

The emerging role of the endocannabinoid system in cardiovascular disease

Pál Pacher · Sabine Steffens

Received: 6 February 2009 / Accepted: 31 March 2009 / Published online: 9 April 2009
© Springer-Verlag 2009

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 CB₁ and CB₂. 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 CB₁ receptors. Furthermore, tonic activation of CB₁ 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 CB₂ receptors in immune cells exerts various immunomodulatory effects, and the CB₂ 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.

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 CB₁ and CB₂ [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].

P. Pacher (✉)

Section on Oxidative Stress Tissue Injury,
Laboratory of Physiological Studies,
National Institutes of Health/NIAAA,
5625 Fishers Lane, MSC-9413,
Bethesda, MD 20892-9413, USA
e-mail: pacher@mail.nih.gov

S. Steffens (✉)

Division of Cardiology, Department of Internal Medicine,
University Hospital, Foundation for Medical Researches,
Avenue Roseraie 64,
1211 Geneva, Switzerland
e-mail: Sabine.Steffens@unige.ch

Endocannabinoids bind to both CB₁ and CB₂ receptors. Anandamide has higher affinity for CB₁ and CB₂ receptors, whereas 2-AG has higher CB₁ and CB₂ efficacy than anandamide. Endocannabinoids may also exert various CB-receptor-independent effects (e.g., AEA may also bind to vanilloid VR₁ (TRPV₁) receptors) [10]. Both CB₂ and CB₁ 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 CB₂ receptors are mainly expressed in immune and hematopoietic cells mediating various immunomodulatory effects, while CB₁ receptors are primarily distributed in the central nervous system and are responsible for psychoactive properties of cannabis. However, recent studies have also demonstrated CB₁ 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 CB₂ 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 CB₂ receptors (rank order for messenger RNA (mRNA): B cells>NK cells>monocytes>polymorphonuclear neutrophils (PMNs)>T cells [22]) also express CB₁ 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, Δ⁹-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 Human	CB ₁ CB ₁ , CB ₂	RT-PCR IFL, WB, RT-PCR	[16, 156]
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 CB₁ and TRPV₁ 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 CB₂ 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]. CB₂ receptor activation also attenuates the TNF- α -induced human coronary artery smooth muscle cell proliferation (Fig. 1) [15]. As already mentioned above, CB₁ 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 CB₁ antagonist AM251, implicating the existence of a CB₁ receptor-dependent negative inotropy in the myocardium [11]. Consistent with in vitro CB₁-mediated negative inotropy, HU-210 decreases left ventricular developed pressure in isolated perfused rat hearts most likely through CB₁ receptor activation [41, 57] and also decreases myocardial contractility in vivo (measured by the analyses of the pressure–volume relations) without

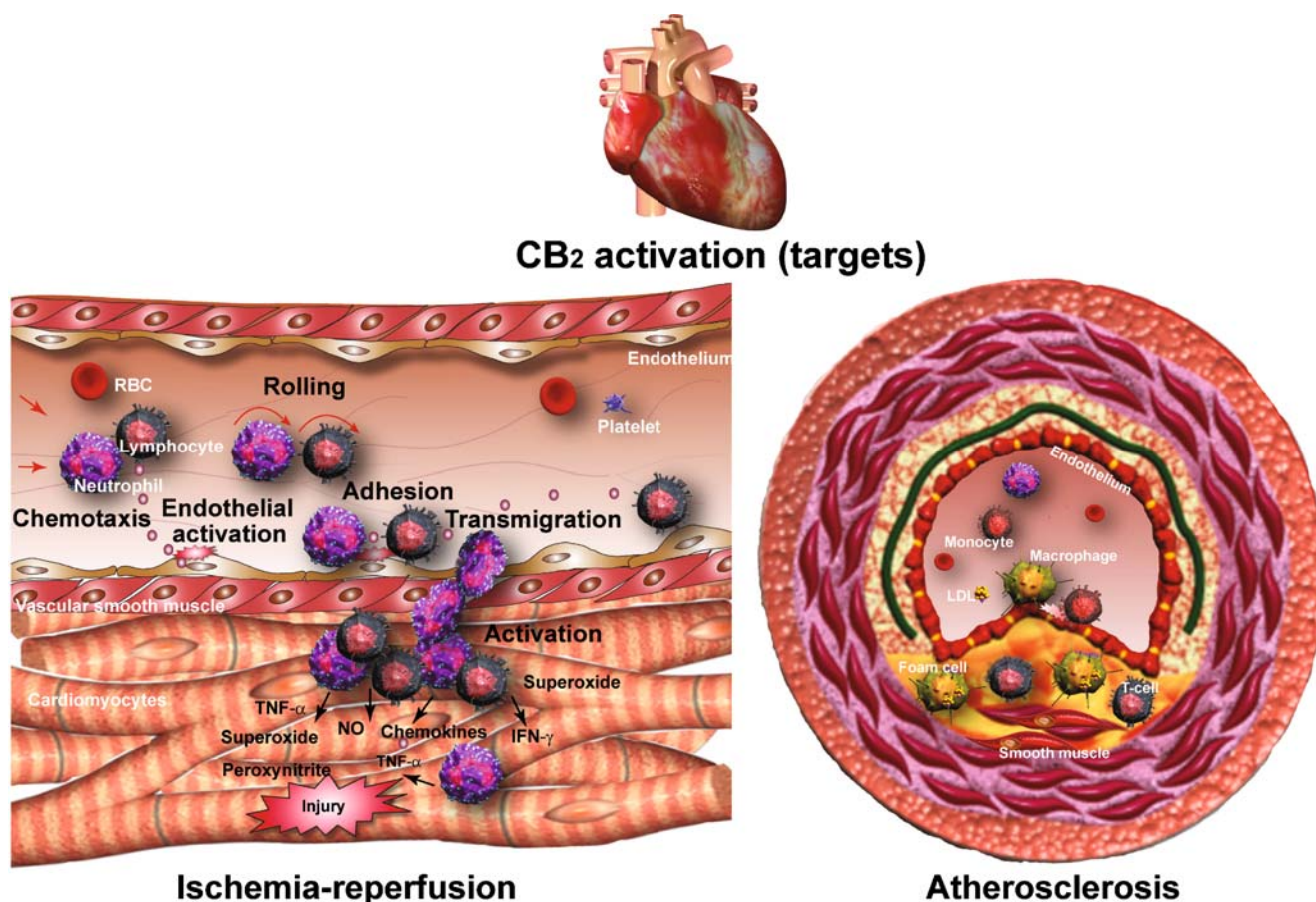


Fig. 1 Therapeutic targets of CB₂ receptor stimulation in ischemia/reperfusion injury and atherosclerosis. CB₂ agonists decrease endothelial cell activation and inflammatory response, chemotaxis triggered by inflammatory stimuli/chemokines, and adhesion of inflammatory cells (lymphocytes, neutrophils, and/or monocytes) to activated endothelium,

transendothelial migration of inflammatory cells, attachment to parenchymal cells, and activation. In addition, CB₂ 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]. CB₁ receptors may also play an important role in vascular smooth muscle cell proliferation [58]. Despite evidence on the presence of CB₂ 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 over-activated and may contribute to hypotension/cardiodepression through cardiovascular CB₁ 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 Ca²⁺ 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 CB₂ receptors with CB₂ antagonist SR144528 was shown to abolish bacterial lipopolysaccharide (LPS) or heat-stress-induced preconditioning against myocardial ischemia, while the CB₁ 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 CB₂ receptors. A subsequent study, however, reported that preconditioning induced by short-term ischemia could be blocked by either CB₁ or CB₂ antagonism [59]. Conversely, delayed cardioprotection induced by nitric oxide was reversed by the CB₁ antagonist AM251 but not the CB₂ 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 CB₂ receptors has been recently involved in the cardioprotective effects of remote ischemic preconditioning, induced by mesenteric artery occlusion and

reperfusion [75]. Systemic pretreatment with CB₂ antagonist AM630, but not the CB₁ 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 CB₂ antagonism with SR144528 totally abolished the beneficial effects of PEA and 2-AG [69]. The CB₁ antagonist rimonabant only partially blocked the effect of 2-AG. However, selective agonists for both CB₁ and CB₂ 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 CB₁- and CB₂-independent pathways. Previous findings may suggest that the CB₂ 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 PPAR- 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 CB₁ or CB₂ antagonism, using rimonabant or SR144528, respectively [78]. However, CB₁ and CB₂ 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 CB₂ 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 CB₂ antagonist AM630, but not the CB₁ antagonist AM251 abolished the effect of WIN55,212-2. In support of the cardioprotective role of CB₂ receptor activation, recent findings have demonstrated that a single dose of the CB₂ 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 CB₂ activation on cardiomyocytes and an anti-inflammatory effect on neutrophils. Consistently, selective CB₂ 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 CB₁ and CB₂ 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 CB₁ 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 CB₁ 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-CB₁-receptor-mediated effects, given the suboptimal doses of agonists and antagonists used in these studies.

More recent evidence suggests a cardioprotective effect of CB₁ 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 CB₁ antagonists rimonabant or AM281 [13]. CB₁ 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 CB₁ antagonists, since neither CB₁ or CB₂ agonists nor CB₂ antagonists were protective. In addition, the CB₁ 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 CB₁ and CB₂ 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 CB₁ receptors which leads to cardiodepression and cell death in cardiomyocytes. The underlying cellular mechanisms of the cytoprotective effect of CB₁ 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 CB₁ 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

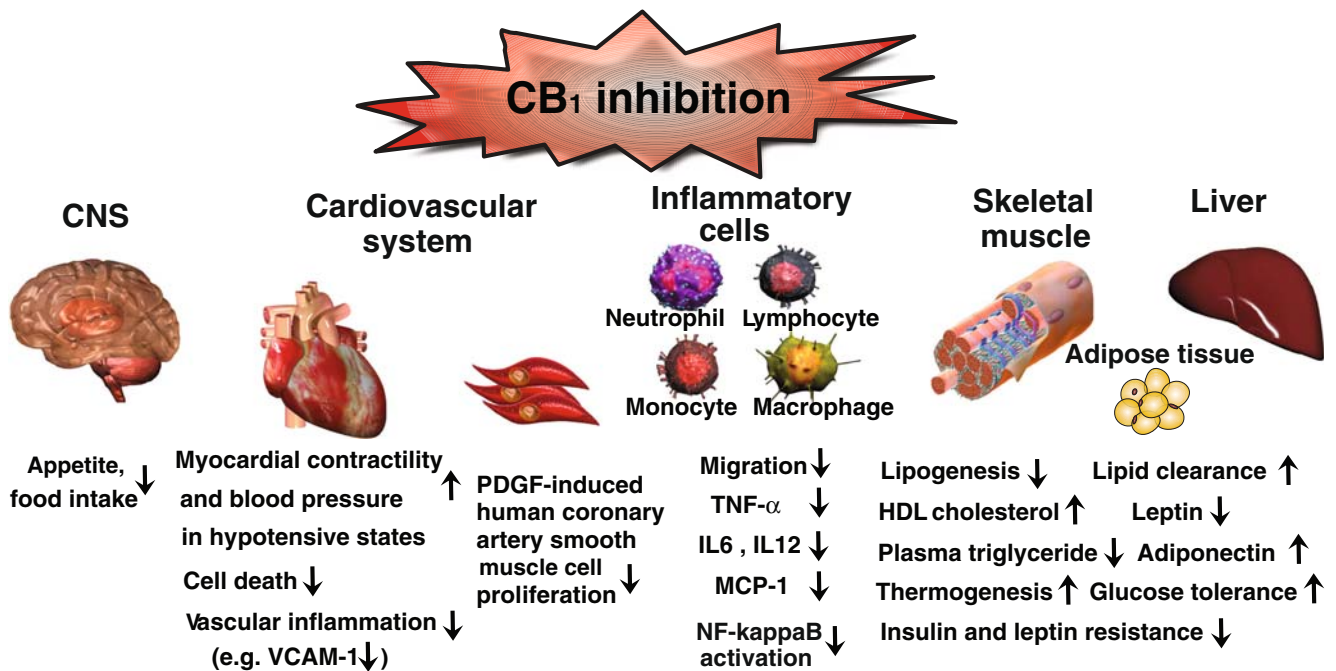


Fig. 2 Selected beneficial effects of CB₁ receptor blockade with rimonabant observed in preclinical/clinical studies with potential relevance to atherosclerosis and cardiovascular diseases/risk. Modified with the permission of the American Heart Association from [94]

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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁-deficient mice. A recent experimental study further demonstrated that the upregulation of CB₁ expression, together with adipocyte differentiation, is directly inhibited by PPAR- δ [123]. Exercise reduced visceral fat mass, adipocyte size, and CB₁ expression in this rat model of high-fat-diet-induced obesity.

Clinical trials with rimonabant, a selective antagonist of the CB₁ 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 HDL₂-C and HDL₃-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 CB₁ receptors may overcome the safety problems associated with currently available CB₁ antagonists such as rimonabant [94, 126]. A recently published experimental study conducted with diet-induced obese rats supports the notion that selective peripheral CB₁ antagonism mediates metabolic benefits, while also reducing food intake and body weight [127]. On the other hand, specific central CB₁ 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 CB₁ 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 CB₁ 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 CB₁ 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 CB₁ receptors, targeting CB₂ receptors might be a promising therapeutic approach for atherosclerosis, given the well-known immunomodulatory effects of CB₂ activation. Treatment with the plant-derived cannabinoid THC has been shown to inhibit atherosclerotic plaque progression in mice, whereas CB₂ 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]. CB₁ 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 CB₁ expression was reported in the unstable angina group. The immunohistological CB₁ staining was particularly 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 CB₁ receptors, during monocyte to macrophage differentiation. In addition, rimonabant was found to reduce LPS-induced proinflammatory cytokine and matrix metalloproteinase 9 expression in human macrophages. On the basis of experiments using CB₁ receptor antagonists AM251 and AM281, as well as CB₁ receptor knockdown with small interfering RNA, the authors concluded that the observed anti-inflammatory effects in macrophages were CB₁ 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 CB₂ 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 CB₂ 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 CB₂ deficiency in an atherosclerotic background may help to clarify a possible causal role of endocannabinoid-mediated CB₂ signaling in atherosclerosis.

Role of CB₁

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 CB₁ activation in atherosclerosis has been recently provided in an experimental mouse study. The authors report antiatherosclerotic effects of the CB₁ 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 chemoattractant 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 CB₁^{-/-} mice were used, suggesting a CB₁-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 CB₁ 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 CB₁-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, CB₁ 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 CB₁ and CB₂ 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 CB₁ 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 CB₁ signaling in the pathogenesis of atherosclerosis, suggesting that CB₁ antagonism may represent a promising therapeutic strategy for the treatment of this life-threatening disease.

Role of CB₂

Cannabinoids are well known for their immunomodulatory properties, which have been mainly attributed to CB₂ receptors in the past. CB₂ 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 CB₂ 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 CB₂ 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 CB₂ 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 CB₂ 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 CB₂ activation, in vitro treatment with the synthetic CB₂ agonists JWH-133 and HU-308 reduced the TNF- α -induced activation of human coronary artery endothelial cells [14]. In particular, CB₂ stimulation attenuated the TNF- α -induced nuclear factor kappa B (NF- κ B) 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 CB₂ activation with selective CB₂ 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 CB₂ 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 CB₂ deficiency on oxidized LDL-induced apoptosis [147]. They found that the apoptosis rate was

significantly reduced in peritoneal macrophages from CB₂ knockout mice as compared to wild-type animals. They further provided evidence implicating the Akt survival pathway in CB₂-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 CB₂, 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.

CB₁- and CB₂-independent cannabinoid effects

CBD is a nonpsychoactive *Cannabis* component with potent anti-inflammatory and antioxidant properties and low affinity for CB₁ and CB₂ 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- κ B 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 CB₁ antagonism (with rimonabant or AM281) nor CB₂ 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.

Acknowledgements S.S. is supported by grants from the Swiss National Science Foundation, the Novartis Foundation for Biomedical Research, Prevot and Wolferrmann-Naegeli Foundation. P.P. is supported by the Intramural research program of the NIH/NIAAA.

References

- Mechoulam R, Fride E, Di Marzo V (1998) Endocannabinoids. *Eur J Pharmacol* 359:1–18
- Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 58:389–462
- Milman G, Maor Y, Abu-Lafi S et al (2006) *N*-arachidonoyl L-serine, an endocannabinoid-like brain constituent with vasodilatory properties. *Proc Natl Acad Sci USA* 103:2428–2433
- Howlett AC (2005) Cannabinoid receptor signaling. *Handb Exp Pharmacol* 168:53–79
- Begg M, Pacher P, Batkai S et al (2005) Evidence for novel cannabinoid receptors. *Pharmacol Ther* 106:133–145
- Lauckner JE, Jensen JB, Chen HY et al (2008) GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci USA* 105:2699–2704
- Liu J, Wang L, Harvey-White J et al (2008) Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* 54:1–7
- Di Marzo V (2008) Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol* 160:1–24
- Ahn K, McKinney MK, Cravatt BF (2008) Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev* 108:1687–1707
- Starowicz K, Nigam S, Di Marzo V (2007) Biochemistry and pharmacology of endovanilloids. *Pharmacol Ther* 114:13–33
- Bonz A, Laser M, Kullmer S et al (2003) Cannabinoids acting on CB1 receptors decrease contractile performance in human atrial muscle. *J Cardiovasc Pharmacol* 41:657–664
- Batkai S, Pacher P, Osei-Hyiaman D et al (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* 110:1996–2002
- Mukhopadhyay P, Batkai S, Rajesh M et al (2007) Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol* 50:528–536
- Rajesh M, Mukhopadhyay P, Batkai S et al (2007) CB2-receptor stimulation attenuates TNF- α -induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol* 293:H2210–H2218
- Rajesh M, Mukhopadhyay P, Hasko G et al (2008) CB(2) cannabinoid receptor agonists attenuate TNF- α -induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol* 153:347–357
- Cota D, Marsicano G, Tschop M et al (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112:423–431
- Engeli S, Bohnke J, Feldpausch M et al (2005) Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 54:2838–2843
- Osei-Hyiaman D, DePetrillo M, Pacher P et al (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 115:1298–1305
- Mallat A, Lotersztajn S (2008) Endocannabinoids and liver disease. I. Endocannabinoids and their receptors in the liver. *Am J Physiol Gastrointest Liver Physiol* 294:G9–G12
- Van Sickle MD, Duncan M, Kingsley PJ et al (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310:329–332
- Pacher P, Gao B (2008) Endocannabinoid effects on immune cells: implications for inflammatory liver diseases. *Am J Physiol Gastrointest Liver Physiol* 294:G850–G854
- Klein TW, Newton C, Larsen K et al (2003) The cannabinoid system and immune modulation. *J Leukoc Biol* 74:486–496
- Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* 5:400–411
- Di Marzo V, Petrosino S (2007) Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* 18:129–140
- Mackie K (2006) Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol Toxicol* 46:101–122
- Di Marzo V (2008) Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* 7:438–455
- Pacher P, Batkai S, Osei-Hyiaman D et al (2005) Hemodynamic profile, responsiveness to anandamide, and baroreflex sensitivity of mice lacking fatty acid amide hydrolase. *Am J Physiol Heart Circ Physiol* 289:H533–541
- Pacher P, Batkai S, Kunos G (2005) Blood pressure regulation by endocannabinoids and their receptors. *Neuropharmacology* 48:1130–1138
- Varga K, Lake K, Martin BR et al (1995) Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 278:279–283
- Varga K, Lake KD, Huangfu D et al (1996) Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension* 28:682–686
- Lake KD, Martin BR, Kunos G et al (1997) Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension* 29:1204–1210
- Pacher P, Batkai S, Kunos G (2004) Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. *J Physiol* 558:647–657
- Gardiner SM, March JE, Kemp PA et al (2002) Complex regional haemodynamic effects of anandamide in conscious rats. *Br J Pharmacol* 135:1889–1896
- Wheal AJ, Bennett T, Randall MD et al (2007) Cardiovascular effects of cannabinoids in conscious spontaneously hypertensive rats. *Br J Pharmacol* 152:717–724
- Pacher P, Mukhopadhyay P, Mohanraj R et al (2008) Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension* 52:601–607
- Ishac EJ, Jiang L, Lake KD et al (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br J Pharmacol* 118:2023–2028
- Malinowska B, Godlewski G, Bucher B et al (1997) Cannabinoid CB1 receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn Schmiedeberg Arch Pharmacol* 356:197–202
- Szabo B, Nordheim U, Niederhoffer N (2001) Effects of cannabinoids on sympathetic and parasympathetic neuroeffector transmission in the rabbit heart. *J Pharmacol Exp Ther* 297:819–826
- Niederhoffer N, Szabo B (2000) Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. *J Pharmacol Exp Ther* 294:707–713
- Pfister T, Niederhoffer N, Szabo B (2004) Central effects of the cannabinoid receptor agonist WIN55212-2 on respiratory and cardiovascular regulation in anaesthetised rats. *Br J Pharmacol* 142:943–952
- Maslov LN, Lasukova OV, Krylatov AV et al (2004) Selective cannabinoid receptor agonist HU-210 decreases pump function of isolated perfused heart: role of cAMP and cGMP. *Bull Exp Biol Med* 138:550–553

42. Sterin-Borda L, Del Zar CF, Borda E (2005) Differential CB1 and CB2 cannabinoid receptor-inotropic response of rat isolated atria: endogenous signal transduction pathways. *Biochem Pharmacol* 69:1705–1713
43. Gebremedhin D, Lange AR, Campbell WB et al (1999) Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca²⁺ channel current. *Am J Physiol* 276:H2085–2093
44. Jarai Z, Wagner JA, Varga K et al (1999) Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* 96:14136–14141
45. Wagner JA, Jarai Z, Batkai S et al (2001) Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB(1) receptors. *Eur J Pharmacol* 423:203–210
46. Wagner JA, Abesser M, Karcher J et al (2005) Coronary vasodilator effects of endogenous cannabinoids in vasopressin-precontracted unpaced rat isolated hearts. *J Cardiovasc Pharmacol* 46:348–355
47. Randall MD, Harris D, Kendall DA et al (2002) Cardiovascular effects of cannabinoids. *Pharmacol Ther* 95:191–202
48. Mendizabal VE, Adler-Graschinsky E (2007) Cannabinoids as therapeutic agents in cardiovascular disease: a tale of passions and illusions. *Br J Pharmacol* 151:427–440
49. Hillard CJ (2000) Endocannabinoids and vascular function. *J Pharmacol Exp Ther* 294:27–32
50. Ralevic V, Kendall DA, Randall MD et al (2002) Cannabinoid modulation of sensory neurotransmission via cannabinoid and vanilloid receptors: roles in regulation of cardiovascular function. *Life Sci* 71:2577–2594
51. Pacher P, Batkai S, Kunos G (2005) Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol* 168:599–625
52. Batkai S, Osei-Hyiaman D, Pan H et al (2007) Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *FASEB J* 21:1788–1800
53. Rajesh M, Pan H, Mukhopadhyay P et al (2007) Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *J Leukoc Biol* 82:1382–1389
54. Montecucco F, Burger F, Mach F et al (2008) CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol* 294:H1145–H1155
55. Mach F, Montecucco F, Steffens S (2008) Cannabinoid receptors in acute and chronic complications of atherosclerosis. *Br J Pharmacol* 153:290–298
56. Pacher P, Hasko G (2008) Endocannabinoids and cannabinoid receptors in ischaemia–reperfusion injury and preconditioning. *Br J Pharmacol* 153:252–262
57. Krylatov AV, Maslov LN, Lasukova OV et al (2005) Cannabinoid receptor antagonists SR141716 and SR144528 exhibit properties of partial agonists in experiments on isolated perfused rat heart. *Bull Exp Biol Med* 139:558–561
58. Rajesh M, Mukhopadhyay P, Hasko G et al (2008) Cannabinoid CB1 receptor inhibition decreases vascular smooth muscle migration and proliferation. *Biochem Biophys Res Commun* 377:1248–1252
59. Bouchard JF, Lepicier P, Lamontagne D (2003) Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci* 72:1859–1870
60. Montecucco F, Lenglet S, Brauersreuther V et al (2009) CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol* (in press)
61. Mukhopadhyay P, Mohanraj R, Batkai S et al (2008) CB1 cannabinoid receptor inhibition: promising approach for heart failure? *Congest Heart Fail* 14:330–334
62. Braunwald E (2008) Biomarkers in heart failure. *N Engl J Med* 358:2148–2159
63. Yellon DM, Hausenloy DJ (2005) Realizing the clinical potential of ischemic preconditioning and postconditioning. *Nat Clin Pract Cardiovasc Med* 2:568–575
64. Lange RA, Hillis LD (2002) Reperfusion therapy in acute myocardial infarction. *N Engl J Med* 346:954–955
65. Yellon DM, Hausenloy DJ (2007) Myocardial reperfusion injury. *N Engl J Med* 357:1121–1135
66. Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 58:87–114
67. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87:315–424
68. Lagneux C, Lamontagne D (2001) Involvement of cannabinoids in the cardioprotection induced by lipopolysaccharide. *Br J Pharmacol* 132:793–796
69. Lepicier P, Bouchard JF, Lagneux C et al (2003) Endocannabinoids protect the rat isolated heart against ischaemia. *Br J Pharmacol* 139:805–815
70. Joyeux M, Arnaud C, Godin-Ribuot D et al (2002) Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. *Cardiovasc Res* 55:619–625
71. Varga K, Wagner JA, Bridgen DT et al (1998) Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. *FASEB J* 12:1035–1044
72. Liu J, Batkai S, Pacher P et al (2003) Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-kappa B independently of platelet-activating factor. *J Biol Chem* 278:45034–45039
73. Maccarrone M, De Petrocellis L, Bari M et al (2001) Lipopolysaccharide downregulates fatty acid amide hydrolase expression and increases anandamide levels in human peripheral lymphocytes. *Arch Biochem Biophys* 393:321–328
74. Wagner JA, Abesser M, Harvey-White J et al (2006) 2-Arachidonylglycerol acting on CB1 cannabinoid receptors mediates delayed cardioprotection induced by nitric oxide in rat isolated hearts. *J Cardiovasc Pharmacol* 47:650–655
75. Hajrasouliha AR, Tavakoli S, Ghasemi M et al (2008) Endogenous cannabinoids contribute to remote ischemic preconditioning via cannabinoid CB2 receptors in the rat heart. *Eur J Pharmacol* 579:246–252
76. Calignano A, La Rana G, Giuffrida A et al (1998) Control of pain initiation by endogenous cannabinoids. *Nature* 394:277–281
77. LoVerme J, Russo R, La Rana G et al (2006) Rapid broad-spectrum analgesia through activation of peroxisome proliferator-activated receptor-alpha. *J Pharmacol Exp Ther* 319:1051–1061
78. Underdown NJ, Hiley CR, Ford WR (2005) Anandamide reduces infarct size in rat isolated hearts subjected to ischaemia–reperfusion by a novel cannabinoid mechanism. *Br J Pharmacol* 146:809–816
79. Krylatov AV, Ugdyzhkova DS, Bernatskaya NA et al (2001) Activation of type II cannabinoid receptors improves myocardial tolerance to arrhythmogenic effects of coronary occlusion and reperfusion. *Bull Exp Biol Med* 131:523–525
80. Di Filippo C, Rossi F, Rossi S et al (2004) Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia–reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol* 75:453–459
81. Durst R, Danenberg H, Gallily R et al (2007) Cannabidiol, a nonpsychoactive Cannabis constituent, protects against myocardial ischemic reperfusion injury. *Am J Physiol Heart Circ Physiol* 293:H3602–H3607
82. Ryberg E, Larsson N, Sjogren S et al (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 152:1092–1101

83. Carrier EJ, Auchampach JA, Hillard CJ (2006) Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA* 103:7895–7900
84. Hasko G, Linden J, Cronstein B et al (2008) Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov* 7:759–770
85. Mudd JO, Kass DA (2008) Tackling heart failure in the twenty-first century. *Nature* 451:919–928
86. Cohn JN, Ferrari R, Sharpe N (2000) Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol* 35:569–582
87. Pacher P, Schulz R, Liaudet L et al (2005) Nitrosative stress and pharmacological modulation of heart failure. *Trends Pharmacol Sci* 26:302–310
88. Takimoto E, Kass DA (2007) Role of oxidative stress in cardiac hypertrophy and remodeling. *Hypertension* 49:241–248
89. Wagner JA, Hu K, Bauersachs J et al (2001) Endogenous cannabinoids mediate hypotension after experimental myocardial infarction. *J Am Coll Cardiol* 38:2048–2054
90. Wagner JA, Hu K, Karcher J et al (2003) CB(1) cannabinoid receptor antagonism promotes remodeling and cannabinoid treatment prevents endothelial dysfunction and hypotension in rats with myocardial infarction. *Br J Pharmacol* 138:1251–1258
91. Pacher P, Liaudet L, Bai P et al (2003) Potent metalloporphyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin-induced cardiac dysfunction. *Circulation* 107:896–904
92. Matias I, Di Marzo V (2007) Endocannabinoids and the control of energy balance. *Trends Endocrinol Metab* 18:27–37
93. Engeli S (2008) Dysregulation of the endocannabinoid system in obesity. *J Neuroendocrinol* 20(Suppl 1):110–115
94. Pacher P (2009) Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? *Arterioscler Thromb Vasc Biol* 29:7–9
95. Despres JP, Golay A, Sjostrom L (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 353:2121–2134
96. Pi-Sunyer FX, Aronne LJ, Heshmati HM et al (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA* 295:761–775
97. Scheen AJ, Finer N, Hollander P et al (2006) Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* 368:1660–1672
98. Van Gaal LF, Rissanen AM, Scheen AJ et al (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 365:1389–1397
99. Despres JP, Ross R, Boka G et al (2009) Effect of rimonabant on the high-triglyceride/low-HDL-cholesterol dyslipidemia, intra abdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* 29:416–423
100. Jones D (2008) End of the line for cannabinoid receptor 1 as an anti-obesity target? *Nat Rev Drug Discov* 7:961–962
101. Katagiri H, Yamada T, Oka Y (2007) Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res* 101:27–39
102. Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96:939–949
103. Okamoto Y, Kihara S, Funahashi T et al (2006) Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci (Lond)* 110:267–278
104. Han SH, Quon MJ, Kim JA et al (2007) Adiponectin and cardiovascular disease: response to therapeutic interventions. *J Am Coll Cardiol* 49:531–538
105. Okamoto Y, Folco EJ, Minami M et al (2008) Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherosclerosis. *Circ Res* 102:218–225
106. La Cava A, Matarese G (2004) The weight of leptin in immunity. *Nat Rev Immunol* 4:371–379
107. Hasty AH, Shimano H, Osuga J et al (2001) Severe hypercholesterolemia, hypertriglyceridemia, and atherosclerosis in mice lacking both leptin and the low density lipoprotein receptor. *J Biol Chem* 276:37402–37408
108. Mertens A, Verhamme P, Bielicki JK et al (2003) Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. *Circulation* 107:1640–1646
109. Wu KK, Wu TJ, Chin J et al (2005) Increased hypercholesterolemia and atherosclerosis in mice lacking both ApoE and leptin receptor. *Atherosclerosis* 181:251–259
110. Taleb S, Herbin O, Ait-Oufella H et al (2007) Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. *Arterioscler Thromb Vasc Biol* 27:2691–2698
111. Pagotto U, Marsicano G, Cota D et al (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 27:73–100
112. D'Eon TM, Pierce KA, Roix JJ et al (2008) The role of adipocyte insulin resistance in the pathogenesis of obesity-related elevations in endocannabinoids. *Diabetes* 57:1262–1268
113. Matias I, Gonthier MP, Orlando P et al (2006) Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 91:3171–3180
114. Starowicz KM, Cristino L, Matias I et al (2008) Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. *Obesity (Silver Spring)* 16:553–565
115. Despres JP, Lemieux I, Bergeron J et al (2008) Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol* 28:1039–1049
116. Bluher M, Engeli S, Kloting N et al (2006) Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 55:3053–3060
117. Cote M, Matias I, Lemieux I et al (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)* 31:692–699
118. Spoto B, Fezza F, Parlongo G et al (2006) Human adipose tissue binds and metabolizes the endocannabinoids anandamide and 2-arachidonoylglycerol. *Biochimie* 88:1889–1897
119. Ravinet Trillou C, Amone M, Delgorge C et al (2003) Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 284:R345–R353
120. Ravinet Trillou C, Delgorge C, Menet C et al (2004) CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int J Obes Relat Metab Disord* 28:640–648
121. Osei-Hyiaman D, Liu J, Zhou L et al (2008) Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* 118:3160–3169
122. Tedesco L, Valerio A, Cervino C et al (2008) Cannabinoid type 1 receptor blockade promotes mitochondrial biogenesis through endothelial nitric oxide synthase expression in white adipocytes. *Diabetes* 57:2028–2036
123. Yan ZC, Liu DY, Zhang LL et al (2007) Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-delta. *Biochem Biophys Res Commun* 354:427–433

124. Nissen SE, Nicholls SJ, Wolski K et al (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* 299:1547–1560
125. Van Gaal LF, Scheen AJ, Rissanen AM et al (2008) Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. *Eur Heart J* 29:1761–1771
126. Kunos G, Osei-Hyiaman D, Batkai S et al (2008) Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci* 30:1–7
127. Nogueiras R, Veyrat-Durebex C, Suchanek PM et al (2008) Peripheral, but not central, CB1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. *Diabetes* 57:2977–2991
128. Rosenstock J, Hollander P, Chevalier S et al (2008) SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naive Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naive type 2 diabetes. *Diabetes Care* 31:2169–2176
129. Karsak M, Gaffal E, Date R et al (2007) Attenuation of allergic contact dermatitis through the endocannabinoid system. *Science* 316:1494–1497
130. Wright KL, Duncan M, Sharkey KA (2007) Cannabinoid CB(2) receptors in the gastrointestinal tract: a regulatory system in states of inflammation. *Br J Pharmacol* 153:263–270
131. Batkai S, Rajesh M, Mukhopadhyay P et al (2007) Decreased age-related cardiac dysfunction, myocardial nitrative stress, inflammatory gene expression, and apoptosis in mice lacking fatty acid amide hydrolase. *Am J Physiol Heart Circ Physiol* 293:H909–H918
132. Berdyshev EV, Boichot E, Germain N et al (1997) Influence of fatty acid ethanolamides and delta9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. *Eur J Pharmacol* 330:231–240
133. Hoareau L, Buyse M, Festy F et al (2009) Anti-inflammatory effect of palmitoylethanolamide on human adipocytes. *Obesity (Silver Spring)* 17:431–438
134. Maccarrone M, Bari M, Menichelli A et al (1999) Anandamide activates human platelets through a pathway independent of the arachidonate cascade. *FEBS Lett* 447:277–282
135. MacCarrone M, Bari M, Menichelli A et al (2001) Human platelets bind and degrade 2-arachidonoylglycerol, which activates these cells through a cannabinoid receptor. *Eur J Biochem* 268:819–825
136. Dol-Gleizes F, Paumelle R, Visentin V et al (2009) Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 29:12–18
137. Sugamura K, Sugiyama S, Nozaki T et al (2009) Activated endocannabinoid system in coronary artery disease and anti-inflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* 119:28–36
138. Montecucco F, Matias I, Lenglet S et al (2009) Regulation and possible role of endocannabinoids and related mediators in hypercholesterolemic mice with atherosclerosis. *Atherosclerosis (in press)*
139. Steffens S, Veillard NR, Arnaud C et al (2005) Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 434:782–786
140. Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 6:508–519
141. Di Marzo V (2008) Play an ADAGIO with a STRADIVARIUS: the right patient for CB1 receptor antagonists? *Nat Clin Pract Cardiovasc Med* 5:610–612
142. Gerthoffer WT (2007) Mechanisms of vascular smooth muscle cell migration. *Circ Res* 100:607–621
143. Lusis AJ (2000) Atherosclerosis. *Nature* 407:233–241
144. Jiang LS, Pu J, Han ZH et al (2009) Role of activated endocannabinoid system in regulation of cellular cholesterol metabolism in macrophages. *Cardiovasc Res* 81:805–813
145. Braunersreuther V, Mach F, Steffens S (2007) The specific role of chemokines in atherosclerosis. *Thromb Haemost* 97:714–721
146. Miller AM, Stella N (2008) CB(2) receptor-mediated migration of immune cells: it can go either way. *Br J Pharmacol* 153:299–308
147. Freeman-Anderson NE, Pickle TG, Netherland CD et al (2008) Cannabinoid (CB2) receptor deficiency reduces the susceptibility of macrophages to oxidized LDL/oxysterol-induced apoptosis. *J Lipid Res* 49:2338–2346
148. Kockx MM, Herman AG (2000) Apoptosis in atherosclerosis: beneficial or detrimental? *Cardiovasc Res* 45:736–746
149. Liu J, Thewke DP, Su YR et al (2005) Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. *Arterioscler Thromb Vasc Biol* 25:174–179
150. Libby P, Geng YJ, Aikawa M et al (1996) Macrophages and atherosclerotic plaque stability. *Curr Opin Lipidol* 7:330–335
151. Reinhard W, Stark K, Neureuther K et al (2008) Common polymorphisms in the cannabinoid CB2 receptor gene (CNR2) are not associated with myocardial infarction and cardiovascular risk factors. *Int J Mol Med* 22:165–174
152. Thomas BF, Gilliam AF, Burch DF et al (1998) Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J Pharmacol Exp Ther* 285:285–292
153. Rajesh M, Mukhopadhyay P, Batkai S et al (2007) Cannabidiol attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption. *Am J Physiol Heart Circ Physiol* 293:H610–H619
154. Mechoulam R, Peters M, Murillo-Rodriguez E et al (2007) Cannabidiol—recent advances. *Chem Biodivers* 4:1678–1692
155. Galiegue S, Mary S, Marchand J et al (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 232:54–61
156. Bensaid M, Gary-Bobo M, Esclançon A et al (2003) The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 63:908–914
157. Juan-Pico P, Fuentes E, Javier Bermudez-Silva F et al (2006) Cannabinoid receptors regulate Ca(2+) signals and insulin secretion in pancreatic beta-cell. *Cell Calcium* 39:155–162
158. Carlisle SJ, Marciano-Cabral F, Staab A et al (2002) Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int Immunopharmacol* 2:69–82
159. Coopman K, Smith LD, Wright KL et al (2007) Temporal variation in CB2R levels following T lymphocyte activation: evidence that cannabinoids modulate CXCL12-induced chemotaxis. *Int Immunopharmacol* 7:360–371
160. Ghosh S, Preet A, Groopman JE et al (2006) Cannabinoid receptor CB(2) modulates the CXCL12/CXCR4-mediated chemotaxis of T lymphocytes. *Mol Immunol* 43:2169–2179
161. Kurihara R, Tohyama Y, Matsusaka S et al (2006) Effects of peripheral cannabinoid receptor ligands on motility and polarization in neutrophil-like HL60 cells and human neutrophils. *J Biol Chem* 281:12908–12918
162. Deusch E, Kress HG, Kraft B et al (2004) The procoagulatory effects of delta-9-tetrahydrocannabinol in human platelets. *Anesth Analg* 99:1127–1130 table of contents