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REVIEW

Targeting cannabinoid receptor CB_2 in cardiovascular disorders: promises and controversies

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Cardiovascular disease is the leading cause of death and disability worldwide, which can be largely attributed to atherosclerosis, a chronic inflammation of the arteries characterized by lesions containing immune and smooth muscle cells, lipids and extracellular matrix. In recent years, the lipid endocannabinoid system has emerged as a new therapeutic target in variety of disorders associated with inflammation and tissue injury, including those of the cardiovascular system. The discovery that Δ-9-tetrahydrocannabinol (Δ9-THC), the main active constituent of marijuana, inhibited atherosclerotic plaque progression via a cannabinoid 2 (CB_2) receptor-dependent anti-inflammatory mechanism, and that certain natural and synthetic cannabinoid ligands could modulate the myocardial or cerebral ischaemia–reperfusion-induced tissue damage, have stimulated impetus for a growing number of studies investigating the implication of CB_2 receptors in atherosclerosis, restenosis, stroke, myocardial infarction and heart failure. The aim of this review is to update on recent findings and controversies on the role of CB_2 receptors in cardiovascular disease. Particular emphasis will be placed on novel insights in the potential cellular targets of CB_2 stimulation in cardiovascular system (e.g. endothelial and vascular smooth muscle cells, cardiomyocytes, infiltrating and/or resident monocytes/macrophages and leukocytes, etc.), their interplay and intracellular signalling mechanisms identified, as well as on experimental and clinical studies.

Abbreviations

2-AG, 2-arachidonoylglycerol; Δ9-THC, Δ-9-tetrahydrocannabinol; AEA, anandamide; MCP-1/CCL2, monocyte chemotactic protein-1; OEA, oleoylethanolamine; ROS, reactive oxygen species

Introduction

The cannabinoid 2 (CB_2) receptor was first cloned from the human leukaemia cell line HL-60 in 1993 (Munro *et al.*, 1993). It is highly expressed in spleen and immune cells and at low levels in various peripheral tissues under normal physiological conditions (Galiegue *et al.*, 1995). In the cardiovascular system, CB_2 receptor expression has been established in the rat myocardium (Lepicier *et al.*, 2007) and at low levels in human cardiomyocytes (Weis *et al.*, 2010; Mukhopadhyay *et al.*, 2010a), coronary endothelial cells and smooth muscle cells (Rajesh *et al.*, 2007a; 2008). Under pathophysiological conditions such as inflammatory stimulation or tissue injury, increased CB_2 receptor expression levels have been reported in the cardiovascular system, which probably reflects a protective response to limit cell or tissue injury (Pacher and Mechoulam, 2011). For example, up-regulation of CB_2 receptor expression has been described in primary human endothelial and smooth muscle cells stimulated by pro-inflammatory triggers and/or mitogens (Rajesh *et al.*, 2007a; 2008; Ramirez *et al.*, 2012), in human and mouse atherosclerotic plaques (Steffens *et al.*, 2005), neointimal lesions following balloon injury (Molica *et al.*, 2012) and in the myocardium of chronic heart failure patients (Weis *et al.*,

Table 1

Ranges of K_i values for certain cannabinoid CB_1 and/or CB_2 receptor agonists or antagonists/inverse agonists for the *in vitro* displacement of [^{3}H]CP55,940, [^{3}H]HU243 or [^{3}H]BAY38-7271 from CB_1- and CB_2-specific binding sites (reviewed in Pertwee 2005)

Agonis/ligand	CB_1 K_i value (nM)	CB_2 K_i value (nM)	Reference
Mixed (without marked $CB_{1/2}$ selectivity)			
Anandamide	61–543	279–1940	Pertwee (2005)
2-AG	58–472	145–1400	Pertwee (2005)
CP55,940	0.5–5	0.7–2.8	Pertwee (2005)
HU-210	0.06–0.1	0.2–0.5	Pertwee (2005)
R-(+)-WIN 55,212-2	1.9–123	0.3–16	Pertwee (2005)
Δ^9-THC	5–53	3–75	Pertwee (2005)
CB_1 selective agonists			
ACEA	1.4–5.3	195 to >2000	Pertwee (2005)
R-(+)-methanandamide	17.9–28.3	815–868	Pertwee (2005)
CB_2 selective agonists			
JWH015	383	13.8	Pertwee (2005)
JWH133	677	3.4	Pertwee (2005)
O-1966	4071–6039	20.9–25.1	Wiley *et al.* (2002)
O-3853	1361–1657	3.5–8.5	Zhang *et al.* (2007)
HU-308	>10 000	22.7	Pertwee (2005)
HU-910	1400	6	Horvath *et al.* (2012)
Inverse CB_2 agonist/antagonist			
AM630	5152	31.2	Pertwee (2005)
SR144528	50.3 to >10 000	0.28–5.6	Pertwee (2005)
Inverse CB_1 agonist/antagonist			
SR141716A	1.8–11.8	515–13200	Pertwee (2005)
AM251	7.5	2290	Pertwee (2005)
AM281	12	4200	Pertwee (2005)
LY320135	141	14900	Pertwee (2005)

2010). Conversely, CB_2 immunostaining was significantly reduced in carotid artery plaques of stroke versus asymptomatic patients (Montecucco *et al.*, 2011). Therefore, one can speculate that CB_2 signalling is part of a protective response against human plaque vulnerability, which is impaired in patients with acute vascular events. Further clinical studies are warranted to validate this hypothesis. Similarly, down-regulation of CB_2 was described in kidney biopsies from patients with advanced kidney nephropathy, suggesting impaired protective CB_2 regulation that counteracts the deleterious effects of signalling through CB_1 receptors (Barutta *et al.*, 2011), consistently with protective effects of CB_2 receptor agonists in models of experimental nephropathy (Mukhopadhyay *et al.*, 2010b; Barutta *et al.*, 2011).

As to the experimental evidence for an implication of CB_2 in cardiovascular disease, there is a mounting number of studies suggesting a protective role for CB_2 receptors in mouse models of atherosclerosis (Steffens *et al.*, 2005; Netherland *et al.*, 2010; Zhao *et al.*, 2010a,b; Hoyer *et al.*, 2011), restenosis (Molica *et al.*, 2012) and myocardial (Defer *et al.*, 2009; Montecucco *et al.*, 2009) and cerebral ischaemia/reperfusion injury (Zhang *et al.*, 2007; 2008; Pacher and Hasko, 2008; Murikinati *et al.*, 2010; Zarruk *et al.*, 2012).

The selectivity of the endocannabinoids and synthetic ligands used in various cardiovascular and other related disorders towards $CB_{1/2}$ receptors discussed in the following parts is summarized in Table 1 (reviewed in Pertwee, 2005; Pacher and Mechoulam, 2011).

Intracellular signalling of CB_2

CB_2 receptors are GPCRs that exert their biological effects through heterotrimeric $G_{i/0}$-proteins (Klein *et al.*, 2003; Howlett, 2005). Coupling to $G_i\alpha$ subunits inhibits AC and reduction in cAMP accumulation. This leads to decreased PKA activity and reduced phosphorylation of cAMP response-element binding-protein, thus decreasing gene expression (reviewed in Bosier *et al.*, 2010). In addition, reduction of PKA activity causes decrease in constitutive inhibitory phosphorylation of the MAPK cascade (reviewed in Bosier *et al.*, 2010). Simultaneously, CB_2 activation induces members of the

Table 2

Intracellular signalling in vascular and immune cells

Cell type/tissue	Receptor agonist	Intracellular target	Reference
HL-60	2-AG	MEK 1/2 → ERK 1/2, p38, Rho	(Kobayashi *et al.*, 2001; Kishimoto *et al.*, 2003)
Jurkat	JWH015	ERK 1/2	(Ghosh *et al.*, 2006)
Human monocytes	JWH015	Akt, ERK 1/2	(Montecucco *et al.*, 2008)
Human neutrophils	JWH133	ERK 1/2	(Montecucco *et al.*, 2011)
Human coronary artery EC	JWH133, HU-308	NF-κB, Rho	(Rajesh *et al.*, 2007a)
Human sinusoidal EC	JWH133, HU-308, HU-910	NF-κB?	(Batkai *et al.*, 2007; Rajesh *et al.*, 2007b; Horvath *et al.*, 2012)
Human brain EC	JWH133, O-1966	NF-κB?	(Ramirez *et al.*, 2012)
Human coronary artery SMC		Ras, p38 MAPK, ERK 1/2, SAPK/JNK, Akt	(Rajesh *et al.*, 2008)
Mouse heart	JWH133	MEK 1/2 → ERK 1/2, JAK → STAT-3	(Montecucco *et al.*, 2009)

HL-60, human promyelocytic leukaemia cell line; MEK, p44/42 MAPK (kinase activating ERK 1/2); SAPK, stress-activated protein kinase; '?' depicts studies providing indirect evidence on the CB_2-dependent inhibition of NF-κB activation (e.g. inhibition of the expression of various adhesion molecules).

MAPK family through Gβγ, which triggers expression of genes (e.g. involved in cell survival, proliferation or stress response) (reviewed in Klein *et al.*, 2003; Howlett, 2005) (Table 2). A majority of genes induced by CB_2 activation is associated with nuclear translocation of transcription factor NF-κB in promyelocytic cells HL-60 transfected with the CB_2 receptor (Derocq *et al.*, 2000).

In contrast to CB_1, CB_2 receptor activation does not modulate ion channel function (reviewed in Demuth and Molleman, 2006; Bosier *et al.*, 2010). As a result, CB_2 receptor-mediated Ca^{2+} responses are less pronounced (Felder *et al.*, 1995; Zoratti *et al.*, 2003; Rao *et al.*, 2004; Schuehly *et al.*, 2011) than the potent CB_1 receptor-mediated effects on Ca^{2+} fluxes (reviewed in Bosier *et al.*, 2010). In calf pulmonary artery cells, Zoratti *et al.* (2003) observed CB_2-dependent increases in cytosolic Ca^{2+} via activation of PLC and subsequent release from endoplasmic reticulum stores.

In addition to G-protein-dependent signalling, GPCRs can also mediate G-protein-independent signalling that involves receptor phosphorylation as well as interaction with regulatory proteins mediating receptor internalization or acting as scaffolds to modulate G-protein-mediated signalling (reviewed in Bockaert *et al.*, 2004; Ritter and Hall, 2009; Smith *et al.*, 2010). So far, available data about CB_2 receptor modification and trafficking are limited and mainly based on stably CB_2 expressing cells. Bouaboula *et al.* (1999) reported constitutively active, phosphorylated (at serine 352) and internalized CB_2 receptor expression at basal levels in stably transfected CHO cells. The effect was sensitive to the CB_2 antagonist/inverse agonist SR144528, which blocked phosphorylation and up-regulated CB_2 levels at the cell surface. Conversely, CP55,940 agonist treatment resulted in sustained (>8 h) CB_2 receptor phosphorylation. This is in accordance with the data from Derocq *et al.* (2000) reporting CB_2 phosphorylation in response to CP55,940 activation in CB_2-transfected HL-60 cells, which was maximal after 15 min. In naturally CB_2-expressing microglial cells, receptor expression was shown both internalized and at the cell surface in absence of agonist, while agonist stimulation increased receptor internalisation (Carrier *et al.*, 2004). A recent study also provided evidence for marked functional selectivity of cannabinoid receptor internalization, depending on the cannabinoid ligand used (Atwood *et al.*, 2012).

Chronic exposure of CB_1-expressing cells to agonists results in a loss of response when cells are subsequently challenged after drug washout (Breivogel *et al.*, 1999). This adaptation to prolonged drug exposure is known as receptor desensitization and occurs in part due to uncoupling of receptors from their downstream G-proteins and/or effectors (Smith *et al.*, 2010). In cell lines stably transfected with CB_2, chronic endocannabinoid as well as synthetic agonist treatment resulted in CB_2 receptor desensitization and down-regulation, as determined by the capacity to inhibit AC activity and receptor binding assays (Shoemaker *et al.*, 2005). Following internalization in endocytosis vesicles, it is thought that CB_2 receptors are resensitized by dephosphorylation which allows receptor recycling to the cell surface (Bouaboula *et al.*, 1999; Grimsey *et al.*, 2011).

Whether the above described mechanisms of CB_2 trafficking has physiological relevance in primary vascular and peripheral blood immune cells deserve further investigations.

Intracellular targets of CB_2 activation in vascular and immune cells

The regulation of normal vascular endothelial, smooth muscle and cardiomyocyte cell proliferation, survival, growth and differentiation involves extracellular ligands, growth factors and cytokines that bind to cell surface receptors and activate intracellular signal transduction cascades. In cardiovascular diseases, the role of several MAPK signalling

pathways has been recognized, including ERK, JNK and p38 MAPK (Muslin, 2008).

A number of *in vitro* and *in vivo* studies have demonstrated the capacity of CB_2 agonists to interact with signalling pathways induced by other cell surface receptors under pathophysiological/inflammatory conditions, suggesting a cross-talk between individual signal transduction pathways (Table 2). For example, CB_2 receptors have been implicated in the modulation of immune cell migration (reviewed in Miller and Stella, 2008). In particular, monocytes treated with the CB_2 agonist JWH015 showed significantly reduced chemokine-induced migration, associated with reduced expression of corresponding chemokine receptors CCR2 and CCR1 as well as IFN-γ-induced adhesion molecule ICAM-1 induction (Montecucco *et al.*, 2008). JWH015 also cross-desensitized human monocytes for chemokine-induced migration by its own chemoattractant properties. The underlying pathways involved PI3K/Akt and ERK 1/2, but not p38 MAPK.

There is also evidence for CB_2-mediated modulation of endothelial cell responses to the pro-inflammatory cytokine TNF-α. The healthy endothelium which separates blood and vessel wall is a highly selective permeable barrier with anti-adhesive properties. Endothelial injury results in increased permeability and subendothelial lipid accumulation, adhesion molecule up-regulation, release of cytokines and growth factors, and adherence of platelets and monocytes (reviewed in Libby *et al.*, 2011). In human primary coronary artery (Rajesh *et al.*, 2007a), liver sinusoidal (Batkai *et al.*, 2007; Rajesh *et al.*, 2007b; Horvath *et al.*, 2012) and brain endothelial cells (Ramirez *et al.*, 2012) CB_2 activation with selective synthetic agonists attenuated the bacterial endotoxin- or TNF-α-induced NF-κB and RhoA activation (Rajesh *et al.*, 2007a), ICAM-1 and VCAM-1 up-regulation (Batkai *et al.*, 2007; Rajesh *et al.*, 2007a,b; Horvath *et al.*, 2012; Ramirez *et al.*, 2012), CCL2 release as well as transendothelial migration and/or adhesion of THP-1 monocytes or neutrophils (Batkai *et al.*, 2007; Rajesh *et al.*, 2007a,b; Horvath *et al.*, 2012).

Vascular smooth muscle cell migration and proliferation are key events during vascular development, in response to injury and atherosclerosis (reviewed in Gerthoffer, 2007). Within the atherosclerotic plaque, smooth muscle cells together with connective tissue encapsulate the necrotic lipid core, thus protecting the plaque from rupturing (reviewed in Lusis, 2000). On the other hand, smooth muscle cells, like macrophages, can express a variety of receptors for lipid uptake and can form foam cells, thereby participating in the cellular accumulation of lipids within plaques. They may also contribute to monocyte recruitment through expression of cytokines and adhesion molecules (reviewed in Doran *et al.*, 2008). In the pathogenesis of restenosis in response to endovascular coronary or peripheral artery interventions, smooth muscle cells play a detrimental role through excessive migration and proliferation, leading to critical reduction in blood flow (reviewed in Ferns and Avades, 2000). In human coronary artery smooth muscle cells, CB_2 activation inhibited the TNF-α-induced proliferation and migration via inhibition of TNF-α-induced Ras, p38 MAPK, ERK 1/2, SAPK/JNK and Akt activation (Rajesh *et al.*, 2008).

In vitro role of CB_2 in oxidized LDL-induced apoptosis

Elevated levels of plasma cholesterol, in particular low-density lipoprotein (LDL), are recognized as a major cardiovascular risk factor and lead to higher concentrations in the subendothelial intimal space. In the intima, LDL is oxidatively modified by reactive oxygen species (ROS) produced in endothelial cells, resident macrophages or smooth muscle cells. Oxidized LDL may injure the endothelium and play a role in the increased leukocyte adherence (Maier *et al.*, 1996; Vora *et al.*, 1997; Wang *et al.*, 1997; Aikawa *et al.*, 2002). Furthermore, oxidized LDL accumulates in macrophages and triggers macrophage apoptosis within atherosclerotic lesions (reviewed in Lusis, 2000). 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 (Liu *et al.*, 2005). In advanced lesions, however, apoptosis of macrophage-derived foam cells promotes the formation of a pro-thrombotic central lipid pool whose size correlates with plaque instability. Thus, in advanced lesions, macrophage apoptosis could be considered as a pro-atherogenic factor triggering plaque vulnerability and risk of acute plaque rupture (reviewed in Libby *et al.*, 1996).

There is *in vitro* evidence for a role of CB_2 deficiency in oxidized LDL-induced macrophage apoptosis, which involves modulation of the Akt survival pathway (Freeman-Anderson *et al.*, 2008). The apoptosis rate was significantly reduced in peritoneal macrophages from CB_2 knockout mice as compared with wild-type animals. While oxidized LDL inhibited Akt phosphorylation, the effect was impaired in CB_2-deficient macrophages. In rat peritoneal macrophages, oxidized LDL dose-dependently induced endocannabinoid levels as well as cannabinoid receptor CB_1 and CB_2 expression (Jiang *et al.*, 2009). On the other hand, treatment with a synthetic agonist promoted cholesterol accumulation in these cells in a CB_1-dependent manner.

Effects of plant-derived or synthetic CB_2 agonists in experimental atherosclerosis

The potential implication of CB_2 receptors in atherosclerosis, at least in mice, evolved from the initial discovery that the non-selective agonist Δ9-THC was atheroprotective in a CB_2-dependent matter (Steffens *et al.*, 2005). The inhibitory effect of Δ9-THC on atherosclerotic plaque progression was reversed by the CB_2 antagonist/inverse agonist SR144528. The reduction of plaque size was associated with lower relative plaque macrophage content. *In vitro* proliferative responses and IFN-γ release were inhibited in splenocytes from Δ9-THC-treated mice, and migration of peritoneal macrophages versus CCL2 was also reduced. CB_2 antagonism reversed the anti-migratory effects, and Δ9-THC did not affect migration of $CB_2^{-/-}$ macrophages.

In 2010, Zhao *et al.* provided further evidence for anti-atherosclerotic effects of pharmacological CB_2 activation.

In $ApoE^{-/-}$ mice fed 8 weeks on high-cholesterol diet, daily i.p. injection with the synthetic non-selective agonist WIN55,212-2 during the last 2 weeks before harvest reduced plaque size, macrophage content and expression of adhesion molecules VCAM-1, ICAM-1 and P-selectin (Zhao *et al.*, 2010b). Pharmacologic CB_2 antagonism with AM630 inhibited the atheroprotective effects of WIN55,212-2. In a second study, WIN55,212-2 (administered by daily i.p. injection for 8 weeks before analysis) reduced atherosclerotic plaque formation, lesional macrophage content and mRNA levels of inflammatory markers IL-6, TNF-α and CCL2, as well as NF-κB activation in $ApoE^{-/-}$ mice fed 16 weeks on high-cholesterol diet (Zhao *et al.*, 2010a).

Lessons from experimental atherosclerosis studies employing genetic CB_2 deficiency

So far, the postulated role for CB_2 in anti-atherosclerotic effects was exclusively based on non-selective agonists combined with pharmacological receptor blockade, which paved the way for further studies investigating the impact of genetic CB_2 receptor deficiency in experimental atherosclerosis. Several groups independently studied atherosclerosis development in $LDLR^{-/-}$ or $ApoE^{-/-}$ mouse models, based on double deficiency and/or bone marrow transplantation. Some controversy exists in the reported effects on atherosclerotic plaque size and composition between these studies, which might be only partially explained by the different genetic backgrounds and experimental strategies.

First, Netherland *et al.* (2010) reported accelerated macrophage and smooth muscle cell infiltration and a less stable plaque phenotype without changes in plaque size in $LDLR^{-/-}CB_2^{-/-}$ mice. The $CB_2^{-/-}$ strain used for generation of double knockout mice was from Nancy Buckley *et al.* (2000).

Second, Willecke *et al.* (2011) claimed that neither genetic deficiency nor activation of CB_2 modulated atherogenesis in $LDLR^{-/-}$ mice. In fact, they reported no changes in plaque size but significantly increased macrophage and lipid plaque content in $LDLR^{-/-}CB_2^{-/-}$ mice, which were independently generated with $CB_2^{-/-}$ mice obtained from Jackson Laboratories (Bar Harbor, ME, USA). Surprisingly, they did not observe any effect on atherosclerotic lesion size, plaque composition or immune cell function in $LDLR^{-/-}$ mice treated with CB_2 agonist JWH133. Based on the limited pharmacokinetic data provided in this study, it is difficult to assess if the lack of anti-atherosclerotic effect might be due to insufficient frequency and/or dosage of cannabinoid ligand administration. In an attempt to proof the *in vivo* efficacy of JWH133 administration, the authors performed additional experiments based on thioglycollate-induced peritonitis and found reduced peritoneal macrophage recruitment in JWH133-treated mice. However, no effect was observed on acute TNF-α-induced systemic cytokine release or leukocyte adhesion marker expression. *In vitro* flow chamber assays also failed to show inhibitory effects on peritoneal macrophage adhesion to endothelial cells. The latter could also be related to the possibility that peritoneal macrophages and/or endothelial cells were already activated during the handling, possible presence of high levels of endocannabinoids in the serum used to culture cells (Marazzi *et al.*, 2011), as well as to the potential rapid internalization of the CB_2 receptors (Atwood *et al.*, 2012) and concomitant activation of CB_1 receptors with opposing consequences (reviewed in Pacher, 2009; Pacher and Mechoulam, 2011), by the extremely high concentration of the JWH133 used. In contrast to the above mentioned study, several recent reports using JWH133 and other CB_2 agonists *in vitro, ex vivo* or *in vivo*, confirmed the anti-inflammatory effects of CB_2 activation in endothelium, and its inhibitory effect on monocytes/macrophages and/or leukocyte migration during unrelated pro-inflammatory conditions (Ni *et al.*, 2004; Murikinati *et al.*, 2010; Horvath *et al.*, 2012; Ramirez *et al.*, 2012; Zarruk *et al.*, 2012).

A third study was conducted by Georg Nickenig's group and the $CB_2^{-/-}$ mice from Andreas Zimmer (Buckley *et al.*, 2000), based on the $ApoE^{-/-}$ mouse model of atherosclerosis. The authors employed both double deficiency and bone marrow chimeric approach to demonstrate that CB_2 expression in both vascular as well as immune cells influences atherosclerosis development (Hoyer *et al.*, 2011). $ApoE^{-/-}CB_2^{-/-}$ mice had significantly increased plaque macrophage infiltration and aortic superoxide production, but a non-significant increase in plaque size. Similarly, lethally irradiated $ApoE^{-/-}$ mice reconstituted with $CB_2^{-/-}$ bone marrow had increased macrophage infiltration without significant increase in lesion size. On the other hand, treatment with CB_2 agonist JWH133 significantly reduced atherosclerotic lesion size, macrophage infiltration, superoxide production and improved vascular endothelium-dependent relaxations *ex vivo* in isolated aortic ring preparations. Interestingly, the authors further reported some changes in aortic levels of endocannabinoids and related lipid mediators (i.e. reduced 2-AG and increased OEA levels) in $CB_2^{-/-}$ mice on C57BL6 wild-type background. Unfortunately, whether similar endocannabinoid levels are detectable in CB_2-deficient $ApoE^{-/-}$ mice (on normal chow or high cholesterol diet) remains unclear.

Finally, Delsing *et al.* studied the effect of immune cell CB_2 deficiency in irradiated $LDLR^{-/-}$ mice reconstituted with bone marrow from $CB_2^{-/-}$ mice (from Andreas Zimmer). At the level of the aortic arch but not the aortic sinus, the lesional area was significantly increased in $CB_2^{-/-}$ bone marrow transplanted mice as compared with mice receiving wild-type bone marrow after 12 weeks of high cholesterol (0.15%) diet (Delsing *et al.*, 2011).

Pharmacological CB_2 activation: a new approach for restenosis prevention?

Since the first percutaneous transluminal coronary angioplasty (PTCA) performed in the 1970s (Gruntzig *et al.*, 1979), this intervention has become one of the most common procedures to reopen occluded vessels in coronary artery disease patients. Despite significant advances in this technique based on inflation of a balloon-tipped catheter and the introduction of stents (including drug-eluting stents), restenosis remains the primary limitation of PTCA (reviewed in Bittl, 1996; Douglas, 2007; Finn *et al.*, 2007).

Restenosis is an inflammatory process in response to arterial injury, leading to secretion of cytokines and growth factors, recruitment of inflammatory cells as well as increased migratory, proliferative and secretory responses of vascular smooth muscle cells (reviewed in Ferns and Avades, 2000; Weber *et al.*, 2004; Gerthoffer, 2007). Efforts to limit this constrictive vascular remodelling process have focused on inhibiting smooth muscle cell proliferation and migration, leading to the development of local stent-based delivery of antiproliferative agents such as sirolimus and paclitaxel (reviewed in Finn *et al.*, 2007). Although these drug-eluting stents reduce rates of restenosis compared with bare metal stents, still a significant percentage of higher-risk patients develop in-stent restenosis (reviewed in Douglas, 2007). Moreover, late stent thrombosis due to the lack of complete endothelial repair has emerged as a major safety concern (reviewed in Joner *et al.*, 2006; Finn *et al.*, 2007; Luscher *et al.*, 2007). Therefore, novel strategies should be targeted on restenosis prevention without impairing the arterial healing process.

Since CB_2 receptor activation inhibited inflammatory proliferation and migration of vascular smooth muscle cells in vitro (Rajesh *et al.*, 2008), we subsequently investigated the effect of CB_2 activation in a mouse model of balloon angioplasty (Molica *et al.*, 2012). As reported in other models of organ injury or inflammation (reviewed in Pacher and Mechoulam, 2011), balloon injury increased vascular CB_2 expression in hypercholesterolaemic $ApoE^{-/-}$ mice (Molica *et al.*, 2012). Injured vessels of mice treated with CB_2 agonist JWH133 showed reduced intimal and medial thickening, associated with less in situ proliferation, smooth muscle cells and macrophages. Re-endothelialization was not inhibited by treatment with the CB_2 agonist, according to CD31 immunostaining. Conversely, CB_2 deficiency resulted in increased intima formation compared with wild-type mice, whereas JWH133 did not affect intimal formation in $CB_2^{-/-}$ mice. Apoptosis rates assessed by *in situ* TUNEL staining were significantly higher in the CB_2 knockouts. *In vitro* proliferation rates were significantly increased in $CB_2^{-/-}$ smooth muscle cells compared with wild-type cells. Bone marrow-derived $CB_2^{-/-}$ macrophages showed enhanced adherence and migration compared with $CB_2^{+/+}$ macrophages. The underlying mechanisms involved increased mRNA levels of adhesion molecule ICAM-1, chemokine receptors CCR1 and CCR5, as well as the pro-inflammatory chemokine CCL2.

Implication of CB_2 in myocardial preconditioning based on *ex vivo* or *in vitro* models

An implication of the endocannabinoid system in the cardioprotective mechanisms of preconditioning has been initially described in isolated rat heart models (Lagneux and Lamontagne, 2001; Joyeux *et al.*, 2002; Bouchard *et al.*, 2003; Lepicier *et al.*, 2003). However, whether CB_1, CB_2 or both receptors are involved in these models is controversial; furthermore the clinical relevance of these *ex vivo* model is limited because of the absence of the important inflammatory response (reviewed in Pacher and Hasko, 2008).

Cardioprotective effects of endocannabinoid-mediated CB_2 activation were first reported in LPS-induced preconditioning (Lagneux and Lamontagne, 2001). Perfusion with CB_2 antagonist SR144528 abolished the cardioprotective effect of LPS pretreatment, whereas CB_1 antagonism with rimonabant had no effect. The implication of NO in CB_2-dependent cardioprotection was shown by additional experiments using NOS inhibitor or NO donor respectively. Similarly, blocking of CB_2, but not CB_1 receptors reversed cardioprotection by heat stress-mediated preconditioning (Joyeux *et al.*, 2002). As to endocannabinoid perfusion-mediated cardioprotection, one study reported that only CB_2, but not CB_1 (or only partially) reversed the protective effect (Lepicier *et al.*, 2003). A different study, however, reported inhibition of endocannabinoid-mediated cardioprotection by both CB_1 and CB_2 antagonists (Underdown *et al.*, 2005).

Two studies further investigated the link between endocannabinoid signalling and NO-mediated cardioprotection (Wagner *et al.*, 2006; Lepicier *et al.*, 2007). Both studies suggest the requirement for NO in CB_1, but not CB_2-mediated cardioprotection. By contrast, in isolated neonatal cardiomyocytes preventive effects of the plant-derived cannabinoid Δ9-THC against hypoxia were dependent on NO production and sensitive to CB_2, but not CB_1 antagonism (Shmist *et al.*, 2006).

In vivo role of CB_2 in myocardial ischaemia/reperfusion injury, importance of inflammatory response

A different experimental approach is based on *in vivo* myocardial ischaemia/reperfusion in anaesthetized rodents, pretreated with cannabinoid receptor agonists or antagonists. The synthetic cannabinoid HU-210 decreased the incidence of ventricular arrhythmias following ischaemia/reperfusion in rats through activation of CB_2 receptors (Krylatov *et al.*, 2001). Treatment with the non-selective agonist WIN55,212-2 before ischaemia significantly reduced the infarct size in mouse hearts (Di Filippo *et al.*, 2004). The CB_2 antagonist AM630, but not the CB_1 antagonist AM251 abolished the effect of WIN55,212-2.

In a clinically more relevant experimental setting, CB_2 activation with JWH133 administered at the end of the ischaemic period significantly reduced the infarct size as compared with vehicle-treated mice (Montecucco *et al.*, 2009). Serum levels of the clinical marker cardiac troponin I, which is released from necrotic cardiomyocytes, were significantly lower in mice treated with JWH133. The infarct size reduction was associated with a decrease in ROS production and neutrophil infiltration into the infarcted myocardium, and activation of cardioprotective signalling pathways (Montecucco *et al.*, 2009). Pre-injection with kinase inhibitors for Akt, ERK 1/2, STAT-3 pathways partially abrogated the JWH133-mediated infarct size reduction. *In vitro*, JWH133 inhibited TNF-α induced chemotaxis and integrin CD18/CD11b up-regulation on human neutrophils (Montecucco *et al.*, 2009), suggesting a mechanistic explanation that might contribute to the infarct size reduction and potential relevance for human pathology.

In addition, CB_2 receptors have been involved in the cardioprotective effects of remote ischaemic preconditioning, which is a protective phenomenon induced by preceding ischaemia in other organs or vascular beds (Hajrasouliha *et al.*, 2008). Systemic pretreatment with CB_2 antagonist AM630, but not the CB_1 antagonist AM251, abolished the cardioprotective effects of remote preconditioning on infarct size and arrhythmias.

As to a potential clinical relevance for CB_2 signalling in myocardial infarction, a large case-control study enrolling 1968 individuals addressed the involvement of the gene encoding CB_2, CNR2, in the development of myocardial infarction and several cardiovascular risk factors. In particular, a potential association of genetic variations with the development of myocardial infarction and classic cardiovascular risk factors, including arterial hypertension, obesity, hypercholesterolaemia and diabetes mellitus was investigated (Reinhard *et al.*, 2008). However, none of the 13 investigated single nucleotide polymorphisms in the CNR2 gene was associated with myocardial infarction or any of the investigated risk factors.

CB_2 in post-ischaemic repair and heart failure

Acute myocardial infarction leads to necrosis of cardiac myocytes, which induces repair mechanisms that lead to scar formation (Sun, 2009). This process of post-infarction cardiac remodelling involves adaptive changes in shape, size and function of the ventricle, which may ultimately lead to contractile dysfunction and heart failure. Defer *et al.* (2009) provided substantial evidence for a protective role of CB_2 receptors in ischaemic cardiac myocyte cell death, fibrosis and cardiac dysfunction. Hearts of $CB_2^{-/-}$ mice had larger infarcts in comparison with wild-type mice and more sustained cell loss 3 days after ischaemia, together with accelerated injury and apoptosis in the non-ischaemic remote myocardium. Furthermore, increased infiltration of macrophages was observed in the infarct-surrounding myocardium of $CB_2^{-/-}$ mice. Accelerated cardiac remodelling in $CB_2^{-/-}$ post-ischaemic hearts was documented by higher number of spindle-shaped α-smooth muscle actin-positive myofibroblasts. *In vitro*, $CB_2^{-/-}$ cardiomyocytes and fibroblasts were more susceptible to oxidative stress-induced cell death. Long-term effects of cardiac remodelling in $CB_2^{-/-}$ hearts involved marked fibrosis, accelerated cardiomyocyte hypertrophy, dilative cardiomyopathy and cardiac dysfunction, as reported 4 weeks post infarction. By contrast, wild-type post-ischaemic hearts developed moderate fibrosis and cardiomyocyte hypertrophy, while cardiac function was preserved.

Heart failure is a complication of many diseases that affect the heart, such as coronary artery disease, arterial hypertension, valvular disease or diabetic cardiomyopathy (Jessup and Brozena, 2003; Boudina and Abel, 2007). Recent findings in humans reported an up-regulation of cardiac CB_2 expression and enhanced endocannabinoid AEA and 2-AG levels in patients with chronic heart failure (Weis *et al.*, 2010). Analysis of cannabinoid receptor expression by real-time PCR and immunohistochemistry also revealed a slight but significant down-regulation of cardiac CB_1 receptors in chronic heart failure patients. Concerning potential pathophysiological consequences of CB_2 up-regulation, the authors speculate that it may have a negative inotropic effect due to reduced cAMP levels that may contribute to ventricular weakening. On the other hand, CB_2 receptors might mediate positive inotropic effects through cAMP-independent mechanisms, thus representing a compensatory mechanism to maintain cardiac performance. Moreover, CB_2 up-regulation could represent a protective response to counterbalance chronic heart failure-induced structural changes, as shown in mice (Defer *et al.*, 2009).

In vivo role of CB_2 in cerebral ischaemia/reperfusion injury

Ischaemic stroke is a leading cause of death in developed countries and acquired adult disability. Increased accumulation of CB_2-positive macrophages derived from resident microglia and/or invading monocytes following cerebral ischaemia/reperfusion have previously been reported (Ashton *et al.*, 2007). Numerous recent studies have investigated the role of CB_2 receptors by evaluating the effects of various CB_2 ligands or knockout mice in experimental models of stroke (models of cerebral ischaemia–reperfusion injury) (Ni *et al.*, 2004; Zhang *et al.*, 2007; 2008; Murikinati *et al.*, 2010; Ramirez *et al.*, 2012). Similarly to the effects observed in myocardial ischaemia/reperfusion injury, CB_2 receptor activation limited cerebral infarct size in experimental stroke by attenuating endothelial cell activation, chemokine signalling, inflammatory cell infiltration, glial activation, oxidative/nitrative stress and consequent cell death (Ni *et al.*, 2004; Zhang *et al.*, 2007; 2008; Murikinati *et al.*, 2010; Zarruk *et al.*, 2012). Most of these beneficial effects could be prevented by CB_2 receptor antagonist(s) and/or were absent in CB_2 knockout mice, which often exhibited enhanced injury indicative of the protective role of endocannabinoid system through CB_2 receptors. Principally comparable protection could also be seen in hepatic ischaemia/reperfusion injury models (Batkai *et al.*, 2007; Rajesh *et al.*, 2007b; Horvath *et al.*, 2012), which emphasizes a key role of CB_2 receptors in limiting reperfusion damage in general and also provides a strong rationale for development of this promising approach for clinical use.

Interestingly, recent findings further suggest protective effects of the endocannabinoid-related brain compound N-arachidonoyl-L-serine (araS) in traumatic brain injury (Cohen-Yeshurun *et al.*, 2011). Despite its structural similarity to anandamide, araS exhibits very low affinity at CB_1 and CB_2 receptors (Milman *et al.*, 2006). Nevertheless, treatment with selective CB_2 and transient receptor potential vanilloid 1, but not CB_1 and GPR55 receptor antagonists reversed the protective effects in traumatic brain injury (Cohen-Yeshurun *et al.*, 2011). This raises an intriguing possibility that certain metabolites of araS may exert direct protective effects on these receptors.

Conclusions

Collectively, several lines of evidence discussed above have established that functional CB_2 receptors in cells of the

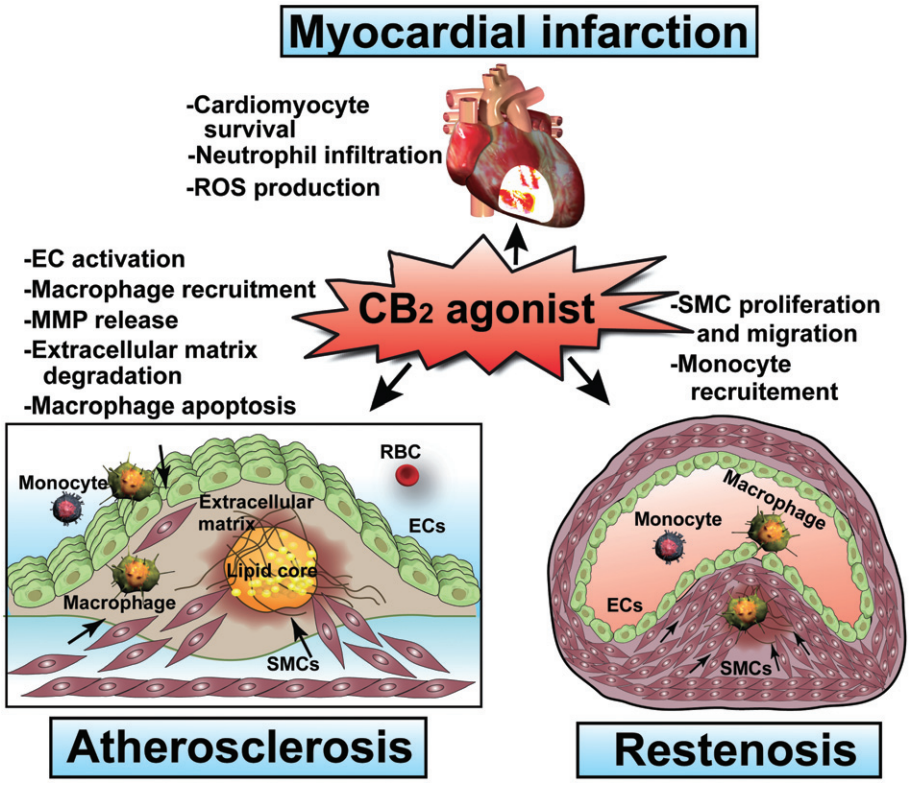

Figure 1

Potential therapeutic targets of CB_2 activation in cardiovascular disorders. EC, endothelial cell; RBC, red blood cell; SMC, smooth muscle cells.

cardiovascular system (e.g. endothelial and vascular smooth muscle cells, cardiomyocytes, fibroblasts and resident immune cells), as well as in infiltrating monocytes/macrophages and leukocytes during various pathological conditions of the cardiovascular system (e.g. atherosclerosis, restenosis, stroke, myocardial infarction and heart failure) may play an important compensatory role in controlling tissue inflammation and injury (Figure 1). In most of the cases, these receptors limit inflammation and associated tissue injury; however, in certain cases or disease states, CB_2 receptor activation may also enhance tissue damage (Pacher and Mechoulam, 2011). On the basis of preclinical results, pharmacological modulation of CB_2 receptors may hold a unique therapeutic potential in stroke, myocardial infarction and atherosclerosis despite some controversies (Figure 1). Nevertheless, the successfully translation of these promising preclinical results to clinical practice requires a better understanding of the underlying pathology, CB_2 pharmacology and signalling, as well as the therapeutic window and long term safety of the use of various CB_2 ligands. Finally, there is an urgent need for better, more selective pharmacological tools that are suitable for clinical application.

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Conflict of interest

None.

References

Aikawa M, Sugiyama S, Hill CC, Voglic SJ, Rabkin E, Fukumoto Y *et al.* (2002). Lipid lowering reduces oxidative stress and endothelial cell activation in rabbit atheroma. Circulation 106: 1390–1396.

Ashton JC, Rahman RM, Nair SM, Sutherland BA, Glass M, Appleton I (2007). Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. Neuroscience Lett 412: 114–117.

Atwood BK, Wager-Miller J, Haskins C, Straiker A, Mackie K (2012). Functional selectivity in CB2 cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB2 ligands. Mol Pharmacol 81: 250–263.

Barutta F, Piscitelli F, Pinach S, Bruno G, Gambino R, Rastaldi MP *et al.* (2011). Protective role of cannabinoid receptor type 2 in a mouse model of diabetic nephropathy. Diabetes 60: 2386–2396.

Batkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P *et al.* (2007). Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. FASEB J 21: 1788–1800.

Bittl JA (1996). Advances in coronary angioplasty. N Engl J Med 335: 1290–1302.

Bockaert J, Roussignol G, Becamel C, Gavarini S, Joubert L, Dumuis A *et al.* (2004). GPCR-interacting proteins (GIPs): nature and functions. Biochem Soc Trans 32: 851–855.

Bosier B, Muccioli GG, Hermans E, Lambert DM (2010). Functionally selective cannabinoid receptor signalling: therapeutic implications and opportunities. Biochem Pharmacol 80: 1–12.

Bouaboula M, Dussossoy D, Casellas P (1999). Regulation of peripheral cannabinoid receptor CB2 phosphorylation by the inverse agonist SR 144528. Implications for receptor biological responses. J Biol Chem 274: 20397–20405.

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.

Boudina S, Abel ED (2007). Diabetic cardiomyopathy revisited. Circulation 115: 3213–3223.

Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, Sim-Selley LJ (1999). Chronic delta9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. J Neurochem 73: 2447–2459.

Buckley NE, McCoy KL, Mezey E, Bonner T, Zimmer A, Felder CC *et al.* (2000). Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. Eur J Pharmacol 396: 141–149.

Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K *et al.* (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Mol Pharmacol 65: 999–1007.

Cohen-Yeshurun A, Trembovler V, Alexandrovich A, Ryberg E, Greasley PJ, Mechoulam R *et al.* (2011). N-arachidonoyl-L-serine is neuroprotective after traumatic brain injury by reducing apoptosis. J Cereb Blood Flow Metab 31: 1768–1777.

Defer N, Wan J, Souktani R, Escoubet B, Perier M, Caramelle P *et al.* (2009). The cannabinoid receptor type 2 promotes cardiac myocyte and fibroblast survival and protects against ischemia/reperfusion-induced cardiomyopathy. FASEB J 23: 2120–2130.

Delsing DJ, Leijten FP, Arts K, van Eenennaam H, Garritsen A, Gijbels MJ *et al.* (2011). Cannabinoid receptor 2 deficiency in haematopoietic cells aggravates early atherosclerosis in LDL receptor deficient mice. Open Cardiovasc Med J 5: 15–21.

Demuth DG, Molleman A (2006). Cannabinoid signalling. Life Sci 78: 549–563.

Derocq JM, Jbilo O, Bouaboula M, Segui M, Clere C, Casellas P (2000). Genomic and functional changes induced by the activation of the peripheral cannabinoid receptor CB2 in the promyelocytic cells HL-60. Possible involvement of the CB2 receptor in cell differentiation. J Biol Chem 275: 15621–15628.

Di Filippo C, Rossi F, Rossi S, D'Amico M (2004). Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. J Leukoc Biol 75: 453–459.

Doran AC, Meller N, McNamara CA (2008). Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol 28: 812–819.

Douglas JS, Jr (2007). Pharmacologic approaches to restenosis prevention. Am J Cardiol 100: 10K–16K.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O *et al.* (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48: 443–450.

Ferns GA, Avades TY (2000). The mechanisms of coronary restenosis: insights from experimental models. Int J Exp Pathol 81: 63–88.

Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK *et al.* (2007). Vascular responses to drug eluting stents: importance of delayed healing. Arterioscler Thromb Vasc Biol 27: 1500–1510.

Freeman-Anderson NE, Pickle TG, Netherland CD, Bales A, Buckley NE, Thewke DP (2008). Cannabinoid (CB2) receptor deficiency reduces the susceptibility of macrophages to oxidized LDL/oxysterol-induced apoptosis. J Lipid Res 49: 2338–2346.

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P *et al.* (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232: 54–61.

Gerthoffer WT (2007). Mechanisms of vascular smooth muscle cell migration. Circ Res 100: 607–621.

Ghosh S, Preet A, Groopman JE, Ganju RK (2006). Cannabinoid receptor CB(2) modulates the CXCL12/CXCR4-mediated chemotaxis of T lymphocytes. Mol Immunol 43: 2169–2179.

Grimsey NL, Goodfellow CE, Dragunow M, Glass M (2011). Cannabinoid receptor 2 undergoes Rab5-mediated internalization and recycles via a Rab11-dependent pathway. Biochim Biophys Acta 1813: 1554–1560.

Gruntzig AR, Senning A, Siegenthaler WE (1979). Nonoperative dilatation of coronary-artery stenosis: percutaneous transluminal coronary angioplasty. N Engl J Med 301: 61–68.

Hajrasouliha AR, Tavakoli S, Ghasemi M, Jabehdar-Maralani P, Sadeghipour H, Ebrahimi F *et al.* (2008). Endogenous cannabinoids contribute to remote ischemic preconditioning via cannabinoid CB2 receptors in the rat heart. Eur J Pharmacol 579: 246–252.

Horvath B, Magid L, Mukhopadhyay P, Batkai S, Rajesh M, Park O *et al.* (2012). A new cannabinoid 2 receptor agonist HU-910 attenuates oxidative stress, inflammation, and cell death associated with hepatic ischemia/reperfusion injury. Br J Pharmacol 165: 2462–2478.

Howlett AC (2005). Cannabinoid receptor signaling. Handb Exp Pharmacol 168: 53–79.

Hoyer FF, Steinmetz M, Zimmer S, Becker A, Lutjohann D, Buchalla R *et al.* (2011). Atheroprotection via cannabinoid receptor-2 is mediated by circulating and vascular cells in vivo. J Mol Cell Cardiol 51: 1007–1014.

Jessup M, Brozena S (2003). Heart failure. N Engl J Med 348: 2007–2018.

Jiang LS, Pu J, Han ZH, Hu LH, He B (2009). Role of activated endocannabinoid system in regulation of cellular cholesterol metabolism in macrophages. Cardiovasc Res 81: 805–813.

Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E *et al.* (2006). Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 48: 193–202.

Joyeux M, Arnaud C, Godin-Ribuot D, Demenge P, Lamontagne D, Ribuot C (2002). Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. Cardiovasc Res 55: 619–625.

Kishimoto S, Gokoh M, Oka S, Muramatsu M, Kajiwara T, Waku K *et al.* (2003). 2-arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. J Biol Chem 278: 24469–24475.

Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L *et al.* (2003). The cannabinoid system and immune modulation. J Leukoc Biol 74: 486–496.

Kobayashi Y, Arai S, Waku K, Sugiura T (2001). Activation by 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, of p42/44 mitogen-activated protein kinase in HL-60 cells. J Biochem 129: 665–669.

Krylatov AV, Ugdyzhekova DS, Bernatskaya NA, Maslov LN, Mekhoulam R, Pertwee RG *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.

Lagneux C, Lamontagne D (2001). Involvement of cannabinoids in the cardioprotection induced by lipopolysaccharide. Br J Pharmacol 132: 793–796.

Lepicier P, Bouchard JF, Lagneux C, Lamontagne D (2003). Endocannabinoids protect the rat isolated heart against ischaemia. Br J Pharmacol 139: 805–815.

Lepicier P, Lagneux C, Sirois MG, Lamontagne D (2007). Endothelial CB1-receptors limit infarct size through NO formation in rat isolated hearts. Life Sci 81: 1373–1380.

Libby P, Geng YJ, Aikawa M, Schoenbeck U, Mach F, Clinton SK *et al.* (1996). Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 7: 330–335.

Libby P, Ridker PM, Hansson GK (2011). Progress and challenges in translating the biology of atherosclerosis. Nature 473: 317–325.

Liu J, Thewke DP, Su YR, Linton MF, Fazio S, Sinensky MS (2005). Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. Arterioscler Thromb Vasc Biol 25: 174–179.

Luscher TF, Steffel J, Eberli FR, Joner M, Nakazawa G, Tanner FC *et al.* (2007). Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. Circulation 115: 1051–1058.

Lusis AJ (2000). Atherosclerosis. Nature 407: 233–241.

Maier JA, Barenghi L, Bradamante S, Pagani F (1996). Induction of human endothelial cell growth by mildly oxidized low density lipoprotein. Atherosclerosis 123: 115–121.

Marazzi J, Kleyer J, Paredes JM, Gertsch J (2011). Endocannabinoid content in fetal bovine sera – unexpected effects on mononuclear cells and osteoclastogenesis. J Immunol Methods 373: 219–228.

Miller AM, Stella N (2008). CB2 receptor-mediated migration of immune cells: it can go either way. Br J Pharmacol 153: 299–308.

Milman G, Maor Y, Abu-Lafi S, Horowitz M, Gallily R, Batkai S *et al.* (2006). N-arachidonoyl L-serine, an endocannabinoid-like brain constituent with vasodilatory properties. Proc Natl Acad Sci U S A 103: 2428–2433.

Molica F, Matter CM, Burger F, Pelli G, Lenglet S, Zimmer A *et al.* (2012). The cannabinoid receptor CB2 protects against balloon-induced neointima formation. Am J Physiol Heart Circ Physiol 302: 1064–1074.

Montecucco F, Burger F, Mach F, Steffens S (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.

Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M *et al.* (2009). CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. J Mol Cell Cardiol 46: 612–620.

Montecucco F, Di Marzo V, da Silva RF, Vuilleumier N, Capettini L, Lenglet S *et al.* (2011). The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. Eur Heart J 33: 846–856.

Mukhopadhyay P, Rajesh M, Batkai S, Patel V, Kashiwaya Y, Liaudet L *et al.* (2010a). CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. Cardiovasc Res 85: 773–784.

Mukhopadhyay P, Rajesh M, Pan H, Patel V, Mukhopadhyay B, Batkai S *et al.* (2010b). Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. Free Radic Biol Med 48: 457–467.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61–65.

Murikinati S, Juttler E, Keinert T, Ridder DA, Muhammad S, Waibler Z *et al.* (2010). Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. FASEB J 24: 788–798.

Muslin AJ (2008). MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. Clin Sci (Lond) 115: 203–218.

Netherland CD, Pickle TG, Bales A, Thewke DP (2010). Cannabinoid receptor type 2 (CB2) deficiency alters atherosclerotic lesion formation in hyperlipidemic Ldlr-null mice. Atherosclerosis 213: 102–108.

Ni X, Geller EB, Eppihimer MJ, Eisenstein TK, Adler MW, Tuma RF (2004). Win 55212-2, a cannabinoid receptor agonist, attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. Mult Scler 10: 158–164.

Pacher P (2009). Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? Arterioscler Thromb Vasc Biol 29: 7–9.

Pacher P, Hasko G (2008). Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion injury and preconditioning. Br J Pharmacol 153: 252–262.

Pacher P, Mechoulam R (2011). Is lipid signaling through cannabinoid 2 receptors part of a protective system? Prog Lipid Res 50: 193–211.

Pertwee RG (2005). Pharmacological actions of cannabinoids. Handb Exp Pharmacol 168: 1–51.

Rajesh M, Mukhopadhyay P, Batkai S, Hasko G, Liaudet L, Huffman JW *et al.* (2007a). CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. Am J Physiol Heart Circ Physiol 293: H2210–H2218.

Rajesh M, Pan H, Mukhopadhyay P, Batkai S, Osei-Hyiaman D, Hasko G *et al.* (2007b). 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.

Rajesh M, Mukhopadhyay P, Hasko G, Huffman JW, Mackie K, Pacher P (2008). CB(2) cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. Br J Pharmacol 153: 347–357.

Ramirez SH, Hasko J, Skuba A, Fan S, Dykstra H, McCormick R *et al.* (2012). Activation of cannabinoid receptor 2 attenuates leukocyte-endothelial cell interactions and blood-brain barrier dysfunction under inflammatory conditions. J Neurosci 32: 4004–4016.

Rao GK, Zhang W, Kaminski NE (2004). Cannabinoid receptor-mediated regulation of intracellular calcium by delta(9)-tetrahydrocannabinol in resting T cells. J Leukoc Biol 75: 884–892.

Reinhard W, Stark K, Neureuther K, Sedlacek K, Fischer M, Baessler A *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.

Ritter SL, Hall RA (2009). Fine-tuning of GPCR activity by receptor-interacting proteins. Nat Rev Mol Cell Biol 10: 819–830.

Schuehly W, Paredes JM, Kleyer J, Huefner A, Anavi-Goffer S, Raduner S *et al.* (2011). Mechanisms of osteoclastogenesis inhibition by a novel class of biphenyl-type cannabinoid CB(2) receptor inverse agonists. Chem Biol 18: 1053–1064.

Shmist YA, Goncharov I, Eichler M, Shneyvays V, Isaac A, Vogel Z *et al.* (2006). Delta-9-tetrahydrocannabinol protects cardiac cells from hypoxia via CB2 receptor activation and nitric oxide production. Mol Cell Biochem 283: 75–83.

Shoemaker JL, Joseph BK, Ruckle MB, Mayeux PR, Prather PL (2005). The endocannabinoid noladin ether acts as a full agonist at human CB2 cannabinoid receptors. J Pharmacol Exp Ther 314: 868–875.

Smith TH, Sim-Selley LJ, Selley DE (2010). Cannabinoid CB1 receptor-interacting proteins: novel targets for central nervous system drug discovery? Br J Pharmacol 160: 454–466.

Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C *et al.* (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature 434: 782–786.

Sun Y (2009). Myocardial repair/remodelling following infarction: roles of local factors. Cardiovasc Res 81: 482–490.

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.

Vora DK, Fang ZT, Liva SM, Tyner TR, Parhami F, Watson AD *et al.* (1997). Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. Circ Res 80: 810–818.

Wagner JA, Abesser M, Harvey-White J, Ertl G (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.

Wang GP, Deng ZD, Ni J, Qu ZL (1997). Oxidized low density lipoprotein and very low density lipoprotein enhance expression of monocyte chemoattractant protein-1 in rabbit peritoneal exudate macrophages. Atherosclerosis 133: 31–36.

Weber C, Schober A, Zernecke A (2004). Chemokines: key regulators of mononuclear cell recruitment in atherosclerotic vascular disease. Arterioscler Thromb Vasc Biol 24: 1997–2008.

Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A *et al.* (2010). Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. J Mol Cell Cardiol 48: 1187–1193.

Wiley JL, Beletskaya ID, Ng EW, Dai Z, Crocker PJ, Mahadevan A *et al.* (2002). Resorcinol derivatives: a novel template for the development of cannabinoid CB(1)/CB(2) and CB(2)-selective agonists. J Pharmacol Exp Ther 301: 679–689.

Willecke F, Zeschky K, Ortiz Rodriguez A, Colberg C, Auwarter V, Kneisel S *et al.* (2011). Cannabinoid receptor 2 signaling does not modulate atherogenesis in mice. PLoS ONE 6: e19405.

Zarruk JG, Fernandez-Lopez D, Garcia-Yebenes I, Garcia-Gutierrez MS, Vivancos J, Nombela F *et al.* (2012). Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. Stroke 43: 211–219.

Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI, Tuma RF (2007). Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. J Cereb Blood Flow Metab 27: 1387–1396.

Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D, Tuma RF (2008). Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. Neuroscience 152: 753–760.

Zhao Y, Liu Y, Zhang W, Xue J, Wu YZ, Xu W *et al.* (2010a). WIN55212-2 ameliorates atherosclerosis associated with suppression of pro-inflammatory responses in ApoE-knockout mice. Eur J Pharmacol 649: 285–292.

Zhao Y, Yuan Z, Liu Y, Xue J, Tian Y, Liu W *et al.* (2010b). Activation of cannabinoid CB2 receptor ameliorates atherosclerosis associated with suppression of adhesion molecules. J Cardiovasc Pharmacol 55: 292–298.

Zoratti C, Kipmen-Korgun D, Osibow K, Malli R, Graier WF (2003). Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. Br J Pharmacol 140: 1351–1362.