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REVIEW

The emerging role of the endocannabinoid system in cardiovascular disease

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Abstract Endocannabinoids are endogenous bioactive lipid mediators present both in the brain and various peripheral tissues, which exert their biological effects via interaction with specific G-protein-coupled cannabinoid receptors, the CB_1 and CB_2. Pathological overactivation of the endocannabinoid system (ECS) in various forms of shock and heart failure may contribute to the underlying pathology and cardiodepressive state by the activation of the cardiovascular CB_1 receptors. Furthermore, tonic activation of CB_1 receptors by endocannabinoids has also been implicated in the development of various cardiovascular risk factors in obesity/metabolic syndrome and diabetes, such as plasma lipid alterations, abdominal obesity, hepatic steatosis, inflammation, and insulin and leptin resistance. In contrast, activation of CB_2 receptors in immune cells exerts various immunomodulatory effects, and the CB_2 receptors in endothelial and inflammatory cells appear to limit the endothelial inflammatory response, chemotaxis, and inflammatory cell adhesion and activation in atherosclerosis and reperfusion injury. Here, we will overview the cardiovascular actions of endocannabinoids and the growing body of evidence implicating the dysregulation of the ECS in a variety of cardiovascular diseases. We will also discuss the therapeutic potential of the modulation of the ECS by selective agonists/antagonists in various cardiovascular disorders associated with inflammation and tissue injury, ranging from myocardial infarction and heart failure to atherosclerosis and cardiometabolic disorders.

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Introduction

Endocannabinoids are endogenous lipid mediators with wide range of biological effects similar to those of marijuana. These lipid mediators can be generated in virtually all cell types (both in the brain as well as in various peripheral tissues) and along with their cellular receptors and several proteins implicated in their synthesis, release, transport, and degradation are parts of a novel signaling system termed the endocannabinoid system (ECS). The two most widely studied endocannabinoids are arachidonoyl ethanolamide or anandamide (AEA) and 2-arachidonoylglycerol (2-AG) [1], but several other similar endogenous substances have also been identified [2, 3]. Endocannabinoids exert their biological effects via two main G-protein-coupled cannabinoid receptors, the CB_1 and CB_2 [2, 4]. Based on pharmacological evidence, additional as yet unidentified cannabinoid receptor candidates have also been proposed recently [5, 6]. The tissue levels of endocannabinoids are determined by the balance between their biosynthesis (involving phospholipase D- and diacylglycerol lipase-dependent and other pathways), cellular uptake, and degradation by fatty acid amide hydrolase (FAAH) and/or monoacylglycerol lipases [2]. The detailed description of the pathways involved in the biosynthesis and metabolism of endocannabinoids is beyond the scope of this synopsis and we would like to refer readers to several excellent recent overviews on this subject [7–9].

Endocannabinoids bind to both CB_1 and CB_2 receptors. Anandamide has higher affinity for CB_1 and CB_2 receptors, whereas 2-AG has higher CB_1 and CB_2 efficacy than anandamide. Endocannabinoids may also exert various CB-receptor-independent effects (e.g., AEA may also bind to vanilloid VR_1 ($TRPV_1$) receptors) [10]. Both CB_2 and CB_1 receptors are negatively coupled to adenylate cyclase through $G_{i/o}$ proteins; however, the cannabinoid receptor signaling appears to be more complex than previously thought. Depending on cell type, it may also involve mitogen-activated protein kinases, protein kinase A and C, and cyclooxygenase-2 pathway, just to name a few [2, 4, 8]. Previously, it was thought that CB_2 receptors are mainly expressed in immune and hematopoietic cells mediating various immunomodulatory effects, while CB_1 receptors are primarily distributed in the central nervous system and are responsible for psychoactive properties of cannabis. However, recent studies have also demonstrated CB_1 receptors in various peripheral tissues (e.g., myocardium) [11–13], human coronary artery endothelial and smooth muscle cells [14, 15], adipose tissue [16, 17], and the liver [17–19]. The presence of CB_2 receptors has also been established in the myocardium [13], human coronary endothelial and smooth muscle cells [14, 15], brain [20], and the liver [19, 21]. It should also be noted that human peripheral blood immune cells in addition to expressing CB_2 receptors (rank order for messenger RNA (mRNA): B cells>NK cells>monocytes>polymorphonuclear neutrophils (PMNs)>T cells [22]) also express CB_1 receptors (B lymphocytes>natural killer (NK) cells≥PMNs≥CD8 lymphocytes>monocytes>CD4 lymphocytes). Importantly, the CB receptor expression in immune cells can be modulated by various inflammatory (e.g., bacterial lipopolysaccharide) and other stimuli resulting in activation of these cells [22], which may also trigger increased production of endocannabinoids (AEA and 2-AG) via activation of various biosynthetic pathways and/or by reducing expression of FAAH, the enzyme responsible for the degradation of AEA (Table 1; overviewed in [23, 24]).

In the past decade, the ECS has been implicated in a growing number of physiological functions of the nervous system and various peripheral organs, and its modulation turned out to hold tremendous therapeutic promise in a wide range of disparate diseases and pathological conditions, ranging from mood and anxiety disorders, movement disorders, neuropathic pain, multiple sclerosis, and spinal cord injury to cancer, glaucoma, osteoporosis, atherosclerosis, myocardial infarction, stroke, hypertension, and obesity/metabolic syndrome to name just a few [2, 25, 26]. Here, we aim to overview the emerging role of endocannabinoid system in cardiovascular regulation in health and disease. We will specifically focus on its role in various cardiovascular disorders/states associated with inflammation and cell death, ranging from myocardial infarction and heart failure to atherosclerosis and cardiovascular risk factors.

Cardiovascular effects of cannabinoids

Cannabinoids and their endogenous and synthetic analogs exert a variety of cardiovascular effects both in vivo and in vitro [27, 28]. In anesthetized rodents, AEA, Δ^9-tetrahydrocannabinol (THC), as well as potent synthetic cannabinoid ligands such as HU-210 evoke hypotension, bradycardia, and depressed cardiac contractility [12, 27, 29–32]. These hemodynamic effects are less pronounced/absent in conscious normotensive animals but are augmented in hypertensive ones [12, 33, 34] (reviewed in [2, 35]). Acute use of marijuana in humans usually causes tachycardia, whereas chronic use may lead to bradycardia and hypotension [2]. The underlying mechanisms are multifaceted, involving

Table 1 Expression of cannabinoid receptors in the cardiovascular system, adipose tissue, and pancreas

Cell type or tissue	Species	Receptor	Detection method	References
Coronary artery endothelial cells	Human	CB_1, CB_2	IFL, WB, RT-PCR	[14]
Coronary artery smooth muscle cells	Human	CB_1, CB_2	IFL, WB, RT-PCR	[15, 58]
Heart	Human, rat, mouse	CB_1, CB_2	IFL, WB, RT-PCR, real-time RT-PCR	[11–13, 59, 60, 90, 155]
Adipocytes	Mouse, rat	CB_1	RT-PCR	[16, 156]
	Human	CB_1, CB_2	IFL, WB, RT-PCR	
Pancreas	Mouse	CB_1, CB_2	IFL, real-time RT-PCR	[157]
Monocytes	Human	CB_1, CB_2	RT-PCR, FACS	[14, 54, 155]
Macrophages	Mouse	CB_1, CB_2	RT-PCR, WB	[158]
T lymphocytes	Human	CB_2	FACS, WB, RT-PCR	[159, 160]
Neutrophils	Human	CB_2	IFL, FACS, RT-PCR	[155, 161]
Platelets	Human	CB_1, CB_2	WB	[162]

FACS, fluorescence activated cell sorting analysis; IFL, immunofluorescence; RT-PCR, reverse transcriptase polymerase chain reaction; WB, western blot

modulation of autonomic outflow through sites of action at presynaptic autonomic nerve terminals [36–39] and in the central [39, 40] nervous system, as well as direct effects on the myocardium [11, 41, 42] and the vasculature [32, 43–46]. As for endocannabinoids, these effects are complicated by their rapid degradation to arachidonic acid that can further be metabolized into multiple vasoactive prostanoids [2, 47, 48]. The vasodilatory effect of endocannabinoids and synthetic cannabinoids in vitro displays tissue and interspecies differences and may involve CB_1 and $TRPV_1$ receptor- and NO-mediated or NO-independent mechanisms, as well as yet undefined endothelial site(s) of action. The detailed discussion of these vasodilatory effects of endocannabinoids and synthetic ligands is beyond the scope of this paper and can be found in several more focused reviews on this subject [5, 47, 49–51]. Activation of CB_2 receptors in human coronary endothelial and various inflammatory cells (e.g., monocytes, neutrophils, etc.) attenuates the tumor necrosis factor alpha (TNF-α)- or other trigger-induced endothelial inflammatory response, chemotaxis, and adhesion of inflammatory cells to the activated endothelium and the consequent release of a variety of proinflammatory mediators [14, 52–54] (key processes involved both in the initiation and progression of atherosclerosis and restenosis, as well as in mediating reperfusion-induced tissue damage) [55, 56]. CB_2 receptor activation also attenuates the TNF-α-induced human coronary artery smooth muscle cell proliferation (Fig. 1) [15]. As already mentioned above, CB_1 receptors are expressed in the myocardium and anandamide, *R*-methanandamide (a stable analog of AEA), likewise a potent synthetic analog HU-210, all dose-dependently decrease contractile performance in isolated electrically paced human atrial muscle, an effect which can be blocked by the CB_1 antagonist AM251, implicating the existence of a CB_1 receptor-dependent negative inotropy in the myocardium [11]. Consistent with in vitro CB_1-mediated negative inotropy, HU-210 decreases left ventricular developed pressure in isolated perfused rat hearts most likely through CB_1 receptor activation [41, 57] and also decreases myocardial contractility in vivo (measured by the analyses of the pressure–volume relations) without

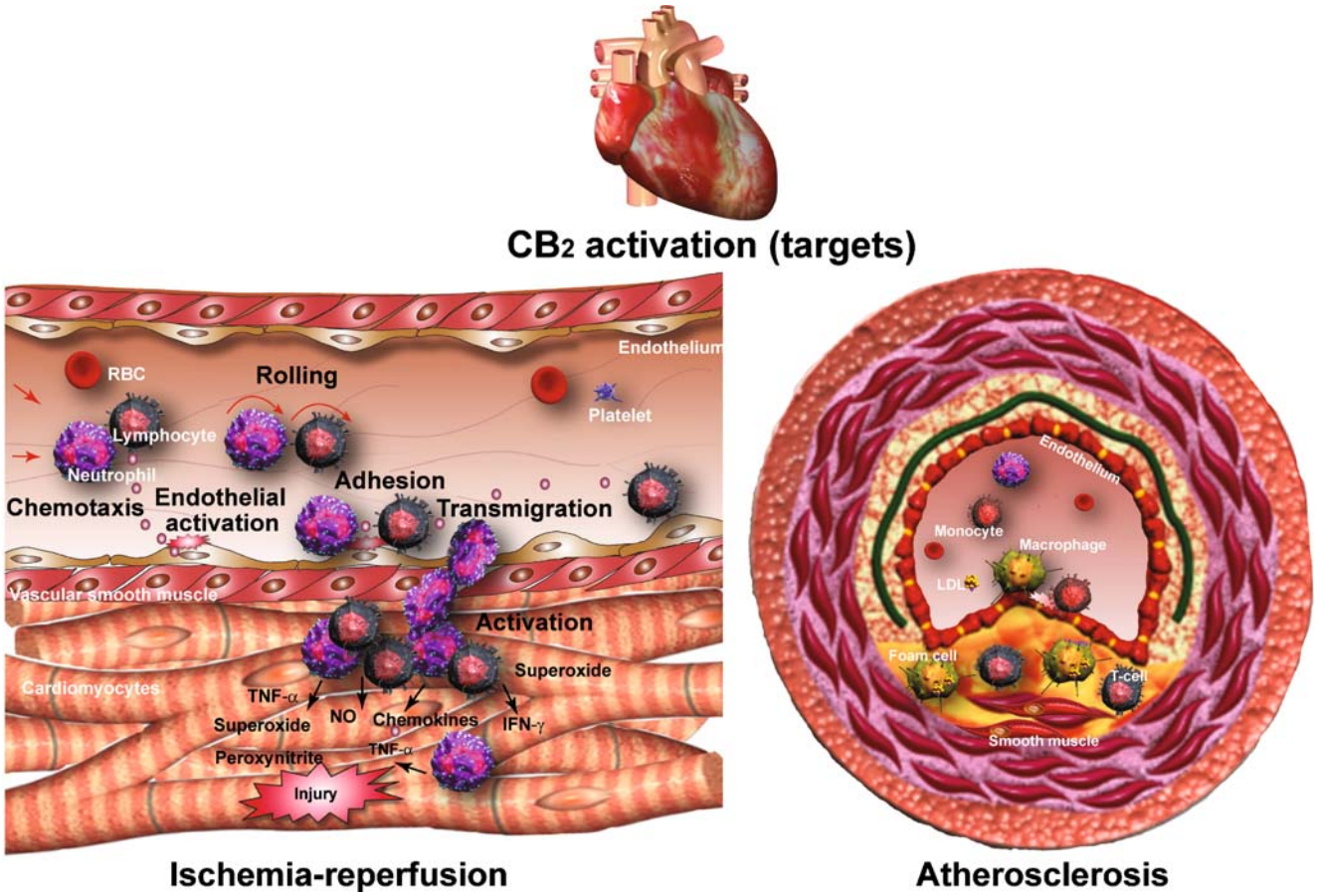

Fig. 1 Therapeutic targets of CB_2 receptor stimulation in ischemia/reperfusion injury and atherosclerosis. CB_2 agonists decrease endothelial cell activation and inflammatory response, chemotaxis triggered by inflammatory stimuli/chemokines, and adhesion of inflammatory cells (lymphocytes, neutrophils, and/or monocytes) to activated endothelium, transendothelial migration of inflammatory cells, attachment to parenchymal cells, and activation. In addition, CB_2 activation may also mediate direct protective effects in cardiomyocytes and decrease smooth muscle cell proliferation/migration. Reproduced with permission from American Heart Association from [35]

major effect on the total peripheral resistance [51]. CB_1 receptors may also play an important role in vascular smooth muscle cell proliferation [58]. Despite evidence on the presence of CB_2 receptors in the myocardium [13, 59, 60] and a few recent studies implicating this receptor in myocardial cardioprotection [59, 60], its role in cardiomyocytes is still elusive. The existence of a functional ECS in the myocardium is further supported by the increased myocardial AEA levels in FAAH knockout mice and by more prolonged AEA-induced hypotensive response in FAAH knockouts compared to their wild-type littermates [27], as well as by elevated myocardial AEA levels in rats following the treatment with an FAAH inhibitor [12]. However, despite the presence of functional cannabinoid receptors, endocannabinoids and their metabolizing enzyme in cardiovascular tissues/cells, and the above-mentioned complex cardiac and vascular effects of endocannabinoids, the ECS appears to play a limited role in cardiovascular regulation under normal physiological conditions, which is also supported by the normal blood pressure and myocardial contractility and baroreflex sensitivity of FAAH knockout mice in spite of the elevated myocardial AEA levels [27].

The endocannabinoid system in cardiac disease

Contrary to the described above, under numerous pathological conditions (e.g., in experimental models of hemorrhagic, endotoxic and septic shock, advanced liver cirrhosis, and heart failure, just to name a few), the ECS may become overactivated and may contribute to hypotension/cardiodepression through cardiovascular CB_1 receptors (overviewed in [2, 51, 61], and see also in parts below). Intriguingly, the ECS may also be activated as a compensatory mechanism in various forms of hypertension to limit pathologically increased blood pressure and myocardial contractility, but the discussion of this is beyond the scope of this synopsis and was recently overviewed [35]. In this review, we will discuss the accumulating evidence both from preclinical and clinical studies forecasting therapeutic benefits of the modulation of the ECS in various cardiovascular diseases/conditions associated with inflammation and tissue injury, ranging from myocardial ischemia–reperfusion and heart failure to atherosclerosis and cardiometabolic risk.

Myocardial ischemia/reperfusion syndrome

Acute myocardial infarction, due to the sudden thrombotic occlusion of a coronary artery, is the leading cause of morbidity and mortality in the adult population of developed and developing nations. The most common complication is the occurrence of left ventricular dysfunction and heart failure. In addition to cardiac overload and injury, an increasing number of biomarkers, such as inflammatory factors, hormones, and biologic substances, as well as genetic factors, appear to play a crucial role in the development of heart failure [62].

Myocardial ischemia is characterized as a state of insufficient oxygen supply, leading to irreversible tissue damage within 20 to 40 min of sustained ischemia [63]. The most effective therapy after an acute myocardial infarction is the rapid restoration of blood flow by mechanical or pharmacological intervention [64]. The early reperfusion is critical for reducing the size of myocardial infarct and improving the clinical outcome. However, reperfusion itself is responsible for myocardial injury which is induced by the preceding ischemic episode, resulting in cardiomyocyte death and increase in infarct size [65]. Potential mediators of reperfusion injury involve reactive oxygen and nitrogen species (ROS/RNS), intracellular and mitochondrial Ca^{2+} overload, complement activation, and the accumulation of inflammatory cells in the infarcted myocardial tissue. Inflammatory processes including leukocyte recruitment play a major role in the extension of myocardial damages after ischemia and reperfusion. Rapidly, after the restoration of blood flow, leukocytes infiltrate the myocardium in response to complement activation and massive release of ROS/RNS. Neutrophils, monocytes, and lymphocytes are the principal immune cells implicated in this process. Once recruited into the tissue, inflammatory cells release proteolytic enzymes and ROS/RNS [66, 67] that contribute to the development of injury.

An implication of the endocannabinoid system in the cardioprotective mechanisms of preconditioning has been initially described in isolated heart models [59, 68, 69]. In these models, perfusion with agonists or antagonists was initiated before the ischemic period and was kept until the end of reperfusion. In the first two studies, blockade of CB_2 receptors with CB_2 antagonist SR144528 was shown to abolish bacterial lipopolysaccharide (LPS) or heat-stress-induced preconditioning against myocardial ischemia, while the CB_1 antagonist rimonabant had no effect in these isolated rat heart models [68, 70]. These findings suggested that LPS or heat shock increase endocannabinoid production, presumably in inflammatory cells [71–73], which in turn activate cardiac CB_2 receptors. A subsequent study, however, reported that preconditioning induced by short-term ischemia could be blocked by either CB_1 or CB_2 antagonism [59]. Conversely, delayed cardioprotection induced by nitric oxide was reversed by the CB_1 antagonist AM251 but not the CB_2 antagonist AM630 [74]. Increased tissue levels of the endocannabinoid 2-AG were found in isolated hearts from preconditioned rats, whereas anandamide levels remained unchanged. In an in vivo rat model, endocannabinoid-mediated activation of CB_2 receptors has been recently involved in the cardioprotective effects of remote ischemic preconditioning, induced by mesenteric artery occlusion and

reperfusion [75]. Systemic pretreatment with CB_2 antagonist AM630, but not the CB_1 antagonist AM251, abolished the cardioprotective effects of remote preconditioning on infarct size and arrhythmias.

In addition, various studies have been performed, again mainly in isolated rat heart models, to clarify the potential cardioprotective role of endocannabinoid and anandamide-related mediator signaling in ischemia/reperfusion injury. Perfusion with palmitoylethanolamide (PEA) or 2-AG, but not anandamide, decreased myocardial damage in isolated rat hearts, while CB_2 antagonism with SR144528 totally abolished the beneficial effects of PEA and 2-AG [69]. The CB_1 antagonist rimonabant only partially blocked the effect of 2-AG. However, selective agonists for both CB_1 and CB_2 receptors, ACEA and JWH-015, also reduced the infarct size in this isolated rat heart model of low-flow ischemia and reperfusion. The effect of SR144528 on PEA-mediated actions is surprising, since it is considered to act through CB_1- and CB_2-independent pathways. Previous findings may suggest that the CB_2 antagonist SR144528 may also antagonize some of the effects mediated by PPAR-α agonists. Indeed, it has been described that the analgesic effects of PEA and other PPPAR-α ligands are antagonized by SR144528 [76, 77]. The above-described findings are somewhat conflicting with a subsequent study published by Underdown and colleagues. In an isolated rat heart model of no-flow ischemia and reperfusion, anandamide perfusion reduced the infarct size, which could be blocked by either CB_1 or CB_2 antagonism, using rimonabant or SR144528, respectively [78]. However, CB_1 and CB_2 agonists (ACPA and JWH-133) could not mimic the effect of anandamide.

Using a clinically more relevant in vivo mouse model of myocardial ischemia/reperfusion, the synthetic cannabinoid HU-210 decreased the incidence of ventricular arrhythmias following ischemia/reperfusion in rats through activation of CB_2 receptors [79]. Again, in an in vivo mouse model, Di Filippo and colleagues showed that preventive treatment with the nonselective agonist WIN55,212-2 before ischemia significantly reduced the extent of infarct size [80]. The CB_2 antagonist AM630, but not the CB_1 antagonist AM251 abolished the effect of WIN55,212-2. In support of the cardioprotective role of CB_2 receptor activation, recent findings have demonstrated that a single dose of the CB_2 agonist JWH-133 given shortly before reperfusion reduced the infarct size in a mouse model of myocardial ischemia and reperfusion [60]. In the myocardial ischemia/reperfusion model, JWH-133 reduced superoxide generation, increased ERK 1/2 and STAT-3 phosphorylation, and inhibited neutrophil recruitment in the infarcted myocardium of drug-treated mice compared to the sham-treated controls. In vitro, JWH-133 inhibited the TNF-α-induced chemotaxis and integrin CD18/CD11b (Mac-1) upregulation on human neutrophils [60]. These data may suggest a direct cardioprotective effect of CB_2 activation on cardiomyocytes and an anti-inflammatory effect on neutrophils. Consistently, selective CB_2 agonists exert potent anti-inflammatory effects in various other models of ischemic–reperfusion injury [52, 53, 56].

Finally, the nonpsychoactive *Cannabis* component with potent anti-inflammatory properties, cannabidiol (CBD), has been shown to be protective in an in vivo rat model of ischemia–reperfusion [81]. The infarct size was significantly reduced in CBD-treated rats as determined after 7 days, together with reduced myocardial inflammation and improved left ventricular function. The cardioprotective effect was absent in isolated hearts, supporting the crucial role of systemic inflammatory processes which are modulated by CBD in the in vivo model. However, the underlying mechanisms of CBD signaling are not very clear. This ligand exhibits only very weak binding activity at CB_1 and CB_2 receptors but has been characterized as an antagonist for the GPR55 receptor [82]. The biological effects of cannabinoid-mediated GPR55 signaling, however, remain to be investigated. On the other hand, a recent in vitro study suggests that the anti-inflammatory effects of CBD may involve enhanced adenosine signaling through inhibition of its uptake in microglia [83]. Release of adenosine is an endogenous mechanism of immunosuppression evoked during cellular stress and inflammation, while uptake of adenosine is a primary mechanism of terminating adenosine signaling [84]. CBD potently decreased uptake of [3H]-adenosine in murine microglia and RAW264.7 macrophages, and binding studies confirmed that CBD efficiently binds to the equilibrative nucleoside transporter 1 [83].

Heart failure and myocardial remodeling

Heart failure is a major health problem and may result from different pathophysiologic conditions such as ischemic cardiac injury, cardiomyopathies, myocarditis, pressure overload, as well as genetic defects [62, 85–87]. Cardiac injury and/or hemodynamic load in association with neurohormonal activation, induced by one of these pathophysiologic conditions, lead to a progressive remodeling process with activation of secondary inflammatory pathways and increased ROS/RNS generation by which the heart changes its size, shape, and function [86–88]. Patients with major remodeling demonstrate progressive worsening of clinical prognosis. Therefore, a major therapeutic goal in order to reduce mortality and preserve cardiac function in heart failure patients is to prevent the progression of remodeling.

The activation of vascular and cardiac CB_1 receptors by endocannabinoids or synthetic ligands exhibits strong cardiovascular effects (induction of hypotension and bradycardia), which have been described in the previous

chapter. In numerous experimental studies, the endocannabinoid system has been linked to hypotension associated with various forms of circulatory shock and advanced liver cirrhosis [51]. As demonstrated by Wagner and colleagues in a rat model of acute myocardial infarction, increased endocannabinoid production by inflammatory cells contributes to hypotension associated with cardiogenic shock [89]. Cardiogenic shock, characterized by inadequate cardiac output, profound hypotension, and systemic hypoperfusion, is a common complication of acute myocardial infarction, associated with poor prognosis. In this study, anandamide and 2-AG were measured in platelets and monocytes isolated 30 min after left coronary artery occlusion in rats. Injection of these isolated monocytes and platelets into rats decreased mean arterial pressure, suggesting the direct contribution of endocannabinoids in postmyocardial infarction hypotension. Pretreatment with rimonabant reduced the mean arterial pressure decline but increased mortality monitored at 2 h after myocardial infarction. In a subsequent study, the same authors reported a deleterious effect of CB_1 antagonism with AM251 on cardiac function in a chronic myocardial infarction model [90]. On the other hand, treatment with the synthetic agonist HU-210 increased left ventricular end-diastolic pressure but prevented endothelial dysfunction in aortic rings isolated from treated rats. A possible explanation for these in part conflicting findings in these two above-described studies might be the involvement of non-CB_1-receptor-mediated effects, given the suboptimal doses of agonists and antagonists used in these studies.

More recent evidence suggests a cardioprotective effect of CB_1 antagonism in doxorubicin-induced cardiotoxicity, a major consequence of antitumor therapy, which may lead to cardiomyopathy and heart failure [13]. The underlying mechanisms of the heart failure involve increased oxidative/nitrosative stress, proteolytic enzyme activity, and changes in cardiomyocyte energetics [87, 91]. Five days after a single doxorubicin injection in mice, hemodynamic measurements revealed severe ventricular dysfunction, which was improved by daily treatment with CB_1 antagonists rimonabant or AM281 [13]. CB_1 antagonism also prevented the doxorubicin-induced cell death in vitro in rat embryonic ventricular myocardial-derived H9c2 cells, as well as in vivo using heart lysates from treated mice. Treatment with antagonists alone had no effect on measured hemodynamic parameters or cell viability. Importantly, the protective effect against doxorubicin-induced cell death was selectively mediated by CB_1 antagonists, since neither CB_1 or CB_2 agonists nor CB_2 antagonists were protective. In addition, the CB_1 agonist by itself markedly increased cell death in cardiomyocytes, which was additive in the presence of doxorubicin. In line with these findings, a significant doxorubicin-induced increase in cardiac endocannabinoid anandamide levels was found, whereas 2-AG levels were not altered. Doxorubicin had no effect on the expression of myocardial CB_1 and CB_2 or on the expression of these receptors in cells exposed to the compound. These findings suggest that doxorubicin induces an activation of the endocannabinoid system, resulting in increased signaling via vascular and cardiac CB_1 receptors which leads to cardiodepression and cell death in cardiomyocytes. The underlying cellular mechanisms of the cytoprotective effect of CB_1 antagonists in doxorubicin-induced cardiotoxicity, however, remain to be clarified.

The endocannabinoid system in cardiometabolic risk

A growing body of evidence supports a role for the endocannabinoid system in the regulation of cardiovascular risk factors, i.e., obesity and obesity-related metabolic disorders, including alterations in lipid profile and glucose homeostasis [92–94]. The implication of the endocannabinoid system in the regulation of food intake and energy metabolism has been well established in the past [92]. Overactivity of the endocannabinoid system promotes excessive food intake and fat accumulation in animal models and humans, suggesting its pharmacological modulation for therapeutic use [92, 93]. Now, recent findings points to its implication in the regulation of insulin sensitivity, glucose homeostasis, and plasma lipid levels, which are closely related to type 2 diabetes. Clinical trials have indicated multiple therapeutic benefits of the pharmacological CB_1 blockade in obesity and associated cardiometabolic disorders, including obese subjects with metabolic syndrome and type 2 diabetes (Fig. 2) [95–99]. However, serious recent concerns have also been raised related to this class of compound because of the increased incidence of anxiety and depression in treated patients [100], see also below later.

Obesity

Obesity, characterized by an excess of adipose tissue mass, is closely associated with an increase in cardiovascular morbidity and mortality, including atherosclerosis. A cluster of common disorders, e.g., hyperglycemia, hyperlipidemia, and hypertension (described as metabolic syndrome), are often found in obese individuals [101]. The adipose tissue has been identified as a source of biologically active substances known as adipokines or adipocytokines [101, 102]. In obesity, the adipose tissue is a source of local inflammation, in which reduced levels of adiponectin (a mediator with anti-inflammatory properties) and increased levels of leptin (with proinflammatory actions) play crucial roles. Reduced plasma levels of adiponectin, which are found in obese patients, are closely associated with obesity-related diseases, including atherosclerotic cardiovascular

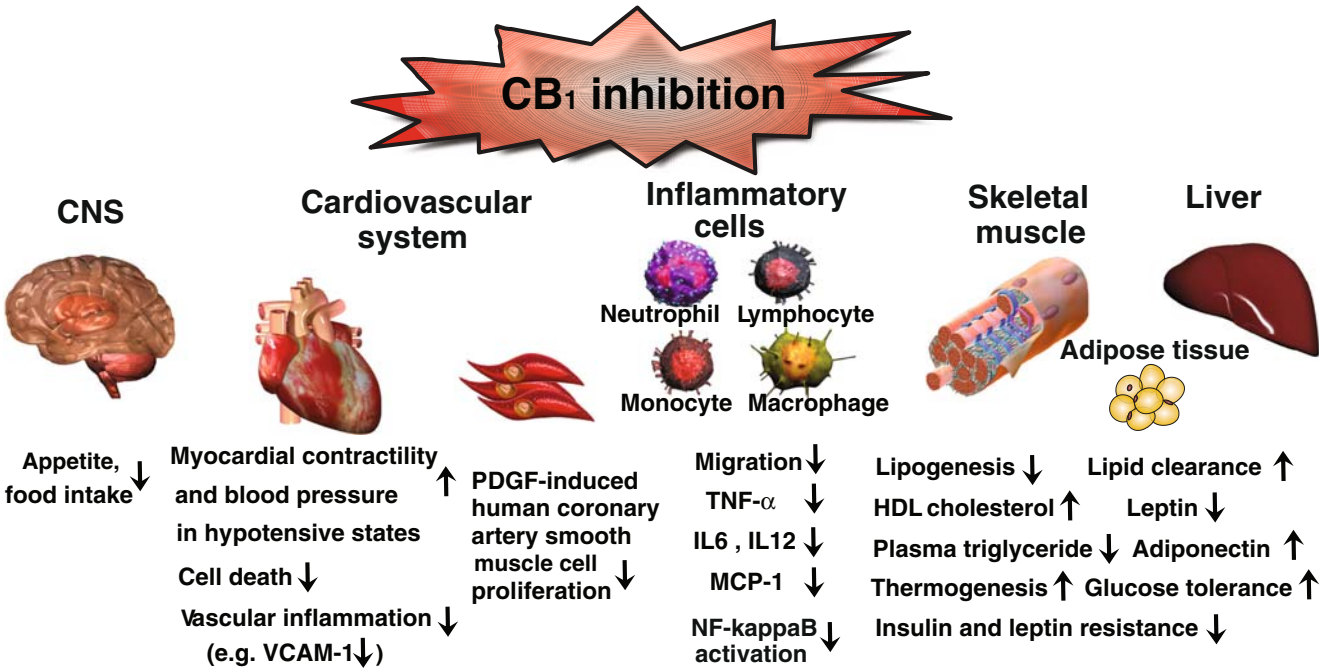

Fig. 2 Selected beneficial effects of CB_1 receptor blockade with rimonabant observed in preclinical/clinical studies with potential relevance to atherosclerosis and cardiovascular diseases/risk. Modified with the permission of the American Heart Association from [94]

diseases, type 2 diabetes, hypertension, and dyslipidemia [103]. There is emerging experimental evidence that adiponectin mediates antiatherogenic and antithrombotic effects through direct protective actions on endothelial cells, smooth muscle cells, macrophages, T lymphocytes, and platelets [101, 102, 104, 105].

The other major adipocytokine leptin, expressed by the *ob* gene, also exhibits direct effects on immune cells, including the promotion of T lymphocyte type 1 helper (Th1) response [106]. Various studies reported that leptin deficiency is protective in atherosclerotic mice, as demonstrated in the low-density lipoprotein receptor knockout (LDLR−/−) or apolipoprotein E (ApoE−/−) background [107–109]. However, these mice have severe hypercholesterolemia, high triglyceride levels, and insulin resistance. In a recent study, atherosclerotic lesion development in leptin-deficient LDLR−/− mice was compared to LDLR−/− mice with similar cholesterol levels [110]. Here, leptin deficiency induced a strong reduction in atherosclerotic lesion development. The authors suggest a role for leptin in the modulation of the regulatory immune response in this experimental model.

The endocannabinoid system is known to play a crucial role in energy balance and substrate metabolism, which involves central hypothalamic and leptin-regulated pathways [92, 111]. In addition to central effects, the endocannabinoid system also regulates food intake and metabolic factors through peripheral CB_1 receptors located at multiple sites throughout the body [93]. Overactivity of the endocannabinoid system promotes excessive food intake and fat accumulation in animal models and in humans [92, 93]. In mice fed with high-fat diet for several weeks, 2-AG endocannabinoid levels are upregulated in epididymal adipose tissue, whereas anandamide and 2-AG levels decrease in the subcutaneous fat [112–114]. In human obese individuals, the abdominal, but not subcutaneous fat mass, which is linked to several cardiometabolic risk factors [115], directly correlates with high local and circulating 2-AG levels, whereas no correlation with anandamide was found [113, 116, 117]. The enzymes involved in endocannabinoid synthesis and degradation are expressed in human and mouse adipose tissue [114, 118]. Changes in their expression levels were found to correlate with a decrease in subcutaneous fat endocannabinoid concentrations in high-fat-diet-fed obese mice [114]. Similarly, decreased mRNA levels of the endocannabinoid-degrading enzyme FAAH and CB_1 receptors were found in human obese subcutaneous and visceral adipose tissue [17, 116].

Insulin has recently been identified as a key regulator of endocannabinoid synthesis in three T3-L1 adipocytes, a regulatory mechanism which fails in insulin-resistant adipocytes [112]. This pathway may explain the increased endocannabinoid levels found in insulin-resistant obese individuals. However, no evidence exists in humans to directly indicate that insulin reduces endocannabinoid levels in the blood or other tissues and therefore that insulin resistance underlies part of the peripheral endocannabinoid overactivity. In rodents, pharmacologic blockade or genetic ablation of CB_1 receptors reduces appetite and

weight and prevents obesity, insulin resistance, and the development of fatty liver [119–121]. The underlying mechanisms which may explain the observed effects of CB_1 blockade may involve changes in endothelial nitric oxide (eNOS) expression, which has been implicated in the mitochondrial biogenesis and function in adipocytes [122], though the expression of eNOS in adipocytes is still a controversial issue requiring further clarification. Treatment with CB_1 antagonist rimonabant was found to increase eNOS expression in murine white adipocytes, together with an increase of mitochondrial mass and function. While high-fat diet decreased eNOS expression in wild-type mice, this effect was reversed by chronic rimonabant treatment and absent in CB_1-deficient mice. A recent experimental study further demonstrated that the upregulation of CB_1 expression, together with adipocyte differentiation, is directly inhibited by PPAR-delta [123]. Exercise reduced visceral fat mass, adipocyte size, and CB_1 expression in this rat model of high-fat-diet-induced obesity.

Clinical trials with rimonabant, a selective antagonist of the CB_1 receptor, have suggested a beneficial effect of this drug in the management of obesity and cardiometabolic risk factors in humans. The Rimonabant in Obesity (RIO) program comprised four 1–2-year placebo-controlled randomized clinical trials, recruiting more than 6,600 overweight/obese patients [95–98]. Rimonabant (20 mg daily) consistently reduced body weight, waist circumference, triglycerides, blood pressure, insulin resistance, and C-reactive protein levels and increased high-density lipoprotein (HDL) cholesterol concentrations in both nondiabetic and type-2 diabetic overweight/obese patients. Adiponectin levels were increased, an effect that correlated with HDL cholesterol augmentation, while small dense LDL cholesterol levels were decreased in patients receiving rimonabant 20 mg compared with those receiving placebo in RIO Lipids.

In addition, data from the ADAGIO Lipids study, a 1-year trial which assessed the effect of rimonabant on cardiometabolic risk factors and intra-abdominal (visceral) and liver fat in 803 obese patients, have been recently published [99]. This study not only confirmed the well-documented properties of rimonabant on several cardiometabolic risk factors but also demonstrated significant effects of rimonabant (20 mg/day) on visceral adiposity and liver fat content. Visceral and liver fat were measured by computed tomography in a subgroup of 231 patients, revealing a significant loss of visceral fat accompanied by a decrease in liver fat in the rimonabant-treated group. In addition to the previously described favorable effects of rimonabant on lipid levels, the ADAGIO Lipids study also revealed an improvement in lipid "quality." Rimonabant induced a shift in LDL particle size, a reduction in apo B to apo A1 ratio, and an increase in HDL_2-C and HDL_3-C levels as well as increased HDL particle size.

These promising outcomes are contrasted by the safety concerns related to the increased incidence of psychiatric adverse events associated with this class of drugs [100, 124, 125]. Although available in Europe since 2006 for overweight patients with associated risk factors, the increasing incidence of psychiatric side effects led to the recent temporary suspension of rimonabant from the market in Europe. For similar reasons, rimonabant failed to secure Food and Drug Administration approval in the US in 2007. In the future, selective targeting of peripheral CB_1 receptors may overcome the safety problems associated with currently available CB_1 antagonists such as rimonabant [94, 126]. A recently published experimental study conducted with diet-induced obese rats supports the notion that selective peripheral CB_1 antagonism mediates metabolic benefits, while also reducing food intake and body weight [127]. On the other hand, specific central CB_1 blockade predominantly affected food intake and body weight in this study but had no beneficial effect on peripheral lipid and glucose metabolism. A challenge for future studies will be to develop peripherally restricted CB_1 antagonists and to study if their biological activity compares with the beneficial cardiometabolic effects of rimonabant.

Diabetes

Emerging evidence suggests a crucial implication of the endocannabinoid system in the regulation of insulin sensitivity, glucose homeostasis, and lipid profile. The pathophysiology of type 2 diabetes is closely related to these disorders. Two clinical studies have been recently published that investigated the glucose-lowering efficacy and safety of CB_1 blocking with rimonabant in type 2 diabetic patients. The RIO Diabetes study enrolled 1,045 overweight subjects with type 2 diabetes [97]. In this study, treatment with rimonabant (20 mg/day for 1 year) significantly reduced body weight and improved glycemic control and HbA_{1C} levels in comparison to placebo. The Study Evaluating Rimonabant Efficacy in Drug-Naive Diabetic Patients enrolled 278 patients. In this study, treatment with 20 mg/day of rimonabant significantly reduced the levels of HbA_{1C}, also independently of weight loss [128].

The endocannabinoid system in atherosclerosis

In the past few years, many different physiologic and pathophysiologic regulatory actions have been attributed to the endocannabinoid system. Changes in endocannabinoid levels and/or receptor expression have been described in pathophysiologic conditions such as circulatory shock, doxorubicin-induced cardiotoxicity, advanced liver cirrhosis, obesity, and allergic contact dermatitis, gastrointestinal

inflammation, and many other inflammatory disorders [2, 13, 129, 130]. A growing body of evidence suggests that endocannabinoids might also be involved in the pathogenesis of atherogenesis; however, their precise role has still been poorly investigated.

Endocannabinoids released from endothelial cells, macrophages, or platelets reduced hypertension in rodents, a major risk factor for atherosclerosis [12]. An experimental study further suggested a possible protective role of anandamide, or other lipid mediators metabolized by FAAH, against age-associated decline of cardiac function and changes in inflammatory gene expression as well as TNF-α-induced monocyte adhesion to endothelial cells [131]. PEA was reported to downregulate inflammatory cytokine release from human peripheral blood mononuclear cells [132] and to exhibit anti-inflammatory effects in human and mouse adipocytes [133]. On the other hand, endocannabinoids might also mediate proatherosclerotic effects by inducing platelet activation [134, 135].

Clinical trials with rimonabant have revealed that blocking of CB_1 receptors not only causes weight reduction but reduces also several metabolic cardiovascular risk factors, suggesting a potential benefit for atherosclerosis [95, 124]. This is supported by a recently published experimental animal study, indicating a potential relevance for the process of atherosclerosis [136]. In line with these findings, latest results demonstrated a modulation of endocannabinoid levels in patients with coronary artery disease as well as in atherosclerotic mice [137, 138]. In addition to CB_1 receptors, targeting CB_2 receptors might be a promising therapeutic approach for atherosclerosis, given the well-known immunomodulatory effects of CB_2 activation. Treatment with the plant-derived cannabinoid THC has been shown to inhibit atherosclerotic plaque progression in mice, whereas CB_2 antagonism reversed the antiatherosclerotic effect [139]. These anti-inflammatory effects may be very important because of the increasing recognition of the role of chronic inflammation in the development and progression of atherosclerosis and other cardiovascular diseases [140].

Endocannabinoid levels

The first evidence for an activation of the endocannabinoid system in human atherosclerosis has been recently published by Sugamura and colleagues [137]. CB_1 mRNA levels in coronary atherectomy samples from a small group of unstable versus stable angina patients were analyzed by real-time reverse transcription polymerase chain reaction. A significant increase of CB_1 expression was reported in the unstable angina group. The immunohistological CB_1 staining was particular abundant in lipid-rich atheromatous plaques. The authors reported higher blood levels of endocannabinoids anandamide and 2-AG in coronary artery disease (CAD) patients versus asymptomatic patients without CAD. In vitro experiments revealed modulated mRNA levels of enzymes involved in endocannabinoid synthesis and degradation, NAPE-PLD and FAAH, together with an upregulation of CB_1 receptors, during monocyte to macrophage differentiation. In addition, rimonabant was found to reduce LPS-induced proinflammatory cytokine and matrix metallopeptidase 9 expression in human macrophages. On the basis of experiments using CB_1 receptor antagonists AM251 and AM281, as well as CB_1 receptor knockdown with small interfering RNA, the authors concluded that the observed anti-inflammatory effects in macrophages were CB_1 dependent [137].

A recent study has investigated the modulation of the endocannabinoid levels during atherosclerosis development in mice by comparing wild-type and apolipoprotein E knockout (ApoE−/−) mice fed either normal chow or high-cholesterol diet [138]. Increased levels of 2-AG in aortas and visceral adipose tissue (VAT) of ApoE−/− mice fed on high-cholesterol diet for 12 weeks were found, whereas no significant difference in 2-AG levels was observed after 8 weeks of diet. No changes in anandamide levels were found in any group. The levels of the anandamide-related mediators with anti-inflammatory or antilipogenic properties, PEA and oleoylethanolamide (OEA), were also investigated. While PEA levels decreased in VAT of ApoE−/− mice fed on high-cholesterol diet, OEA increased in aortas and VAT. The observed changes in 2-AG, PEA, and OEA levels did not appear to be a consequence of the high-cholesterol diet, since they were not found in wild-type mice fed with the same diet. The immunohistological analysis revealed that the endocannabinoid and PEA/OEA-degrading enzymes FAAH and MAGL were expressed by macrophages within mouse atherosclerotic lesions. Additional in vitro experiments with human monocytes and macrophages suggested that enhanced 2-AG and OEA levels in advanced atherosclerotic lesions might trigger the inflammatory process by recruiting more inflammatory cells and inducing extracellular matrix degradation, mainly via CB_2 receptors. On the other hand, PEA was also found to induce monocyte migration but counteracted the proinflammatory chemoattractant effects of both 2-AG and OEA. However, in vivo treatment with CB_2 antagonist SR144528 did not affect plaque progression in ApoE−/− mice, which may depend on the experimental protocol used in this study. Further experiments with genetic CB_2 deficiency in an atherosclerotic background may help to clarify a possible causal role of endocannabinoid-mediated CB_2 signaling in atherosclerosis.

Role of CB_1

The STRADIVARIUS trial studied the effect of rimonabant on atherosclerosis progression in patients with abdominal

obesity and coronary artery disease [124]. Patients received 20 mg/day rimonabant or placebo for 18 months, and atherosclerosis progression was determined using intravascular ultrasound. No statistical difference was observed in the primary end point, the percent atheroma volume (PAV). However, the total atheroma volume, a secondary end point, was significantly improved. In the subgroup analyses, rimonabant also significantly reduced PAV in patients who were not under statin treatment and in those with high baseline triglyceride levels (≥140 mg/ml) [141]. In addition, the Atherosclerosis Underlying Development Assessed by Intima-Media Thickness in Patients on Rimonabant (AUDITOR) trial, an ongoing study which started in 2005, aims to assess the effect of rimonabant on atherosclerosis progression in carotid arteries (ClinicalTrials.gov Identifier: NCT00228176).

Direct evidence for a causal role of endocannabinoid-mediated CB_1 activation in atherosclerosis has been recently provided in an experimental mouse study. The authors report antiatherosclerotic effects of the CB_1 antagonist rimonabant in the low-density lipoprotein receptor knockout (LDLR−/−) mouse model of atherosclerosis, fed a Western-type diet for 3 months [136]. Rimonabant given at a dose of 10 to 50 mg/kg per day in the diet resulted in a dose-dependent inhibition of atherosclerotic lesion development in aorta and aortic sinus, together with a decrease in body weight. However, the observed antiatherosclerotic effect was not related to inhibition of food intake, since the lesion size in pair-fed mice was similar to that in unrestricted Western-diet-fed control mice. A reduction of serum total cholesterol, interleukin (IL)-12, and monocyte chemotactic protein 1 levels, together with increased adiponectin, was only found at the high rimonabant dose (50 mg/kg per day), whereas lower serum triglyceride and leptin levels were also observed at low doses (10 and 30 mg/kg per day). In vitro, rimonabant was shown to inhibit LPS-induced proinflammatory IL-6, TNF-α, and CCL2 gene expression in mouse peritoneal macrophages. Importantly, this effect was still observed when cells from CB_1−/− mice were used, suggesting a CB_1-independent anti-inflammatory effect of rimonabant. These findings are in conflict with the above-discussed study from Sugamara and colleagues, who reported that the anti-inflammatory effects of rimonabant on LPS-induced IL-6 expression were abrogated by experimental CB_1 knockdown using interfering RNA [137]. These discrepancies might be explained by the different experimental settings used in the two studies. In conclusion, the observed antiatherosclerotic effect of rimonabant at low dose (which is independent of serum total cholesterol changes) may be related to reduced inflammation [136]. This latter effect seems to be mediated at least in part via CB_1-independent mechanisms.

Vascular smooth muscle cell migration and proliferation are pivotal events in the pathogenesis of atherosclerosis and are directly implicated in the failure of clinical interventions used to treat patients with coronary heart disease [142]. For example, percutaneous transluminal angioplasty is an invasive procedure aimed to repair a stenotic blood vessel, which often fails with the time because of the development of restenosis. Vascular smooth muscle cells are the principal cell type in restenotic lesions and are also a major component of atherosclerotic lesions. As recently demonstrated in vitro, CB_1 antagonism (with rimonabant) dose-dependently inhibited PDGF-induced proliferation, migration and signal transduction of human coronary artery smooth muscle cells [58].

A recent in vitro study has highlighted a link between the endocannabinoid system and the regulation of cellular cholesterol accumulation in rodent macrophages. Oxidized LDL, a key factor in atherosclerosis [143], increased 2-AG and anandamide levels as well as CB_1 and CB_2 expression, as shown in the mouse macrophage cell line RAW264.7 and rat peritoneal macrophages [144]. The synthetic cannabinoid WIN55,212-2 triggered cholesterol accumulation in RAW264.7 macrophages. This effect was associated with an increase in CD36 and PPAR γ expression, whereas adenosine-triphosphate-binding cassette protein A1 expression was reduced. The CB_1 antagonist AM251 inhibited the observed effects of WIN55,212-2.

In conclusion, an emerging body of in vitro and in vivo findings supports a key role for endocannabinoid-mediated CB_1 signaling in the pathogenesis of atherosclerosis, suggesting that CB_1 antagonism may represent a promising therapeutic strategy for the treatment of this life-threatening disease.

Role of CB_2

Cannabinoids are well known for their immunomodulatory properties, which have been mainly attributed to CB_2 receptors in the past. CB_2 receptors have been implicated in the modulation of immune cell migration, which is an essential step in the development and progression of atherosclerosis. The tethering, rolling, adhesion, and trans-endothelial migration of leukocytes is triggered by local production of chemokines and chemokine receptors as well as adhesion molecules [145]. Cannabinoids have been reported to inhibit chemokine-induced chemotaxis of various cell types [146]. In addition, synthetic and endogenous cannabinoids themselves are potent inducers of immune cell migration, which raises the question if they may also promote inflammation by recruiting immune cells to inflammatory sites [138, 146]. On the other hand, they may reduce inflammation by interfering with the action of other chemoattractants. Recent in vitro findings support this hypothesis [54]. Human monocytes treated with the synthetic CB_2 agonist JWH-015 showed significantly reduced migration versus chemokines CCL2 and CCL3

via downregulation of their cognate receptors CCR2 and CCR1, and inhibition of interferon-gamma-induced intercellular adhesion molecule (ICAM)-1 upregulation. Moreover, JWH-015 cross-desensitized human monocytes for migration in response to CCL2 and CCL3 by its own chemoattractant properties. This may have physiological relevance, as systemic administration of CB_2 agonists might inhibit leukocyte recruitment to local inflammatory sites by desensitizing cells for migration versus chemokine gradients.

The first in vivo evidence for a direct antiatherosclerotic effect of CB_2 receptor activation has been provided in the ApoE−/− mouse model of atherosclerosis. Oral administration of THC resulted in the significant inhibition of plaque development, an effect that could be inhibited by the CB_2 antagonist SR144528 [139]. The antiatherosclerotic effect was associated with reduced lesional macrophage infiltration as well as reduced proliferation and interferon gamma release by splenocytes isolated from THC-treated mice. In support of the antiatherosclerotic role of CB_2 activation, in vitro treatment with the synthetic CB_2 agonists JWH-133 and HU-308 reduced the TNF-α-induced activation of human coronary artery endothelial cells [14]. In particular, CB_2 stimulation attenuated the TNF-α-induced nuclear factor kappa B (NF-κB) and RhoA activation, ICAM-1 and VCAM-1 upregulation, and CCL2 release, as well as transendothelial migration and adhesion of THP-1 monocytes. A recent in vitro study further demonstrated that CB_2 activation with selective CB_2 receptor agonists inhibited the TNF-α-induced proliferation and migration of human coronary artery smooth muscle cells [15]. As described above, smooth muscle cells are crucially involved in the pathogenesis of atherosclerosis and restenosis. Moreover, there is evidence for a role of CB_2 receptors in macrophage apoptosis induced by oxidized LDL [147]. Oxidized LDL is a well-known trigger for atherosclerosis, which accumulates in macrophages within atherosclerotic lesions, resulting in foam cell formation [143]. The capacity of oxidized LDL to induce macrophage apoptosis is likely to play an important role in the progression of atherosclerosis and atherosclerotic plaque stability [148]. Apoptosis of macrophages might be beneficial for plaque stability if apoptotic bodies are removed. Indeed, it has been demonstrated that impaired macrophage apoptosis triggers lesion formation in mice [149]. In advanced lesions, however, apoptosis of macrophage-derived foam cells promotes the formation of a prothrombotic central lipid pool whose size correlates with plaque instability. Thus, macrophage apoptosis, at least in advanced lesions, could be considered as a proatherogenic factor triggering plaque instability and rupture [150]. Freeman-Anderson and colleagues investigated the effect of genetic CB_2 deficiency on oxidized LDL-induced apoptosis [147]. They found that the apoptosis rate was significantly reduced in peritoneal macrophages from CB_2 knockout mice as compared to wild-type animals. They further provided evidence implicating the Akt survival pathway in CB_2-mediated signaling in their in vitro model. However, the in vivo consequences of these findings in the pathophysiology of atherosclerosis remain to be elucidated.

Finally, a recent large case control study enrolling 1,968 individuals addressed the involvement of the gene encoding CB_2, CNR2, in the development of myocardial infarction and several cardiovascular risk factors. In particular, a potential association of genetic variations with the development of myocardial infarction and classic cardiovascular risk factors, including arterial hypertension, obesity, hypercholesterolemia, and diabetes mellitus, was investigated [151]. However, none of the 13 investigated single-nucleotide polymorphisms in the CNR2 gene was associated with myocardial infarction or any of the investigated risk factors.

CB_1- and CB_2-independent cannabinoid effects

CBD is a nonpsychoactive *Cannabis* component with potent anti-inflammatory and antioxidant properties and low affinity for CB_1 and CB_2 receptors [82, 152]. A recent study investigated the effect of CBD on high-glucose-induced activation in human coronary artery endothelial cells, as an in vitro model mimicking endothelial dysfunction in diabetic patients [153]. CBD inhibited all the observed high-glucose-induced effects in endothelial cells, including increased mitochondrial superoxide generation, NF-κB activation and ICAM-1 and VCAM-1 upregulation, as well as transendothelial migration and adhesion of THP-1 monocytes. CBD also reversed the high-glucose-induced decrease of endothelial barrier function. Neither CB_1 antagonism (with rimonabant or AM281) nor CB_2 antagonism (with SR144528 or AM630) inhibited the various effects of CBD on the endothelial cells. These findings suggest that CBD may have a therapeutic potential not only for the treatment of diabetes [154] and diabetic complications but also for atherosclerosis. Future studies should help to clarify the molecular mechanisms underlying the beneficial effects of CBD.

Conclusion

Collectively, the above-mentioned accumulating evidence suggests that the modulation of the endocannabinoid system by selective agonists or antagonists may hold tremendous therapeutic potential in various cardiovascular disorders associated with inflammation and tissue injury, ranging from myocardial infarction and heart failure to atherosclerosis and cardiometabolic disorders.

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