagner et al. 2001a, 2003). The vasodilated state associated with advanced liver cirrhosis appears to be due to a similar mechanism (Bátkai et al. 2001; Ros et al. 2002), which is most likely secondary to the endotoxemia commonly found in patients with late-stage cirrhosis (Lumsden et al. 1988).

In many of these conditions, circulating macrophages and platelets were found to contain elevated levels of endocannabinoids and to elicit SR141716-sensitive hypotension when injected into normal rats, suggesting the involvement of CB_1 receptors. In cirrhosis this was also suggested by the observed increase in CB_1 receptor mRNA and binding sites in vascular endothelial cells from cirrhotic human livers (Bátkai et al. 2001). However, SR141716 can also inhibit a receptor(s) distinct from CB_1 or CB_2 (see above), so the relative role of such receptors versus CB_1 receptors needed to be explored. In a recent study (Bátkai et al. 2004a), we reported that the acute hypotensive response of anesthetized rats to lipopolysaccharide (LPS) is inhibited by SR141716, but not by AM251, an antagonist equipotent with SR141716 at CB_1 receptors (Gatley et al. 1997), but devoid of inhibitory potency at the SR141716-

sensitive endothelial and myocardial receptors described above (Ford et al. 2002; Ho and Hiley 2003; O'Sullivan et al. 2004). Furthermore, LPS caused similar, SR141716-sensitive hypotension in wild-type mice and in mice deficient in CB_1 or both CB_1 and CB_2 receptors. Detailed hemodynamic analysis of the effects of LPS also indicated that the hypotension is primarily due to decreased cardiac contractility rather than decreased peripheral vascular resistance. These findings therefore suggest that receptors distinct from CB_1 or CB_2 are primarily responsible for the acute, SR141716-sensitive hypotensive response to LPS (Bátkai et al. 2004a). Anandamide is a ligand for such receptors and LPS stimulates the synthesis of anandamide in macrophages (Liu et al. 2003). Whether LPS may induce the synthesis of additional endogenous ligands for such receptors remains to be determined. Increased target organ sensitivity to anandamide may also play a role in the hemodynamic effects of LPS, as suggested by the potentiation of the vasodilator effect of anandamide in mesenteric beds isolated from endotoxemic rats (Orliac et al. 2003).

Endocannabinoid-mediated cardiovascular effects appear to have survival value, as indicated by the increased mortality, despite the increase in blood pressure, following blockade of CB_1 receptors in hemorrhagic (Wagner et al. 1997) and cardiogenic shock (Wagner et al. 2001a, 2003). In contrast, treatment with the cannabinoid agonists THC or HU-210 improved endothelial function and increased survival in cardiogenic (Wagner et al. 2001a, 2003) and endotoxic shock (Varga et al., 1998). Endocannabinoids may improve tissue oxygenation in these conditions by counteracting the excessive sympathetic vasoconstriction triggered by hemorrhage or myocardial infarction. In addition, endocannabinoids may mediate important protective mechanisms against hypoxic damage in the heart and vasculature and also exert potent anti-inflammatory effects (reviewed in Hiley and Ford 2003, 2004; Walter and Stella 2004, see also below).

5.2 Role of the Endocannabinergic System in Myocardial Reperfusion Damage

Interest in the investigation of potential cardioprotective effects of cannabinoids has recently been rekindled by a study of Lagneux and Lamontagne (2001) in which they compared the effects of a period of 90 min of low-flow ischaemia, followed by 60 min reperfusion at normal flow, in isolated hearts from rats pre-treated with bacterial endotoxin or saline. Endotoxin pretreatment reduced infarct size and enhanced functional recovery on reperfusion relative to the saline controls (preconditioning). The beneficial effects of endotoxin-induced preconditioning were blocked by the CB_2 receptor antagonist SR144528 but not by the CB_1 antagonist SR141716 (Lagneux and Lamontagne, 2001). Similarly, Joyeux et al. (2002) found that the infarct-size-reducing effect of heat stress preconditioning was also abolished by SR144528 but not by SR141716. In contrast, in an isolated perfused rat heart model in which preconditioning was induced by a brief period of ischemia (5 min), blockade of either CB_1 receptors with SR141716 or CB_2 receptors with SR1445278 abolished the protective effect of preconditioning. Preconditioning (5 min) also preserved the endothelium-dependent vasodilation evoked by serotonin (5-HT) in another study (Bouchard et al. 2003) in which both CB_1 and CB_2 receptors were implicated. Lepicier et al. (2003) have shown that the CB_2 receptor-mediated cardioprotection by endocannabinoids and synthetic cannabinoid ligands involves p38/ERK1/2 and protein kinase C (PKC) activation.

In an anesthetized rat model of ischemia/reperfusion injury (I/R), induced by coronary occlusion/reocclusion, Krylatov et al. have demonstrated that HU-210 and anandamide reduced the infarct size and the incidence of ventricular arrhythmias through activation of CB_2 but not CB_1 receptors (Krylatov et al. 2001; 2002a,b,c; Ugdyzhenkova et al. 2001; 2002). More recently, in an anesthetized mouse model of myocardial I/R, the mixed CB_1/CB_2 receptor agonist WIN55,212-2 significantly reduced the extent of leukocyte-dependent myocardial damage. The protective effect of WIN55,212-2 was abolished by the selective

CB_2 receptor antagonist AM630 and not affected by the selective CB_1 receptor antagonist AM251 (DiFilippo C et al. 2004).

5.3 Role of Endocannabinergic System in Hypertension

As early as in the 1970s, the potential use of cannabinoid ligands as antihypertensive agents had been considered (Archer 1974; Crawford and Merritt 1979; Varma et al. 1975; Zaugg and Kyncl 1983), in the hope that their neurobehavioral and cardiovascular effects could be separated. Although an early study in normotensive rats indicated rapidly developing tolerance to the hypotensive and bradycardic effects of THC (Adams et al. 1976), a subsequent study in spontaneously hypertensive rats (SHR) found no evidence for tolerance for the same effects during a similar, 10-day treatment period (Kosersky 1978).

Following the introduction of selective CB receptor antagonists in the mid 1990s, the finding that treatment of normotensive rats or mice with CB_1 antagonists alone does not affect blood pressure (Lake et al. 1997a; Varga et al. 1995), and that baseline blood pressure is similar in CB_1 knockout mice and their wild-type littermates (Járai et al. 1999; Ledent et al. 1999), indicated the absence of an endocannabinergic “tone” in the maintenance of normal levels of blood pressure. This is also suggested by the lack of significant hypotension following inhibition of anandamide transport (Calignano et al. 1997), in agreement with the relatively modest hypotensive effect of anandamide in normotensive animals (Lake et al. 1997a; Varga et al. 1995). However, SHR respond with greater and longer-lasting hypotension than normotensive rats to both THC (Kosersky 1978) and anandamide (Lake et al. 1997b; Bátkai et al. 2004b). THC inhalation evokes a greater and longer-lasting decrease of arterial blood pressure in hypertensive as compared to normotensive individuals (Crawford and Merritt 1979). Although the mechanism underlying this increased sensitivity has not been explored, it could suggest a role for the endocannabinoid system in regulating cardiovascular functions in hypertension. In a recent study we used three different models of experimental hypertension to explore this possibility (Bátkai et al. 2004b). The results document a significant endocannabinergic tone in hypertension that limits increases in blood pressure and cardiac contractile performance through tonic activation of cardiac and vascular CB_1. They also indicate that upregulation of cardiac and vascular CB_1 contributes to this tone, the potentiation of which, by inhibiting the inactivation of endogenous anandamide, can normalize blood pressure and cardiac contractile performance in hypertension. These findings raise the interesting possibility of the therapeutic use of inhibitors of fatty acid amide hydrolase in the treatment of hypertension.

6 Conclusions

Functional CB_1 receptors are present in vascular tissue as well as the myocardium, and cannabinoid agonists and endocannabinoids exert major hypotensive and cardiodepressor effects in vivo through the stimulation of CB_1 receptors. There is evidence for the existence of an as-yet-undefined endothelial and cardiac receptor or receptors that mediate certain endocannabinoid-induced cardiovascular effects. Vanilloid TRPV1 receptors can be activated by anandamide, but the role of these receptor in in vivo hemodynamic effects appears to be limited to the transient activation of the Bezold-Jarisch reflex by very high initial plasma concentrations of anandamide.

Endocannabinoids play important roles in a variety of pathophysiological conditions including hemorrhagic, endotoxic, and cardiogenic shock, and in the hemodynamic sequelae of advanced liver cirrhosis. Furthermore, pharmacological manipulation of the endocannabinoid system may offer novel therapeutic approaches in hypertension and ischemic heart disease.

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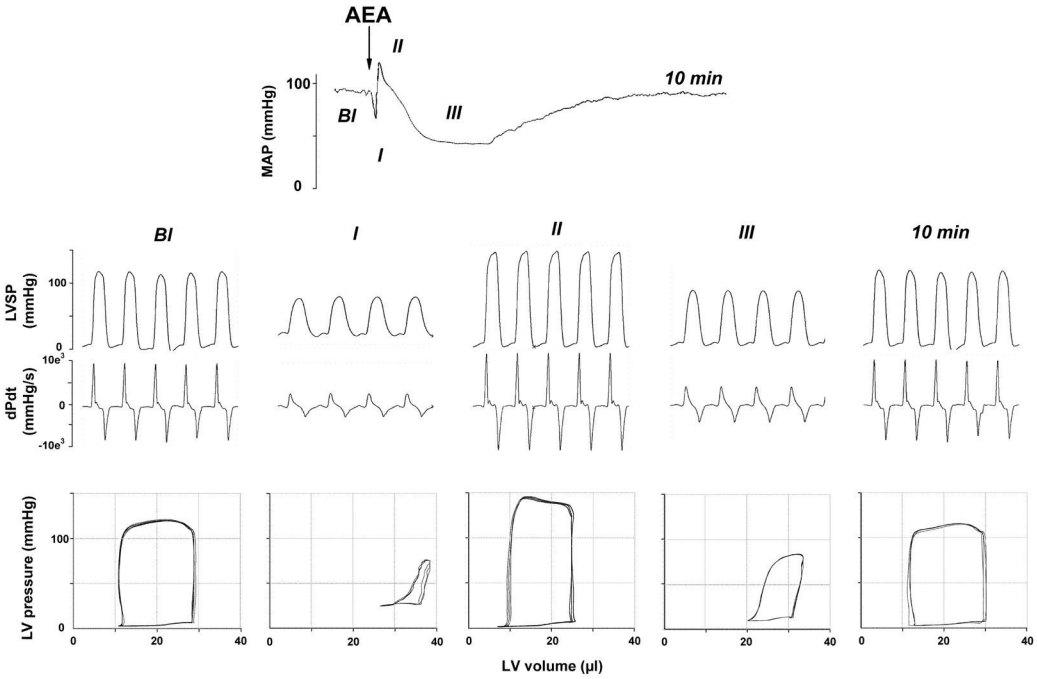

Fig. 1.

Hemodynamic effects of anandamide in anesthetized mice. Representative recordings of the effects of anandamide [20 mg/kg i.v., *N*-arachidonoyl-ethanolamine (*AEA*)] on mean arterial pressure (*MAP*, *top panel*), cardiac contractility (left ventricular systolic pressure (*LVSP*) and d*P*/d*t* (*dPdt*); *middle panel*) and pressure-volume relations (*bottom panel*) in a pentobarbital-anesthetized C57BL6 mouse. The five parts of the *middle* and *bottom panels* represent baseline conditions (*Bl*), phase I (*I*), phase II (*II*), and phase III (*III*) of the anandamide response, and recovery *10 min* following the injection. The *arrow* indicates the injection of the drug. The decrease of the amplitude of PV loops and shift to the right indicate decrease of cardiac contractile performance

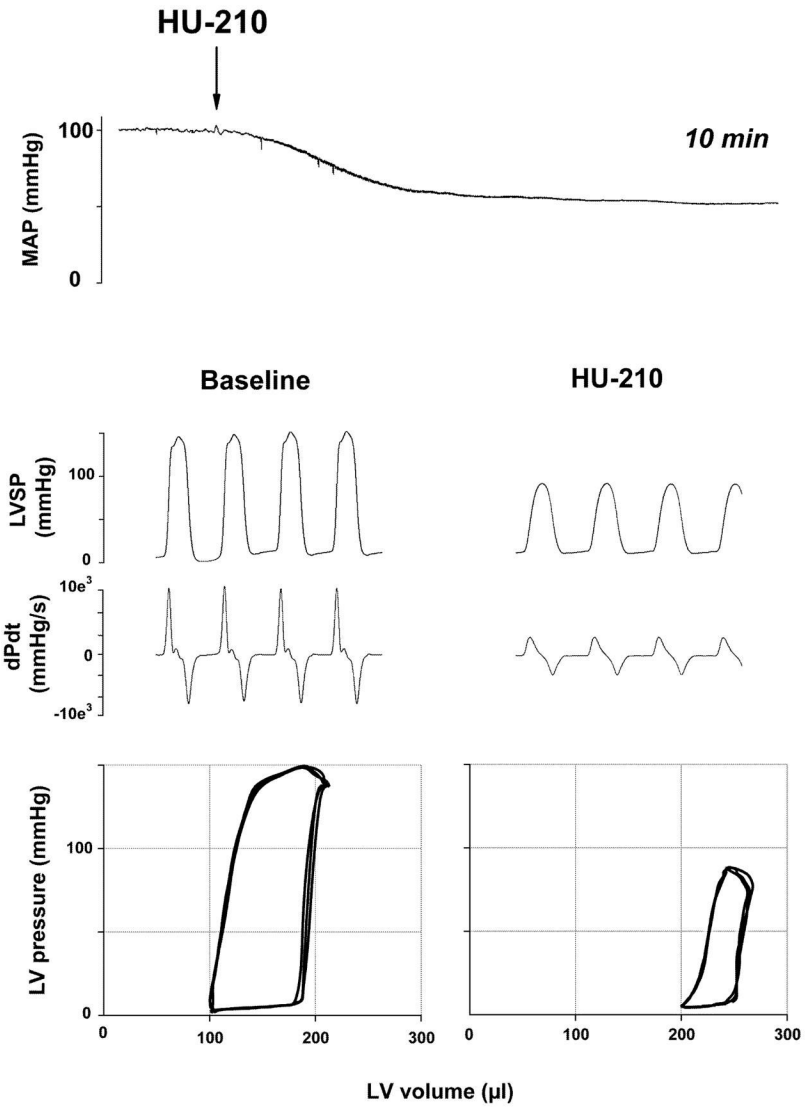

Fig. 2.

Hemodynamic effects of HU-210 in anesthetized rat. Representative recordings of the effects of HU-210 (30 μg/kg i.v.) on mean arterial pressure (*MAP*, *top panel*), cardiac contractility (*LVSP* and d*P*/d*t; middle panel*), and pressure-volume (*PV*) relations (*bottom panel*) in a pentobarbital-anesthetized rat. The *arrow* indicates the injection of the drug. The decrease of the amplitude of PV loops and their shift to the right are indicative of decreased cardiac contractile performance