Endocannabinoids and the Cardiovascular System in Health and Disease

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Contents

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Abstract

The endocannabinoid system is widely distributed throughout the cardiovascular system. Endocannabinoids play a minimal role in the regulation of cardiovascular function in normal conditions, but are altered in most cardiovascular disorders. In shock, endocannabinoids released within blood mediate the associated hypotension through CB_1 activation. In hypertension, there is evidence for changes in the expression of CB_1, and CB_1 antagonism reduces blood pressure in obese hypertensive and diabetic patients. The endocannabinoid system is also upregulated in cardiac pathologies. This is likely to be cardioprotective, via CB_2 and CB_1 (lesser extent). In the vasculature, endocannabinoids cause vasorelaxation through activation of multiple target sites, inhibition of calcium channels, activation of potassium channels, NO production and the release of vasoactive substances. Changes in the expression or function of any of these pathways alter the vascular effect of endocannabinoids. Endocannabinoids have positive (CB_2) and negative effects (CB_1) on the progression of atherosclerosis. However, any negative effects of CB_1 may not be consequential, as chronic CB_1 antagonism in large scale human trials was not associated with significant reductions in atheroma. In neurovascular disorders such as stroke, endocannabinoids are upregulated and protective, involving activation of CB_1, CB_2, TRPV1 and PPARα. Although most of this evidence is from preclinical studies, it seems likely that cannabinoid-based therapies could be beneficial in a range of cardiovascular disorders.

Keywords

Artery • Atherosclerosis • Blood • Blood pressure • Blood–brain barrier • Heart • Hypertension • Vein

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
ARA-S	*N*-arachidonoyl-L-serine
BBB	Blood–brain barrier
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
COX	Cyclooxygenase

eNOS	Endothelial nitric oxide synthase
FAAH	Fatty acid amide hydrolase
MAGL	Monoacylglycerol lipase
NADA	*N*-arachidonoyl dopamine
NO	Nitric oxide
OEA	Oleoylethanolamide
PEA	Palmitoylethanolamide
PPAR	Peroxisome proliferator-activated receptors
PTX	Pertussis toxin
SHR	Spontaneously hypertensive rat
TBI	Traumatic brain injury
THC	Delta-9-tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid 1

1 Introduction

Cannabinoid receptors are widely distributed throughout the cardiovascular system. The CB_1 receptor is expressed in myocardium, human coronary artery, endothelial and smooth muscle cells and on pre-synaptic sympathetic nerve terminals innervating the cardiovascular system. CB_2 receptors have also been identified in the myocardium and in human coronary endothelial and smooth muscle cells. Endocannabinoids are produced in endothelial and smooth muscle cells and in cardiac tissue, and circulating levels of endocannabinoids are detectable in blood. Despite this, under normal conditions, it is unlikely that the endocannabinoid system plays a major role in the regulation of cardiovascular function. The evidence for this is that animals in whom either CB_1 (Mukhopadhyay et al. 2007), CB_2 (Batkai et al. 2007) or fatty acid amide hydrolase (FAAH, the main endocannabinoid degradation enzyme) (Pacher et al. 2005) has been knocked down have no major changes in cardiovascular function. However, it is clear that in many pathological conditions of the cardiovascular system, the endocannabinoid system is upregulated and appears to play an important, possibly protective, role. For example, mice in which FAAH has been knocked-out (which will increase endocannabinoid levels due to decreased degradation) have a reduced decline in age-related cardiac dysfunction and increased susceptibility to atherosclerosis (Batkai et al. 2007). Mice in which the CB_1 receptor has been knocked-out are more susceptible to chronic heart failure (Liao et al. 2013), and stroke (Batkai et al. 2007). CB_2-deficient mice have increased susceptibility to atherosclerosis (Netherland et al. 2010; Hoyer et al. 2011), stroke (Zhang et al. 2008) and cardiomyopathy (Duerr et al. 2014).

In the following article, I will review the role for the endocannabinoid system in cardiovascular function in health and disease, starting from the in vivo haemodynamic (changes in blood pressure and heart rate) effects of endocannabinoids, the role of endocannabinoids in modulating cardiac function, vascular and haematological (blood) function as well as neurovascular function. I will also discuss the evidence

for endocannabinoid involvement in hypertension, cardiovascular shock, myocardial infarction (heart attack), atherosclerosis, stroke and traumatic brain injury.

2 In Vivo Haemodynamic Response to Endocannabinoids

2.1 Blood Pressure and Heart Rate

Endocannabinoids have a complex effect on blood pressure and heart rate in animal studies, and the response observed is dependent on whether the animal is anaesthetised or conscious. Differences between anaesthetised and conscious responses may be due to altered basal levels of sympathetic activity observed with anaesthesia (Neukirchen and Kienbaum 2008), as many of the in vivo responses to endocannabinoids are mediated by modulation of the autonomic nervous system, through changes in both vagal and sympathetic activity.

In anaesthetised animals, application of anandamide (AEA) causes a triphasic response; rapid and transient bradycardia (fall in heart rate), a rapid and transient pressor response (increase in blood pressure) and a prolonged hypotensive phase (see Malinowska et al. 2012 for a detailed review). The initial bradycardic response to AEA is absent in transient receptor potential vanilloid 1 (TRPV1) knockout mice (Pacher et al. 2004) and is brought about by vagal activation (Varga et al. 1995). The mechanisms behind the brief pressor response are more complex and are likely to involve TRPV1, *N*-methyl-D-aspartate (NMDA) and beta2 (β_2) adrenoceptors (Malinowska et al. 2012). The prolonged hypotensive response to AEA in anaesthetised animals has been best characterised. This response is absent in CB_1 knockout mice (Jarai et al. 1999), and the location of the CB_1 receptors involved is likely to be on nerve terminals of the sympathetic nervous system inhibiting function at the level of the heart and vasculature. Endocannabinoids inhibit noradrenaline release via CB_1 activation in arteries (Deutsch et al. 1997) and in the mesenteric arterial bed (Ralevic et al. 2002). 2-arachidonoylglycerol (2-AG) administration to anaesthetised rats also causes a fall in blood pressure, although accompanied by tachycardia. Unlike AEA, the response to 2-AG is CB_1-independent and more likely to involve cyclooxygenase-catalysed metabolism to other vasoactive compounds (Jarai et al. 2000).

In contrast to its effects on anaesthetised animals, in conscious rats, AEA causes profound bradycardia, with a transient hypotension followed by a longer lasting pressor effect accompanied by vasoconstriction of the renal and mesenteric vascular beds (Stein et al. 1996; Gardiner et al. 2001, 2002, 2009). This is also accompanied by a hindquarter β_2-adrenoceptor-mediated vasodilator response (Gardiner et al. 2002). Gardiner and colleagues (2001, 2002) showed this complex haemodynamic effect results from increased circulating adrenaline acting via β_2-adrenoceptors and a CB_1-mediated increase in sympathetic activity. In contrast to data obtained from anaesthetised rats, there appears to be no role for TRPV1 activation (Gardiner et al. 2009). Looking at other endocannabinoid agonists in conscious animals, *N*-arachidonoyl dopamine (NADA) causes a similar triphasic

response to that seen in anaesthetised animals but with tachycardia accompanying the hypotensive phase, mediated by TRPV1 (Wang and Wang 2007). Oleamide has no effect on haemodynamics (Huitron-Resendiz et al. 2001).

The endocannabinoid system appears to be involved in the central control of blood pressure via the brainstem baroreceptor complex. The nucleus tractus solitarius is one site of termination of baroreceptor afferent fibres from arterial baroreceptors and cardiac mechanoreceptors. Cannabinoid CB_1 receptors are functionally expressed in the nucleus tractus solitarius (Himmi et al. 1998), and microinjection of AEA prolongs reflex inhibition of renal sympathetic nerve activity, suggesting an increase in baroreflex sensitivity, probably due to inhibition of GABAergic tone (Rademacher et al. 2003). AEA concentrations in the nucleus tractus solitarius increase after a phenylephrine-induced rise in blood pressure, supporting the physiological relevance of the endocannabinoid control of baroreflex activity (Seagard et al. 2004). Interestingly, this effect of AEA is blunted in hypertensive rats, possibly contributing to impaired baroreflex sensitivity (Brozoski et al. 2009).

It is worth remembering that animals in whom the CB_1, CB_2 or FAAH proteins have been knocked out have normal cardiovascular function, suggesting the endocannabinoid system plays a minimal role in the regulation of blood pressure and cardiac function under normal conditions. Similarly antagonists of CB_1 or FAAH/monoacylglycerol lipase (MAGL) inhibitors do not affect blood pressure and heart rate in conscious, normotensive animals (later in this review I will discuss how this is different in hypertensive animals), also shedding doubt on the role of the endocannabinoid system in the regulation of haemodynamics. However, recent evidence has pointed to a role for CB_1 in modulating sleep–wake cardiorespiratory control (Silvani et al. 2014). Mice lacking the CB_1 receptor had a significantly enhanced blood pressure and heart rate response to changes in sleep–wake cycles, and irregular breathing rhythms during sleep, suggesting further research is required to fully understand the role of the endocannabinoid system in all aspects of cardiovascular control.

There is very little evidence for a potential role of the endocannabinoid system in regulating blood pressure in humans in non-pathological situations. To my knowledge, there are no studies that have examined the acute haemodynamic effects of endocannabinoids in humans, although administration of a CB_1 receptor antagonist does not affect resting blood pressure in normotensive humans (Ruilope et al. 2008). However, a FAAH gene variant is associated with lower blood pressure in young males, suggesting a potential endocannabinoid role (Sarzani et al. 2008).

2.2 Endocannabinoids and Hypertension

In anaesthetised hypertensive rats, the prolonged hypotensive effect of AEA is enhanced compared to normotensive rats (Lake et al. 1997). CB_1 receptor agonists or FAAH inhibition also decreases contractility and normalises blood pressure in anaesthetised hypertensive animals (Bátkai et al. 2004). However, a similar

experiment with conscious, freely moving animals showed only a modest response to AEA, although the hypertensive rats had a CB_1-mediated bradycardic response to AEA not seen in the normotensive animals (Wheal et al. 2007). Nonetheless, Bátkai and colleagues (2004) also showed that CB_1 expression was significantly greater in both cardiac tissue and the aortic endothelium of spontaneously hypertensive rats (SHRs) than in normotensive controls, indicating at least that the expression of the endocannabinoid system is altered in hypertension.

Clinical studies with the CB_1 antagonist, Rimonabant, in the Rimonabant in Obesity trials demonstrated minimal effects on blood pressure in normotensive subjects, but much greater reductions in blood pressure in obese hypertensives and patients with type II diabetes (Ruilope et al. 2008) suggesting that excessive endocannabinoid activation of CB_1 receptors could underlie the patients' hypertension. To support this theory, several studies have shown positive correlations between circulating endocannabinoid levels and blood pressure. One study looking at potential correlations between circulating AEA and obstructive sleep apnea found that circulating AEA was a stronger determinant of blood pressure than sleep apnea severity, obesity, insulin resistance or inflammation (Engeli et al. 2012). Another study in females with depression showed that diastolic and mean arterial blood pressures were positively correlated with serum levels of AEA and 2-AG (Ho et al. 2012).

2.3 Endocannabinoids in Shock

Shock is characterised by a reduction in cardiac output, significantly reduced blood pressure and poor tissue perfusion. In 1997, Wagner and colleagues showed that a CB_1 receptor antagonist could prevent the fall in blood pressure associated with haemorrhagic shock and that AEA and 2-AG synthesised by monocytes and platelets were responsible for CB_1 activation in shock (Wagner et al. 1997). The same group went on to show a similar role for CB_1 in endotoxic shock (Varga et al. 1998) and in cardiogenic shock after a myocardial infarction (Wagner et al. 2001). The effects of the CB_1 receptor antagonist were not observed when administered centrally, indicating a peripheral mechanism of action. Activation of CB_1 on arteries to directly produce vasodilatation (see Sect. 4), as well as inhibition of sympathetic neurotransmitter release (see Malinowska et al. 2008 for a review), brings about vasodilatation and the drop in blood pressure. It is suggested that the release of endocannabinoids may play an important role in mediating cardioprotection in shock (see Sect. 3.1), however, it has also been shown that CB_1 antagonists decrease mortality in models of shock (Kadoi et al. 2005).

2.4 Summary on Haemodynamic Effects of Endocannabinoids

Endocannabinoids play a minimal role in the regulation of cardiovascular function in normal conditions, with the exception of modulation of baroreflex sensitivity and sleep–wake cardiorespiratory control at a central level. In various forms of shock, there is a clear role for activation of the CB_1 receptor by endocannabinoids released within blood in mediating the associated hypotension. In hypertension, there is evidence for both upregulation (cardiac tissue and the aortic endothelium) and downregulation (nucleus tractus solitarius) of the CB_1 receptor involved in the maintenance of high blood pressure and reduced baroreflex sensitivity. Human studies have shown that CB_1 antagonism reduces blood pressure in obese hypertensive and diabetic patients.

3 Endocannabinoids and Cardiac Function

The endocannabinoid system is expressed throughout the myocardium, and endocannabinoids are detected in cardiac tissue (see Tuma and Steffens 2012 for a review), playing roles in various aspects of cardiac function including contractility and regulation of coronary tone. AEA decreases contractile performance in human atrial muscle via CB_1 receptors (Bonz et al. 2003). This may be related to the finding that CB_1 activation in the heart decreases noradrenaline release, so less β_1 receptors will then be activated (Molderings et al. 1999). It is also related to the fact that AEA inhibits the function of voltage-dependent Na^+ and L-type Ca^{2+} channels in rat ventricular myocytes (Al Kury et al. 2014). AEA causes endothelium-dependent vasorelaxation of rat or sheep coronary arteries via CB_1 activation, with no role for endocannabinoid metabolites, CB_2 or TRPV1 (White et al. 2001; Ford et al. 2002; Grainger and Boachie-Ansah 2001). In contrast, palmitoylethanolamide (PEA) did not relax precontracted rat coronary arteries (White et al. 2001).

3.1 Cardioprotective Effects of Endocannabinoids

In cardiac pathologies, the endocannabinoid system is altered, and the majority of evidence suggests the increases in endocannabinoid levels in cardiac disorders are protective. AEA levels are transiently increased during ischaemia/reperfusion (Duerr et al. 2014). Patients with aortic stenosis have higher concentrations of AEA (Duerr et al. 2013). Chronic heart failure patients have elevated AEA and 2-AG levels (Weis et al. 2010). Cardiac levels of 2-AG, but not AEA, are increased in preconditioning (Wagner et al. 2006). However, acute stress was recently shown to decrease cardiac endocannabinoid levels (Holman et al. 2014), which might indicate that an upregulation of the endocannabinoid system in the heart is a chronic effect. Upregulation of cannabinoid receptors has been shown in cardiac pathologies, particularly CB_2, which is upregulated in chronic heart failure (Weis

et al. 2010), in aortic stenosis (Duerr et al. 2013) and in ischaemia/reperfusion (Duerr et al. 2014). CB_2 expression is under the control of microRNA-665 (miR-665), whose expression is increased in heart failure (Mohnle et al. 2014).

The hypothesis that endocannabinoids are protective in cardiac dysfunction comes from multiple pieces of evidence. Exogenous application of 2-AG (Wagner et al. 2006), PEA (Lepicier et al. 2003) or AEA (Underdown et al. 2005; Hydock et al. 2009; Li et al. 2013a) confers cardiac protection after various stressors in animal models. The majority of studies suggest that this is a CB_2 receptor-mediated event, although AEA has been shown to also have cardioprotective actions through CB_1. The importance of CB_2 in cardioprotection was highlighted in a recent paper which found that CB_2-deficient mice showed greater damage in response to repetitive periods of ischaemia/reperfusion leading to cardiomyopathy (Duerr et al. 2014). This was because the hearts of the CB_2 knockout mice had increased inflammatory responses, adverse remodelling, increased rates of apoptosis and an inability to turn on anti-oxidative enzymes (Duerr et al. 2014). CB_1 knockout mice are also more susceptible to a chronic heart failure model (Liao et al. 2013). Similarly, mice in whom FAAH has been knocked-out have reduced age-related cardiac dysfunction, indicating a cardioprotective role for locally produced endogenous cannabinoids (Batkai et al. 2007). In humans, a polymorphism of FAAH is associated with an increased risk of a myocardial infarction (Chmelikova et al. 2014).

In the heart, mild stress confers protection leading to a reduction in infarct size in response to subsequent stressors. This is known as preconditioning. A role for the endocannabinoid system has been well established in mediating cardiac preconditioning. Endotoxin preconditioning (Lagneux and Lamontagne 2001) and heat stress preconditioning (Joyeux et al. 2002) are attenuated by CB_2 receptor blockade, suggesting a protective role for locally produced endocannabinoids. Delayed preconditioning is also sensitive to CB_1 receptor blockade (Wagner et al. 2006).

Cardiac protection is conferred not only by endocannabinoids locally synthesised in the heart but also by circulating endocannabinoids. Remote ischaemic preconditioning is defined as transient brief episodes of ischaemia at a remote site before a subsequent prolonged ischaemia/reperfusion injury of the target organ. In the heart, remote ischaemic preconditioning reduces subsequent infarct volume, and this was inhibited by a CB_2, but not CB_1, antagonist, implicating a role for circulating endocannabinoids (Hajrasouliha et al. 2008).

The mechanisms by which endocannabinoids are cardioprotective include decreased neutrophil infiltration, decreased inflammation, decreased oxidative stress and increased activation of cardioprotective signalling pathways, through activation of CB_1 and CB_2 (Tuma and Steffens 2012). The cardioprotective effects of AEA involve the induction of heat shock protein 72 through the PI3K/Akt signalling pathway via CB_2 (Li et al. 2013a). CB_2 activation also inhibits mitochondria-mediated apoptosis via PI3K/Akt signalling in the myocardium after ischaemia/reperfusion injury (Li et al. 2013b).

3.2 Cardiodeleterious Effects of Endocannabinoids

There are also studies suggesting CB_1 receptor activation has negative effects on cardiac function. For example, CB_1 receptor antagonism reduces, and AEA enhances, the cardiotoxic effects of the chemotherapy drug doxorubicin in human cardiomyocytes (Mukhopadhyay et al. 2010). CB_1 activation by AEA in human coronary artery endothelial cells activates cell death (Rajesh et al. 2010). Daily treatment with the CB_1 antagonist Rimonabant has also been shown to reduce infarct size, and this effect was absent in $CB_1^{-/-}$ mice (Lim et al. 2009). Daily treatment with Rimonabant also improves systolic and diastolic heart function after permanent ligation of the left coronary artery (Slavic et al. 2013).

3.3 Endocannabinoids and Arrhythmias

CB_2 receptor activation reduces the incidence of ventricular arrhythmias during coronary occlusion (Krylatov et al. 2001). AEA also reduces epinephrine-induced arrhythmias, although this was CB_1 and CB_2 independent (Ugdyzhekova et al. 2001). However, more recently, neither AEA nor 2-AG were found to affect ischaemia-induced ventricular fibrillation, although a CB_1 antagonist (but not CB_2 antagonist) alone did have some positive effects during the later stage of acute ischaemia (Andrag and Curtis 2013). In isolated sinoatrial node samples from rabbits, AEA shortens the action potential duration and amplitude via CB_1 (Zhang et al. 2013). A similar effect of AEA, that resulted from an inhibitory effect on the functioning of voltage-dependent Na^+ and L-type Ca^{2+} channels, has been observed on the action potential of rat ventricular myocytes, although in these cells, the effect was independent of CB_1 and CB_2 receptors (Al Kury et al. 2014).

3.4 Summary of Cardiac Effects of Endocannabinoids

There is much evidence that the endocannabinoid system is upregulated in cardiac pathologies. The majority of evidence indicates this is likely to be cardioprotective, mainly through CB_2 activation, but with a role also for CB_1 activation. However, the role of CB_1 is controversial because in some situations, CB_1 activation may be detrimental in the heart.

4 Endocannabinoids and the Vasculature

CB_1 and CB_2 are widely distributed in the vasculature, observed in vascular smooth muscle and endothelial cells (Sugiura et al. 1998; Liu et al. 2000; Rajesh et al. 2007; Rajesh et al. 2008). The first in vitro report of endocannabinoid-induced vasorelaxation of isolated arteries and arterial beds came from Ellis and colleagues (1995) who showed that AEA and Δ^9-tetrahydrocannabinol (THC) cause

vasorelaxation of rabbit cerebral arteries, associated with an increase in vasoactive prostanoids. Many studies have since shown acute vasorelaxant responses (within minutes of application) to other endocannabinoid and endocannabinoid-like compounds including 2-AG, NADA, oleoylethanolamine (OEA), PEA, *N*-arachidonoyl-L-serine (ARA-S), *N*-arachidonoyl glycine and oleamide in a range of different arterial beds from different species (see Stanley and O'Sullivan 2014a). The mechanisms underlying these responses involve the activation of some, but not necessarily all, of the following targets/actions: CB_1, TRPV1, a site on the endothelium and modulation of ion channels. Some endocannabinoids also cause a time-dependent (over hours) vasorelaxant effect mediated by peroxisome proliferator-activated receptors (PPARs; O'Sullivan et al. 2009; Romano and Lograno 2012). The evidence for each of the pathways involved will now be discussed.

4.1 Role for CB_1

A potential role for CB_1 activation is one of the most commonly investigated mechanisms of action for the vascular effects of cannabinoids, and we know this underpins the hypotensive effects of endocannabinoids in shock. Vasorelaxation to AEA is inhibited by CB_1 receptor antagonism in renal arterioles (Deutsch et al. 1997; Koura et al. 2004), rat mesenteric arteries (White and Hiley 1998; O'Sullivan et al. 2004a), the perfused mesenteric bed (Wagner et al. 1999), bovine ophthalmic arteries (Romano and Lograno 2006), cat cerebral arteries (Gebremedhin et al. 1999) and the rabbit aorta (Mukhopadhyay et al. 2002). However, other studies have shown that CB_1 antagonism does not affect AEA-induced vasorelaxation in rat mesenteric arteries (Plane et al. 1997), the rat mesenteric bed (Peroni et al. 2004), rat hepatic arteries or guinea pig basilar arteries (Zygmunt et al. 1999) or the rat aorta (O'Sullivan et al. 2005). AEA is also capable of causing vasorelaxation of the same magnitude in the mesenteric bed of $CB_1^{-/-}$ as $CB_1^{+/+}$ mice (Jarai et al. 1999), suggesting other pathways can compensate when CB_1 is blocked or absent. Vasorelaxation induced by NADA, OEA and oleamide are all at least partly mediated by CB_1 (see Stanley and O'Sullivan 2014a). The mechanism by which CB_1 activation brings about relaxation is likely to involve numerous pathways. Gebremedhin et al. (1999) showed that AEA decreases Ca^{2+} currents via CB_1 in smooth muscles cells from cat cerebral microvasculature. Other studies have shown that CB_1 activation in the vasculature is coupled to nitric oxide (NO) release (Deutsch et al. 1997; Poblete et al. 2005).

In humans, AEA-induced vasorelaxation of isolated mesenteric arteries is inhibited by CB_1 antagonism (Stanley and O'Sullivan 2012). However, in the same arteries, the vasorelaxant effect of 2-AG was not CB_1 mediated (Stanley and O'Sullivan 2014b). AEA and virodhamine-induced vasorelaxation of the human pulmonary artery is also not dependent on CB_1 (Kozlowska et al. 2007; Kozlowska et al. 2008; Baranowska-Kuczko et al. 2014).

4.2 Role for CB_2

Most studies have found that there is no involvement of CB_2 in mediating the vascular responses to endocannabinoids in animals or humans (see Stanley and O'Sullivan 2014a). However, there are a couple of exceptions to this. AlSuleimani and Hiley (2013) showed a role for CB_2 in OEA-induced vasorelaxation of small resistance arteries of the mesenteric bed. AEA also causes vasorelaxation of rat coronary arteries that is inhibited by CB_2 antagonism (Mair et al. 2010). It is more likely that CB_2 plays a role in other functions of the endothelium such as the regulation of adhesion molecules, monocyte adhesion and endothelial permeability (see Sect. 4.10).

4.3 Role for CB_e

Early indications of an endothelial cannabinoid receptor that is distinct from CB_1 and CB_2 came from the works of Jarai and colleagues (1999) who showed that AEA was able to cause endothelium-dependent vasorelaxation of the mesenteric vasculature equally in CB_1/CB_2 knockouts as in wild-type mice, suggesting the involvement of receptors other than CB_1 or CB_2 located on the endothelium. This has become known as the endothelial cannabinoid receptor, or CB_e. Activation of this receptor by AEA has been confirmed in numerous studies. In rabbit aortic rings, AEA causes vasorelaxation through a pertussis toxin (PTX)-sensitive endothelial receptor (Mukhopadhyay et al. 2002), and in the rat aorta, AEA-induced relaxation is sensitive to endothelium denudation, PTX and O-1918 (a proposed antagonist of CB_e that has no affinity for CB_1 or CB_2 receptors), but not to CB_1 or CB_2 antagonism (Herradon et al. 2007). Similar results have been obtained in rat resistance mesenteric arteries (O'Sullivan et al. 2004a). Other endocannabinoids or endocannabinoid-like compounds suggested to activate CB_e include NADA in rat mesenteric arteries (O'Sullivan et al. 2004b), OEA in rat mesenteric arteries and the aorta (Wheal et al. 2010; AlSuleimani and Hiley 2013), oleamide in rat mesenteric resistance arteries (Hoi and Hiley 2006) and ARA-S (Milman et al. 2006) and *N*-arachidonoyl glycine (Parmar and Ho 2010) in rat mesenteric arteries. However, there is no role for CB_e in the vasorelaxant effects of 2-AG (Kagota et al. 2001) or PEA (White and Hiley 1998). Vasorelaxation induced by the activation of CB_e may involve the release of endothelium-derived hyperpolarising factor (Jarai et al. 1999; O'Sullivan et al. 2004b), BK_{ca} channel modulation (Hoi and Hiley 2006) and NO production (Mukhopadhyay et al. 2002; Herradon et al. 2007; McCollum et al. 2007).

In human pulmonary and mesenteric arteries, AEA causes endothelium-dependent vasorelaxation that can be inhibited using the proposed CB_e antagonist O-1918 (Stanley and O'Sullivan 2012; Baranowska-Kuczko et al. 2014). Similarly, in the human pulmonary artery, the vasorelaxant effects of virodhamine are inhibited by O-1918 (Kozlowska et al. 2007, 2008). This suggests that this proposed

endothelial target site for endocannabinoids is also present and functional in human vasculature.

4.4 Role for Other Uncloned CB Receptors

Some pharmacological evidence suggests there may be other cannabinoid receptors in the vasculature that remain to be identified. For example, 2-AG-induced vasorelaxation of the rabbit mesenteric arteries is inhibited by 3 μM but not 1 μM Rimonabant and is not affected by removal of the endothelium. This is not consistent with a role for either CB_1 or CB_e and suggests that another target for 2-AG may exist on the vascular smooth muscle (Kagota et al. 2001). ARA-S-induced vasorelaxation of rat mesenteric arteries is inhibited by O-1918 (even in denuded arteries) but not PTX (Milman et al. 2006), which casts doubt on the specificity of action of O-1918 at CB_e if it inhibits responses in endothelial-denuded arteries. In the rat aorta, vasorelaxation by AEA or NADA is inhibited by PTX, but not by antagonism of either CB_1 or CB_2 or removal of the endothelium (O'Sullivan et al. 2005), again suggesting another receptor for these endocannabinoids is located on vascular smooth muscle. Similarly, vasorelaxation of the rat aorta by ARA-S is inhibited by PTX but not O-1918, or CB_1 or CB_2 antagonism (Milman et al. 2006). Together, these studies suggest that further sites of action for endocannabinoids may exist on vascular smooth muscle.

4.5 Role for TRPV1

Zygmunt and colleagues (1999) were the first to show that the vasorelaxant effects of AEA, but not 2-AG or PEA, could be blocked by capsaicin pre-treatment (to deplete sensory neurotransmitters) or inhibited by a TRPV1 antagonist. They showed this involves the release of calcitonin gene-related peptide (CGRP) causing vasorelaxation through activation of CGRP receptors (Zygmunt et al. 1999). AEA induced vasorelaxation though TRPV1 is also reported to be linked to NO production in the rat mesenteric vascular bed (Poblete et al. 2005). Many studies have confirmed the role of TRPV1 in AEA-induced vasorelaxation (Harris et al. 2002; Ho and Hiley 2003; O'Sullivan et al. 2004b; Peroni et al. 2004). Other endocannabinoids or endocannabinoid-like compounds that cause vasorelaxation through TRPV1 activation include NADA (O'Sullivan et al. 2004a) and OEA (Ho et al. 2008; Wheal et al. 2010; AlSuleimani and Hiley 2013). However, in rat coronary arteries and rat pulmonary arteries, AEA-induced vasorelaxation is not affected by incubation with capsaicin or a TRPV1 antagonist (White et al. 2001; Baranowska-Kuczko et al. 2012), which may reflect differences in sensory innervations or TRP expression between vascular beds. In isolated human mesenteric arteries and pulmonary arteries, capsaicin pre-treatment does not inhibit AEA-, 2-AG- or virodhamine-induced vasorelaxation (Kozlowska et al. 2008; Stanley and O'Sullivan 2014b; Baranowska-Kuczko et al. 2014), possibly suggesting species

differences in the role or expression of TRP channels in the vasculature or the ability of endocannabinoids to activate these sites.

4.6 Role for PPARs

In addition to the acute vascular responses to endocannabinoids, a time-dependent (over hours) vasorelaxant response can be seen after a single application of AEA and NADA, but not PEA (O'Sullivan et al. 2009). This effect was mediated by PPARγ. Romano and Lograno (2012) showed a similar time-dependent vasorelaxant response to AEA and PEA in the bovine ophthalmic artery that could be inhibited by a PPARα (but not PPARγ) antagonist. As PPAR activation in the vasculature mediates other effects such as anti-inflammatory and anti-atherosclerotic actions, the possibility exists that the endocannabinoid system and production of endocannabinoids, in endothelial or smooth muscle cells, could bring about some of these effects through PPAR activation.

4.7 Metabolic Products of Cannabinoids

Some of the vascular effects of endocannabinoids are mediated by their metabolic products. This is evidenced by the fact that the vasorelaxant effects of AEA and 2-AG can be inhibited by FAAH, MAGL, cyclooxygenase (COX) and cytochrome P450 inhibition (Ellis et al. 1995; Fleming et al. 1999; Gauthier et al. 2005; Herradon et al. 2007; Awumey et al. 2008; Czikora et al. 2012; Stanley and O'Sullivan 2014b). The metabolites produced include arachidonic acid, prostaglandins and epoxyeicosatrienoic acids (Pratt et al. 1998; Stanke-Labesque et al. 2004), which can themselves have direct vascular effects, or be further metabolised into vasoactive substances. For example, metabolic products of AEA metabolism activate the prostacyclin receptor in the rat and human pulmonary artery (Baranowska-Kuczko et al. 2012, 2014). It is likely that for some endocannabinoids, their vascular responses are brought about by a combination of effects of the compounds themselves (through CB_1, TRPV or PPAR activation) and vascular effects of their metabolites. Some of these metabolites formed from endocannabinoids or endocannabinoid-like compounds can also have vasoconstrictor effects. For example, metabolites of AEA can induce vasoconstriction in the rabbit lung via the prostanoid EP_1 receptor (Wahn et al. 2005), and metabolites of 2-AG (Stanke-Labesque et al. 2004) and OEA (Wheal et al. 2010) cause vasoconstriction via the thromboxane receptor. Therefore, it is worth considering that the vascular effects of endocannabinoids might be altered in pathologies where the expression of enzymes involved (FAAH, MAGL or COX) and of the receptors activated might be altered.

4.8 Vascular Responses to Endocannabinoids in Disease Situations

The vascular responses to endocannabinoids are altered in some disease situations. Wheal et al. (2007) showed an enhanced vasorelaxant response to AEA in perfused mesenteric beds of rats made hypertensive by chronic NO synthase inhibition. A subsequent study with this model showed this was abolished by capsaicin pre-treatment, suggesting an increased sensory nerve involvement (Wheal and Randall 2009). However, in the SHR, the vasorelaxant effects of AEA were reduced in the perfused mesenteric bed and were enhanced in aortic rings (Wheal and Randall 2009). The enhanced response in SHR aortae was endothelium-dependent (Wheal and Randall 2009). Hopps et al. (2012) also showed that the vasorelaxant response to oleamide was enhanced in the aorta of SHRs, and that this could be abolished by capsaicin pre-treatment, again suggesting an increased role for sensory nerve activation by endocannabinoids in hypertension. In contrast, the COX-sensitive component of the response to oleamide was lost in SHRs (Hopps et al. 2012).

Domenicali and colleagues (2005) showed that the vasorelaxant response to AEA was enhanced in cirrhotic rats, and that this was associated with an increase in CB_1 and TPRV1 receptor expression. Similarly, Moezi et al. (2006) showed that AEA increases mesenteric arteriole diameter in cirrhotic rats but not control rats, and that this was blocked by a CB_1 antagonist and associated with increased CB_1 and TPRV1 receptor protein. By contrast, the vasorelaxant responses to AEA are reduced in mesenteric arteries from young obese Zucker rats, and this is associated with decreased CB_1 and CB_2 expression (Lobato et al. 2013). We have also shown that the responses to AEA and 2-AG are reduced in the Zucker diabetic model, which appears to be brought about by enhanced metabolism of these endocannabinoids, including the production of vasoconstrictor metabolites acting at the thromboxane receptor (Wheal et al. 2012).

4.9 Endocannabinoids and Veins

Despite the wealth of literature on the direct effects of endocannabinoids on arteries, there are few studies on the effects of endocannabinoids in veins. Although many authors have used human umbilical vein endothelial cells, this has been as a model of endothelial cell function, rather than to examine the effects of endocannabinoid on venous function. Only two studies have looked at this. Stefano et al. (1998) showed that acute treatment with AEA increased NO release in human saphenous vein, and this was associated with decreased monocyte adherence. However, chronic treatment of human saphenous veins with AEA led to increased monocyte adherence because of a desensitisation to AEA-induced NO release (Stefano et al. 1998). In isolated rings of human umbilical vein (Pelorosso et al. 2009), 150 min (but not 15 min) exposure to AEA decreases the contractile response to bradykinin via the CB_1 receptor and not the CB_2 receptor.

4.10 Endocannabinoids and Atherosclerosis

Many studies have investigated the role of the endocannabinoid system in atherosclerosis (see Steffens and Pacher 2015; Carbone et al. 2014 for reviews). Increased expression of CB_1 has been observed in human coronary atherectomy samples and CB_1 expression was greater in lipid-rich atheromatous plaques than in fibrous plaques (Sugamura et al. 2009). Increased levels of 2-AG have also been observed in the aorta of a mouse model of atherosclerosis (Montecucco et al. 2009). Plasma levels of AEA and 2-AG are raised in patients with coronary artery disease (Sugamura et al. 2009). As in cardiac pathologies, the assumption is that up-regulation of the endocannabinoid system in atherosclerosis is protective. Accordingly, FAAH knockout mice show increased monocyte adhesion to endothelial cells (Batkai et al. 2007), and genetic deletion of CB_2 worsens atherogenesis in hyperlipidic mice (Hoyer et al. 2011).

Given the anti-inflammatory effects of CB_2 activation, it is not surprising that many studies have indicated a protective role of CB_2 agonists/activation in vivo in animal models of atherosclerosis. The effects of CB_2 activation in vivo include decreased plaque development, decreased vascular smooth muscle cell proliferation, improved endothelial function, decreased expression of adhesion molecules, decreased oxidative stress, and decreased macrophage infiltration (Steffens et al. 2005; Zhao et al. 2010; Hoyer et al. 2011). In endothelial cell studies, AEA and CB_2 agonists decrease TNFα and adhesion molecules, and chemotaxis and neutrophil adhesion (Rajesh et al. 2007). CB_2 agonists also decrease the proliferation and migration of human vascular smooth muscle cells (Rajesh et al. 2008).

The role of CB_1 in atherosclerosis is more controversial, with evidence suggesting both a pro- and anti-atherosclerotic effect of receptor activation. Rimonabant has been shown to reduce atherosclerotic lesions and decrease cytokine release in a mouse model (Dol-Gleizes et al. 2009), and cell studies have shown that CB_1 blockade decreases inflammatory cytokines in macrophages (Sugamura et al. 2009; Han et al. 2009). Also, CB_1 activation causes endothelial cell injury (Rajesh et al. 2007). In contrast to these studies, the STRADIVARIUS trial studying the effect of Rimonabant on atherosclerosis progression in patients with abdominal obesity and coronary artery disease did not see a significant difference in their primary outcome measure, atheroma volume (Nissen et al. 2008). Similarly, the AUDITOR study (Atherosclerosis Underlying Development assessed by Intima-media Thickness in patients On Rimonabant) saw no difference in atherosclerosis progression in patients receiving Rimonabant for 30 months (O'Leary et al. 2011), casting doubt on a contributory role for CB_1 activation in atherosclerosis. Furthermore, a screening of 2411 patients looking at 19 different polymorphisms of the gene encoding CB_1 did not reveal any association with coronary heart disease (de Miguel-Yanes et al. 2011). However, the G1359A polymorphism of CNR1 (the gene encoding CB_1) does contribute to the genetic risk of coronary artery disease in a Chinese Han population with type 2 diabetes (Wang et al. 2012).

4.11 Summary of Vascular Effects of Endocannabinoids

Endocannabinoids cause acute and time-dependent vasorelaxation of arteries in animal and human studies through activation of CB_1, CB_e, TRPV and PPARs, coupled to inhibition of calcium channels, activation of potassium channels, NO and vasoactive metabolite production and the release of other vasoactive substances such as CGRP. Changes in the expression of any of these components alters the vascular effects of endocannabinoids, with both enhancement and reductions in the response to endocannabinoids observed in hypertension, cirrhosis, obesity and diabetes. Endocannabinoids can have positive and negative effects on the progression of atherosclerosis. Most evidence suggests a protective role for CB_2 activation and a negative effect of CB_1 activation. However, any negative CB_1-mediated effects may not be consequential, as chronic CB_1 antagonism in large scale human trials was not associated with significant reductions in atheroma volume.

5 Endocannabinoids and Blood

Circulating levels of endocannabinoids are altered in a multitude of disorders including (but not limited to) obesity (Blüher et al. 2006), diabetes and insulin resistance (Cote et al. 2007; Abdulnour et al. 2014), obstructive sleep apnea (Engeli et al. 2012) and post-traumatic stress (Hauer et al. 2013). In many studies, it has been shown that plasma levels of AEA and 2-AG are correlated with metabolic and cardiovascular risks (Weis et al. 2010; Quercioli et al. 2011), although it is not clear whether there is a causal link between these factors. It is also not clear what the source of circulating endocannabinoids are, although in situations like cardiogenic shock, it is likely that endocannabinoids are derived from platelets and macrophages (Varga et al. 1998), while in obesity, it is suggested that they might arise from adipose tissue.

Looking first at the effects of endocannabinoids and endocannabinoid-like compounds on the formation of blood cellular components, AEA, 2-AG and PEA have been shown to stimulate mouse haematopoietic cell growth and differentiation into granulocyte, erythrocyte, macrophage and megakaryocyte colonies (Valk et al. 1997; Patinkin et al. 2008) through activation of the CB_2 receptor (Valk et al. 1997). 2-AG can also increase the formation and maturation of platelets from human megakaryoblasts (Gasperi et al. 2014).

AEA can easily pass through the cell membrane of red blood cells (erythrocytes) (Bojesen and Hansen 2005), and in red blood cells, AEA increases cytosolic Ca^{2+} activity, leading to cell shrinkage and cell membrane scrambling of mature erythrocytes, and this was inhibited by cyclooxygenase inhibitors (Bentzen and Lang 2007). This ability of AEA to stimulate red blood cell death is beneficial in infections in which erythrocytes get infected, and inducing cell death maintains a healthy red blood cell population (Bobbala et al. 2010).

Both AEA (Maccarrone et al. 1999) and 2-AG (Maccarrone et al. 2001) activate platelets, albeit at very high concentrations. However, the platelet levels of

endocannabinoids may also be very high, suggesting this activation is likely to be physiologically relevant. Activation of platelets by endocannabinoids has been ascribed to their metabolism to arachidonic acid (Braud et al. 2000) or to cannabinoid receptor activation (Maccarrone et al. 2001). Interestingly, CB_1 and CB_2 have been detected in human platelets, within the cell membrane (Catani et al. 2010a). More recently, virodhamine and 2-AG, but not AEA, were shown to share the ability of arachidonic acid to induce human platelet aggregation (Brantl et al. 2014). This could be blocked by inhibitors of their metabolism by MAGL or COX, and was not mimicked by CB_1 or CB_2 agonists, suggesting it is metabolites of virodhamine and 2-AG that mediate their effects. 2-AG can also increase platelet formation and maturation (Gasperi et al. 2014). Similarly, AEA can extend platelet survival through CB_1-dependent Akt signalling (Catani et al. 2010b), indicating that there are many aspects of platelet function that can be modulated by endocannabinoids.

In human peripheral blood mononuclear cells (lymphocytes, monocytes and macrophages), endocannabinoids decrease cytokine production and regulate many aspects of white blood cell function and immunity. Immune system modulation by endocannabinoids is discussed in detail in this volume in Cabral et al., "Endocannabinoids and the immune system in health and disease".

6 Endocannabinoids and Neurovascular Function

Endocannabinoids are neuroprotective, an effect brought about by decreased excitotoxicity, decreased oxidative stress, anti-inflammatory actions and the induction of hypothermia (see Fernández-Ruiz et al., "Endocannabinoids and neurodegenerative disorders: Parkinson's disease, Huntington's chorea, Alzheimer's disease, and others" in this volume). As well as these neurological actions, endocannabinoids also affect vascular function in the brain. As in other arteries, endocannabinoids cause vasorelaxation of cerebral arteries through the production of vasoactive prostanoids (Ellis et al. 1995). Activation of the CB_1 receptor in cat cerebral vascular smooth muscle cells inhibits the influx of Ca^{2+} through L-type Ca^{2+} channels, helping to bring about vasorelaxation (Gebremedhin et al. 1999). 2-AG reduces the effects of endothelin-1 and thus reduces cerebral vasoconstriction in human cerebral endothelial cells, mediated by CB_1 (Chen et al. 2000). AEA also inhibits the vasoconstrictor effects of endothelin-1 in rabbit basilar arteries (Dogulu et al. 2003). There appears to be a relationship between endocannabinoids and cerebral vasoconstriction, as another study showed that the thromboxane mimetic, U-46619, significantly increased AEA and 2-AG content of the middle cerebral artery, whereas serotonin decreased AEA and 2-AG content (Rademacher et al. 2005). U46619-induced contractions of the rat middle cerebral artery could also be enhanced by antagonism of the CB_1 receptor. This may help to explain the potential beneficial effects of endocannabinoids in migraine (see Greco et al. 2010 for a review).

The blood–brain barrier (BBB) is formed by brain endothelial cells that line the cerebral microvasculature, capillary basement membranes and astrocyte end feet, which surround 99 % of the BBB endothelia and play an important role in maintaining BBB integrity. Increased BBB permeability associated with multiple

sclerosis is decreased by AEA (Mestre et al. 2011). We recently investigated the effects of various endocannabinoids and endocannabinoid-like compounds on BBB permeability using an in vitro model in which human brain microvascular endothelial cells and human astrocytes were co-cultured (Hind et al. 2015). We found that only AEA and OEA affected BBB permeability in control conditions and that they both decreased BBB permeability (i.e. increased resistance). This was mediated by CB_2, TRPV1 and CGRP receptors (for AEA) and PPARα (for OEA). In contrast, oleamide has been shown to inhibit gap junction coupling in pig brain microvascular endothelial cells, thus increasing barrier permeability in vitro (Nagasawa et al. 2006). However, we saw no effect of oleamide on BBB permeability in our human in vitro model (Hind et al. 2015).

Given the knowledge that endocannabinoids are neuroprotective, cause cerebral vasorelaxation and reduce BBB permeability, it is not surprising that they have been shown to be protective in neurovascular disorders such as traumatic brain injury (TBI) and cerebral ischaemia/reperfusion injury (stroke).

6.1 Endocannabinoids and Traumatic Brain Injury

TBI occurs when an external force traumatically injures the brain. This type of brain injury has been shown to increase 2-AG levels up to tenfold within hours and to last for at least 24 h post-injury (Panikashvili et al. 2001). The hypothesis that this increase in 2-AG might be protective was proven when it was found that administration of 2-AG enhanced the recovery from TBI, associated with a decrease in infarct volume, neuronal loss and inflammation (Panikashvili et al. 2001). TBI is known to disrupt the BBB, and in this study, 2-AG limited the increase in BBB permeability, and thus reduced the associated oedema. The effect of 2-AG was inhibited by CB_1 receptor antagonism and absent in CB_1 knockout mice. The effects of TBI are worse in CB_1 knockout mice, suggesting a CB_1-mediated protective role for endogenous endocannabinoid production in TBI. However, there is probably also a contribution of the CB_2 receptor, as a synthetic CB_2-selective agonist can also ameliorate TBI outcomes, which can be inhibited by CB_2 antagonism (Elliott et al. 2011). The endocannabinoid-like substance *N*-arachidonoyl-L-serine also improves TBI outcomes, and for this compound, the effects were inhibited by antagonists of CB_2 and TRPV1, but not CB_1 (Cohen-Yeshurun et al. 2013). More recently, PEA has been shown to have a beneficial effect in reducing oedema and infarct size in TBI (mechanisms of action not probed) (Ahmad et al. 2012a).

6.2 Endocannabinoids and Cerebral Ischaemia/Stroke

The expression of cannabinoid receptors is upregulated in the rat brain following cerebral ischaemia (stroke), indicating that the endocannabinoid system may play an important role in the endogenous response to stroke (see Hillard 2008; Tuma and

Steffens 2012). Human and animal in vivo data have shown increases in neurological and circulating plasma levels of AEA, 2-AG, OEA and PEA after stroke (Schabitz et al. 2002; Hillard 2008; Naccarato et al. 2010). As in other cardiovascular disorders, the hypothesis is that upregulation of the endocannabinoid system is protective in stroke, and this is supported by numerous studies showing that 2-AG (Wang et al. 2009), AEA (Wang et al. 2009) as well as the endocannabinoid-like compounds, OEA (Sun et al. 2007; Zhou et al. 2012) and PEA (Schomacher et al. 2008; Garg et al. 2010; Ahmad et al. 2012b), offer protection against ischaemic/reperfusion injury. *N*-acylethanolamine compounds such as lauroylethanolamide and linoleoylethanolamide have also been shown to be protective against stroke (Garg et al. 2011). In a recent systematic review and meta-analysis, we reported that endocannabinoids significantly reduced infarct volume in several models of experimental stroke (England et al. 2015).

There are multiple target sites at which endocannabinoids may act in this regard. Mice that are lacking the CB_1 receptor are more susceptible to stroke (Parmentier-Batteur et al. 2002), and CB_1 has been shown to mediate the protective effects of AEA and 2-AG (Wang et al. 2009). CB_1 activation increases neurotrophic factors, reduces excitotoxicity, reduces oxidative stress and causes the induction of hypothermia (see Tuma and Steffens 2012 for a review). CB_2 activation is also important in cerebral ischaemic injury by decreasing the release of pro-inflammatory cytokines, decreasing neutrophil recruitment, decreasing leukocyte adhesion to cerebral vessels and increasing brain-derived neurotrophic factor (Choi et al. 2013). Mice that lack the CB_2 receptor are also more susceptible to stroke (Zhang et al. 2008). In addition, the protective effects of OEA have been shown to be mediated by PPARα (Sun et al. 2007), while the protective effects of PEA are independent of CB_1 or TRPV1 (Garg et al. 2010). We have found that OEA and PEA decreased ischaemia/reperfusion-induced increases in BBB permeability in vitro and that this was PPARα mediated (Hind et al. 2015). The vasodilatory effects of endocannabinoids in the cerebral vasculature may also play a role in maintaining and restoring blood flow after a stroke.

6.3 Summary

In neurovascular disorders such as TBI and stroke, endocannabinoids are produced and the endocannabinoid system is upregulated in a protective manner, as shown by the ability of various endocannabinoid agonists to reduce damage in TBI and stroke. This protection involves CB_1, CB_2, TRPV1 and PPARα activation, and both vascular tissue (vasorelaxation, inhibition of vasoconstriction and reductions of BBB permeability and oedema) and neuronal tissue.

7 Conclusions and Closing Comments

It is clear that the endocannabinoid system has important roles in the cardiovascular system, particularly in cardiovascular pathologies. However, although much research has been carried out with AEA and 2-AG, comparatively little is known about the role and effect of other endocannabinoids and endocannabinoid-like compounds in the cardiovascular system and cardiovascular pathologies. When probing possible mechanisms of action, many studies have focussed on the potential role of CB_1 and CB_2 activation, and less is therefore known about the impact on cardiovascular pathologies of the activation by endocannabinoids of other targets, such as CB_e, and the vascular receptors, PPARs, GPR55 and $5HT_{1A}$. Furthermore, the majority of work in this area has been carried out in animals, and more research is required in humans to establish the importance of the endocannabinoid system (including as yet unidentified targets on the endothelium and vascular smooth muscle), especially in cardioprotection and atherosclerosis, both areas of unmet medical needs. Despite this, is seems likely from the evidence presented in this review that greater understanding of the role and effects of the endocannabinoid system in cardiovascular regulation in humans will lead to new target sites of action for drug discovery.

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