Endocannabinoids and the cardiovascular response to stress

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Abstract

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system (SNS), resulting in cardiovascular responses. The endocannabinoid system (ECS), a ubiquitously expressed lipid signalling system, modulates both HPA and SNS activity. The purpose of this review is to explore the possible involvement/role of the ECS in the cardiovascular response to stress. The ECS has numerous cardiovascular effects including modulation of blood pressure, heart rate, the baroreflex, and direct vascular actions. It is also involved in a protective manner in response to stressors in cardiac preconditioning, and various stressors (for example, pain, orthostasis and social stress) increase plasma levels of endocannabinoids. Given the multitude of vascular effects of endocannabinoids, this is bound to have consequences. Beneficial effects of ECS upregulation could include cardioprotection, vasodilatation, CB_2-mediated anti-inflammatory effects and activation of peroxisome proliferator-activated receptors. Negative effects of endocannabinoids could include mediation of the effects of glucocorticoids, CB_1-mediated metabolic changes, and metabolism to vasoconstrictor products. It is also likely that there is a central role for the ECS in modulating cardiovascular activity via the HPA and SNS. However, much more work is required to fully integrate the role of the ECS in mediating many of the physiological responses to stress, including cardiovascular responses.

Keywords

Cardiovascular system, endocannabinoid, stress

Introduction

Stress can be defined as a state in which homeostasis is threatened resulting in adaptive physiological changes mediated by activation of the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic nervous system (SNS). If these responses are too small or too large, or of an inappropriate duration, homeostasis remains disrupted, possibly resulting in disease (Chrousos et al., 2009). The roles of the endocannabinoid system in mediating neurological responses to stress have been reviewed elsewhere (Hill and Gorzalka, 2009; Patel and Hillard, 2008). The purpose of this short review is to explore the involvement of the ubiquitous endocannabinoid (EC) signalling system in the stress response, particularly in relation to the cardiovascular system, and to discuss whether its role is predominantly protective or harmful, and if there are therapeutic opportunities available through manipulation of endocannabinoid function.

Stress and the cardiovascular system

There are a number of physiological responses that commonly, if not inevitably, follow the application of different stressors. These are mainly endocrine and autonomic changes, including increases in glucocorticoid levels, heart rate and blood pressure (McCormick et al., 2010; Taveres and Correa, 2006). The acute increase in cardiovascular performance in reaction to stress is part of the well-known *fight-or-flight* effect which primes the organism's physical capacity to respond. The increase in cardiac performance with sympathetic stimulation is co-ordinated by a combination of intrinsic inotropic, lusitropic and chronotropic effects, mediated mostly by activation of β-adrenoceptors which are expressed throughout the cardiac tissues along with sympathetic nerve terminals. A combination of bio-messengers, largely catecholamines and nitric oxide, control the complex vascular stress response largely designed to increase blood flow in the skeletal muscles and skin (Joyner and Casey, 2009).

Under stressful situations, the sympathetic–adrenal medullary system and the HPA axis are activated (Axelrod and Reisine, 1984). The perception of a stressor leads to activation of sensory and limbic brain structures (Pacak and Palkovits, 2001), resulting in stimulation of hypothalamic paraventricular neurones (PVN), secretion of corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and ultimately corticosteroids from the adrenal cortex.

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Activation of the sympathetic adrenal medullary system produces elevation of circulating catecholamines. This combined effect of stressors is largely protective to the organism, but hyperactivity is associated with a variety of mood disorders (Pariante and Lightman, 2008) and systemic disease risks.

Although stress itself is not a disease, it may increase the susceptibility of individuals to a wide variety of different diseases (McEwen and Stellar, 1993). It is a 'well-known fact' that chronic stress increases the risk of cardiovascular diseases, including ischaemic heart disease, myocardial infarction (MI), heart failure, arrhythmias, and sudden death (Davis and Natelson, 1993). The factors underlying the pathological basis of stress-induced cardiovascular disease are not completely understood, but excessive amounts of catecholamines released from the SNS during stress can produce cardiac dysfunction by inducing intracellular Ca^{2+} overload in cardiomyocytes. Catecholamines can also be oxidized to form aminolutins and promote the generation of oxyradicals which produce coronary spasm, arrhythmias, and cardiac dysfunction, again by inducing inappropriate Ca^{2+} handling and defects in mitochondrial energy production. Glucocorticoids amplify the effects of SNS activation by increasing plasma levels of catecholamines, by inhibiting their extraneuronal uptake and inducing β-adrenoceptor supersensitivity (Adameova et al., 2009).

There are some differences in cardiovascular stress responses, for example, to mental or emotional stress and exercise stress. Heart rate and systolic blood pressure are elevated more by physical than by mental stress, largely due to the need for increased metabolic activity to support exercise. On the other hand, mental stress increases diastolic blood pressure and systemic vascular resistance, which are unaffected or even decreased in response to dynamic exercise (Soufer and Burg, 2007). Short-term stress does not seem to be a cause of disease and, indeed, low levels of acute stressors can actually provide cardiac protection (see discussion of preconditioning below). However, long-term stress can have severe negative consequences for cardiovascular health. Longitudinal studies of more than 3000 European adults have found that chronic stress over several years predicts high blood pressure throughout the 3–7-year period of follow-up (Steptoe and Marmot, 2005). The American CARDIA study similarly showed that impatience and time pressure stress predicted hypertension 15 years later (Yan et al., 2003), and the INTERHEART study (24,000 adults in 52 countries) showed that MI was strongly associated with chronic psychosocial stress (Rosengren et al., 2004). Psychosocial stress is now believed to be as important in magnitude as smoking, obesity, diabetes, and hypertension in relation to MI risk. However, the mechanisms underlying the connections between stress and cardiovascular disease are far from clear, but it is possible that the endogenous cannabinoid signalling system is involved.

Endocannabinoid signalling

The ECs are a family of ubiquitous, small lipid messengers that have modulatory roles in most biological systems including the central nervous, endocrine and cardiovascular systems (see Di Marzo, 2009, and Figure 1 for a summary of the components of the EC system). The EC signalling system consists of endogenous agonists (and perhaps antagonists), the enzymes that control their synthesis and catabolism, and the receptors which recognize them and transduce their signals into cellular actions.

The archetypal EC, anandamide (arachidonoylethanolamide, AEA), was first isolated from pig brain in 1992 (Devane et al., 1992), and has since been joined by a number of other fatty acid-containing molecules, most notably 2-archidonoylglycerol (2-AG), which is probably the most important member of the family in the central nervous system where it functions as a retrograde transmitter, dampening synaptic transmission (Kano et al., 2009). The evidence supporting physiological roles for other putative fatty acid ECs, dihomo-γ-linolenoyl ethanolamide, docosatetraenoyl ethanolamide (Hanus et al., 1993), 2-arachidonyl glycerol ether (noladin ether) (Hanus et al., 2001), *O*-arachidonoylethanolamine (virodhamine) (Porter et al., 2002), and *N*-arachidonoyl dopamine (NADA) (Walker et al., 2002), is much less convincing. A number of other related acylethanolamines such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are widely distributed in the central nervous system and periphery. These agents do not activate cannabinoid receptors, but are suggested to influence EC signalling by competing for catabolic enzymes with AEA: a so-called 'entourage effect' (Fowler, 2003). Recently, the existence of α-haemoglobin-derived peptides, which function as cannabinoid receptor agonists that can be metabolized to an antagonist, has been described in mouse brain (Gomes et al., 2009), although their physiological/pathophysiological roles are, as yet, uncertain.

There are complex biochemical routes for the synthesis and catabolism of AEA and 2-AG (see Alexander and Kendall, 2007). Generation of AEA and related ethanolamide ECs is thought to occur primarily via hydrolysis of a minor membrane phospholipid, *N*-acylphosphatidylethanolamine (NAPE) (Di Marzo et al., 1994), by a phospholipase D (NAPE-PLD), which is able to produce the whole range of endogenous fatty acid ethanolamines (Okamoto et al., 2004). Other indirect routes of synthesis of the acylethanolamines have also been described, including phospholipase C hydrolysis of NAPE and the consequent production of acylethanolamine-*O*-phosphates, which may be hydrolysed by a selective phosphatase, PTPN22 (Liu et al., 2006). An enzyme with the ability to hydrolyse ECs, termed fatty acid amide hydrolase (FAAH), has been cloned from mouse and human sources (Giang and Cravatt, 1997), and this is probably the most important catabolic route for AEA, although oxidation via cyclooxygenases and lipoxygenases also occurs (Vandevoorde and Lambert, 2007). 2-AG synthesis is probably the result of the sequential action of phospholipase C and diacylglycerol lipase via the production of the intermediate diacylglycerol (Ben-Shabat et al., 1998; Parrish and Nichols, 2006), which is well known as an output from the phosphoinositide signalling cycle and a stimulus for protein kinase C activation (Berridge and Irvine, 1984). This gives the potential for generating 2-AG via stimulation of any G protein-coupled receptor whose activation leads to phosphoinositide hydrolysis. Monoacylglycerol lipase (MAGL) is widely agreed to be the most important enzyme for 2-AG degradation (Dinh et al.,

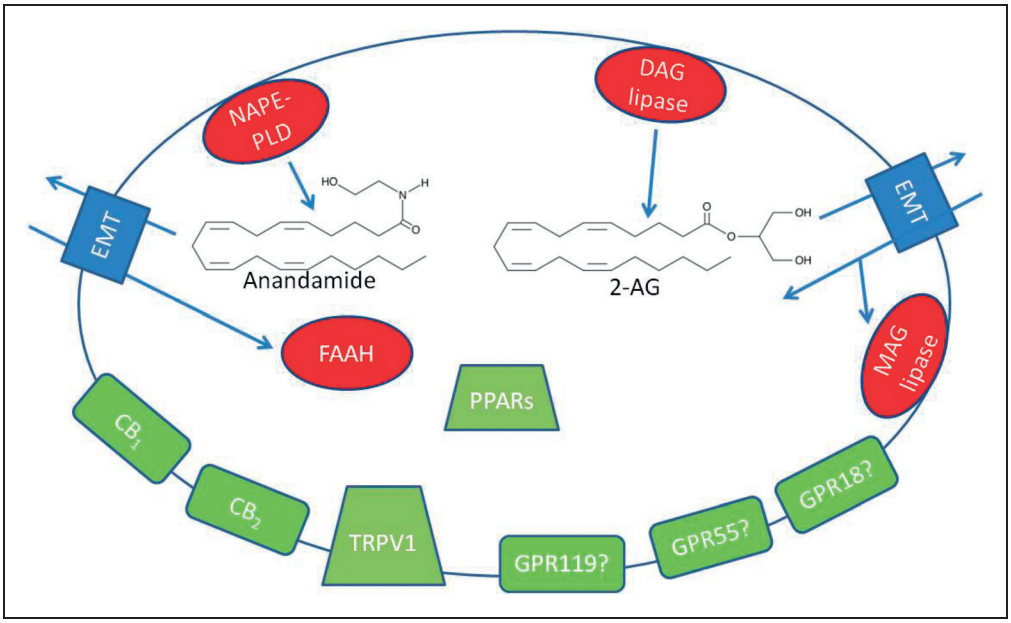

Figure 1. Major components of the endocannabinoid signalling system. The endocannabinoid system comprises endogenous compounds such as anandamide and 2-AG, their synthesizing enzymes (mainly membrane-bound NAPE-PLD for anandamide, and DAG lipase for 2-AG), endocannabinoid membrane transport (EMT) processes, and degradation enzymes (mainly cytosolic FAAH for anandamide and membrane-bound MAG lipase for 2-AG). Extracellular target sites of action for endocannabinoids include the CB_1 and CB_2 receptor (both G protein-coupled receptors) and potentially a number of orphan G protein-coupled receptors (GPR119, GPR55 and GPR18). Intracellular target sites of action include the PPAR nuclear receptors and the binding site of members of the transmembrane TRP ion channel family (including TRPV1). DAG, diacylglycerol; EMT, endocannabinoid membrane transport; FAAH, fatty acid amide hydrolase; MAG, monoacylglycerol; NAPE-PLD, *N*-acylphosphatidylethanolamine-phospholipase D; PPAR, peroxisome proliferator-activated receptor.

2002), although the less well-characterized catabolic enzyme ABHD6 (Blankman et al., 2007) has recently been reported to have a rate-limiting role in 2-AG signalling (Marrs et al., 2010).

There is no evidence for storage and exocytotic release of ECs, and the widely held belief is that they are synthesized in tissues on demand in an activity-dependent manner. Since the rate-limiting steps in EC synthesis are suggested to be Ca^{2+} dependent, it is thought that many Ca^{2+}-mobilizing stimuli can result in EC production (Cadas et al., 1997), possibly acting as a braking mechanism in excitable cells.

ECs can be recognized by, and activate representatives of, three of the four receptor super-families. The most widely studied cannabinoid receptors are the G protein-coupled receptor (GPCR), predominantly neuronal CB_1 receptor and the predominantly immune system-localized CB_2 receptor. In recent years, three additional 'orphan' GPCRs, GPR18, GPR55 and GPR119, have also been proposed to be members of the cannabinoid family, although this is a matter of some controversy (Brown, 2007). Activation of CB_1 and CB_2 receptors results in similar post-receptor signalling; reduced cyclic AMP formation, activation of K^+ channels and inhibition of Ca^{2+} channels, all contributing to reduced cell excitability, although the consequences of activating the orphan receptors are more diverse and uncertain (Godlewski et al., 2009; Kapur et al., 2009).

Rapid AEA signalling can also be achieved by activating the cation-gating TRPV1 (vanilloid) receptor channels on sensory nerve endings (Zygmunt et al., 1999). The ligand binding site is thought to be on the inside of the plasma membrane, making it readily accessible to intracellularly generated AEA, and it has been proposed that AEA can, therefore, act as an amplifier of Ca^{2+} signalling, with an initial rise in intracellular Ca^{2+} concentration activating AEA synthesis which then opens TRPV1 channels, allowing more Ca^{2+} entry in a virtuous cycle (Van der Stelt et al., 2006). There is also evidence for interactions between ECs and other TRP channels including TRPV4 (Plant and Strotman, 2007) which might play a role in vascular function.

Long-term cannabinoid signalling can be achieved through alterations in gene transcription via the peroxisome proliferator-activated receptors (PPARs) (see O'Sullivan, 2007 for a review). PPARs are nuclear receptors that regulate cell differentiation and lipid metabolism (Bishop-Bailey, 2000; Ferre, 2004; Glass, 2006). Since PPARs are activated by fatty acids and their derivatives, it was not surprising to discover that many members of the EC family can activate both the alpha and gamma isoforms of PPARs. OEA and PEA regulate feeding and body weight, stimulate fat utilization and have neuroprotective and anti-inflammatory effects via activation of PPARα (see Jhaveri et al., 2008; O'Sullivan, 2007). AEA and 2-AG similarly have anti-inflammatory properties mediated by PPARγ (O'Sullivan, 2007). AEA and NADA have also been recently shown to have vascular properties mediated by PPARγ (O'Sullivan et al., 2009a).

Endocannabinoid functions in the cardiovascular system

Cannabinoid receptors are widely distributed throughout the cardiovascular system, with CB_1 expression in myocardium (Bonz et al., 2003; Kunos et al., 2002), human coronary artery, endothelial and smooth muscle cells (Mukhopadhyay et al., 2007). CB_2 receptors have also been identified in the myocardium and in human coronary endothelial and smooth muscle cells (Rajesh et al., 2007). CB_1 receptors are also present on pre-synaptic sympathetic nerve terminals and their activation is reported, at least on the basis of *in vitro* studies, to decrease sympathetic outflow by inhibition of noradrenaline release (Niederhoffer and Szabo, 1999). These various sites of action provide the potential for control by cannabinoids at many different levels within the cardiovascular system. However, increasing the availability of endocannabinoids with the use of a transport inhibitor or a FAAH inhibitor had no effect on cardiovascular parameters in normotensive animals, although FAAH inhibition did reduce blood pressure, cardiac contractility, and vascular resistance in spontaneously hypertensive animals (Bátkai et al., 2004; Calignano et al., 1997). Similarly, genetic deletion of FAAH failed to produce baseline cardiovascular modification (Pacher et al., 2005).

Overall, therefore, there is little evidence supporting significant EC-mediated tonic control over the cardiovascular system in normal physiology, although its involvement in disease conditions is highly probable (for reviews see Hiley, 2009; Pacher and Steffens, 2009; Pacher et al., 2008). Indeed, clinical studies with the CB_1 antagonist rimonabant in the Rimonabant in Obesity (RIO) trials demonstrated minimal effects on blood pressure in normotensive subjects, but much greater reductions in blood pressure in obese hypertensives and in subjects with type II diabetes (Ruilope et al., 2008), suggesting that excessive EC activation of CB_1 receptors (at some undetermined location) could underlie these patients' hypertension.

Exogenous application of cannabinoids has been shown to have numerous cardiovascular effects. In conscious rats, anandamide causes profound bradycardia, with a transient hypotension followed by a longer-lasting pressor effect (Stein et al., 1996). The bradycardic effect was sensitive to cyclooxygenase inhibition, indicating mediation by eicosenoids. The complex haemodynamic effects of intravenously injected anandamide in conscious rats (Gardiner et al., 2002) were insensitive to the CB_1 antagonist AM251, and appeared to result from increased circulating adrenaline acting via β2-adrenoceptors. Studies using synthetic cannabinoids such as WIN 55,212-2 on normotensive and hypertensive rats have also demonstrated a CB_1-mediated pressor effect linked to increases in sympathetic activity (Gardiner et al., 2001). It should be noted that in anaesthetized animals, application of cannabinoid agonists causes prolonged hypotension (see Randall et al., 2004, for a review of the complex cardiovascular effects of cannabinoids), which may be due to altered basal sympathetic activity observed with anaesthesia (Neukirchen and Kienbaum, 2008). In human studies, smoking cannabis has been observed in some subjects to cause hypotension and/or tachycardia, both of which can be inhibited with rimonabant, indicating a role for CB_1 receptor activation (Gorelick et al., 2006; Huestis et al., 2007).

Given the widespread distribution of cannabinoid receptors, pharmacological studies based on systemic administration can be difficult to interpret, but more discrete drug application can be instructive. One such study has indicated that the EC system appears to be directly involved in the central control of blood pressure via the brainstem baroreceptor complex. The nucleus tractus solitarius (NTS) is one site of termination of baroreceptor afferent fibres from arterial baroreceptors and cardiac mechanoreceptors. Baroreceptor activation, which results, for example, from stress-induced elevation of heart rate and blood pressure, excites the caudal ventrolateral medulla, via glutamatergic fibres, which in turn inhibit, via GABAergic neurones, activity in the rostral ventolateral medulla (RVLM). The RVLM is the primary regulator of the SNS, via glutamatergic excitation of sympathetic pre-ganglionic neurons located in the intermediolateral nucleus of the spinal cord. Cannabinoid CB_1 receptors are functionally expressed in the NTS (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). In the latter study, AEA concentration in the NTS was observed to increase after a phenylephrine-induced rise in blood pressure, supporting the physiological relevance of the EC control. These studies were recently extended, and demonstrated that the EC-mediated baroreceptor response was significantly blunted in spontaneously hypertensive rats, suggesting that reduced CB_1 receptor density in the NTS could contribute to the elevated sympathetic tone characteristic of this hypertensive model (Brozoski et al., 2009). It should, however, be emphasized that these electrophysiological studies were necessarily conducted in anaesthetized animals, with the likelihood that basal SNS activity was affected.

In the vasculature, ECs have direct vasorelaxant effects when measured in isolated arteries *in vitro* (see Lopez-Miranda et al., 2008; Randall et al., 2004). The mechanisms underlying this response involve the activation of some, but not necessarily all, of the following pathways: activation of the CB_1 receptor, CB_2 receptor, TRPV1, the endothelium and modulation of ion channels. Cannabinoids have also been recently shown to have vascular effects mediated by PPARγ, causing a time- and nitric oxide-dependent vasorelaxation (O'Sullivan et al., 2009a, 2009b).

The EC system has been implicated to be involved in several cardiovascular disease states. For instance, activation of cardiac CB_1 receptors by endogenous anandamide contributes to the reduced cardiac contractility in liver cirrhosis (Bátkai et al., 2007), and excessive cannabinoid tone might also be involved in cardiogenic shock which frequently accompanies MI and is characterized by inadequate cardiac output and profound hypotension (Wagner et al., 2001). There is also evidence that the EC system is upregulated in hypertension, and that CB_1 receptor antagonists reduce blood pressure in hypertension, although this is likely to be due to decreased cardiac contractility (see Pacher et al., 2008 for a recent review). A good deal of attention has also been focussed on the potential beneficial role of ECs in

atherosclerosis. Increased levels of 2-AG have been observed in aortae in models of atherosclerosis, and CB_2 agonists have been shown to reduce atherosclerosis (see Pacher and Steffens, 2009 for a review). Conversely, the STRADIVARIUS trial studying the effect of rimonabant on atherosclerosis progression in patients with abdominal obesity and coronary artery disease found some positive effects in terms of their total atheroma volume after 18 months of treatment (Nissen et al., 2008). Similarly, in a mouse model of atherosclerosis, rimonabant has been shown to reduce atherosclerotic lesions (Dol-Gleizes et al., 2009). Thus ECs could have both positive and negative effects on atherosclerosis mediated by the CB_2 and CB_1 receptor.

Endocannabinoids and cardiac stress

The term *hormesis* refers to beneficial actions resulting from a mild activation of the stress system triggering positive, adaptive effects that allow resistance to a more severe stress that might otherwise cause dysfunction or disease. In the heart, this means that mild stress confers protection leading, for instance, to a reduction in infarct size in response to subsequent stressors; this is known as preconditioning. A role for the EC system has been well established in cardiac preconditioning (see Hiley, 2009 for a review). In brief, endotoxin preconditioning (Lagneux and Lamontagne, 2001), heat stress preconditioning (Joyeux et al., 2002) and remote ischaemic preconditioning (Hajrasouliha et al., 2008) are all attenuated by CB_2 receptor blockade, suggesting a protective role for locally produced ECs. Delayed preconditioning is also sensitive to CB_1 receptor blockade (Wagner et al., 2006). Furthermore, tissue levels of 2-AG, but not AEA, are increased in the heart in preconditioning (Wagner et al., 2006), and exogenous application of either 2-AG (Wagner et al., 2006), PEA (Lépicier et al., 2003), or anandamide (Underdown et al., 2005) have all been shown to confer cardiac protection after various stressors. The molecular mechanisms underlying EC preconditioning are not totally clear but might involve cannabinoid receptor-mediated activation of ERK-MAP kinase (Zhang et al., 2010). Collectively, the evidence strongly suggests that ECs have a cardioprotective effect in stressful conditions, although excessive cannabinoid receptor activation can be damaging.

A number of studies have reported that endogenous CB_1 receptor activation can promote cardiovascular inflammation and oxidative stress (Sugamura et al., 2009; Rajesh et al., 2010; Mukhopadhyay et al., 2010; Tiyerili et al., 2010) although this might be counterbalanced by CB_2 receptor activation (Han et al., 2009).

The effects of stress on endocannabinoid production

Painful stressors tend to reduce local EC levels; for example, Jhaveri et al. (2008) demonstrated significantly reduced levels of AEA and PEA in the hindpaws of rats injected with carrageenan. Similarly, Maione et al. (2007) reported decreased levels of AEA, 2-AG and PEA in the hindpaws of animals given intraplantar formalin injections. Such decreases are not anatomically universal, and formalin-induced pain is accompanied by increased EC levels in the spinal cord (Walker et al., 1999), presumably as part of the adaptive response attempting to alleviate the pain by modulating descending pain control pathways. Chronic pain stress, as present in persistent models such as the neuropathic rat, is associated with increased EC levels in the spinal cord and dorsal root ganglia (Mitrirattanakul et al., 2006).

Unfortunately, it is very rare for circulating plasma levels of ECs to be measured in preclinical models of stress. However, plasma levels of AEA are reported to be elevated in patients with complex regional pain syndrome (Kaufmann et al., 2009). Interestingly, 2-AG, but not AEA, plasma levels have been shown to be increased in orthostatic stress, but the increase in 2-AG was not correlated with heart rate or noradrenaline responses (Schroeder et al., 2009), challenging the concept that the EC system directly contributes to the response to haemodynamic stress and/or sympathetic stimulation of peripheral tissues. Hill et al. (2009) have also shown that a social stress test leads to an increase in plasma 2-AG levels (but not AEA, PEA or OEA) in women with depression. Obesity, which is a significant physical stressor, is also associated with increased 2-AG levels (Côté et al., 2007; Engeli et al., 2005). Conversely, anaesthesia was associated with a decrease in plasma AEA levels, while cardiopulmonary bypass was associated with an increase in 2-AG levels (Weis et al., 2010). Although the source of ECs during these stressors cannot be identified, it is not inconceivable that a systemic rise in circulating EC might affect local cardiovascular tissues. However, it has yet to be established what effect this might have on the vasculature. We have preliminary data in a rat model of obesity to show that 2-AG, which normally causes vasorelaxation in a lean rat, causes vasoconstriction of the femoral artery and aorta in obese animals, suggesting high circulating 2-AG levels may actually contribute to increase vascular tone in these animals (O'Sullivan et al., unpublished). This effect could be inhibited by indomethacin, suggesting COX-derived vasoconstrictor metabolites of 2-AG bring about this effect. However, more work is required to understand the physiological and pathological effects of the EC system on the cardiovascular system under different forms of stress.

Endocannabinoids and control of the HPA axis

There is good evidence supporting a role for the EC system in control of the HPA axis. Disruption of EC signalling either by CB_1 receptor blockade or genetic ablation increases the activity of the HPA axis (Cota et al., 2007; Steiner et al., 2008). The selective CB_1 receptor antagonist rimonabant increases ACTH and corticosterone release in rats (Manzanares et al., 1999), and plasma ACTH concentrations are reported to be increased in CB_1-null mice compared with wild type. Conversely, activation of EC signalling using the CB_1/CB_2 receptor agonist CP55940, the EC transport inhibitor AM404, or by blocking EC catabolism using the FAAH inhibitor URB597, decreased or eliminated restraint stress-induced corticosterone release in mice (Patel et al., 2004). The

same authors showed differences in EC system responses to acute and chronic stress, in that acute restraint reduced hypothalamic 2-AG content compared with the control value, but 5 days of the same repeated stress, which resulted in a reduced HPA axis activation, increased 2-AG levels. The central mechanism underlying EC control over HPA axis function has been suggested to be via CB_1-mediated inhibition of glutamate release onto PVN neurones (Di et al., 2003). Chronic environmental stress has also been reported to reduce both 2-AG content and CB_1 expression within the hippocampus, and this appears to be associated with cognitive defects reversible by cannabinoid administration (Hill et al., 2005), suggesting that the reductions are functionally relevant.

Habituation of the HPA axis response to repeated stressors, particularly of the same type, is necessary to prevent the damaging effects of chronically raised levels of glucocorticoids. Hill et al. (2010) have recently shown that the EC system is essential for such adaptation. In their rat model, repeated restraint stress increased basal corticosterone levels, but reduced steroid hormone responses to successive individual applications of the stressor. The reductions in corticosterone responses were strongly associated with increased 2-AG signalling in the amygdala, and CB_1 receptor antagonism prevented the habituation of the stress response. Interestingly, the involvement of 2-AG and AEA in regulation of habituation appeared to differ, in that repeated stress persistently decreased AEA content throughout the corticolimbic stress circuit, whereas 2-AG was elevated specifically within the amygdala, in a stress-dependent manner.

It seems likely, therefore, that EC signalling could underpin a negative feedback system with stress-induced glucocorticoids enhancing EC synthesis, the products of which apply a brake to HPA axis activity. For such a feedback system to be practicable, glucocorticoids, or another component of the HPA axis, must be able to enhance EC synthesis rapidly. In fact, glucocorticoids are able to produce a rapid, non-genomic enhancement of EC synthesis in neuroendocrine cells of the hypothalamus via activation of a cAMP signalling pathway (Malcher-Lopes et al., 2006). This is suggested to be supported by a glucocorticoid-mediated shift in membrane lipid metabolism away from arachidonic acid and eicosenoid generation towards anandamide and 2-AG synthesis, assisted by a post-transcriptional reduction in cyclooxygenase activity (Malcher-Lopes et al., 2008). Indeed, EC synthesis might mediate many of the non-genomic effects of glucocorticoids (see commentary by Hill and McEwen, 2009), although is not known whether the steroids have similar effects on EC turnover in other, non-neuronal cells, for example in the cardiovascular system.

Corticosteroids and cardiovascular disease

Excessive corticosteroids resulting from Cushing's syndrome, treatment for a variety of conditions, or perhaps chronic stress, are well known to produce hypertension. This is widely attributed to activation of the mineralocorticoid receptor by cortisol, promoting excess re-absorption of sodium and water at the level of the kidney, but there is now clear evidence of the involvement of glucocorticoid receptors in the vascular smooth muscle mediating at least part of the hypertension (Goodwin et al., 2008). Given the corticosteroid-mediated stimulation of EC synthesis, discussed in the previous section, it is interesting to speculate that the potential hypertensive effects of the steroids resulting from chronic stress might be offset by local production of vasodilator ECs in the vasculature (see Randall et al., 2004). An increase in the activity of the EC system in the vasculature has certainly been suggested to be a protective mechanism against hypertension (see Pacher et al., 2008).

The metabolic syndrome is a collection of metabolic disorders including obesity, insulin resistance, hypertension and disordered lipid metabolism that increases the risk for type 2 diabetes mellitus and vascular disease. There is now increasing evidence suggesting that patients with the metabolic syndrome show hyperactivity of the HPA axis, resulting in functional hypercortisolism, the causes for which are unknown, but might be associated with chronic stress (Anagnostis et al., 2009). The EC system is multifunctional in the regulation of food intake and energy balance, and there has been a great deal of interest in testing the potential of EC system inhibition in the metabolic syndrome. Clinical trials have shown that CB_1 receptor antagonism reduces food intake, abdominal fat and its metabolic consequences (Matias et al., 2008; Ruilope et al., 2008). It is also possible that CB_1 antagonists might have some positive effects through modulation of the HPA axis. In fact, very recent evidence suggests that CB_1-mediated signalling is crucial in mediating the metabolic effects of glucocorticoids. Hill et al. (2010) recently reported that the negative metabolic effects associated with chronic glucocorticoid administration (increased body weight, hepatic steatosis and increased triglycerides, insulin and leptin) were abolished in a CB_1-deficient mouse. Unfortunately, no cardiovascular measurements were made in these animals, so whether CB_1 activation plays a role in the vascular dysfunction associated with glucocorticoids remains to be established.

In addition to chronic stress in adulthood generating cardiovascular risk, an adverse prenatal environment due to stress in pregnancy, possibly coupled to defects in 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) (the foetoplacental barrier to maternal glucocorticoids) can also induce persistent risks of cardiovascular, metabolic, neuroendocrine and psychiatric disorders lasting into adulthood (Seckl and Holmes, 2007). Interestingly, the consequences of prenatal stress are similar to many effects of prenatal cannabinoid exposure (Fride et al., 2009), which may suggest that prenatal stress impacts on the EC system and that, vice versa, prenatal cannabinoid exposure may interfere with the ability of the foetus to cope with the stress. Early life stress in rats, due to social isolation, alters the expression of a number of genes associated with EC signalling in different brain areas, but whether this extends to cardiovascular tissues or the HPA axis is not known.

Corticosteroids, PPARs and cardiovascular disease

Not surprisingly, most attention has been paid to those effects of ECs and other cannabinoids that are mediated via the G

protein-coupled CB_1 and CB_2 receptors, but, as described above, a variety of cannabinoids have agonist potency at different PPARs (see O'Sullivan, 2007) and it is possible that these receptors could mediate some of the physiological/pathophysiological effects of ECs and, thereby, corticosteroid responses. There certainly seems to be a direct link between the HPA axis and PPARs, since both immobilization stress and dexamethasone injection in adult rats have been reported to be effective stimulators of PPARα expression, at least in the liver (Lemberger et al., 1994, 1996).

In addition to their well-recognized metabolic effects, recent studies have identified roles of PPARα, γ and β in regulation of cardiac inflammation, extracellular matrix remodelling, oxidative stress, and regulation of cardiac hypertrophy. Whether any of these effects involve ECs has yet to be investigated, but AEA and NADA have both been shown to cause a time-dependent vasorelaxant effect in rat aortae that involves increases in superoxide dismutase (SOD) activity (increased nitric oxide bioavailability) mediated by PPARγ (O'Sullivan et al., 2009a). This is in agreement with other work showing that PPARγ ligands cause the induction of Cu/Zn-SOD (Hwang et al., 2005), and with numerous studies showing that PPARγ ligands increase nitric oxide production and bioavailability *in vitro* and *in vivo* (Bagi et al., 2004; Cho et al., 2004). It is also interesting to note that analgesia in a rat model of inflammatory pain following the elevation of ECs by the FAAH inhibitor URB597 is mediated, at least in part, by PPARα (Jhaveri et al., 2008; Sagar et al., 2008). PPARα and glucocorticoid receptor (GRα) both have key anti-inflammatory properties inhibiting cytokine expression, largely via inhibition of nuclear factor κB (NF-κB)-driven gene expression. Indeed, co-activation of PPARα and GRα additively enhances transrepression of NF-κB-driven gene expression and repression of cytokine production, but, unexpectedly, PPARα agonists *inhibit* the expression of some classical glucocorticoid response element (GRE)-driven genes (Bougarne et al., 2009a). The mechanism underlying this appears to depend upon interference with the recruitment of GRα, and subsequently of RNA polymerase II, to GRα-coupled gene promoters. This antagonist effect has been demonstrated *in vivo* such that the PPARα agonist fenofibrate was shown to prevent glucocorticoid-induced hyperinsulinaemia of mice fed a high-fat diet (Bougarne et al., 2009b). Whether cannabinoid PPAR ligands are able to replicate the effects of the high-potency PPARα agonists GW647 and WY-14643 employed by these authors remains to be investigated, but the possibility of EC regulation of glucocorticoid effects in addition to modulation at the level of HPA axis activity is intriguing.

Cannabidiol and the cardiovascular response to stress

Cannabidiol is a major component of the cannabis plant, *Cannabis sativa*, but lacks the psychotropic effects of the better known active agent, Δ^9-tetrahydrocannabinol (THC). Cannabidiol is anxiolytic (Moreira et al., 2006; Resstel et al., 2006) and may antagonize the psychotropic effects of THC in the clinically available cannabis-based medicine, Sativex (Russo and Guy, 2006). The only study, to the best of our knowledge, that has investigated directly the effects of a cannabinoid on cardiovascular responses to stress employed cannabidiol is Resstel et al., 2009. In this study, cannabidiol dose-dependently decreased the blood pressure and heart rate response to restraint stress, and these effects were blocked by a 5-HT_{1A} receptor antagonist. Whether this is a mechanism by which ECs could affect cardiovascular stress responses remains to be established.

Therapeutic potential of EC system manipulation

Given its involvement at so many levels in the HPA axis, SNS and the cardiovascular system, there would seem, at first sight, to be huge potential for therapeutic alterations in EC function to benefit stress and associated cardiovascular disease. Some of these points of interaction are indicated in Figure 2.

However, it is clear from this review that upregulation of the EC system, as appears to occur under many situations of stress, may have multiple cardiovascular consequences. As already discussed, positive effects of EC upregulation could include cardioprotection, activation of vasodilator pathways, CB_2-mediated anti-inflammatory effects in the vasculature and activation of vascular PPARα and/or γ. Negative effects of increased circulating ECs could include mediation of some of the effects of glucocorticoids on the cardiovascular system and metabolism to vasoconstrictor products. In addition, CB_1 receptor antagonism has numerous positive cardiovascular effects, suggesting a negative role for CB_1 activation in the vasculature in metabolic disorders. The balance of CB_1, CB_2 and PPAR activation by various EC/EC-like compounds may be critical in cardiovascular health. Further studies are required to fully elucidate these effects in various models of stress.

The ubiquitous nature of the EC system creates significant selectivity problems with regard to therapeutic potential. This is exemplified by the withdrawal of rimonabant due to its propensity to precipitate adverse psychiatric responses in some patients (Jones, 2008), despite encouraging results in respect of its primary metabolic targets. The design of CB_1 antagonists restricted to the periphery would be an advance and the same goes for receptor agonists, since the psychoactive effects of cannabinoids are generally undesirable. However, a central anxiolytic effect could obviously be a very useful property in combating stress-related disease, so peripheral restriction is not a drug development panacea. As an alternative to receptor antagonism, there might be benefits from reducing activation of all of the EC receptor targets by developing inhibitors of EC synthesis but, at the present time, the biochemistry of the processes is imperfectly understood and no selective blockers exist.

There is genuine promise in the development of CB_2 receptor agonists, given their ability to reduce immune cell migration and cell adhesion molecule expression (Ahn et al., 2009), essential steps in the development of atherosclerosis, and their lack of obvious central effects. There is currently great interest in the development of FAAH inhibitors (Ahn et al., 2009) on the grounds that the increased endogenous agonist activity should be confined to those locations at which the endogenous processes have already been activated. However, given

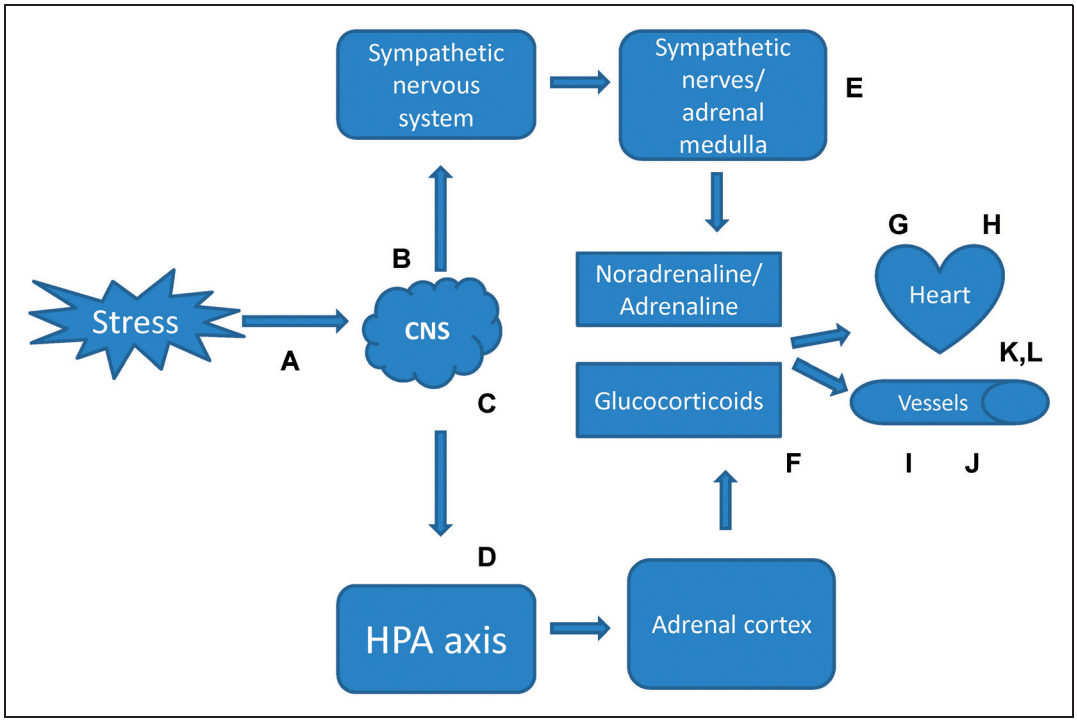

Figure 2. Points of interaction between the endocannabinoid (EC) signalling system, the stress axis and the cardiovascular system. Stressful stimuli simultaneously activate the sympathetic nervous system and the HPA axis, leading to elevated circulating levels of catecholamines and glucocorticoids that impact on the heart and blood vessels. Letters indicate points of interaction between the systems (details in the text). **A**, Stressors increase peroxisome proliferator-activated receptor (PPAR) expression; **B**, anandamide (arachidonoylethanolamide, AEA) increases baroreceptor sensitivity in the nucleus tractus solitarius; **C**, Pain stress increases ECs in the spinal cord; **D**, ECs reduce HPA activity (activity enhanced by CB_1 receptor blockade); **E**, ECs reduce catecholamine release; **F**, Glucocorticoids increase EC synthesis; **G**, AEA reduces inotropy (cirrhosis); **H**, CB_1 and CB_2 receptors mediate cardiac preconditioning; **I**, vasorelaxation mediated by CB_1, CB_2, CB_{other}, TRPV1, PPAR; **J**, CB receptor modulation of atherosclerosis; **K**, CB_1 antagonists reduce blood pressure; **L**, phytocannabinoids (e.g. cannabidiol) reduce blood pressure and heart rate responses to stress. CNS, central nervous system; HPA, hypothalamic–pituitary–adrenal.

the multiplicity of catabolic routes for the ECs, it remains to be seen if long-term treatment with FAAH inhibitors is able to avoid the re-direction of EC substrates down alternative enzymatic pathways. Since a variety of other potential fatty acid substrates (OEA, PEA, etc) would also be preserved by these drugs, more needs to be known about the roles of these other agents in order to predict possible additional benefits or unwanted side effects. Given that the roles of AEA and 2-AG are not universally co-incident and that 2-AG appears to be the 'senior partner' in a variety of EC functions, there is potential value in developing selective MAGL inhibitors, although the same argument as for FAAH inhibitors relating to alternative routes of catabolism applies.

Concluding remarks

Stress is a double-edged sword, providing acute protection against environmental challenges but carrying with it an accompanying threat to health, notably to cardiovascular health, if the stress persists inappropriately. The EC signalling system is intimately involved, not only in the stress axis but also in the many organ systems affected by stress, and the relationships are too complex to allow simple statements of whether EC system activation is a good or bad thing to be meaningful. Despite the phenomenal activity in EC research in the last two decades, much still remains to be clarified before the therapeutic potential of the EC system can be fully exploited.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

Adameova A, Abdellatif Y and Dhalla NS (2009) Role of the excessive amounts of circulating catecholamines and glucocorticoids in stress-induced heart disease. *Can J Physiol Pharmacol* 87: 493–514.

Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, et al. (2009) Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chem Biol* 16: 411–420.

Alexander SP and Kendall DA (2007) The complications of promiscuity: endocannabinoid action and metabolism. *Br J Pharmacol* 152: 602–623.

Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A and Mikhailidis DP (2009) Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab* 94: 2692–2701.

Axelrod J and Reisine TD (1984) Stress hormones: their interaction and regulation. *Science* 224: 452–459.

Bagi Z, Koller A and Kaley G (2004) PPARgamma activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with type 2 diabetes. *Am J Physiol* 286: H742–H748.

Bátkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P and Kunos G (2007) Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *Am J Physiol Heart Circ Physiol* 293: H1689–H1695.

Bátkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, et al. (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* 110: 1996–2002.

Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z, et al. (1998) An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* 353: 23–31.

Berridge MJ and Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312: 315–321.

Bishop-Bailey D (2000) Peroxisome proliferator-activated receptors in the cardiovascular system. *Br J Pharmacol* 129: 823–834.

Blankman JL, Simon GM and Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 14: 1347–1356.

Bonz A, Laser M, Kullmer S, Kniesch S, Babin-Ebell J, Popp V, et al. (2003) Cannabinoids acting on CB1 receptors decrease contractile performance in human atrial muscle. *J Cardiovasc Pharmacol* 41: 657–664.

Bougarne N, Paumelle R, Caron S, Hennuyer N, Mansouri R, Gervois P, et al. (2009a) PPARalpha blocks glucocorticoid receptor alpha-mediated transactivation but cooperates with the activated glucocorticoid receptor alpha for transrepression on NF-kappaB. *Proc Natl Acad Sci U S A* 106: 7397–7402.

Bougarne N, Paumelle R, Haegeman G, Staels B and De Bosscher K (2009b) Circumventing glucocorticoid-mediated hyperinsulinemia via the activation of PPARalpha. *Cell Cycle* 8: 2311–2312.

Brown AJ (2007) Novel cannabinoid receptors. *Br J Pharmacol* 152: 567–575.

Brozoski DT, Dean C, Hopp FA, Hillard CJ and Seagard JL (2009) Differential endocannabinoid regulation of baroreflex-evoked sympathoinhibition in normotensive versus hypertensive rats. *Auton Neurosci* 150: 82–93.

Cadas H, di Tomaso E and Piomelli D (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J Neurosci* 17: 1226–1242.

Calignano A, La Rana G, Beltramo M, Makriyannis A and Piomelli D (1997) Potentiation of anandamide hypotension by the transport inhibitor, AM404. *Eur J Pharmacol* 337: R1–R2.

Cho DH, Choi YJ, Jo SA and Jo I (2004) Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone: evidence for involvement of peroxisome proliferator-activated receptor (PPAR) gamma-dependent and PPARgamma-independent signalling pathways. *J Biol Chem* 279: 2499–2506.

Chrousos GP, Kino T and Charmandari E (2009) Evaluation of the hypothalamic–pituitary–adrenal axis function in childhood and adolescence. *Neuroimmunomodulation* 6: 272–283.

Cota D, Steiner MA, Marsicano G, Cervino C, Herman JP, Grübler Y, et al. (2007) Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. *Endocrinology* 148: 1574–1581.

Côté M, Matias I, Lemieux I, Petrosino S, Alméras N, Després JP, et al. (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)* 31: 692–699.

Davis AM and Natelson BH (1993) Brain–heart interactions. The neurocardiology of arrhythmia and sudden cardiac death. *Tex Heart Inst J* 20: 158–169.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949.

Di S, Malcher-Lopes R, Halmos KC and Tasker JG (2003) Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J Neurosci* 23: 4850–4857.

Di Marzo V (2009) The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol Res* 60: 77–84.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, et al. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372: 686–691.

Dinh TP, Freund TF and Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* 121: 149–158.

Dol-Gleizes F, Paumelle R, Visentin V, Marés AM, Desitter P, Hennuyer N, et al. (2009) Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 29: 12–18.

Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, Bátkai S, et al. (2005) Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 54: 2838–2843.

Ferre P (2004) The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes* 53: S43–S50.

Fowler CJ (2003) Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents. Non-psychoactive cannabinoids, 'entourage' compounds and inhibitors of N-acyl ethanolamine breakdown as therapeutic strategies to avoid psychotropic effects. *Brain Res Brain Res Rev* 41: 26–43.

Fride E, Gobshtis N, Dahan H, Weller A, Giuffrida A and Ben-Shabat S (2009) The endocannabinoid system during development: emphasis on perinatal events and delayed effects. *Vitam Horm* 81: 139–158.

Gardiner SM, March JE, Kemp PA and Bennett T (2001) Regional haemodynamic responses to the cannabinoid agonist, WIN 55212-2, in conscious, normotensive rats, and in hypertensive, transgenic rats. *Br J Pharmacol* 133: 445–453.

Gardiner SM, March JE, Kemp PA and Bennett T (2002) Complex regional haemodynamic effects of anandamide in conscious rats. *Br J Pharmacol* 135: 1889–1896.

Giang DK and Cravatt BF (1997) Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci U S A* 94: 2238–2242.

Glass CK (2006) Going nuclear in metabolic and cardiovascular disease. *J Clin Invest* 116: 556–560.

Godlewski G, Offertaler L, Osei-Hyiaman D, Mo FM, Harvey-White J, Liu J, et al. (2009) The endogenous brain constituent N-arachidonoyl L-serine is an activator of large conductance Ca2+-activated K+ channels. *J Pharmacol Exp Ther* 328: 251–261.

Gomes I, Grushko JS, Golebiewska U, Hoogendoorn S, Gupta A, Heimann AS, et al. (2009) Novel endogenous peptide agonists of cannabinoid receptors. *FASEB J* 23: 3020–3029.

Goodwin JE, Zhang J and Geller DS (2008) A critical role for vascular smooth muscle in acute glucocorticoid-induced hypertension. *J Am Soc Nephrol* 19: 1291–1299.

Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET and Huestis MA (2006) The cannabinoid CB1 receptor antagonist rimonabant attenuates the hypotensive effect of smoked marijuana in male smokers. *Am Heart J* 151: 754.e1–754.e5.

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.

Han KH, Lim S, Ryu J, Lee CW, Kim Y, Kang JH, et al. (2009) CB1 and CB2 cannabinoid receptors differentially regulate the production of reactive oxygen species by macrophages. *Cardiovasc Res* 84: 378–386.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, et al. (2001) 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci U S A* 98: 3662–3665.

Hanus L, Gopher A, Almog S and Mechoulam R (1993) Two new unsaturated fatty acid ethonolamides in brain that bind to the cannabinoid receptor. *J Med Chem* 36: 3032–3034.

Hiley CR (2009) Endocannabinoids and the heart. *J Cardiovasc Pharmacol* 53: 267–276.

Hill MN and Gorzalka BB (2009) The endocannabinoid system and the treatment of mood and anxiety disorders. *CNS Neurol Disord Drug Targets* 8: 451–458.

Hill MN and McEwen BS (2009) Endocannabinoids: The silent partner of glucocorticoids in the synapse. *Proc Natl Acad Sci U S A* 106: 4579–4580.

Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TT, Gray JM, et al. (2010) Endogenous cannabinoid signaling is essential for stress adaptation. *Proc Natl Acad Sci U S A* 107: 9406–9411.

Hill MN, Miller GE, Carrier EJ, Gorzalka BB and Hillard CJ (2009) Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. *Psychoneuroendocrinology* 34: 1257–1262.

Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, et al. (2005) Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology* 30: 508–515.

Himmi T, Perrin J, El Ouazzani T and Orsini JC (1998) Neuronal responses to cannabinoid receptor ligands in the solitary tract nucleus. *Eur J Pharmacol* 359: 49–54.

Huestis MA, Boyd SJ, Heishman SJ, Preston KL, Bonnet D, Le Fur G, et al. (2007) Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. *Psychopharmacology* 194: 505–515.

Hwang J, Kleinhenz DJ, Lassegue B, Griendling KK, Dikalov S and Hart CM (2005) Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. *Am J Physiol* 288: 899–905.

Jhaveri MD, Richardson D, Robinson I, Garle MJ, Patel A, Sun Y, et al. (2008) Inhibition of fatty acid amide hydrolase and cyclooxygenase-2 increases levels of endocannabinoid related molecules and produces analgesia via peroxisome proliferator-activated receptor-alpha in a model of inflammatory pain. *Neuropharmacology* 55: 85–93.

Jones D (2008) End of the line for cannabinoid receptor 1 as an anti-obesity target? *Nat Rev Drug Discov* 7: 961–962.

Joyeux M, Arnaud C, Godin-Ribuot D, Demenge P, Lamontagne D and 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.

Joyner MJ and Casey DP (2009) The catecholamines strike back. What NO does not do. *Circ J* 73: 1783–1792.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M and Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 889: 309–380.

Kapur A, Zhao P and Sharir H (2009) Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem* 284: 29, 817–29, 827.

Kaufmann I, Hauer D, Huge V, Vogeser M, Campolongo P, Chouker A, et al. (2009) Enhanced anandamide plasma levels in patients with complex regional pain syndrome following traumatic injury: a preliminary report. *Eur Surg Res* 43: 325–329.

Kunos G, Batkai S and Offertaler L (2002) The quest for a vascular endothelial cannabinoid receptor. *Chem Phys Lipids* 121: 45–56.

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

Lemberger T, Saladin R, Vazquez M, Assimacopoulos F, Staels B, Desvergne B, et al. (1996) Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. *J Biol Chem* 271: 1764–1769.

Lemberger T, Staels B, Saladin R, Desvergne B, Auwerx J and Wahli W (1994) Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. *J Biol Chem* 269: 24,527–24,530.

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

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. (2006) A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A* 103: 13,345–13,350.

Lopez-Miranda V, Herradón E and Martín MI (2008) Vasorelaxation caused by cannabinoids: mechanisms in different vascular beds. *Curr Vasc Pharmacol* 6: 335–346.

Maione S, de Novellis V, Cappellacci L, Palazzo E, Vita D, Luongo L, et al. (2007) The antinociceptive effect of 2-chloro-2'-C-methyl-N6-cyclopentyladenosine (2'-Me-CCPA), a highly selective adenosine A1 receptor agonist, in the rat. *Pain* 131: 281–292.

Malcher-Lopes R, Di S, Marcheselli VS, Weng FJ, Stuart CT, Bazan NG, et al. (2006) Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci* 26: 6643–6650.

Malcher-Lopes R, Franco A and Tasker JG (2008) Glucocorticoids shift arachidonic acid metabolism toward endocannabinoid synthesis: a non-genomic anti-inflammatory switch. *Eur J Pharmacol* 583: 322–339.

Manzanares J, Corchero J and Fuentes JA (1999) Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of delta(9)-tetrahydrocannabinol in rats. *Brain Res* 839: 173–179.

Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. (2010) The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* 13: 951–957.

Matias I, Petrosino S, Racioppi A, Capasso R, Izzo AA and Di Marzo V (2008) Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: Effect of high fat diets. *Mol Endocrinol* 286: S66–S78.

McCormick CM, Mathews IZ, Thomas C and Waters P (2010) Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain Cogn* 72: 73–85.

McEwen BS and Stellar E (1993) Stress and the individual. *Mechanisms leading to disease*. Arch Intern Med 153: 2093–2101.

Mitrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H, et al. (2006) Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. *Pain* 126: 102–114.

Moreira FA, Aguiar DC and Guimarães FS (2006) Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Prog Neuropsychopharmacol Biol Psychiatry* 30: 1466–1471.

Mukhopadhyay P, Batkai S and Rajesh M (2007) Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol* 50: 528–536.

Mukhopadhyay P, Rajesh M, Bátkai S, Patel V, Kashiwaya Y, Liaudet L, et al. (2010) 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.

Neukirchen M and Kienbaum P (2008) Sympathetic nervous system: evaluation and importance for clinical general anesthesia. *Anesthesiology* 109: 1113–1131.

Niederhoffer N and Szabo B (1999) Involvement of CB1 cannabinoid receptors in the EDHF-dependent vasorelaxation in rabbits. *Br J Pharmacol* 126: 1383–1386.

Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J, Cannon CP, Deanfield JE, et al. (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* 2299: 1547–1560.

O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 152: 576–582.

O'Sullivan SE, Kendall DA and Randall MD (2009a) Time-dependent vascular effects of endocannabinoids mediated by peroxisome proliferator-activated receptor gamma (PPARgamma). *PPAR Res* 2009: 425289.

O'Sullivan SE, Sun Y, Bennett AJ, Randall MD and Kendall DA (2009b) Time-dependent vascular actions of cannabidiol in the rat aorta. *Eur J Pharmacol* 612: 61–68.

Okamoto Y, Morishita J, Tsuboi K, Tonai T and Ueda N (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem* 279: 5298–5305.

Pacak K and Palkovits M (2001) Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocr Rev* 22: 502–548.

Pacher P and Steffens S (2009) The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol* 31: 63–77.

Pacher P, Bátkai S, Osei-Hyiaman D, Offertáler L, Liu J, Harvey-White J, et al. (2005) Hemodynamic profile, responsiveness to anandamide, and baroreflex sensitivity of mice lacking fatty acid amide hydrolase. *Am J Physiol Heart Circ Physiol* 289: H533–H541.

Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Batkai S and Kunos G (2008) Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension* 52: 601–607.

Pariante CM and Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 31: 464–468.

Parrish JC and Nichols DE (2006) Serotonin 5-HT2A receptor activation induces 2-arachidonoylglycerol release through a phospholipase c-dependent mechanism. *J Neurochem* 99: 1164–1175.

Patel S and Hillard CJ (2008) Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. *Eur J Neurosci* 27: 2821–2829.

Patel S, Roelke CT, Rademacher DJ, Cullinan WE and Hillard CJ (2004) Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Endocrinology* 145: 5431–5438.

Plant TD and Strotmann R (2007) TRPV4. *Handb Exp Pharmacol* 179: 189–205.

Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J, et al. (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* 301: 1020–1024.

Rademacher DJ, Patel S, Hopp FA, Dean C, Hillard CJ and Seagard JL (2003) Microinjection of a cannabinoid receptor antagonist into the NTS increases baroreflex duration in dogs. *Am J Physiol Heart Circ Physiol* 284: H1570–H1576.

Rajesh M, Mukhopadhyay P, Batkai S, Hasko G, Liaudet L, Huffman JW, et al. (2007) 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: 2210–2218.

Rajesh M, Mukhopadhyay P, Haskó G, Liaudet L, Mackie K and Pacher P (2010) Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol* 160: 688–700.

Randall MD, Kendall DA and O'Sullivan S (2004) The complexities of the cardiovascular actions of cannabinoids. *Br J Pharmacol* 142: 20–26.

Resstel LB, Joca SR, Moreira FA, Corrêa FM and Guimarães FS (2006) Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behav Brain Res* 172: 294–298.

Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM and Guimarães FS (2009) 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol* 156: 181–188.

Rosengren A, Hawken S, Ounpuu S, Sliwa K, Zubaid M, Almahmeed WA, et al. (2004) Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364: 953–962.

Ruilope LM, Despres JP, Scheen A, Pi-Sunyer X, Mancia G, Zanchetti A, et al. (2008) Effect of rimonabant on blood pressure in overweight/obese patients with/without co-morbidities: analysis of pooled RIO study results. *J Hypertens* 26: 357–367.

Russo E and Guy GW (2006) A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypotheses* 66: 234–246.

Sagar DR, Kendall DA and Chapman V (2008) Inhibition of fatty acid amide hydrolase produces PPAR-alpha-mediated analgesia in a rat model of inflammatory pain. *Br J Pharmacol* 155: 1297–1306.

Schroeder C, Batkai S, Engeli S, Tank J, Diedrich A, Luft FC, et al. (2009) Circulating endocannabinoid concentrations during orthostatic stress. *Clin Auton Res* 19: 343–346.

Seckl JR and Holmes MC (2007) Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nature Clin Prac Endocrinol Metab* 3: 479–488.

Soufer R and Burg MM (2007) The heart–brain interaction during emotionally provoked myocardial ischaemia: implications of cortical hyperactivation in CAD and gender interactions. *Cleve Clin J Med* 74: 59–62.

Stein EA, Fuller SA, Edgemond WS and Campbell WB (1996) Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. *Br J Pharmacol* 119: 107–114.

Steiner MA, Wanisch K, Monory K, Marsicano G, Borroni E, Bächli H, et al. (2008) Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice. *Pharmacogenomics J* 8: 196–208.

Steptoe A and Marmot M (2005) Impaired cardiovascular recovery following stress predicts 3-year increases in blood pressure. *J Hypertens* 23: 529–536.

Sugamura K, Sugiyama S, Nozaki T, Matsuzawa Y, Izumiya Y, Miyata K, et al. (2009) Activated endocannabinoid system in coronary artery disease and anti-inflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* 119: 28–36.

Taveres RF and Correa FM (2006) Role of the medial prefrontal cortex in cardiovascular responses to acute restraint in rats. *Neuroscience* 143: 231–240.

Tiyerili V, Zimmer S, Jung S, Wassmann K, Naehle CP, Lütjohann D, et al. (2010) CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. *Basic Res Cardiol* 105: 465–477.

Underdown NJ, Hiley CR and 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.

Van der Stelt M, Trevisani M, Vellani V, De Petrocellis L, Schiano-Moriello A, Campi B, et al. (2006) Anandamide acts as an intracellular messenger amplifying Ca2+ influx via TRPV1 channels. *EMBO J* 24: 3026–3037.

Vandevoorde S and Lambert DM (2007) The multiple pathways of endocannabinoid metabolism: a zoom out. *Chem Biodivers* 4: 1858–1881.

Wagner JA, Abesser M, Harvey-White J and 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.

Wagner JA, Hu K, Bauersachs J, Karcher J, Wiesler M, Goparaju SK, et al. (2001) Endogenous cannabinoids mediate hypotension after experimental myocardial infarction. *J Am Coll Cardiol* 38: 2048–2054.

Walker JM, Hohmann AG, Martin WJ, Strangman NM, Huang SM and Tsou K (1999) The neurobiology of cannabinoid analgesia. *Life Sci* 65: 665–673.

Walker JM, Krey JF, Chu CJ and Huang SM (2002) Endocannabinoids and related fatty acid derivatives in pain modulation. *Chem Phys Lipids* 121: 159–172.

Weis F, Beiras-Fernandez A, Hauer D, Hornuss C, Sodian R, Kreth S, et al. (2010) Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid concentrations during cardiac surgery. *Br J Anaesth* 105: 139–144.

Yan LL, Liu K, Matthews KA, Daviglus ML, Ferguson TF and Kiefe CI (2003) Psychosocial factors and risk of hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *JAMA* 290: 2138–2148.

Zhang J, Bian HJ, Li XX, Liu XB, Sun JP, Li N, et al. (2010) ERK-MAPK signaling opposes rho-kinase to reduce cardiomyocyte apoptosis in heart ischemic preconditioning. *Mol Med* 16: 307–315.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, et al. (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452–457.