



Cannabinoids and Cardiovascular System

5

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Abstract

Cannabinoids influence cardiovascular variables in health and disease via multiple mechanisms. The chapter covers the impact of cannabinoids on cardiovascular function in physiology and pathology and presents a critical analysis of the proposed signalling pathways governing regulation of cardiovascular function by endogenously produced and exogenous cannabinoids. We know that endocannabinoid system is overactivated under pathological conditions and plays both a protective compensatory role, such as in some forms of hypertension, atherosclerosis and other inflammatory conditions, and a pathophysiological role, such as in disease states associated with excessive hypotension. This chapter focuses on the mechanisms affecting hemodynamics and vasomotor effects of cannabinoids in health and disease states, highlighting mismatches between some studies. The chapter will first review the effects of marijuana smoking on cardiovascular system and then describe the impact of exogenous cannabinoids on cardiovascular parameters in humans and experimental animals. This will be followed by analysis of the impact of can-

nabinoids on reactivity of isolated vessels. The article critically reviews current knowledge on cannabinoid induction of vascular relaxation by cannabinoid receptor-dependent and -independent mechanisms and dysregulation of vascular endocannabinoid signaling in disease states.

Keywords

Cannabis · Endocannabinoids ·
Cannabinoid receptors · Endothelial cells ·
Vascular

Abbreviations

2-AG	2-Arachidonoylglycerol
ACPA	arachidonylcyclopropylamide
BK _{Ca}	large conductance calcium-activated potassium channel, KCa1.1
CB1	cannabinoid receptor type 1
CB2	cannabinoid receptor type 2
CBe	endothelial cannabinoid receptor
CGRP	calcitonin gene-related peptide
COX	cyclooxygenase, prostaglandin-endoperoxide synthase

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DOC salt hypertension	deoxycorticosterone acetate-induced hypertension
EDHF	endothelium-derived hyperpolarizing factor
FAAH	fatty acid amide hydrolase
IK _{Ca}	intermediate conduc- tance calcium-acti- vated potassium channel, KCa3.1
K _{ATP}	ATP-sensitive potas- sium channel
NAGly	N - a r a c h i d o n o y l glycine
NCX	Na ⁺ -Ca ²⁺ exchanger
NO	nitric oxide
PPAR	peroxisome prolifera- tor-activated receptor
SHR	spontaneously hyper- tensive rats
TASK	TWIK-related acid- sensitive potassium channel
THC	Δ ⁹ -tetrahydrocanna- binol
TRPA	transient receptor potential cation chan- nel subfamily A (ankyrin)
TRPV	transient receptor potential cation chan- nel subfamily V (vanilloid)

blood pressure, vasomotor control, cardiac contractility, vascular inflammation, preconditioning and angiogenesis are controlled by cannabinoids. Changes in the levels of circulatory cannabinoids and cannabinoid receptor expression in the vasculature as well as the associated perturbations in cannabinoid signalling have been detected under a number of pathophysiological conditions including obesity, diabetes, advanced liver cirrhosis, cardiotoxicity, circulatory shock, atherosclerosis and hypertension [1–8]. Consequently, the endocannabinoid system is widely accepted to represent an attractive therapeutic target to tackle a range of abnormalities including cardiovascular disorders [9–11].

Because of psychotropic and pain-relieving effects, the members of the plant family Cannabaceae have a long history of cultivation and human use both for recreational and medical purposes, rooting through several thousand years [12]. Recent boosted recreational marijuana abuse and accessibility of a growing number of synthetic psychoactive cannabinoids with greatly increased potencies as compared to that of Δ⁹-tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana, are coincided with the reported serious cardiovascular events such as myocardial infarction, cardiomyopathy, arrhythmias and stroke with documented fatalities even among young and relatively healthy men [13–15]. While the mechanisms for these events are still not entirely clear, these observations emphasize a casual association between recreational cannabis abuse and cardiovascular abnormalities, calling for a need to advance our understanding of the fundamental mechanisms so severely affecting cardiovascular function by cannabis. Besides the principal phytocannabinoid THC, which binds to cannabinoid receptors and determines psychoactive properties of the plant, cannabis contains an extensive number of non-psychoactive phytocannabinoids with low affinity binding at cannabinoid receptors type 1, CB1, and type 2, CB2, such as cannabidiol, cannabinol, cannabidivarin and cannabigerol and others. Because of beneficial effects in a range of disorders and a lack of psychoactivity, therapeutic

5.1 Introduction

Cannabinoids influence the function of many organs and systems and apart from the well-known neurobehavioral and analgesic effects, cannabinoids exert a profound effect on cardiovascular, immune, digestive, reproductive function, and influence cell fate, body temperature, bone formation and other aspects of human physiology. Key cardiovascular parameters such as

tic effects of these compounds and their mechanisms of action is a subject of intense research [10].

The discovery of THC, the main psychoactive phytocannabinoid contained in Cannabis plant [16], was the first major step in the recognition of the role of the endocannabinoid system in health and disease. Cannabinoid research received a strong impetus following the identification and characterization of CB1 [17] and CB2 receptors [18, 19]. The latter was initially identified as peripherally restricted receptor expressed by immune cells. Subsequent studies have shown that the CB2 receptors are also distributed in vascular cells [20, 21] and central and peripheral nervous system [22–25].

The endogenous ligands for cannabinoid receptors, anandamide and 2-arachidonoyl glycerol (2-AG) were detected initially in the brain [26, 27] and gut [28]. Subsequent studies showed that endocannabinoids are synthesized at the plasma membranes of virtually all cell types including vascular [29–32], cardiac cells [33], monocytes and platelets [34]. Cardiovascular pathologies, such as coronary circulatory dysfunction, myocardial infarction, hypertension, atherosclerosis and diseases accompanied by vascular dysfunction, such as diabetes, obesity and cirrhosis, are associated with alterations in cannabinoid signaling and increased plasma levels of 2-AG and anandamide [1, 5, 34–38].

Under normal conditions, CB1 receptors have been detected in different vascular beds, including endothelial cells from rat mesentery [37, 39, 40], rat [2, 32] and human aorta [41], human hepatic artery endothelial cells [42], rat aortic smooth muscle cells [43], pointing at engagement of the endocannabinoid system in regulation of vascular function. Indeed, endocannabinoids and their synthetic analogues exert hypotensive and cardiodepressant effects, control cardiac contractile reactions [1, 11, 44]. The chapter will review the impact of cannabinoids on cardiovascular function.

5.2 Effects of Cannabinoids on Cardiovascular Parameters

Highly diverse actions of cannabinoids are mediated via surprisingly wide number of targets, spanning from classical G protein-coupled cannabinoid receptors, non-CB1/CB2 targets, including G-protein coupled receptors GPR18, GPR35, GPR55 and GPR119 [45–50], and a broad number of ion transport systems [51–54]. The reactions of cardiovascular variables in response to cannabinoids depend on several factors and besides the type of predominantly stimulated cannabinoid receptors, there are many other determinants of the reaction, such as direct targeting of ion channels and transporters located in plasmalemma, engagement in the response of non-CB1/CB2 receptors, intracellular ion channels [55, 56] or intracellularly located receptors for cannabinoids [57–59]. Via stimulation of specific cannabinoid receptors, cannabinoids may attenuate or intensify cardiovascular pathological states and, accordingly, play a protective or pathophysiological role.

The molecular mechanisms underlying diverse effect of cannabinoids and their synthetic analogues on vascular function, although progressively unveiled in the last two decades, are not yet entirely clear. In vivo effects of cannabinoids involve sites of action in the central [60, 61] and the peripheral nervous system [62, 63] as well as both cannabinoid receptor-dependent and –independent targets located on cardiac myocytes [64–66], vascular smooth muscle [40, 67–71] and endothelial cells [6, 40, 52, 53, 72–75].

Apart from regulation of vasoactivity, cannabinoids influence cardiac performance and modulate ischemia-reperfusion injury [8, 76–78], endothelial [79], and smooth muscle cell migration [71], angiogenesis [80, 81], vascular wall inflammation and atherogenesis [3, 82]. We will briefly review the literature describing the impact of smoked cannabis and intravenous THC, as well as central and peripheral regulation of cardiovascular parameters by cannabinoids.

5.2.1 Effects of Marijuana Smoking on Cardiovascular Parameters

The cardiovascular effects elicited by marijuana smoking largely depend on chemical composition of the plant, specifically, the THC content, the dose inhaled and the smoking method. The impact of cannabinoids on cardiovascular parameters has been studied since early 1970s. The studies were mostly directed on examining the effect of THC as the principle active ingredient of *Cannabis sativa* [83, 84]. From the very beginning of investigation of the effects of constituents of cannabis on cardiovascular parameters, the differences in the effects of THC and cannabidiol both on heart rate and some psychological reactions were noticed [85]. Early reports on the effects of cannabis on humans were focused mainly on psychotropic effects and pointed at pharmacological difference between oral injection and inhalation of smoke of cannabis products [86]. Smoking cannabis was shown to lead to a potent bronchodilation of human airways [87] and an immediate increase in heart rate up to 90 beats per minute that may last more than 1 h and an increase in limb blood flow [88, 89]. These responses were not observed after administration of propranolol, a beta-adrenergic blocker, pointing for beta-adrenergic stimulation [89]. However, repeated users within several days or weeks develop a tolerance to the initial effects and experience bradycardia and hypotension. Most of these studies conclude that THC alters autonomic control of the cardiovascular system resulting in parasympathetic dominance. Early [90, 91] and more recent [92] studies have shown that marijuana smoking is associated with increased cerebral blood flow. Systematic reviews of the reported cases indicate that marijuana smoking is linked to increased likelihood of development of severe cardiovascular events including atrial fibrillation, enhanced left ventricular systolic function, transient loss of consciousness and a fall, ventricular arrhythmias, coronary artery disease, severe stroke development, peripheral arteritis [15, 93–95].

5.2.2 Effects of Cannabinoids on Blood Pressure and Heart Rate

5.2.2.1 Human Studies

In healthy men volunteers, 30 mg of THC received orally increased the heart rate and blood pressure [85, 96]), while cannabidiol (15–60 mg) produced no effect when administered alone and blocked the effect of THC when the drugs were administered together [85]. Acute administration of cannabidiol at higher dose (600 mg) was reported to reduce resting blood pressure and the blood pressure increase elicited by exercise and mental stress [97]. The uncovered differences in the impacts of THC and cannabidiol on heart rate and blood pressure in early 1970s [85, 96, 98] initiated an extensive research in this field aiming to identify the mechanisms underlying the impact of cannabinoids on cardiovascular system. While acute administration of THC generally results in an increase in blood pressure and heart rate, repeated administration of THC decreases blood pressure and heart rate. In conscious humans, an acute intravenous administration of THC in the dose of 25 µg/kg elicits tachycardia without significant alterations in systolic and diastolic blood pressures [99]. Prior beta adrenergic blockade partially inhibited this response. Acute oral or intravenous administration of THC at higher dose (0.2–0.3 mg/kg) resulted in an increase in both heart rate and blood pressure in healthy volunteers [96, 100]. The reactions, however, switched to the opposite when THC was injected for a prolonged time [101]. These data point at complex mechanisms involved in bidirectional regulation of cardiovascular parameters by cannabinoids.

5.2.2.2 Animal Studies

The hemodynamic effects of cannabinoids in conscious rats are quite different from those observed in anesthetized rats [102, 103]. In anaesthetized rats, the most prominent response to anandamide and THC infusion is a long-lasting hypotension and bradycardia, generally ascribed as phase 3 of the triphasic response [104–106]. The long-lasting hypotension and bradycardia evoked by anandamide infusion to

anesthetized rats is preceded by an immediate brief drop in blood pressure and heart rate ascribed as phase 1, which was followed by a brief (30–60 s) pressor response and tachycardia, ascribed as phase 2. The long lasting depressor effect of anandamide, but not the two initial transient phases, is inhibited by rimonabant [105, 107] and after transection of the cervical spinal cord or blockade of alfa-adrenergic receptors [103, 104, 107], suggesting that the depressor response is due to CB1 receptor-mediated inhibition of norepinephrine release from peripheral sympathetic nerve terminals in the heart and vasculature and subsequent inhibition of catecholamine release [108]. It was shown also that in anaesthetized rats, cannabidiol and its synthetic analogue O-1918, which have low affinity to CB1 and CB2 receptors, elicit a prolonged decrease in blood pressure, heart rate and mesenteric and renal blood flow, masking/reducing the similar cardiovascular effects of anandamide that are normally observed in the absence of cannabidiol and O-1918 [109]. The authors attributed the hypotensive effects of anandamide in anesthetized rats to stimulation of the third type cannabinoid receptor sensitive to O-1918 [109]. In the earlier study of Malinowska et al [110], the anandamide-evoked decrease in heart rate and blood pressure in anaesthetized rats (phase 3) has been attributed to stimulation of the CB1 and TRPV1 receptors.

The phases 1 and 2 are absent in TRPV1-deficient mice [106, 107, 111], pointing for the involvement of TRPV1 receptor. A TRPV1 agonist capsaicin was shown to be more potent than methanandamide and anandamide at eliciting an immediate short-lasting decrease in heart rate and blood pressure that is inhibited by a selective TRPV1 antagonist capsazepine [110], suggesting that a short-lived depressor effect of anandamide is evoked by the Bezold-Jarisch reflex [112]. A brief pressor response (phase 2) is enhanced after alfa-receptor blockade or cervical cord transection [104].

Unlike the responses elicited by THC and anandamide, the hypotension and bradycardia elicited by 2-AG is insensitive to rimonabant and is preserved in CB1 knock-out mice [113].

However, cardiovascular effects of 2-AG were found to be masked by a rapid degradation of the endocannabinoid by a monoacylglycerol lipase with generation of arachidonic acid. A metabolically stable 2-AG analogue 2-AG ether was found to elicit hypotension that is sensitive to rimonabant and absent in CB1 knock-out mice [113], suggesting the CB1 receptor-mediated signalling, possibly through sympathetic nerves innervating the resistance vessels.

In contrast to anaesthetized rats, in conscious rats, intravenous administrations of anandamide, its stable analogue methanandamide, THC and WIN55212-2 fail to produce a prolonged hypotension, but result in a brief pressor response, that was potentiated by rimonabant [105], indicating that the CB1 receptor-dependent signaling attenuates the pressor response. Consistent with the idea that stimulation of CB1 receptor results in vasodilation, intravenous administration of a synthetic CB1 and CB2 receptor agonist WIN55512-2 to pithed, conscious rabbits in which the sympathetic outflow was continuously stimulated electrically was shown to decrease blood pressure and heart rate [114] and the effect was antagonized by rimonabant.

In other study performed on conscious rats, anandamide (75–1250 µg/kg) elicited a short-lived increase in arterial blood pressure associated with vasoconstriction in renal, mesenteric and hindquarters vascular beds [115]. When anandamide was administered at the higher dose (2.5 mg/kg) to conscious rats, a pressor response was preceded by a transient fall in arterial blood pressure. After high dose of anandamide, the hindquarters vasoconstriction was followed by vasodilation. Intriguingly, in conscious rats, none of the hemodynamic responses to anandamide were found to be influenced by antagonism of CB1 receptors with AM251 [115], suggesting that the anandamide-evoked responses may not involve CB1 receptors. In contrast, in the presence of beta2 adrenoceptor antagonist ICI 118551 the hindquarters vasodilation was inhibited and the pressor response prolonged. Similar to anandamide, WIN55212-2 and HU-210 evoked a pressor response associated with renal and mesenteric vasoconstriction and hindquarters vasodi-

lation that was antagonized by AM251 and the beta2 receptor antagonist ICI 118551 [116], a finding consistent with the involvement of beta2 adrenoreceptors in the CB1 receptor-mediated hindquarters vasodilation. Notably, AM251 [116] has no noticeable effects on resting hemodynamics and blood pressure, suggesting negligible role of CB1 receptor-dependent signaling in cardiovascular hemodynamics under normal conditions.

Conclusively, studies on anesthetized and conscious rats demonstrate complexity of hemodynamic effects of cannabinoids. While in anesthetized rats, cannabinoids evoke a triphasic response, the most prominent of which is a sustained vasodilation, in conscious rats, cannabinoids evoke dose-dependent brief pressor response. In the absence of anesthetics, the only vascular bed that shows vasodilation is the hindquarters.

5.3 Effects of Cannabinoids on Reactivity of Isolated Vessels

The mechanisms of action of cannabinoids and cannabinoid-like substances on vascular cells have been widely studied in isolated vessel preparations with the use of wire myography. In a great number of isolated pre-contracted vascular preparations, cannabinoids produce vasodilation of varying degree, however, constriction responses have also been reported [117], emphasizing complex vascular cannabinoid pharmacology. While it is established that both CB1 and CB2 are distributed in both endothelial and vascular smooth muscle cells [21, 32, 40, 41], a link between stimulation of vascular cannabinoid receptors and vasodilation remains controversial, with the prevailing conclusions that the relaxation of healthy arteries may not require stimulation of vascular cannabinoid receptors. In fact, the mechanisms affecting vasomotor activity elicited by topically applied cannabinoids independently of CB1 and CB2 receptor stimulation are extremely versatile and seem to be predominantly responsible for both endothelium-depen-

dent and -independent relaxation in a vast number of vascular beds.

Numerous studies indicate that the mechanisms of cannabinoid-induced vasodilation vary between species, vessel type and have regional differences [70, 118–120]. Conclusions of different research groups on the involvement of CB1 receptor in the responses of isolated vessels to anandamide and participation of endothelium-dependent mechanisms in these responses sometimes are controversial even with regard to the same vascular bed, adding some confusion into the topic [119, 121]. Thus, in isolated rat mesenteric artery, the relaxation to anandamide has been identified as endothelium- and CB1 receptor-independent [121, 122], whereas other groups showed endothelium-dependency of the response in the same artery with [119] or without [123] CB1 receptor involvement. It is possible that the choice of different constricting agents, unspecific CB1 antagonists and the method of de-endothelization influenced the results obtained.

Anandamide at 30 μM was shown to relax U-46619-pre-contracted rat aortic rings by 22% in endothelium-independent manner [124]. The relaxation was unaffected by rimonabant, AM251 and capsaicin, but was reduced to 13% in pertussis toxin-pre-treated preparations, allowing the authors to suggest the involvement of yet unidentified non-CB1/CB2 cannabinoid receptor located on smooth muscle cells [124]. In other study [125], anandamide (100 μM) gradually relaxed rat aortic rings pre-contracted with phenylephrine by 51% within 7–10 min before reaching a plateau and the relaxation was reduced to 20% following removal of the endothelium. The relaxation was insensitive to pre-treatment with rimonabant and the CB2 antagonist SR144528, but was inhibited by O-1918, allowing the authors to suggest the involvement of unidentified non-CB1/CB2 cannabinoid receptor located on endothelial cells [125]. In rat aortic rings pre-contracted with the combined presence of U-46619 and methoxamine, the endothelium-dependent relaxation to anandamide was shown to be insensitive to CB1 and CB2 receptor antagonists, but inhibited by peroxisome proliferator-activated receptor (PPAR) gamma antagonist

GW9662 [59], suggesting that the relaxation is mediated by stimulation of nuclear PPAR gamma receptor. In phenylephrine-pre-contracted aortic rings isolated from normotensive sham-operated rats subjected to excision of the left renal artery without clipping, the maximal relaxation to 30 μM anandamide amounted 4% only [20]. As could be seen from these studies, the proposed mechanisms governing the anandamide-evoked relaxation of the same vascular preparations principally differ between different research groups. The reasons for the discrepancies are unclear but might be related to variations in wire myography protocols or the constricting agent used (a thromboxane mimetic U-46619 vs. phenylephrine).

Obviously, varying outcomes of the studies as for the requirement for the given cannabinoid receptor in the vasodilation to cannabinoids are unlikely to be solely explained by intrinsic variations in the cannabinoid receptor expression between the vascular beds. A part of the problem is that the pharmacology and molecular modes of action of cannabinoid receptor agonists and antagonists developed and widely used in wire myography studies as selective have not been clearly defined. Mounting number of studies points for additional, cannabinoid-receptor-independent effects and targets, such as PPAR, TRPV4 and large conductance calcium-activated potassium (BK_{Ca}) channels. Employment of highly selective cannabinoid receptor agonists and antagonists with precise molecular mechanisms of action is essential to delineate possible role of cannabinoid receptors in vascular effects of cannabinoids in health and disease. Another possible contributor into variable outcomes of the studies is the choice of technique to capture the mechanisms of vascular cannabinoid signalling and clarify whether the response requires specific receptor. Electrophysiological studies on isolated endothelial and smooth muscle cells and intact vascular preparations allowed to identify a large number ion-transporting systems targeted by cannabinoids and cannabinoid-like substances, including BK_{Ca} channels [72, 126, 127], intermediate conductance calcium-activated potassium (IK_{Ca}) channels [53], voltage-gated Ca^{2+} channels of L and T type [128–130], TRPV4 [74],

TRPA1 [131, 132], and the TASK subfamily of two pore domain K^{+} channels responsible for background K^{+} currents [133–136], $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger (NCX) [65, 127, 137, 138] and $\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$ [52, 139, 140]. All these players are present in the vasculature, determining the membrane potential of endothelial and smooth muscle cells, the release of variety of endothelium-derived vasoactive substances and contractile responses to cannabinoids.

5.3.1 Endothelium-Independent Relaxation to Cannabinoids

The earliest study describing the effect of locally applied endocannabinoids on vascular reactivity showed that in anesthetized rabbits, topically applied anandamide and THC dilate cerebral arterioles [141]. The dilation was suggested to be mediated by the release of endogenous arachidonic acid. The study initiated an intense research into the effects and the mechanisms of action of cannabinoids on blood vessels. In experiments performed on pre-contracted rat superior mesenteric arterial bed, anandamide was found to induce endothelium-independent relaxation that was suppressed by the CB1 receptor inhibitor rimonabant [39]. Relaxations to carbachol and Ca^{2+} ionophore A23187 were also sensitive to rimonabant, suggesting that carbachol and anandamide share the mechanisms of action and that anandamide is an endothelium-derived hyperpolarizing factor (EDHF), an entity responsible for endothelium-dependent relaxation under conditions of inhibition of nitric oxide (NO) synthesis. This mechanism was also proposed to govern the relaxation in rat coronary artery [142]. Since these observations, much attention has been given to investigation of the mechanisms of vasoactive effects of anandamide and other cannabinoids. However, in the rat isolated perfused mesenteric vascular bed, HU-210, WIN55212-2 and THC failed to cause vasodilation and even produced constriction [143]. The possibility that anandamide acts as a mediator of NO-independent vasodilation to endothelium-dependent vasodilators was extensively evaluated in late 1990s in

different vascular beds, including isolated rat mesenteric arteries [70, 118, 144, 145], rat hepatic [146] and rat coronary arteries [147], guinea-pig basilar artery [148] and in anaesthetized rabbits [149] with general conclusion that anandamide is not EDHF in these vascular beds.

5.3.1.1 Potential Role of Cannabinoid Receptors in Vasodilation

The involvement of cannabinoid receptors in the vasodilation to anandamide and other cannabinoids in different vascular beds has been most extensively investigated using wire myography approach with the help of pharmacological modulators of cannabinoid receptors. Less often, pressure myography is used. In a typical wire myography protocol, the excised vessels are cut into approximately 2 mm-long segments and the rings are attached to hooks mounted in a Mulvany-Halpern wire myograph [150] and then properly stretched to achieve a largest contractile response to a submaximal dose of one of the contractile agents such as phenylephrine, methoxamine or U-46619, a stable thromboxane A2 receptor agonist. Following stabilization of the tone, the substances of interest are applied and the vasorelaxation is computed as the ratio to the imposed contraction. In wire myography, tension is measured under isometric conditions. The limitation of the method is that optimum resting tension is not the same as physiological and the attachment of vessel ring leads to nonphysiological geometry and loading [151, 152]. It should be noted, that in the absence of imposed contraction, i.e. in the absence of stimulated Ca^{2+} entry into smooth muscle cells, most of the cannabinoids fail to cause a significant changes in the baseline tension and THC elicits a significant contraction [124].

While the majority of studies concluded that neither CB1, nor CB2 receptors are involved in endothelium-dependent or -independent vasodilation to cannabinoids in healthy arteries [69, 70, 122, 123, 144, 153–155], some limited number of reports point for engagement of CB1 receptors [37, 43, 67, 119, 156–158] or CB2 receptors [37, 40] in the vasodilation. In many early and recent vascular myography studies, rimonabant and

structurally very close compound AM251 have been the most widely employed CB1 receptor antagonists. Rimonabant was first described by Sanofi Aventis as a selective and orally active inverse CB1 receptor agonist with CB1 affinity in low nanomolar range [159]. It should be noted that although the low nM concentrations of rimonabant and AM251 are required to block CB1 receptors, these compounds are frequently used in low micromolar (1–3 μM) concentrations in assessing the role of CB1 receptor in the vasodilation [124, 125, 158, 160, 161]. At these concentrations, these CB1 antagonists display a number of CB1 receptor-independent effects. Thus, at 1 μM rimonabant inhibits cannabinoid-induced hypotension and mesenteric vasodilation via a target distinct from CB1 receptor, as the effect is observed in CB1 and CB1/CB2 knockout mice [123]. The unspecific effects of rimonabant include inhibition of myo-endothelial gap junctions [162], endothelium-dependent relaxation to carbachol, acetylcholine, bradykinin, Ca^{2+} ionophor A23187 and ionomycin [39, 142, 143, 157, 162], direct suppressive action on the BK_{Ca} channel function [163] and Ca^{2+} entry mechanism [164]. In the isolated quinea pig carotid artery, rimonabant at concentrations 0.1–10 μM hyperpolarizes vascular smooth muscle cells by up to 10 mV and significantly inhibits the smooth muscle cell hyperpolarization evoked by acetylcholine [70, 118]. Rimonabant at 10 μM was shown to strongly attenuate the relaxation of cannulated pre-contracted rat mesenteric artery induced by levcromacalim, an opener of ATP-sensitive K^+ (K_{ATP}) channel [122]. Similar to rimonabant, AM251 was found to have a number of non-specific effects [54, 131, 163, 165–167]. Interestingly, the activation of TRPV1 channels by anandamide was reported to be antagonized by rimonabant and AM251, although at concentrations higher than those required for CB1 antagonism. Collectively, the wealth of data points for non-specific effects of widely used CB1 receptor antagonists rimonabant and AM251, warranting considerations in interpretation of the relevant data. Cannabinoid receptor-independent targets for CB1 antagonists rimonabant and AM251 and for the cannabidiol

Table 5.1 Cannabinoid receptor-independent targets for rimonabant, AM251 and O-1918 in the vasculature

CB antagonists	Site of action	Action	References
Rimonabant	Myo-endothelial gap junctions	Inhibition	[162]
Rimonabant	BKCa	Inhibition	[163]
Rimonabant	Unidentified Ca ²⁺ entry	Inhibition	[164]
Rimonabant	Resting MP of SMC	Hyperpolarization	[70]
Rimonabant	Ach-evoked SMC hyperpolarization	Inhibition	[118]
Rimonabant	KATP opener-evoked SMC hyperpolarization	Inhibition	[122]
AM251	TRPA1	Activation	[131]
AM251	BKCa	Inhibition	[163]
O-1918	BKCa	Inhibition	[163]
O-1918	NCX	Inhibition	[127]

analogue O-1918 widely used as a “selective” antagonist of “endothelial cannabinoid receptor” are listed in Table 5.1.

Vasoactive properties of anandamide are most frequently studied among other cannabinoids. There is a general consensus that this endocannabinoid elicits a relaxation in a vast number of isolated pre-contracted vascular preparations with both endothelium-independent and –dependent mechanisms. The results published are somewhat controversial even when the studies from the same vascular bed are compared.

Anandamide was shown to elicit endothelium-independent relaxation of pre-contracted rat small mesenteric artery [39, 121, 143, 145, 157, 168, 169], rat coronary artery [168], rat aorta [124]. In another study performed on isolated rat mesentery pre-contracted with phenylephrine, de-endothelization slightly but significantly reduced the relaxation to anandamide [143]. Removal of the endothelium fails to inhibit the relaxation of pre-contracted rat gastric arteries in response to the stable anandamide analogue methanandamide [69] and the anandamide-evoked smooth muscle cell hyperpolarization in the rat small mesenteric artery [118]. In other study performed on isolated rat small mesenteric artery, the hyperpolarization to anandamide was endothelium-dependent and sensitive to a selective inhibitor of ATP-sensitive K⁺ (K_{ATP}) channel glibenclamide, but not rimonabant [70].

In a number of isolated pre-contracted vascular preparations, including those from human [170] and rat pulmonary arteries [171], rabbit

[162] and human mesenteric arteries [172], bovine [153] and sheep coronary artery [173], anandamide produces endothelium-dependent relaxation. In contrast, in isolated porcine coronary artery with or without endothelium, anandamide (30 μM) did not modify the tension and the membrane potential of smooth muscle cells [70].

Reports on the effect of 2-AG on reactivity of isolated vessels point for engagement of both endothelium-dependent [174] and –independent mechanisms [117, 174, 175]. 2-AG can dually modulate the contractile reactions, as both the relaxing and constricting responses have been described. Consistent with the described hypotensive effect [113, 176], 2-AG was shown to cause relaxation of isolated vascular preparations [30, 67, 174, 175, 177]. In isolated pre-contracted bovine coronary arteries, the relaxation to 2-AG depends on the intact endothelium and is blocked by inhibition of phospholipase C, FAAH, cyclooxygenase (COX) and cytochrome C450 [30]. In contrast, in rabbit pre-contracted mesenteric arteries, the relaxation to 2-AG is endothelium-independent but sensitive to rimonabant [175]. In mesenteric arteries isolated from patients undergoing surgical treatment of bowel carcinoma and inflammatory bowel disorders, the relaxation to 2-AG is endothelium-independent and is insensitive to the antagonists of CB1 and CB2 receptors AM251 (100 nM) and AM-630 (100 nM) [177]. De-sensitization of TRPV1 channels and FAAH inhibition failed to affect the dilation that was reduced by the COX-1 inhibitors [177].

In isolated rat aorta, 2-AG is ineffective at influencing the basal tone, but in pre-contracted rings, the endocannabinoid induces a biphasic response consisting of a transient relaxation followed by a sustained constriction [117], the responses being unaffected by endothelial denudation and inhibition of both types of cannabinoid receptors. Unlike in human mesenteric artery, in rat aorta, pretreatment with COX-1 inhibitor indomethacin failed to inhibit the weak relaxation to 2-AG, however, abolished the contraction phase both in endothelium-intact and denuded rings.

Experimental data derived from the study of the effect of THC, a CB1 agonist, on vascular contractility, also fails to support the engagement of CB1 receptor in the dilator response. In pre-contracted rat superior mesenteric artery, 3 μM THC elicited a marginal relaxation amounting 4% only. The relaxation was enhanced to 16% in the presence of the COX inhibitor indomethacin [120], pointing that the stimulated release of vasoconstrictor prostanoids masks the vasodilation. De-endothelization and 100 nM rimonabant had no effect on the relaxation. Higher concentrations of THC (10–100 μM) elicit a rimonabant-sensitive vasoconstriction that was converted to a weak relaxation following de-endothelization [120]. In third order branches of mesenteric artery, however, THC was shown to elicit endothelium-independent vasorelaxation, sensitive to charybdotoxin, a dual inhibitor of BK_{Ca} and IK_{Ca} channels, and apamin, a selective blocker of small conductance Ca^{2+} -activated K^{+} channel, but insensitive to antagonists of CB1 and TRPV1 receptors. In these arteries, THC was shown to inhibit the contractile response elicited by Ca^{2+} re-addition, indicating for inhibition of Ca^{2+} influx into smooth muscle cells [120].

5.3.1.2 The Role of TRPV1 Channels

Vasorelaxation to anandamide may involve stimulation of TRPV1 receptor as first demonstrated in isolated rat hepatic and mesenteric arteries and guinea pig basilar arteries [155]. The relaxation was shown to be abolished after treatment with capsazepin, a TRPV1 antagonist, and involves calcitonin gene-related peptide released from

perivascular sensory nerves [155]. In that study, rimonabant (0.3 μM) failed to inhibit the vasodilator effect of anandamide. Neither 2-AG, nor synthetic CB1 and CB2 agonists were able to mimic the effect of anandamide [155], strongly indicating that neither of the known cannabinoid receptors mediate the relaxation of these arterial beds. However, the anandamide-induced vasodilation was abolished by capsazepine, a selective TRPV1 antagonist. The vasodilation was not reproduced following TRPV1 desensitization by pretreatment with capsaicin, a selective TRPV1 agonist. Following this original observation, several other studies confirmed, at least partial engagement of this mechanism in the dilation to anandamide [119, 121, 122]. However, no evidences for TRPV1 role in vasodilation to anandamide were obtained in the rat pulmonary [171] and rat coronary [168] arteries. The role of TRPV1 in relaxation to anandamide was further confirmed in pre-contracted small mesenteric artery, where anandamide was shown to produce endothelium-independent relaxation that was reduced following capsaicin pre-treatment [119, 121]. In the study of Ho and Hiley [121], the relaxation to anandamide, although was sensitive to 3 μM rimonabant, was unaffected by AM251 (3 μM) or CB2 receptor inhibitor SR144528, suggesting that perivascular TRPV1, but not CB1, plays a major role in the relaxation. In contrast, in the study of O'Sullivan et al. [119] the relaxation of small mesenteric arteries to anandamide was partially endothelium-dependent and reduced by both rimonabant (100 nM) and AM251 (100 nM), implicating activation of CB1 receptors. The only difference between the studies is that in the study of Ho and Hiley [121] the arteries were pre-contracted with $\alpha 1$ -adrenergic receptor agonist methoxamine, while in the study of O'Sullivan [119], U-46619, a thromboxane A_2 receptor agonist, was used as a constrictor. Similarly, in isolated rat gastric arteries, methanandamide, a metabolically stable analogue of anandamide, induces endothelium-independent relaxation [69] insensitive to AM251 and the CB2 receptor antagonists AM-630 and SR144528 when applied either alone or in combination. The authors found that the addition of exogenous

CGRP relaxed pre-contracted arteries and capsaicin- and capsazepine pre-treatment only slightly inhibited the relaxation to methanandamide. Ca^{2+} -induced vasodilation was found to be inhibited in the presence of methanandamide, suggesting that the endocannabinoid induces smooth muscle relaxation by CB1 and CB2 receptor-independent inhibition of Ca^{2+} entry [69]. In rat mesenteric and gastric arteries, methanandamide and anandamide induce a slowly developing smooth muscle cell hyperpolarization that is reproduced by exogenous CGRP and abolished by capsazepine.

The expression of the mRNA coding for the TRPV1 receptor was detected also in endothelial cells from rat mesenteric artery, where anandamide at nanomolar concentrations was shown to elicit an acute release of NO secondary to stimulation of endothelial TRPV1 [178].

5.3.1.3 The Role of KCa Channels in Endothelium-Independent Vasodilation

Several lines of evidences point for involvement of Ca^{2+} -dependent K^+ (KCa) channels in endothelium-independent relaxation to cannabinoids occurred independently on cannabinoid receptors. In isolated pre-contracted segments of rat superior mesenteric artery, the relaxation to anandamide is associated with the smooth muscle cell hyperpolarization due to activation of BK_{Ca} and, likely, IK_{Ca} channels [144]. The relaxation was found to be endothelium- and CB1 receptor-independent, as rimonabant even at 5 μM failed to modify the relaxation, and HU-210, a selective CB1 receptor agonist, and WIN55212-2, a non-selective cannabinoid receptor agonist, failed to reproduce the effect of anandamide [144]. This observation is in line with electrophysiological studies performed on isolated rat mesenteric artery, where anandamide-evoked smooth muscle cell hyperpolarization is unaffected by 1 μM rimonabant [70, 118]. In addition, neither the synthetic CB1 receptor agonists HU-210 and WIN55212-2, nor palmitoylethanolamide, a CB2 receptor agonist, affected the membrane potential

of the smooth muscle cells [70, 118]. In smooth muscle cells of the main mesenteric artery [118], guinea pig carotid artery and porcine coronary artery [70], anandamide at up to 30 μM failed to produce shifts in the membrane potential.

In isolated pre-contracted rat aortic rings, arachidonylcyclopropylamide (ACPA), a selective CB1 receptor agonist, elicits a weak relaxation (~20%) occurred independently of the presence of endothelium [43]. The relaxation was reduced to 9% in the presence of iberiotoxin, a selective BK_{Ca} inhibitor. In rat superior mesenteric artery, the relaxation to 30 μM ACPA was reduced by endothelial denudation from 18% to 8% [40]. In rings with intact endothelium, iberiotoxin diminished the relaxation to 9%, while in endothelium-free rings, the BK_{Ca} inhibitor fully inhibited the relaxation [40].

5.3.1.4 Inhibition of Ca^{2+} Entry as a Mechanism of Relaxation to Cannabinoids

In order to observe the relaxation to cannabinoids, they are administered to pre-contracted arterial segments, i.e under conditions of pre-stimulated Ca^{2+} entry into smooth muscle cells. Clearly, any interference with any of the Ca^{2+} entry pathways would decrease the tone. In a number of vascular myography studies an inhibition of Ca^{2+} -induced relaxation by cannabinoids and their analogues was observed, indicating that one of the mechanisms of endothelium-independent vasorelaxation to these compounds is an inhibition of Ca^{2+} entry into smooth muscle cells [69, 121, 157, 179]. These observations are supported by electrophysiological studies that identified anandamide as a direct inhibitor of NCX [65, 127], and voltage gated Ca^{2+} channel of L- and T- type [180, 181]. Noteworthy, both NCX and L- type Ca^{2+} channels are crucial determinant of the contraction imposed by norepinephrine and phenylephrine [182, 183] and targeting these systems by cannabinoids should be considered in assessing the mechanisms of vasodilation elicited by cannabinoids.

5.3.2 Endothelium-Dependent Relaxation to Cannabinoids

Endothelium-dependent component of relaxation to anandamide and some other endogenous lipid signaling molecules belonging to N-acyl amino acids, such as N-arachidonoyl glycine, NAGly, [184, 185] and N-arachidonoyl L-serine [186], has been reported in a number of vascular beds with the dominated but yet unproven hypothesis that a novel endothelial G-protein coupled cannabinoid receptor, the so called endothelial cannabinoid receptor (CBe) also referred in literature as abnormal cannabidiol or endothelial atypical cannabinoid receptor, underlies the dilation [109, 123, 143, 154, 161, 172, 187]. Despite some inconsistencies in the proposed signalling mechanisms reviewed earlier [188], the observed sensitivity of cannabinoid-evoked vasodilation to high (micromolar) concentration of CB1 receptor blockers rimonabant and AM251, and the cannabidiol analogue O-1918 was explained by a possibility that these compounds selectively target CBe receptor that remained to be identified [73, 109, 119, 123, 143, 160, 170, 172, 184, 189–191]. Stimulation of CBe was postulated to be coupled to EDHF [119] and NO [184] release, leading to a delayed hypotension in anesthetized rats [109].

The “endothelial cannabinoid receptor” hypothesis has been challenged by recent electrophysiological demonstrations that cannabinoids, cannabinoid-like substances and synthetic analogues at concentrations that are commonly considered to be CBe specific, bidirectionally efficiently affect the BK_{Ca} activity in cell-free patches excised from native cells and cells heterologously expressing BK_{Ca} α , $\alpha+\beta 1$ or $\alpha+\beta 4$ subunits [72, 126, 163, 192–195], the effect being dependent on the plasma membrane cholesterol content [126, 194]. The topic of lipid- and cannabinoid-induced regulation of BK_{Ca} channel function is covered in a review of Bukiya and Dopico [196]. Cholesterol, an essential component of eucariotic plasma membranes, plays an important role in regulation of the activity of a number of membrane proteins [197–199], including TRP [200, 201], BK_{Ca} channels [202, 203] and canna-

binoid signaling [204]. The latter is achieved via direct interaction between cholesterol and anandamide molecules by the establishment of a hydrogen bond and via regulation of interaction between anandamide and CB1 receptor [205, 206]. Consistent with view that direct cannabinoid-BK_{Ca} targeting is responsible for the relaxation, the hyperpolarizing effect of NAGly, a proposed ligand for GPR18 and a candidate for CBe [189, 207], was shown to be fully intact in endothelial cells following intracellular dialysis with GPR18 antibody [163]. A number of studies have provided evidences against the GPR18 involvement in the action of NAGly [127, 208–210], prompting a reconsideration of the concept of existence of a third type cannabinoid receptor required for endothelium-dependent vasodilation to cannabinoids.

Apart of direct targeting BK_{Ca} channels, NCX, TRPV1, V4, PPAR gamma and voltage-dependent Ca²⁺ channels, vasodilation to cannabinoids may also require cannabinoid interaction with vascular CB1 and CB2 receptors. In human mesenteric arteries collected during surgical operations from patients suffering from bowel carcinoma and inflammatory bowel disease, the CB1 receptor was concluded to mediate endothelium-dependent vasodilation to anandamide, with no evidence for engagement of CB2 receptor [172]. Cannabidiol, a phytocannabinoid with low affinity to CB1 and CB2 receptors that exhibits high potency as an antagonist of both CB₁ and CB₂ receptors (Thomas et al. [244]), was shown to induce endothelium-dependent vasodilation of human mesenteric arteries sensitive to blockers of CB1 receptor and TRPV1 channels [172]. A synthetic CB1 receptor agonist CP55,940 produced a greater relaxation compared to that evoked by anandamide. In endothelium-intact rings obtained from rat superior mesenteric arteries, a highly potent CB1 receptor agonist ACPA (EC₅₀ = 2.2 nM) used at extremely high concentration 30 μ M was shown to relax pre-contracted arteries by 18% in NO-dependent manner [40] and the relaxation was partially BK_{Ca}-dependent. Endothelial denudation reduced the vasodilator effect of ACPA to 7.7%. In this study, a CB2 receptor agonist JWH-133 (30 μ M) relaxed pre-

Table 5.2 Summary of the proposed cannabinoid receptor-dependent and -independent sites governing relaxation to cannabinoids

Target	Ligand	Cell type	Vascular bed	References
CB1R	AEA	SMC	RMA	[190]
CB1R	AEA, CP55,940, HU-210	SMC	RMA	[157]
CB1R	ACPA	SMC	RA	[43]
CB1R	AEA	EC, SMC	RSMA	[120]
CB1R	AEA, ACEA	EC, SMC	RMA	[37]
CB1R	ACPA	EC	RMA	[40]
CB1R	2-AG	SMC	RPA	[67]
CB2R	AEA, JWH-015	EC, SMC	RMA	[37]
CB2R	JWH-133	SMC	RMA	[40]
TRPV1	AEA	SMC	RSMA, RMA, RHA, GPBA	[122, 123, 146]
TRPV4	2-AG	EC	RSMA, RA	[75]
Cav1.2	ACPA	SMC	RA	[43]
Cav1.2	AEA	CM		[180]
Cav3.1/3.2	AEA	Cloned channels		[54]
NCX	AEA, NAGly, LPI	EC, EA.hy926	MA	[128, 137]
NCX	AEA	CM		[180]
BKCa	ACPA	SMC	RMA	[40]
BKCa	AEA, NAGly	EC, EA.hy926	MA	[6, 128, 163, 194]
BKCa	NAS	Transfection model		[4]
IKCa	LPI	EA.hy926		[53]
Na-K ATPase	LPI	EA.hy926		[52]
TASK-1	AEA	SMC	RMA, RPA, HPA	[135, 136]
PPAR gamma	AEA	AEA	RA	[59]

CM cardiac myocytes, EC endothelial cells, HPA human pulmonary artery, PPAR peroxisome proliferator-activated receptor, RA rat aorta, RMA rat mesenteric artery, RSMA rat small mesenteric artery, RabPA rabbit pulmonary artery, SMC smooth muscle cells, RPA rat pulmonary artery, MA mouse aorta

contracted mesenteric arteries by 14% in NO-dependent manner. Similar results were obtained in another study performed in isolated endothelium-intact rat mesenteric arteries [37], where anandamide, ACPA and the CB2 agonist JWH-015 elicited concentration-dependent vasodilation. Blockade of TRPV1 channels partially decreased the relaxation, while the blockade of CGRP receptors completely inhibited the relaxation to anandamide. Pre-treatment of vascular preparations with AM251 and the CB2 antagonist AM630, only slightly decreased the relaxation responses to anandamide allowing to conclude that CB1, CB2 and TRPV1 mediate the relaxation to anandamide [37]. The conclusion of the two aforementioned studies on involvement

of both CB1 and CB2 receptors in the vasorelaxation of rat mesenteric artery to cannabinoids contrasts with that derived by others [70, 118, 125, 143, 144]. Electrophysiological studies addressing the effect of CB2 receptor agonists on electrical responses of vascular cells are quite limited. In healthy arteries, CB2 receptor agonists failed to elicit measurable shifts in membrane potential either in smooth muscle [70, 118] or endothelial cells [6]. The action potential-driven endocannabinoid release in hippocampal pyramidal cells is accompanied by a long-lasting CB2-dependent hyperpolarization [25]. Table 5.2 summarizes the key findings on the cannabinoid receptor-dependent and -independent targets governing relaxation to cannabinoids.

5.4 Dysregulation of Vascular Endocannabinoid Signaling in Disease States

Circulating levels of endocannabinoids and cannabinoid receptor expression are altered in disease states. Under various pathophysiological conditions accompanied by vascular abnormalities, the vasodilator potencies of cannabinoid receptor agonists differ from those observed in healthy arteries. Cannabinoids may exert both the beneficial and deleterious effects on cardiovascular system [38, 82]. The majority of beneficial effects of cannabinoids have been ascribed to CB2 receptor stimulation. Signalling via CB1 and CB2 receptors differentially affects vascular inflammation. Stimulation of vascular and cardiac CB1 receptors contributes to pathophysiology of various cardiovascular diseases via promotion of oxidative and nitrosative stress, activation of mitogen-activated protein kinase and cell demise [66, 211–214]. In contrast, a wealth of experimental data indicate that stimulation of CB2 receptors displays cardioprotective effect [215–218], reduces cerebral ischemic injury [219, 220], limits inflammation, oxidative/nitrosative stress, cell demise [221], progression of atherosclerosis [3, 214, 222–224], prevents nephrotoxicity [225]. An increased expression level of CB2 receptors in the cardiovascular system under pathophysiological conditions, such as inflammation and tissue injury, is considered to represent a compensatory protective mechanism [216, 226–228]. In the vasculature, CB2 receptor protein expression was shown to be up-regulated under some pathophysiological conditions, including atherosclerosis, inflammatory insults and DOCA-salt hypertension model [21, 226, 229–231].

In mesenteric arteries excised from young obese rats, anandamide produced an attenuated endothelium-dependent relaxation [37]. The reduction was accompanied by a decreased CB1, CB2, but not TRPV1, protein expression level. In this model, the relaxation responses to acetylcholine and CB1 and CB2 receptor agonists ACPA and JWH-015 were also decreased. The responses were, however, restored following pre-incubation

of the arteries with FAAH inhibitor URB597, indicating that an increased anandamide degradation is responsible for attenuation of the relaxant responses.

Overactivation of cannabinoid system contributes to an increased vasodilation and hypotension. Thus, in patients with cirrhosis, the plasma anandamide and the CB1 receptor expression levels in vascular endothelial cells are elevated [42, 232]. The vasodilator state in chronic liver cirrhosis and hypotension in advanced cirrhosis is determined by activation of vascular endothelial cells cannabinoid CB1 receptors by endogenous cannabinoids and is reversed by the rimonabant treatment [42]. In cirrhotic mesenteric vessels, an increased relaxation to anandamide is mediated by an enhanced signalling via CB1 receptors, perivascular TRPV1 channels and vascular KCa channels [233, 234]. However, while in cirrhotic liver tonic CB1 stimulation plays a pathophysiological role determining chronic vasodilator state, in spontaneously hypertensive rats, chronic CB1 activation seems to represent a part of protective mechanism directed to reduction of blood pressure. In normotensive rats, CB1 receptor antagonism had no effect on blood pressure and other hemodynamic parameters. However, in spontaneously hypertensive rats (SHR), the expression of CB1 receptor is increased in heart and aortic endothelium as compared with Wistar-Kyoto rats, and rimonabant elicited a further increase in blood pressure and myocardial contraction with no change in heart rate [2], pointing at protective effect of tonic CB1 receptor activation. As intracerebroventricular microinjection of rimonabant did not influence blood pressure, it is highly expected that the effect of intravenous rimonabant is mediated by peripheral mechanisms [2]. Similar hypertensive effects were observed with AM251 when administered to either hypertensive salt-sensitive Dahl rats maintained on high salt diet, or to rats with angiotensin II-induced hypertension. Elevation of the endogenous anandamide level by FAAH inhibitor URB597 had no detectable hemodynamic effect in control rats, however, decreased arterial blood pressure in hypertensive rats [2, 235]. The hypotensive effects of WIN55212-2

are described in conscious rats with several forms of experimental hypertension, including SHR rats and Wistar rats made acutely hypertensive by infusion of angiotensin II and arginine vasopressin [235, 236], but not in transgenic hypertensive rats [102]. Consistent with the upregulated role of endocannabinoid system in blood pressure regulation in hypertension, anandamide dose-dependently decrease the mean arterial blood pressure in conscious hypertensive, but not normotensive, rats [235]. While in normotensive rats WIN55212-2 elevates blood pressure, in acutely hypertensive rats WIN55212-2 produces hypotensive effect that was attenuated by AM251 [235]. In conscious hypertensive transgenic rats, however, the pressure and vasoconstrictor effect of WIN55212-2 are little affected [102].

In hypertension, alterations in cannabinoid signalling are model-specific. Thus, while in SHR both cardiac and plasma levels of anandamide and 2-AG are decreased, in DOCA-salt model, the endocannabinoid levels are elevated [231]. In both models, the CB1 receptor expression is higher in the heart and aortic endothelium [2, 33, 231]. However, higher CB2 receptor expression was detected only in DOCA-salt model [231]. Injection of THC (1.5 mg/kg) to rats with experimental renal hypertension elicited a significant decrease in blood pressure and heart rate [237]. The reaction developed within 15 min and in 24 h the parameters returned to the initial levels. However, daily THC injections for 3–5 weeks did not produce any difference in the heart rate and the systolic blood pressure between the control and hypertensive groups.

Numerous beneficial cardiovascular effects have been reported for *in vivo* cannabidiol treatment in a number of disorders. It was shown that cannabidiol treatment improves endothelium-dependent relaxation in mesentery of diabetic fatty rats and leads to improvement of serum biomarkers [238], prevents cerebral infarction [239], is cerebroprotective via cannabinoid receptor-independent pathway [240, 241], attenuates cardiac dysfunction and vascular inflammation, reduces infarct size, oxidative stress and inflammatory pathway in diabetic cardiomyopathy [242, 243].

5.5 Concluding Remarks

Cannabinoids, through central and local mechanisms, affect key cardiovascular parameters in health and disease, such as heart rate, blood pressure, vascular and cardiac contractility and inflammation. Studies over the last decades demonstrated that endocannabinoid system is overactivated under pathological conditions and plays both a pathophysiological role, such as in disease states associated with excessive hypotension, and a protective compensatory role, such as in some forms of hypertension and inflammatory conditions. Mechanisms of local regulation of vascular reactivity by cannabinoids include modulation of a number of ion transporting systems. This modulation may be accomplished either in cannabinoid receptor-dependent or -independent mechanisms. A main emphasis on the local regulation of cardiovascular function by endocannabinoids has been devoted to examining the role of specific type of cannabinoid receptors in vasodilation. Ironically, cannabinoid receptor antagonists of first generation designed and widely used in functional myography assays as selective were later found to be of low selectivity, displaying off-target effects on a number of ion transporting systems. Consequently, the conclusions yielded are at times speculative and controversial, often overlooking receptor-independent effects of cannabinoids and cannabinoid receptor blockers. To advance our understanding of cannabinoid actions on the vasculature, more information is essentially needed on the impact of selective cannabinoid receptor stimulation in disease states and the mechanisms of direct action of cannabinoids on the function of endothelial and smooth muscle cell ion channels and how these effects are translated into mechanotransduction, regulation of inflammation, angiogenesis, etc. Similar to steroids, general anesthetics and alcohols, cannabinoids modulate the function of a number of ion channels. An important still unanswered question is whether the given effect of cannabinoids requires direct interactions between cannabinoid molecules with specific channel protein subunit or the effect is indirect, due to change in lipid composition of the plasma membrane and chang-

ing the physical parameters of plasmalemma. Taking into account the vital role of potassium and TRP channels in physiology and pathophysiology of cardiovascular system, better insights into the intrinsic mechanisms of modulation of the channel function by cannabinoids, would not only advance our basic knowledge of local modulation of vascular function by cannabinoids, but pave the way for development of new selective cannabinoid receptor and ion channel modulators and their therapeutic application.

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