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REVIEW

Cannabinoid receptors in acute and chronic complications of atherosclerosis

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Atherosclerosis is a chronic inflammatory disease that is the primary cause of myocardial infarction and stroke, which occur after sudden thrombotic occlusion of an artery. A growing body of evidence suggests that cannabinoid signalling plays a fundamental role in atherosclerosis development and its clinical manifestations. Thus, CB_2 receptors are protective in myocardial ischaemia/reperfusion and implicated in the modulation of chemotaxis, which is crucial for the recruitment of leukocytes during inflammation. Delta-9-Tetrahydrocannabinol (THC)-mediated activation has been shown to inhibit atherosclerotic plaque progression in a CB_2 dependent manner. Although CB_1 and CB_2 expression has been reported on platelets, their involvement in thrombus formation is still controversial. While several reports suggest that CB_1 receptors may have a relevant role in neuroprotection after ischaemic stroke, recent studies show the protective effects in various forms of neuroprotection are not related to CB_1 stimulation, and a protective role of CB_1 blockade has also been reported. In addition, vascular and myocardial CB_1 receptors contribute to the modulation of blood pressure and heart rate. It is tempting to suggest that pharmacological modulation of the endocannabinoid system is a potential novel therapeutic strategy in the treatment of atherosclerosis. For these purposes, it is important to better understand the complex mechanisms of endocannabinoid signalling and potential consequences of its pharmacological modulation, as it may have both pro- and anti-atherosclerotic effects.
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Abbreviations: ICAM-1, intercellular adhesion molecule-1; I/R, ischaemia/reperfusion; THC, delta-9-tetrahydrocannabinol

Introduction

Atherosclerosis is an inflammatory disease characterized by arterial lesions containing cholesterol, immune infiltrates and connective-tissue elements (Libby, 2002; Osterud and Bjorklid, 2003; Hansson, 2005; Hansson and Libby, 2006). It is responsible for major mortality causes, that is, ischaemic heart disease and cerebrovascular disease. Prevention and current treatments for atherosclerosis are mainly based on drugs that lower plasma cholesterol concentration and high blood pressure. In particular, statins have proven to reduce cardiovascular events significantly, not only by their cholesterol-lowering properties but also by their more recently identified anti-inflammatory and immunomodulatory effects (Mach, 2004). Nevertheless, atherosclerosis remains the primary cause of heart disease and stroke, accounting for up to 50% of deaths in the Western countries. The identification and development of potential promising novel anti-inflammatory therapies is thus of great interest for the medical community.

Cannabinoids such as delta-9-tetrahydrocannabinol (THC) modulate immune functions and therefore have a therapeutic potential for the treatment of inflammatory diseases (Klein, 2003, 2005). It is thought that the immunomodulatory effects of cannabinoids are mediated by CB_2 receptors expressed on immune cells. A growing body of evidence suggests that endocannabinoid signalling plays a critical role in the pathogenesis of atherogenesis and its clinical manifestations (Figure 1). We have recently provided the first experimental evidence for a possible role of CB_2 receptors in atherosclerosis progression (Steffens *et al.*, 2005). Using an experimental mouse model of atherosclerosis, oral administration of THC resulted in significant inhibition of plaque development, an effect that could be reversed by the CB_2 antagonist SR144528. In addition, cannabinoids are known to exhibit complex cardiovascular actions, although the findings are in part controversial due to different species

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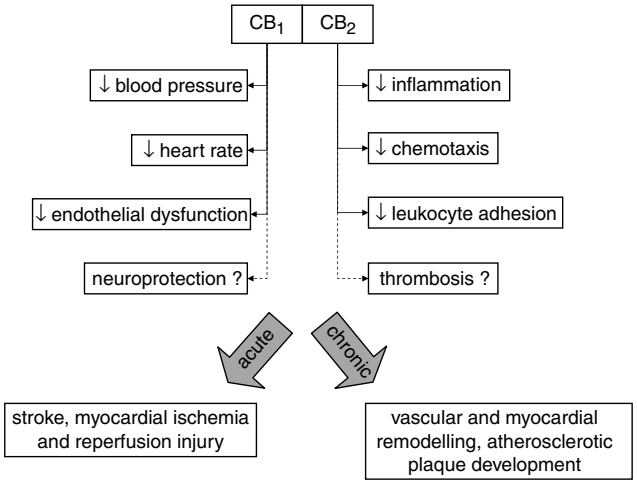

Figure 1 Cannabinoid signalling via CB_1 and CB_2 receptors mediates differential cardiovascular effects, suggesting their implication in acute and chronic complications of atherosclerosis.

and methodologies. The predominant experimental evidence indicates that CB_2 receptor activation via endocannabinoids is protective in myocardial ischaemia/reperfusion (I/R), an acute complication of atherosclerosis (Lagneux and Lamontagne, 2001; Joyeux *et al*., 2002; Lépicier *et al*., 2003; Di Filippo *et al*., 2004). On the other hand, CB_1 receptors contribute to the modulation of blood pressure and heart rate, thus representing an attractive target for therapeutic intervention to reduce cardiovascular risk factors such as hypertension (Bátkai *et al*., 2004; Pacher *et al*., 2005a, b; Mendizábal and Adler-Graschinsky, 2007).

Atherosclerosis and inflammation

Early atherosclerosis is characterized by endothelial dysfunction enhanced by many risk factors. Beyond genetic risk factors, hyperlipidaemia, diabetes, hypertension, obesity and smoking are the main cardiovascular risk factors, which enhance endothelial injury (Lusis, 2000; Glass and Witztum, 2001). Under normal conditions, endothelial cells inhibit platelet and leukocyte adhesion to the vascular surface. In the setting of unfavourable risk factors, an inflammatory response is initiated in the artery wall. The endothelial inflammatory response induces the surface expression of several types of leukocyte adhesion molecules and secretion of chemoattractant molecules (Braunersreuther and Mach, 2006; Hansson and Libby, 2006). For example, the chemokine monocyte chemoattractant protein-1 /CCL2 was found at high expression levels within atherosclerotic lesions, and thus considered as a key player in monocyte recruitment into the arterial wall (Nelken *et al*., 1991; Ylä-Herttuala *et al*., 1991; Yu *et al*., 1992). Other CC family chemokines such as macrophage inflammatory protein-1α /CCL3, macrophage inflammatory protein-1β/CCL4, regulated on activated normal T-cell expressed and secreted/CCL5, as well as a number of more recently discovered chemokines have also been detected in atherosclerotic lesions (Schecter *et al*., 2000; von Hundelshausen *et al*., 2001; Veillard *et al*., 2004).

The local production of chemokines, chemokine receptors and adhesion molecules from activated endothelial cells and inflammatory cells causes leukocyte rolling along the vascular surface and cell adhesion at the site of activation (Braunersreuther and Mach, 2006). Endothelial dysfunction occurs preferentially at the sites of haemodynamic strain. Leukocytes recruited to the subendothelial space secrete chemokines and cytokines, thus promoting the ongoing chronic inflammatory process. This results in the presence of a large number of inflammatory and immune cells within atherosclerotic lesions.

Besides the regulation of leukocyte trafficking during inflammation, chemokines are also involved in the activation of platelets (Abi-Younes *et al*., 2000; Kowalska *et al*., 2000). Platelets are anucleated cellular fragments that circulate in the blood. In addition to their well-recognized role in haemostasis and acute thrombus formation, platelets are also thought to have proinflammatory and growth-regulatory properties that contribute to progression of atherosclerosis (Gawaz *et al*., 2005; Nieswandt *et al*., 2005; Weber, 2005). Platelet activation releases multiple growth factors and inflammatory mediators, including chemokines, such as platelet factor 4/CXCL4) and regulated on activated normal T cell expressed and secreted into the microenvironment (Scheuerer *et al*., 2000; Weber *et al*., 2004; Weber, 2005).

At more advanced stages of atherosclerosis, the release of proinflammatory cytokines and chemokines promotes the proliferation and migration of smooth-muscle cells from the media to the intima. Within the intima, smooth-muscle cells secrete extracellular matrix components, leading to the accumulation of collagen and proteoglycans, key factors implicated in plaque stability (Newby, 2005). Conversely, the secretion of matrix metalloproteinases by vascular and inflammatory cells degrades matrix components such as collagen, gelatin or elastin within atherosclerotic lesions. Depending on the stability of the lesion, the plaque may rupture and induce thrombosis, leading to acute vascular events such as myocardial infarction or stroke.

Role of cannabinoids in acute complications of atherosclerosis

Generally, the acute complications of atherosclerosis are due to the sudden thrombotic occlusion of an artery (Libby and Aikawa, 2002). Physical disruption of the plaque is the most frequent cause of thrombotic occlusion (Libby and Aikawa, 2002). The pathophysiology of this process is mainly regulated by endothelial cells, macrophages, T cells, mast cells and platelets, which produce cytokines and proteases, responsible for conferring to the plaque the susceptibility to rupture and consequent thrombosis (Hansson and Libby, 2006). In areas without collateral vessels (mainly heart and brain), thrombi arising from ruptured plaque can cause infarction of the tissues, with devastating clinical consequences (Hansson and Libby, 2006).

Synthetic and endogenous cannabinoids are known to exhibit complex cardiovascular actions as described in a vast number of *in vitro* and *in vivo* studies, with both increases and decreases in blood pressure being reported (Pacher *et al*., 2006). Clearly, the cardiovascular cannabinoid effects

strongly depend on the pharmacology of the compound, the way and frequency of administration, the vehicle, use of anaesthesia, as well as the experimental *in vitro* model or species used. Given these investigative limitations, it is difficult to draw firm conclusions for a therapeutic use in humans. Nevertheless, synthetic and endogenous cannabinoids may represent an emerging target for pharmacotherapy for reducing acute cardiovascular complications such as stroke and acute myocardial infarction in multiple risk factor patients (Pacher *et al*., 2006).

Cardiovascular effects of cannabinoids

The acute administration of cannabinoids in humans is typically associated with tachycardia (Beaconsfield, 1974; Kanakis *et al*., 1979; Hollister, 1986) and increased supine blood pressure (Jones, 2002), but also marked orthostatic hypotension were observed (Mathew *et al*., 1992; Jones, 2002; Sidney, 2002). In contrast, acute administration of cannabinoids in laboratory animals is accompanied by bradycardia and hypotension (Fredericks *et al*., 1981; Dewey, 1986). Prevention of endocannabinoid anandamide degradation by an inhibitor of fatty-acid amide hydrolase was shown to lower blood pressure and heart rate in hypertensive rodents through reductions in both cardiac contractility and vascular resistance (Bátkai *et al*., 2004). These effects were prevented by CB_1 antagonists. Fatty acid amide hydrolase-knockout mice, however, have normal blood pressure and cardiac function, indicating that under normal conditions anandamide does not play a major role in cardiovascular regulation (Pacher *et al*., 2005c). Consistent with these observations, THC inhalation was found to result in a greater and longer lasting decrease of arterial blood pressure in hypertensive as compared with normotensive individuals (Crawford and Merritt, 1979). These findings suggest that the endocannabinoid system represents a therapeutic target for treatment of hypertension (Pacher *et al*., 2005a).

The differences between humans and animals may be due to at least three different reasons. First, methodological differences in drug administration do not allow a direct comparison of human and animal data. The data on cardiovascular effects in humans mostly refer to marijuana smokers, whereas cannabinoid administration to animals is performed systemically or orally. Second, human populations are usually limited to only young men who represent the predominant population of marijuana smokers, while there are only a few data on older people or women (Mittleman *et al*., 2001; Jones, 2002). Similarly, animal experiments are usually performed with young male populations. Third, the doses of THC or cannabinoid given in the animal experiences are probably too high and do not reflect the concentrations found in humans after drug exposure (Dewey, 1986; Jones, 2002).

Stroke

Ischaemic stroke is caused by a transient interruption of blood flow to the brain, due to a thrombotic acute occlusion of the blood vessels. It represents one of the most important causes of death and disability in the industrialized countries (Klijn and Hankey, 2003; Pinto *et al*., 2004). Several *in vitro* and *in vivo* models of cerebral ischaemia have been developed to identify effective agents against atherosclerotic plaque rupture, the consequent thrombus formation and neurodegeneration.

There is a general consensus on considering cannabinoids as neuroprotective agents. (R +)-WIN 55,212-2, anandamide and 2-AG were found capable of protecting *in vitro* cultured rat neurons against hypoxia and glucose deprivation (Nagayama *et al*., 1999; Sinor *et al*., 2000). CB_1, which is more highly expressed in the brain compared to CB_2, is thought to be crucial for neuroprotection (Galve-Roperh *et al*., 2007). Indeed, alterations of CB_1 receptor expression levels were found to be associated with neuroprotection (Galve-Roperh *et al*., 2007; Hayakawa *et al*., 2007). In different animal models of brain injury and neurodegenerative diseases, endocannabinoid signalling was implicated in the regulation of microglial cell and neuron proliferation and differentiation. After stroke, CB_1 receptor agonists increase the production of neurotrophic factors in mouse neural progenitors (Aguado *et al*., 2007). In order to investigate the underlying molecular mechanisms of CB_1-dependent neuroprotection, Panikashvili *et al*. (2005) showed the involvement of nuclear factor-kappa B inhibition, key regulator of the inflammatory response after brain injury. In this interesting study, the authors showed that activation of nuclear factor-kappa B in injured CB_1-knockout mouse brain was three to fourfolds higher than in the respective non-injured CB_1 knockout mice. Exogenous 2-AG treatment significantly improved neurobehavioral function in wild-type mice after brain injury, whereas no improvement was observed in CB_1 knockout mice. While one study suggested a comparable role for endogenous and exogenous cannabinoids in neuroprotection (Parmentier-Batteur *et al*., 2002), the study by Panikashvili and co-workers showed that only the exogenous compound was neuroprotective. As mentioned previously, the dose and route of cannabinoid administration might strongly affect the results obtained in various experimental designs and explain the discrepant findings.

In addition, the neuroprotective effects of other cannabinoids, such as dexanabinol (HU-211; 4–4.5 $mg\,kg^{-1}$) (Bar-Joseph *et al*., 1994; Leker *et al*., 1999; Lavie *et al*., 2001), WIN 55,212-2 (0.03–1 $mg\,kg^{-1}$) (Nagayama *et al*., 1999), BAY38-7271 (0.1 $\mu g\,kg^{-1}$) (Mauler *et al*., 2003), HU-210 (45 $\mu g\,kg^{-1}$) (Leker *et al*., 2003), as well as 2-AG (1 $mg\,kg^{-1}$) administered together with 2-palmitoyl-glycerol (5 $mg\,kg^{-1}$) and 2-linoeoyl-glycerol (10 $mg\,kg^{-1}$) (Panikashvili *et al*., 2001), have been demonstrated in various animal models of ischaemic, traumatic or compressive brain injuries. Finally, it should be noted that several studies contradict the neuroprotective role of CB_1 receptor activation, but support the protective role of CB_1 blockade (Berger *et al*., 2004; Muthian *et al*., 2004; Sommer *et al*., 2006).

Besides the controversially discussed role of CB_1 in neuroprotection, CB_2 activation was recently shown as being crucial in microglial cell proliferation and migration in ischaemic brain areas (Carrier *et al*., 2004; Ashton *et al*., 2007). The reactivity of these phagocytozing and antigen-presenting cells in the central nervous system is decreased by

CB_2 activation, which may help to reduce neuronal death in response to microglial macrophage infiltration.

On the other hand, the role of cannabinoids on intra-arterial thrombus formation is still controversial, although both CB_1 and CB_2 are known to be expressed on human platelets (Deusch *et al*., 2004). One study reported an inhibiting effect of high concentrations of THC ($\geqslant 10^{-5}$ M) on agonist-induced human and rabbit platelet aggregation (Formukong *et al*., 1989). Other studies found increased spontaneous aggregate formation in the presence of THC at similar concentrations, suggesting a procoagulatory role (Levy *et al*., 1976; Deusch *et al*., 2004). It is also important to note that the function of other cell types involved in thrombus formation (mainly leukocytes and endothelial cells) is modulated by cannabinoids. It is well known that cannabinoids modulate inflammatory cytokine secretion and chemotaxis of immune cells, such as macrophages, microglial cells and T lymphocytes (Sacerdote *et al*., 2000; Klein *et al*., 2003; Sacerdote *et al*., 2005; Ghosh *et al*., 2006; Coopman *et al*., 2007). The implication of CB_2 receptors in leukocyte/endothelial interaction and their expression on platelets strongly suggests their crucial role in thrombus formation.

Acute myocardial infarction

Ischaemic heart disease is the leading cause of death among patients in the United States, Europe and the world (Murray and Lopez, 1997). Therefore, the acute coronary syndrome burden has to be considered as a central field for medical research. Acute myocardial infarction is caused by the coronary microvascular obstruction after plaque rupture, with the interruption of blood flow and the consequent ischaemia and necrosis of myocardium. The mechanisms underlying this acute event and the subsequent reperfusion injury, caused by the treatment of coronary artery occlusion (coronary interventions, focused to re-establish the blood flow to the myocardium), are subject of ongoing research. Various reports have highlighted a role for the endocannabinoid system in the physiopathology of I/R myocardial injury. However, a substantial difference between humans and animals has been reported. Several reports indicate an association of chronic THC intake and elevated risk of myocardial infarction in humans (Mittleman *et al*., 2001). Marijuana smoking in people with cardiovascular disease poses increased health risks (Jones, 2002) due to the increased cardiac work, catecholamine levels, carboxyhemoglobin and postural hypotension (Jones, 2002). To date, no studies investigating the role of cannabinoids in post-ischaemic reperfusion injury in humans are reported.

Conversely, in animals, cannabinoids have been shown to reduce I/R injuries. The endocannabinoid system was found to be implicated in the mechanisms by which lipopolysaccharide limits infarct size, mortality and preconditioning against myocardial I/R injury (Lagneux and Lamontagne, 2001). In this study, the authors showed that pretreatment with the CB_2 antagonist SR144528 abolished the lipopolysaccharide-mediated cardioprotective effects. In support of these findings, a different study performed with isolated rat hearts obtained similar results (Joyeux *et al*., 2002). In addition, Di Filippo *et al*. (2004) showed that WIN55,212-2 significantly reduced the extent of infarct size in a mouse model of myocardial I/R. This effect was abolished by the CB_2 antagonist AM630. In another study, anandamide and HU-210 both decreased the incidence of ventricular arrhythmias and reduced the infarct size through activation of CB_2 receptors (Krylatov *et al*., 2001). In conclusion, numerous animal studies suggest that cannabinoids are cardioprotective in experimental models by activation of CB_2 receptors. If similar mechanisms of cannabinoid actions might apply to acute myocardial ischaemia in humans needs to be investigated.

Only few studies have shown a possible role of CB_1 receptors in I/R injuries. In ischaemic preconditioning, which mediated preservation of endothelium dependent vasodilation in isolated rat hearts, either CB_1 or CB_2 receptor blockade abolished the protective effect of preconditioning (Bouchard *et al*., 2003). In the same model, the selective CB_1 agonist ACEA and the CB_2 agonist JWH-015 both reduced the infarct size after I/R (Lépicier *et al*., 2003). Another study reported that the anandamide-induced reduction of infarct size in isolated perfused rat hearts was equally antagonized by CB_1 and CB_2 antagonists (Underdown *et al*., 2005).

The various studies suggest distinct roles for CB_1 and CB_2 receptors in the I/R syndrome. CB_1 receptors are probably involved in coronary vasodilation (Bátkai *et al*., 2004), while CB_2 receptors orchestrate leukocyte recruitment, responsible for the reperfusion injury in the infarcted myocardium (Sacerdote *et al*., 2000, 2005; Di Filippo *et al*., 2004; Ni *et al*., 2004; Ghosh *et al*., 2006; Lunn *et al*., 2006; Coopman *et al*., 2007). In conclusion, a broad body of evidence indicates that cannabinoids (mainly endocannabinoids) protect against myocardial I/R injury in animal models, predominantly via activating CB_2 receptors.

Role of cannabinoid receptors in chronic complications of atherosclerosis

A growing body of evidence suggests that endocannabinoid signalling plays a critical role in the pathogenesis of atherogenesis and its clinical manifestations. We have recently provided a first experimental evidence for a possible role of CB_2 receptors in atherosclerotic plaque progression (Steffens *et al*., 2005). Moreover, an increasing number of studies reported the modulation of endocannabinoid levels, receptors and related enzymes of biosynthesis and degradation in different inflammatory conditions. This knowledge evolved the concept of blocking endocannabinoid signalling with selective receptor antagonists for therapeutic use. Rimonabant is the first CB_1 antagonist studied and approved as anti-obesity drug in Europe and is under review in the United States. Large randomized trials with rimonabant have demonstrated efficacy in treatment of obesity (Després *et al*., 2005; Van Gaal *et al*., 2005). In addition, multiple other cardiometabolic parameters were improved in the treatment groups, including increased levels of high-density lipoprotein cholesterol and reduced triglycerides, as well as improved glycemic control in prediabetic patients and in type 2 diabetic patients (Gelfand and Cannon, 2006). This

novel medication may become an important therapeutic option to reduce cardiovascular risk factors; however, long-term clinical trials (which are ongoing) are warranted to determine if the improvement in metabolic parameters translates into improved morbidity and mortality and to reveal the rates of potential adverse effects.

Involvement of the endocannabinoid system in pathophysiological conditions

The first study to implicate the endocannabinoid system in a pathophysiological disorder was performed in a rat model of haemorrhagic shock (Wagner *et al*., 1997). In a more recent study, the levels of anandamide and 2-AG were found to increase in sera of patients with endotoxic shock (Wang *et al*., 2001). Activation of CB_1 receptors by endocannabinoids secreted from platelets and/or macrophages appear to be responsible for the hypotension accompanying haemorrhagic or endotoxic shock (Varga *et al*., 1998; Liu *et al*., 2003). Consistently, endocannabinoid signalling via vascular CB_1 receptors has been implicated in the vasodilated state in advanced liver cirrhosis (Bátkai *et al*., 2001), while activation of cardiac CB_1 receptors by endogenous anandamide contributes to the reduced cardiac contractility associated with this condition (Bátkai *et al*., 2007a). In a mouse model of liver I/R injury, hepatic anandamide and 2-AG levels are significantly increased, and CB_2 has a protective role (Bátkai *et al*., 2007b). Moreover, both CB_1 and CB_2 receptors have been implicated in liver fibrosis, promoting both pro- and antifibrogenic effects (Julien *et al*., 2005; Teixeira-Clerc *et al*., 2006). Endocannabinoid signalling has also been reported in periodontal inflammation, as both cannabinoid receptors CB_1 and CB_2 as well as anandamide were upregulated under pathological conditions (Nakajima *et al*., 2006). In a mouse model of colonic inflammation, blocking of endocannabinoid signalling with selective receptor antagonists revealed that CB_1 receptors mediate intrinsic protective signals that counteract proinflammatory responses (Massa *et al*., 2004). A very recent report demonstrates a crucial role of the endocannabinoid system in controlling cutaneous contact hypersensitivity (Karsak *et al*., 2007). Various experimental studies have targeted the endocannabinoid system for the treatment of multiple sclerosis. Using the experimental autoimmune encephalomyelitis model, Maresz *et al*. (2007) recently demonstrated a differential role of CB_1 and CB_2 in the central nervous system autoimmune inflammation. While CB_1 on neurons was responsible for THC-mediated suppression of neuronal symptoms, endocannabinoid activation of CB_2 on effector T cells was crucial for controlling inflammation. Very recent findings further support the protective role of endocannabinoids in inflammatory disorders such as atherosclerosis. Enhanced anandamide levels in mice lacking fatty acid amide hydrolase were protective against age-associated decline in cardiac function, inflammation, oxidative/nitrative stress and apoptosis (Bátkai *et al*., 2007c).

Modulation of leukocyte/endothelial cell interaction

The recruitment of inflammatory cells (mainly monocytes and T lymphocytes) in the intima is an essential step in the development and progression of atherosclerosis. The tethering, rolling, adhesion and transendothelial migration of leukocytes are triggered by local production of chemokines and chemokine receptors as well as adhesion molecules (Braunersreuther and Mach, 2006). It has been reported that treatment of rat macrophages with CP55,940 reduced both spontaneous and formyl–methionyl–leucine–phenylalanine-induced chemotaxis (Sacerdote *et al*., 2000). The non-psychoactive marijuana component cannabidiol was shown to inhibit murine macrophage chemotaxis *in vitro* and *in vivo* in a CB_2 receptor-dependent manner (Sacerdote *et al*., 2005). In a different study, cannabidiol attenuated the high glucose-induced transendothelial migration of THP-1 monocytes, and monocyte-endothelial adhesion in human coronary artery endothelial cells, as well as the disruption of the endothelial barrier function (Rajesh *et al*., 2007a). Moreover, cannabidiol attenuated the high glucose-induced mitochondrial superoxide generation, nuclear factor-kappa B activation, nitrotyrosine formation as well as upregulation of inducible nitric oxide synthase and adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule. The attenuation of all these high glucose-induced effects via cannabidiol was independent of CB_1 and CB_2 receptors. These data are particularly relevant to atherosclerosis, since endothelial dysfunction is a crucial event in the initiation and progression of this pathogenesis.

Both endogenous as well as synthetic agonists 2-AG, CP55,940 and WIN55,212-2, as well as the CB_2-selective agonists JWH-015 and JWH-133, caused a significant inhibition of the chemokine CXCL12-induced and CXCR4-mediated chemotaxis of Jurkat or primary human T cells (Ghosh *et al*., 2006; Coopman *et al*., 2007). In addition, in an experimental autoimmune encephalomyelitis mouse model, WIN 55,212-2 attenuated leukocyte rolling and adhesion on endothelial cells through the activation of CB_2 receptors (Ni *et al*., 2004). Furthermore, the novel CB_2-selective inverse agonist Sch.336 potently inhibited leukocyte chemotaxis to 2-AG, HU-210 or monocyte chemoattractant protein-11/CCL2 *in vitro* and *in vivo* (Lunn *et al*., 2006). In a mouse model of liver I/R injury, the CB_2-selective agonist JWH-133 protected against I/R damage by decreasing inflammatory cell infiltration, tissue and serum tumour necrosis factor-α, macrophage inflammatory protein-1α/CCL3 and macrophage inflammatory protein-2/CXCL2 levels, and expression of ICAM-1 (Bátkai *et al*., 2007b). *In vitro*, JWH-133 was shown to attenuate the tumour necrosis factor-α-induced ICAM-1 and vascular cell adhesion molecule expression in human liver sinusoidal endothelial cells and the adhesion of human neutrophils to human liver sinusoidal endothelial cells. Similar results were obtained with the CB_2-selective agonist HU-308 (Rajesh *et al*., 2007c). In a different study, anandamide dose dependently attenuated the tumour necrosis factor-α-induced ICAM-1 and vascular cell adhesion molecule expression in human coronary artery endothelial cells, and the adhesion of THP-1 monocytes to human coronary artery endothelial cells in a CB_1- and CB_2-dependent manner (Bátkai *et al*., 2007b). Likewise, the two CB_2-selective agonists JWH-133 and HU-308 inhibited the tumour necrosis factor-α-induced nuclear factor-kappa B and RhoA activation, upregulation of adhesion molecules ICAM-1 and vascular

cell adhesion molecule, increased expression of monocyte chemoattractant protein-11, enhanced transendothelial migration of THP-1 monocytes and augmented monocyte–endothelial adhesion (Rajesh *et al*., 2007b). Finally, we have shown that THC inhibited murine peritoneal macrophage chemotaxis in response to monocyte chemoattractant protein-11/CCL2 and reduced the expression of the chemokine receptor CCR2 on splenocytes (Steffens *et al*., 2005). These effects were blocked by the CB_2 receptor antagonist SR144528 or when cells from CB_2-knockout mice were used.

CB_2-dependent effects of THC on atherosclerotic lesion progression
Based on the well-known immunomodulatory properties of cannabinoids, we tested the anti-atherosclerotic potential of THC in a murine model and found that THC inhibited progression of established atherosclerotic lesions (Steffens *et al*., 2005). The anti-atherosclerotic effect was associated with reduced proliferation and interferon-γ secretion of lymphoid cells, as well as reduced macrophage infiltration into atherosclerotic lesions. Moreover, we detected CB_2 receptor expression on macrophages and T lymphocytes within human and mouse atherosclerotic lesions. These findings are promising; however, it is not possible to draw firm conclusions about the potential therapeutic effects in humans. Additional *in vitro* experiments with human vascular cells and clinical data are required to examine the underlying mechanisms of cannabinoid actions in humans.

Post-ischaemic heart failure
Heart failure occurs when the heart is unable to supply sufficient blood flow and thus oxygen delivery to the periphery. Besides coronary artery disease, a major cause of heart failure is myocardial infarction. Non-infarcted regions of the myocardium must compensate the lacking activity of the infarcted tissue, which can lead to changes in the morphology and size of the heart. This process known as remodelling may, over time, cause functional changes leading to cardiac failure.

In an attempt to study the long-term effects of endogenous and exogenous cannabinoids on cardiac remodelling and vascular function, Wagner *et al*. (2003) subjected rats to left coronary artery occlusion without reperfusion, followed by a 12-week CB_1 antagonist (AM-251) or HU-210 treatment. Chronic CB_1 antagonism had a deleterious effect on cardiac performance volume in rats, with large myocardial infarction (necrotic area $>40\%$ of left ventricle), suggesting that endocannabinoids prevent cardiac remodelling in this model. This study is in conflict with recently published findings, demonstrating that treatment with the CB_1 antagonist rimonabant or AM-281 markedly improved cardiac dysfunction in doxorubicin-induced cardiotoxicity (Mukhopadhyay *et al*., 2007). A possible explanation for this discrepancy might be the use of low doses of the CB_1 antagonist AM-251 ($0.5\,mg\,kg^{-1}$) in the previously mentioned study (Wagner *et al*., 2003), as well as the different species and methodologies used. In the long-term study, the non-selective cannabinoid agonist HU-210 increased the left ventricular end-diastolic pressure, but prevented hypotension (Wagner *et al*., 2003). In addition, HU-210 reduced endothelial dysfunction, as determined by vasodilator response of isolated aortic rings. Myocardial CB_1 expression was not altered during remodelling. Remarkably, HU-210 appeared to be of benefit after small infarcts ($\leqslant 40\%$), since cardiac index and stroke volume index were increased, while the total peripheral resistance was decreased. This suggests that the benefit of exogenous cannabinoid intervention might be limited by infarct size. In a different report, the same authors show that the cannabinoids anandamide, R-methanandamide and HU-210 decrease contractile performance in human atrial muscle via CB_1 receptors (Bonz *et al*., 2003). Maslov and co-workers studied the effect of HU-210 or cannabinoid receptor antagonist pretreatment on contractility in the isolated Langendorff-perfused rat heart model of I/R. They found that HU-210 transiently increased the degree of reperfusion-induced cardiac contractile dysfunction, whereas endogenous cannabinoids seem not involved in this process (Maslov *et al*., 2006).

Apart from this limited number of studies, little is known about the cannabinoid-mediated effects on cardiac performance. Therefore, further investigations with particular regard to long-term effects are needed to clarify this important aspect.

Concluding remarks

Synthetic and endogenous cannabinoids are known to exhibit complex cardiovascular actions, although the findings in humans and animal models are in part controversial, likely due to methodological and species differences. Over the centuries, the medical use of cannabinoids has been very limited, mainly due to the psychotropic effects associated with marijuana use. Now, synthetic non-psychotropic cannabinoids or pharmacological modulation of the endocannabinoid system may represent an emerging target for reducing acute and chronic cardiovascular complications in multiple risk factor patients. A future challenge for the scientific community is to overcome these experimental limitations in order to better understand the complex cardiovascular effects of cannabinoids in humans. This may allow the precise modulation of the endocannabinoid system via pharmacological intervention, without triggering detrimental cardiovascular side effects.

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Conflict of interest

The authors state no conflict of interest.

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