British Journal of Pharmacology (2007) 151, 427–440
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0007–1188/07 $30.00
www.brjpharmacol.org

REVIEW

# Cannabinoids as therapeutic agents in cardiovascular disease: a tale of passions and illusions

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In addition to their classical known effects, such as analgesia, impairment of cognition and learning and appetite enhancement, cannabinoids have also been related to the regulation of cardiovascular responses and implicated in cardiovascular pathology. Elevated levels of endocannabinoids have been related to the extreme hypotension associated with various forms of shock as well as to the cardiovascular abnormalities that accompany cirrhosis. In contrast, cannabinoids have also been associated with beneficial effects on the cardiovascular system, such as a protective role in atherosclerosis progression and in cerebral and myocardial ischaemia. In addition, it has also been suggested that the pharmacological manipulation of the endocannabinoid system may offer a novel approach to antihypertensive therapy. During the last decades, the tremendous increase in the understanding of the molecular basis of cannabinoid activity has encouraged many pharmaceutical companies to develop more potent synthetic cannabinoid analogues and antagonists, leading to an explosion of basic research and clinical trials. Consequently. not only the synthetic THC dronabinol (Marinol) and the synthetic THC analogue nabilone (Cesamet) have been approved in the United States, but also the standardized cannabis extract (Sativex) in Canada. At least three strategies can be foreseen in the future clinical use of cannabinoid-based drugs: (a) the use of $CB_1$ receptor antagonists, such as the recently approved rimonabant (b) the use of $CB_2$-selective agonists, and (c) the use of inhibitors of endocannabinoid degradation. In this context, the present review examines the effects of cannabinoids and of the pharmacological manipulation of the endocannabinoid system, in cardiovascular pathophysiology.
*British Journal of Pharmacology* (2007) **151,** 427–440; doi:10.1038/sj.bjp.0707261; published online 23 April 2007

**Keywords:** cannabis; medical use; shock; endocannabinoids; hypertension; rimonabant

**Abbreviations:** THC, $\Delta^9$-tetrahydrocannabinol; CGRP, calcitonin gene-related peptide; 2-AG, 2-arachidonoyl glycerol; $TRPV_1$, transient receptor potential vanilloid type 1 receptors; NOS, nitric oxide synthase; LPS, lipopolysaccharides; SHR, spontaneously hypertensive rats; FAAH, fatty acid amino hydrolase; EDHF, endothelium-derived hyperpolarizing factor; abn-cbd, abnormal cannabidiol

## Introduction

The therapeutic use of cannabinoids has been extensively examined and reviewed, especially within the last few years (see Di Marzo and De Petrocellis, 2006; Mackie, 2006; Pacher *et al*., 2006). This is probably a consequence of the significant increase in the understanding of their pharmacological actions that has given rise to the view that cannabinoids might be used in the treatment of a growing number of pathologies. Nevertheless, the medical use of cannabis is still the focus of contentious debate due mostly to their known psychotropic effects.

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Received 21 November 2006; revised 5 February 2007; accepted 23 February 2007; published online 23 April 2007

More and more evidence indicates that cannabinoids play a major role in the control of physiopathological functions in the cardiovascular system. In recent years, many authors have elucidated the complex actions that both synthetic and endogenous cannabinoids have in the regulation of blood pressure and heart rate (see Hillard, 2000; Randall *et al*., 2002; Pacher *et al*., 2005a, b). Moreover, in an attempt to resolve the issues raised by apparent contradictions between *in vitro* and *in vivo* studies, some authors have also illuminated this complexity by a thorough comparison of the key findings under different experimental conditions (Randall *et al*., 2004). In addition, the involvement of the endocannabinoid system in cardiovascular pathology has also been examined (Wagner *et al*., 1998; Lamontagne *et al*., 2006; Steffens and Mach, 2006).

In the context of the recent approval of the standardized cannabis extract Sativex and of the $CB_1$ receptor antagonist

rimonabant, the aim of the present review is to summarize the major findings and to analyse the potential of cannabinoid-based drugs as therapeutic agents in cardiovascular diseases.

*From ancient medical uses of cannabinoids to scientific research-based therapies*

Evidence of the medical use of cannabis can be found as early as 5000 years ago in an herbarium published during the reign of the Chinese Emperor Chen Nung and, 2600 years ago, in an Assyrian tablet. In the traditional Indian medicine, many of its uses, such as a sedative, relaxant, anxiolytic, analgesic and appetite stimulator, were similar to those for which it is advocated in our own society today (Kalant, 2001).

In Western medicine, *Cannabis sativa* appeared about 60 AD in the Pharmacopoeia of Dioscorides, a Roman army physician living in the times of Claudius and Nero and is considered as the father of pharmaceutical science. During the sixteenth century, the Herbal of John Gerard (1597) in England recommended cannabis for easing the earache, and the Herbal of Nicholas Culpeper (1653) recommended its use to alleviate inflammations and ease the pain of gout and tumours (House of Lords, 1998).

Nevertheless, it was not until the 1960s that the pharmacological effects of cannabis-derived compounds began to be systematically studied, when $\Delta^9$-tetrahydrocannabinol (THC), the main active compound of marihuana, was first isolated and identified (Mechoulam and Gaoni, 1967). Hence, the extensive work by the group of Mechoulam in Israel, led to the complete synthesis of the pure compounds, the establishment of their molecular structures and the study of their structure–activity relationships. Later on, the chemical synthesis of new potent cannabinoid derivatives and analogues that do not exist in nature, allowed Devane *et al*. (1988) to identify specific binding sites in the brain. This finding finally confirmed that the pharmacological actions of cannabinoids were mediated through specific receptors and put aside the long-standing belief that they were mediated by perturbation of cellular membranes. Definite proof of their existence came from the molecular cloning of two proteins: $CB_1$ receptors expressed primarily in the brain (Matsuda *et al*., 1990), but also in various peripheral tissues including the heart and vasculature (Gebremedhin *et al*., 1999; Liu *et al*., 2000; Bonz *et al*., 2003), and $CB_2$ receptors identified in immune cells (Munro *et al*., 1993). Since cannabinoids themselves do not exist in the brain, the existence of the receptors implied that some other endogenous substance in the brain normally binds to them. Thus, as occurred in the case of the opioid peptides, the discovery of cannabinoid receptors soon led to the identification of endocannabinoids. Devane *et al*. (1992) isolated and described the structure of the brain constituent, arachidonoylethanolamide, that was named as anandamide based on the Sanskrit word *ananda* meaning *bringer of inner bliss*. Despite their different chemical structures, both anandamide – formed locally in the brain – and THC bind to cannabinoid receptors and share several common pharmacological properties. A simliar example of receptor activation through endogenous as well as through exogenous compounds arises from the opioid system where endorphins as well as morphine, although chemically unrelated, activate the same opioid receptors (Terenius, 2000). Further support to the view that endocannabinoids are part of an endogenous cannabinoid system is based on the presence of specific pathways for the biosynthesis (Bisogno *et al*., 1997), the enzymatic degradation (Deutsch and Chin, 1993) and the facilitated uptake of endocannabinoids (Beltramo *et al*., 1997). This tremendous increase in the understanding of cannabinoid pharmacology encouraged some pharmaceutical companies and several research laboratories to develop more potent synthetic cannabinoid analogues and antagonists, leading to an explosion of basic research and clinical trials during the last decades.

Although limited by their potential for abuse and dependence, the use of the synthetic THC dronabinol (Marinol) and the synthetic THC analogue nabilone (Cesamet) was approved in the US for the treatment of nausea and vomiting associated with chemotherapy as well as an appetite stimulant in acquired immunodeficiency syndrome (AIDS). Moreover, the standardized cannabis extract Sativex (GW Pharmaceuticals Plc, Salisbury, Wiltshire, UK) is licensed in Canada and has been submitted for approval regulation in several European countries as an adjuvant therapy for the symptomatic relief of neuropathic pain in multiple sclerosis.

Regarding pharmacological intervention strategies on endocannabinoid system, different molecules have been developed and are being tested both in preclinical and clinical studies. For instance, $CB_2$-selective agonists are being assayed against inflammatory and neuropathic pain and inhibitors of endocannabinoid degradation are promising tools in preclinical studies of epilepsy and anxiety. On the other hand, $CB_1$ receptor antagonists have been approved for the treatment of morbid obesity and are under evaluation for tobacco dependence.

## The complexity of the effects of cannabinoids in the cardiovascular system: a need for caution?

Cardiovascular effects of cannabinoids were recognized as early as the 1960s and the great advance in the understanding of cannabinoid biology has elucidated their role in cardiovascular pathophysiology. However, the extensive literature published in this area of research within the last 15 years has also revealed the complexity of their effects in the cardiovascular system (for recent review see Randall *et al*., 2004; Pacher *et al*., 2005a). For example, preclinical studies performed *in vivo* have shown discrepancies according to the state of the experimental animals (anaesthetized vs conscious), the route of administration (central vs peripheral) and even the doses of the compound employed. Moreover, the effects of cannabinoids may be influenced by either the nature of the experiment (*in vitro* vs *in vivo*) or the type of *in vitro* preparation (isolated vessel vs perfused vascular bed). Further complexity is added by the observation that endogenous cannabinoids, such as anandamide, may exert effects by interacting not only with classic

cannabinoid receptors, but also with vanilloid and with other, not yet identified, cannabinoid receptors.

*What have* in vivo *studies revealed about the cardiovascular effects of cannabinoids?*

In spite of the great variability in the cardiovascular responses observed under different experimental conditions as well as among species, hypotension and bradycardia are the most important features elicited by the systemic administration of cannabinoids. The *in vivo* actions of this group of compounds may involve the modulation of the autonomic outflow in both central and peripheral nervous systems as well as direct effects on the myocardium and the vasculature. However, their peripheral effects appear to predominate in cardiovascular control, at least upon systemic administration (Randall *et al*., 2002).

As reported for THC (Siqueira *et al*., 1979), the intravenous administration of anandamide to anaesthetized rats elicits bradycardia and a triphasic blood pressure response (Varga *et al*., 1995), that includes a transient drop, a brief increase and a third phase of a more prolonged decrease in blood pressure. As it is abolished by either atropine administration or cervical vagotomy, the first drop in blood pressure appears to be vagally mediated (Lake *et al*., 1997). In contrast, the second depressor effect is believed to be mediated by $CB_1$ receptors inducing prejunctional inhibition of sympathetic outflow in the periphery (Varga *et al*., 1995; Lake *et al*., 1997). Nevertheless, evidence that an additional direct vasorelaxant effect on the blood vessels is also involved is provided by the fact that the synthetic cannabinoid HU-210 lowers blood pressure even after sympathetic blockade (Vidrio *et al*., 1996; Lake *et al*., 1997).

Unlike the hypotensive phases, the pressor response is not blocked by $CB_1$ receptor antagonists and even persists in $CB_1$ knockout mice (Jarai *et al*., 1999), as well as in the presence of α-adrenoceptor blockade (Varga *et al*., 1995). It has been suggested that this pressor component may be the consequence of vasoconstriction in certain vascular beds, such as the spleen (Wagner *et al*., 2001a).

As in the case of other drugs with actions on blood pressure, the acute effects of cannabinoids not only result from changes in peripheral vascular resistance, but also in cardiac output. Thus, the predominant hypotensive response to anandamide is associated with a decrease in total peripheral resistance and with reductions in cardiac contractility (Pacher *et al*., 2004; Bátkai *et al*., 2004a), effects that can be completely blocked by the $CB_1$ receptor antagonist SR141716.

Perhaps the most significant discrepancy observed in studies performed *in vivo* is the very different profile in haemodynamic responses to systemic cannabinoid administration between anaesthetized and conscious animals. The prolonged decrease in blood pressure that characterizes the typical triphasic blood pressure response elicited by intravenous administration of anandamide or THC in anaesthetized animals is weak or absent in conscious animals. It has been suggested that this lack of a hypotensive phase under physiological conditions might reflect differences in sympathetic activity between conscious and anaesthetized states (Randall *et al*., 2004). On the other hand, the possibility that anaesthetic agents directly influence the responses has also been proposed based on the observation that anandamide can inhibit anaesthetic-sensitive potassium channels (Maingret *et al*., 2001). Another possibility is that the central effects of cannabinoids might be more susceptible to inhibition by general anaesthetics, which could, in its turn, explain the more pronounced hypotensive phase observed in anaesthetized animals. This is because several findings have provided evidence that central effects of cannabinoids may oppose their peripheral effects. For instance, whereas a hypotensive response mainly mediated by the inhibition of sympathetic outflow is the most important feature elicited by the intravenous administration of anandamide, other cannabinoids induced sympathoexcitation and pressor responses when applied intracisternally in conscious rabbits (Niederhoffer and Szabo, 2000), and increased sympathetic activity and hypertension when applied into the rostral ventrolateral medulla oblongata of anaesthetized rats (Padley *et al*., 2003). Nevertheless, an hypotensive response to intrathecally administered anandamide has been reported for urethane-anaesthetized rats (García *et al*., 2003, 2006)

Another inconsistency observed in the literature arises from comparing the results obtained in experimental animals with those found in human studies. For instance, the acute administration of cannabinoids in man is associated with pronounced tachycardia, opposite to the bradycardia reported in animals in both conscious and anaesthetized states (see Dewey, 1986; Jones, 2002). These differences in the patterns of cardiovascular change observed between humans and animals may be either the consequence of the higher doses used in animal studies or may reflect differences in the level of arousal between human volunteers and laboratory animals (Jones, 2002). In this line, the importance of the baseline level of arousal in cardiovascular cannabinoid response was demonstrated in an experiment performed with conscious monkeys tested in an extremely quiet and predictable environment. Under these conditions, THC induced tachycardia as commonly seen in humans, whereas in monkeys tested under more typical laboratory conditions, the same dose of THC induced bradycardia (Fredericks *et al*., 1981).

Moreover, discrepancies are also found in human studies when acute and chronic administrations are compared. The acute administration of cannabinoids is associated with tachycardia and a small pressor effect, whereas its long-term use is associated with bradycardia and hypotension (Benowitz and Jones, 1975; Benowitz *et al*., 1979). In this regard, tolerance to many of the effects of cannabinoids can be revealed after a few repeated doses. For instance, tolerance to increased heart rate and blood pressure changes can be found after only 1 or 2 days of frequent exposure, but it is rapidly lost when THC administration is stopped (Benowitz and Jones, 1975, 1981). Hence, special attention should be given to checking that experimental and laboratory conditions as well as the doses and the history of drug use, are similar when comparing and interpreting the cardiovascular *in vivo* effects of cannabinoids in human and animal studies.

Considering the significant cardiovascular effects of exogenously administered cannabinoids, it is rather surprising that basal blood pressure and heart rate were normal in $CB_1$ knockout mice (Ledent *et al*., 1999) and that fatty acid amino

hydrolase (FAAH)-deficient mice had a normal haemodynamic profile (Pacher *et al*., 2005b). Accordingly, pharmacological blockade of $CB_1$ receptors with rimonabant, at doses reported to abolish the sympathoinhibitory effects of exogenous cannabinoid receptor agonists, did not affect sympathetic tone, blood vessel tone or heart rate in pithed rats (Pfitzer *et al*., 2005). Taken together, these results indicate that endogenous cannabinoids do not exert a tonic control of cardiovascular responses and therefore do not seem to play a major role in cardiovascular regulation, at least under normal conditions. In contrast, little information is available regarding the possible role of endocannabinergic tone under physiological situations in which plasma concentrations of catecholamines are increased, such as during an adrenergic discharge, or under pathological conditions in which sympathetic activity is enhanced, such as in some types of human essential hypertension. In this regard, it has been suggested that, under hypertensive states, an endocannabinoid tone may limit the elevation of blood pressure and cardiac contractile responses through tonic activation of $CB_1$ receptors (Pacher *et al*., 2006).

*What is the contribution of* in vitro *studies to the understanding of the cardiovascular actions of cannabinoids?*

Whereas *in vivo* studies with cannabinoids have revealed how the different mechanisms involved in cardiovascular responses combine to provide an overall physiological effect, *in vitro* experiments, although reductionists in nature, have significantly contributed to the dissection of these mechanisms. Thus, *in vitro* studies have provided evidence that the cardiovascular actions of cannabinoids are mediated through the regulation of sympathetic neurotransmission, direct vasodilating effects and a modulatory role in sensory nerves.

The hypothesis that the hypotensive and bradycardic effects of cannabinoids result from the inhibition of sympathetic outflow was put forward many years ago (Hardman *et al*., 1971; Vollmer *et al*., 1974). Nevertheless, it was not until more recent years that mRNA for $CB_1$ receptors was detected (Buckley *et al*., 1998) and that they were proposed to mediate the inhibition of peripheral sympathetic neurotransmission (Niederhoffer and Szabo, 1999). Accordingly, most of the *in vitro* studies performed in heart and blood vessels have provided further evidence to support the view that this mechanism may be responsible for the hypotensive action of cannabinoids observed *in vivo*. For instance, either anandamide or THC inhibit noradrenaline release in the rat-isolated atria (Ishac *et al*., 1996) and different chemical classes of cannabinoids inhibit sympathetic neurotransmission in the rat mesenteric arterial bed (Ralevic and Kendall, 2002). In addition, experiments with anandamide and the synthetic cannabinoid HU210 performed in isolated Langendorff rat hearts and in isolated, electrically stimulated human atrial appendages (Ford *et al*., 2002; Bonz *et al*., 2003) have revealed a negative inotropic effect of cannabinoids that may underlie the ability of anandamide and HU-210 to decrease cardiac output as observed in studies performed *in vivo* (Wagner *et al*., 2001a).

A second line of investigation has taken up the direct vasodilating effects of this group of compounds based on the finding that some cannabinoids such as HU-210 lower blood pressure even after sympathetic blockade. As in the case of studies performed *in vivo*, these studies have added further complexity to the understanding of cannabinoid effects in the cardiovascular system. Although there is consensus about the direct vasodilation caused by cannabinoids, as revealed by a great majority of the studies performed in isolated vessels, neither a common mechanism nor a common site of action is likely to underlie this effect (for detailed overview of *in vitro* effects see Kunos *et al*., 2000; Högestatt and Zygmunt, 2002; Randall *et al*., 2002). Moreover, the magnitude of their vascular actions was found to vary widely among species. For instance, anandamide causes 20% maximal relaxation in the rat aorta (O'Sullivan *et al*., 2004), and 80% in the rabbit aorta (Mukhopadhyay *et al*., 2002). The mechanisms involved in the direct vasodilating effects of cannabinoids seem to depend on the vascular bed and the experimental conditions employed, probably reflecting the involvement of different vascular receptors and different receptor coupling. This could suggest that each vascular bed may have a particular local regulation of vascular tone that could, in its turn, differentially contribute to the global haemodynamic effects of cannabinoids.

Since the first report providing evidence that anandamide caused indomethacin-sensitive vasodilation in rat cerebral arterioles (Ellis *et al*., 1995), further studies have revealed that the generation of arachidonic acid and its subsequent metabolism by cyclooxygenase is not a major mechanism involved in the direct vasodilating effects of cannabinoids. Although anandamide can act through the products formed via epoxygenase and cyclooxygenase pathways, as reported for bovine and ovine coronary arteries (Pratt *et al*., 1998; Grainger and Boachie-Ansah, 2001), in most blood vessels anandamide can act directly, for instance in the rat mesenteric vasculature (Randall *et al*., 1996).

As in the case of anandamide-induced increase in prostanoid formation, the participation of vasorelaxant agents such as nitric oxide (NO) on anandamide effects do not seem to be a major mechanism for direct vasodilatory effects of cannabinoids since it may also depend on the tissue studied. Hence, NO has been shown to mediate responses to anandamide in rat renal arteries (Deutsch *et al*., 1997) as well as in a range of human vessels (Bilfinger *et al*., 1998) but not in most of the studies performed in other vascular beds or other species (Harris *et al*., 2002).

The interest in the direct vasodilating actions of endocannabinoids was further stimulated by the original proposal by Randall *et al*. (1996) that anandamide might be an endothelium-derived hyperpolarizing factor (EDHF). Based on the finding that EDHF-mediated responses were sensitive to the $CB_1$ receptor antagonist SR141716A (Randall *et al*., 1996), and on the observations that anandamide-induced relaxations were abolished by raised extracellular $K^+$ (Randall *et al*., 1996) and decreased by $K^+$ channel blockers (Plane *et al*., 1997; White and Hiley, 1997; Mendizábal *et al*., 2001), it was suggested that endocannabinoids could play a physiological role as an EDHF. Nevertheless, inhibition of EDHF-induced vasorelaxation by SR141716 was confirmed in some (White and Hiley 1997) but not in other studies (Chataigneau *et al*., 1998; Fulton and Quilley 1998; Pratt

*et al*., 1998; Niederhoffer and Szabo 1999). Moreover, the observation that only the endothelium-dependent component of anandamide-induced vasodilation is sensitive to inhibition by the $CB_1$ receptor antagonist SR141716, as shown in rabbit (Chaytor *et al*., 1999) and in rat mesenteric vessels (Wagner *et al*., 1999), argues against anandamide itself being EDHF.

Finally, a third mechanism of action of cannabinoids in the cardiovascular system was revealed by another set of *in vitro* experiments. The controversial results involving $CB_1$ receptors in the effects of anandamide on the vasculature, together with the fact that this endocannabinoid shares structural similarities with the vanilloid agonist olvanil, lead to assessment of the role of vanilloid receptors in the vascular actions of anandamide. Zygmunt *et al*. (1999) demonstrated that anandamide, but neither 2-arachidonoyglycerol (2-AG), nor palmitoylethanolamide or synthetic cannabinoid receptor agonists, could induce relaxation acting at transient receptor potential vanilloid type 1 receptors ($TRPV_1$) through the release of the potent vasodilator calcitonin gene-related peptide (CGRP) from sensory nerves. Nevertheless, the findings that the hypotension caused by anandamide is absent in mice lacking $CB_1$ receptors (Ledent *et al*., 1999) and that mice lacking $TRPV_1$ have a normal cardiovascular profile (Pacher *et al*., 2004), suggest that the interaction with vanilloid receptors on sensory nerves is of minor importance in the haemodynamic profile induced by systemically administered cannabinoids, at least under physiological conditions. In contrast, this mechanism has been proposed to be significant in pathophysiological situations such as septic shock (Orliac *et al*., 2003) in which high concentrations of cannabinoids are produced (Varga *et al*., 1998; Wang *et al*., 2001).

Moreover, the participation of vanilloid receptors could also be relevant when considering possible sex-linked differences in the relaxant effects of anandamide. For instance, anandamide-induced relaxations have been reported to be greater in mesenteric beds isolated from female Sprague–Dawley rats, compared to those obtained in males (Peroni *et al*., 2004). It was recently proposed that the higher relaxation caused by anandamide in female mesenteries is critically dependent on the presence of oestrogens and involves the participation of relaxing factors such as CGRP and prostacyclin (Peroni *et al*., 2007). Whether the greater incidence of hypertension and coronary artery disease in men and postmenopausal women compared with that in premenopausal women could be, at least in part, related to this mechanism remains to be established. Since the experiments providing evidence that the genetic ablation of $TRPV_1$ is unrelated to significant changes in cardiovascular responses have been performed in male mice (Pacher *et al*., 2004), it would be of interest to test if female mice lacking $TRPV_1$ also have a normal cardiovascular profile and a predominant $CB_1$-dependent response to anandamide, as reported for males.

### *Evidence for novel non-$CB_1$/non-$CB_2$ cannabinoid receptors involved in cardiovascular responses*

Several lines of evidence indicate that although the cardiovascular depressor effects of cannabinoids are mediated mainly by peripherally localized $CB_1$ receptors, they may exert effects by interacting not only with classic cannabinoid receptors, but also with other, not yet identified, receptor sites. Studies performed with mice lacking $CB_1$ and/or $CB_2$ receptors provide evidence to support the view that, apart from the cloned $CB_1$ and $CB_2$ receptors, at least two additional cannabinoid receptors may be regulating vascular and neuronal functions (for review see Begg *et al*., 2005; Mackie and Stella 2006).

The possible existence of cannabinoid receptors distinct from $CB_1$ or $CB_2$ was first suggested by Wagner *et al*. (1999) on the basis that neither THC nor synthetic cannabinoid agonists elicit vasodilation in the rat mesenteric vascular bed, a preparation in which anandamide and methanandamide have strong vasodilator activity. When tested on anandamide responses in the mesenteric vasculature, the $CB_1$ receptor antagonists were either ineffective, as in the case of AM251, or less potent, as in the case of rimonabant, in comparison with the concentrations reported to act at classical $CB_1$ receptors. Moreover, the inhibitory activity of SR141716 depended on intact vascular endothelium and was lost following endothelial denudation (Chaytor *et al*., 1999; Jarai *et al*., 1999; Wagner *et al*., 2001a). Taken together, these findings suggest that an endothelial site distinct from $CB_1$ or $CB_2$ receptors, yet somewhat sensitive to inhibition by SR141716, is involved in the vasodilator effect of anandamide in the rat mesenteric circulation. It has been proposed that the activation of this endothelial receptor may be coupled to the release of NO, culminating in the opening of potassium channels on vascular smooth muscle and leading to relaxation and vasodilation (Begg *et al*., 2005).

A second line of *in vitro* experiments supporting the existence of a novel endothelial receptor is based on the observation that the cannabidiol analogue, abnormal cannabidiol (abn-cbd), caused SR141716-sensitive, endothelium-dependent vasodilation in rat-isolated mesenteric beds, although it did not bind to either $CB_1$ or $CB_2$ receptors (Jarai *et al*., 1999). In addition, both cannabidiol and O-1918, a synthetic cannabidiol analogue, blocked the vasodilator actions of abn-cbd. Thus, it appears that whereas abn-cbd acts as a selective agonist of the endothelial cannabinoid receptor, cannabidiol and O-1918 may be considered as specific antagonists acting at this novel receptor (Offertáler *et al*., 2003). Moreover, evidence obtained in rat-isolated hearts suggests that, in addition to its action at classical $CB_1$ receptors (Bonz *et al*., 2003), this non$CB_1$/non$CB_2$ mechanism is also implicated in the negative inotropy and coronary vasodilatation caused by anandamide (Ford *et al*., 2002).

In line with *in vitro* experiments, SR141716-sensitive effects that persist in $CB_1$ knockout mice have also been described for *in vivo* paradigms. For instance, *in vivo* studies have shown that abd-cbd causes hypotension in $CB_1$ knockout mice (Jarai *et al*., 1999). Furthermore, a similar ability of SR-141716 to prevent endotoxin-induced hypotension was reported in animal models of septic shock developed in wild-type mice as well as in mice deficient in $CB_1$ or in both $CB_1$ and $CB_2$ receptors (Bátkai *et al*., 2004a). Hence, the fact that this latter effect had been preserved in knockout mice not only gives further support to the existence of additional non $CB_1$/non $CB_2$ receptors in the vasculature, but it also opens

the possibility of developing new therapeutic strategies in the treatment of septic shock.

Regarding neuronal function, a non-$CB_1$/non-$CB_2$ site was also postulated to exist on glutamatergic terminals in the mouse hippocampus, where its activation by cannabinoids inhibits glutamatergic transmission and excitatory postsynaptic potentials (Hájos *et al*., 2001). Although there are strong pharmacological parallels between the novel receptors in the vasculature and the hippocampus, there are some notable differences that seem to support the view that they represent two different receptor entities. For instance, the vascular receptor is insensitive to potent synthetic cannabinoids such as WIN55,212-2 and CP55,940, whereas the neuronal receptor is insensitive to abd-cbd but sensitive to WIN55,212-2 and CP55,940. Nevertheless, the possibility that such differences between these two putative receptors arise from either the specific cellular context in which the receptor is expressed or from receptor dimerization, cannot be ruled out until molecular cloning of these novel receptors has been achieved.

## Cannabinoids in cardiovascular pathology: cure or disease?

In recent years, several studies have provided evidence that elevated levels of endocannabinoids could play an important role in pathological conditions associated with extreme hypotension such as various forms of shock, and also in the cardiovascular abnormalities that accompany cirrhosis. On the other hand, cannabinoids have also been associated with cardiovascular beneficial effects, such as a protective role in the progression of atherosclerosis and after myocardial ischaemia. In addition, it has been proposed that an increased endocannabinergic tone in hypertension could limit increases in blood pressure and cardiac contractile performance through tonic activation of cardiac and vascular $CB_1$ receptors (Pacher *et al*., 2006). Hence, the pharmacological manipulation of the endocannabinoid system may offer novel therapeutic approaches in a variety of cardiovascular disorders.

### *Possible involvement of cannabinoids in the pathogenesis of cardiovascular disease*

The first evidence of the key role that endocannabinoids may play in situations of extreme hypotension was found for a rat model of haemorrhagic shock (Wagner *et al*., 1997), where macrophage production of anandamide appeared to be involved in a systemic hypotension that could be overcome by the selective $CB_1$ receptor antagonist, SR141716A. In a further study from the same laboratory, it was found that the pretreatment with the $CB_1$ receptor antagonist SR141716A also prevented the prolonged hypotension elicited by the administration of lipopolysaccharides (LPS) in the rat (Varga *et al*., 1998).

Macrophages are the primary cellular targets of LPS, a cell wall component ubiquitous to Gram-negative bacteria that is involved in the initiation of endotoxic shock. Since macrophages produce anandamide (Di Marzo *et al*., 1996; Schmid *et al*., 1997), these cells might be the source of the endocannabinoids, that acting at $CB_1$ receptors induce the pronounced decrease in blood pressure associated with septic shock (Parrillo, 1993). Accordingly, LPS stimulated the production of 2-AG in platelets and induced the production of anandamide in macrophages when administered *in vitro* in cell culture (Varga *et al*., 1998). Moreover, an SR141716-sensitive hypotensive response similar to that observed in LPS-treated rats was obtained in normotensive rats treated with macrophages plus platelets isolated from the blood of an LPS-treated donor rat (Varga *et al*., 1998). Taken together, these findings suggested a novel paracrine mechanism of vasodilation in endotoxic shock, where macrophage-derived anandamide and platelet-derived 2-AG were likely to be responsible for the activation of vascular $CB_1$ cannabinoid receptors.

Based on the observation that in the anaesthetized rat LPS-induced vasodilatation was preserved when the sympathetic tone was removed by phentolamine, it was proposed that hypotension is independent of autonomic innervation and occurs solely through the activation of vascular $CB_1$ receptors (Varga *et al*., 1998). In contrast, data obtained on pithed rats suggested that $CB_1$ receptors are located presynaptically on the sympathetic nerve fibres innervating the resistance vessels (Godlewski *et al*., 2004). The reason for the discrepancy between both studies may be related to differences in either the experimental model (pithed vs anaesthetized rat), or the dose of LPS (4 vs 15 mg/kg) or the different time course of the hypotension (15 vs 120 min).

Several studies have provided further evidence of the key role played by endocannabinoids in endotoxin-induced hypotension not only in animal experimental models (Bátkai *et al*., 2004a; Godlewski *et al*., 2004), but also in patients with endotoxic shock, where significant increases in anandamide and 2-AG levels have been detected in sera (Wang *et al*., 2001). Moreover, Orliac *et al*. (2003) have shown that the relaxant effects of the endocannabinoid anandamide in the mesenteric bed of the rat are potentiated at early stages after LPS treatment, when no changes in blood pressure are observed. Although no conclusions can be drawn regarding a link between the decrease in blood pressure in advanced stages of septic shock and the observed potentiation of anandamide effects at early stages of endotoxemia, this latter finding suggests that an increased target organ sensitivity to anandamide through vanilloid receptor overexpression may also play a role in the haemodynamic effects of LPS (Orliac *et al*., 2003). Nevertheless, the fact that the effects of LPS on blood pressure were not modified by the $TRPV_1$ receptor antagonist capsazepine in experiments performed in pithed rats, seems to argue against the possible involvement of vanilloid receptors in endotoxic-induced hypotension (Godlewski *et al*., 2004). In this regard, it would be of interest to test whether the genetic ablation of $TRPV_1$ receptors reveals similar results to those observed after their pharmacological blockade.

In addition to their involvement in haemorrhagic and septic shock, an overproduction of endocannabinoids has also been described in other pathological conditions associated with extreme hypotension, such as the cardiogenic shock, developed in a percentage of patients within the first

few hours after myocardial infarction, and the cardiomyopathy associated with advanced liver cirrhosis. Indeed, Wagner *et al*. (2001b) found that activated vascular $CB_1$ receptors contribute to severe hypotension after experimental myocardial infarction in rats. In addition, circulating monocytes and platelets were found to increase the production of anandamide and 2-AG during cardiogenic shock.

Regarding cirrhosis, it was found that the decrease in blood pressure, obtained in an experimental model developed in rats, was acutely reversed by selective $CB_1$ receptor antagonists (Bátkai *et al*., 2001; Ros *et al*., 2002). In addition, monocytes isolated from the blood of cirrhotic patients that were found to contain elevated levels of anandamide, caused $CB_1$-receptor mediated hypotension when injected into normal rats (Bátkai *et al*., 2001). Dealing with a possible involvement of endocannabinoids in cirrhotic cardiomyopathy, Gaskari *et al*. (2005) provided the first evidence for the existence of a $CB_1$ receptor-mediated tonic inhibition of $\beta$-adrenergic responsiveness of isolated cardiac ventricular muscle in a rat model of biliary cirrhosis. On the basis of these findings, the authors proposed that an increased local endocannabinoid synthesis in the hearts taken from cirrhotic animals could play an important role in the blunted contractile responsiveness associated with cirrhotic cardiomyopathy.

The possible involvement of $CB_1$ receptors in the extreme hypotension associated with different kinds of shock such as haemorrhagic, septic and cardiogenic shock is supported by the observation that this effect is sensitive to the $CB_1$ antagonist SR141716. Nevertheless, this proposal deserves further studies. This is because SR141716 can also inhibit a novel cardiac cannabinoid receptor, that it appears to differ from $CB_1$ and $CB_2$ (Ford *et al*., 2002). In this regard, Bátkai *et al*. (2004a) have shown that the hypotension induced by LPS was counteracted by SR-141716 not only in wild-type mice, but also in $CB_1$ and $CB_1/CB_2$ knockout mice. Moreover, this potential beneficial effect of SR141716 on blood pressure contrasts with the fact that this cannabinoid antagonist causes increases rather than decreases in mortality rates in animal models of haemorrhagic (Wagner *et al*., 1997) and cardiogenic shock (Wagner *et al*., 2001b, 2003). These latter findings seem to indicate that endocannabinoid-mediated cardiovascular effects appear to have a survival value. Accordingly, pretreatment with cannabinoid agonists, such as THC or HU-210 improved endothelial dysfunction and survival both in cardiogenic (Wagner *et al*., 2001b, 2003) and endotoxic shock (Varga *et al*., 1998), probably as the result of an improvement of tissue oxygenation. Taken together, these findings could suggest that the dual role of cannabinoids observed in different kinds of shock could be reflecting the involvement of two SR141716-sensitive receptor subtypes. This is, a non-$CB_1$/non-$CB_2$ mechanism mediating the extreme hypotensive responses, and a $CB_1$-mediated mechanism involved in improving survival. The pharmacological dissection of these mechanisms could be of potential interest in developing new therapeutical approaches to treat different kinds of shock.

In summary, it seems clear that an overproduction of endocannabinoids such as anandamide and 2-AG may be implicated in the severe hypotension associated to various kinds of shock and cirrhotic cardiomyopathy. Although there is substantial evidence to support the view that SR141716-sensitive receptors could represent the main target for the action of these endocannabinoids, the involvement of more than one subtype of cannabinoid receptors cannot be ruled out. Moreover, the relative contribution of different mechanisms of action in each pathological situation is likely to depend on the experimental conditions employed and certainly deserves further studies.

*Involvement of cannabinoids in cardioprotection and atherosclerosis progression*

Several stimuli, such as heat stress or LPS pretreatment are known to trigger delayed endogenous protective mechanisms against myocardial ischaemia-reperfusion injury (Bolli, 2000). Since LPS treatment can induce the production of endocannabinoids, it has been proposed that these mediators could play a role in the cardioprotection induced by LPS in experimental models of heart ischaemia. In this regard, it has been reported that the specific $CB_2$ receptor antagonist SR 144528 abolishes the protective effects of LPS against ischaemia in the rat heart (Lagneux and Lamontagne, 2001). Moreover, endocannabinoids acting through $CB_2$ receptors have been involved in the reduction of infarct size conferred by heat stress preconditioning on isolated rat hearts (Joyeux *et al* 2002). In another study, THC exerted cardioprotection in cardiomyocyte cells subjected to hypoxia, via $CB_2$ receptors and NO production, suggesting that specific $CB_2$ agonists might be useful for cardioprotection (Shmist *et al*., 2006). In contrast, other studies have also highlighted that either $CB_1$ receptors or novel cannabinoid receptors might also mediate cardioprotection from ischaemia-reperfusion injury. For instance, 2-AG acting at $CB_1$ receptors reduced infarct size and mimicked the cardioprotective effects of NO-mediated preconditioning, when administered 30 min before ischaemia/reperfusion in unpreconditioned hearts (Wagner *et al*., 2006). Thus, it was suggested that NO application can increase the production of the endocannabinoid 2-AG, which in turn, may elicit protective effects against myocardial infarction via $CB_1$ cannabinoid receptors. On the other hand, in another study performed in rat-isolated hearts, anandamide and methanandamide limit infarct size induced by ischaemia-reperfusion injury and the pharmacological profile of this response fails to match with any of the previously known mechanisms of cannabinoid action (Underdown *et al*., 2005). Since the infarct-limiting action of anandamide was blocked by either rimonabant or the $CB_2$ receptor antagonist SR144528, the authors of this study suggested that anandamide is acting at both $CB_1$ and $CB_2$ receptors or that, alternatively, it limits the cardiac infarction associated with ischaemia-reperfusion by activation of one or more novel cannabinoid receptors. However, since neither $CB_1$ nor $CB_2$ receptor-selective agonists used individually or in combination affected infarct size, the involvement of a novel site seems to be the most likely explanation (Underdown *et al*., 2005). In further support of this view is the observation that palmitoylethanolamide, an endocannabinoid supposedly inactive at $CB_1$ and $CB_2$ receptors (Lambert *et al*., 1999) and suggested to act

at novel cannabinoid receptors (Mackie and Stella, 2006), also protects the rat-isolated heart against ischaemia (Lepicier *et al*., 2003). Definite proof regarding the involvement of novel receptors in this cardioprotective effect of endocannabinoids will come from studies performed in mice lacking $CB_1$ and/or $CB_2$ receptors in which infarct size-reducing properties of endocannabinoids should be preserved, sorting out the controversial results involving either $CB_2$ or $CB_1$ receptors.

As cannabinoids have also been related to immunomodulatory properties, recent research has focused on the possibility that they could be of therapeutic benefit to the pharmacological management of atherosclerosis, a process in which chronic inflammation is a key player (reviewed by Libby and Theroux, 2005). For instance, Steffens *et al*. (2005) found that low doses of THC inhibited the progression of established atherosclerotic lesions in a murine model of atherosclerosis. In support of the proposal that the immunomodulatory effects of cannabinoids are mediated by the $CB_2$ receptor expressed on immune cells (Buckley *et al*., 2000), it was found that the inhibitory effect of THC on these lesions was blocked by the specific $CB_2$ receptor antagonist SR144528 (Steffens *et al*., 2005). Regarding the mechanisms implicated in the anti-atherosclerotic properties of THC, this latter study also provided evidence that they may be associated with a reduction of the T-helper type 1 response and an inhibition of monocyte/macrophage migration to the site of inflammation, two features playing a major role during early atherosclerosis development. Further implication of $CB_2$ receptors in these effects of THC is supported by the *in vitro* experiments performed after either the pharmacological blockade or the genetic ablation of these receptors. For instance, THC-induced inhibition of macrophage migration was completely blocked by the $CB_2$ antagonist SR144528, and was also absent when peritoneal macrophages isolated from $CB_2$ knockout mice were used, demonstrating that effects of THC on chemoattraction are in fact $CB_2$ receptor-dependent (Steffens *et al*., 2005). Interestingly, the authors also detected $CB_2$ receptor expression within human and mouse atherosclerotic lesions, whereas no $CB_2$ receptors were detected in non-diseased arteries. These data strongly suggest that $CB_2$ receptors agonists may offer a new approach in the treatment of atherosclerosis. Nevertheless, additional *in vivo* studies employing selective $CB_1$ and $CB_2$ receptor antagonists or cannabinoid receptor-deficient mice are warranted to clarify the role of the endocannabinoid system during atherosclerosis.

### *Role of endocannabinoids under hypertensive states and during exercise*

Studies of the hypotensive effects of THC carried out in the 1970s encouraged scientists to propose cannabinoids as new potential antihypertensive agents (Archer, 1974). However, progress in this direction was hindered not only by the difficulty in separating cardiovascular and psychotropic effects but also by the finding that the hypotensive and bradycardic effects of THC developed rapid tolerance (Adams *et al*., 1976). Thus, it was not until the 1990s that the resurgent interest in the study of the cardiovascular effects of cannabinoids was combined with the discovery of the specific receptors and their endogenous ligands.

Although the present evidence does not seem to support the view that endocannabinoids are relevant to cardiovascular regulation under normal conditions, several studies indicate that the endocannabinoid system could be relevant in the cardiovascular regulation of hypertensive states. For instance, the decrease in blood pressure induced by both THC (Kosersky, 1978) and anandamide (Lake *et al*., 1997; Bátkai *et al*., 2004b) was higher and lasted longer in spontaneously hypertensive rats (SHRs) than in normotensive rats. Moreover, in SHR, blockade of $CB_1$ receptors increased blood pressure and cardiac contractility, whereas the elevation of anandamide levels by interference of either anandamide degradation or uptake restored these variables to the values observed in normotensive animals (Bátkai *et al*., 2004b). Taken together, these results suggest the existence of an endocannabinoid tone in hypertension that, according to Pacher *et al*. (2006), limits the elevation of blood pressure and cardiac contractile responses through tonic activation of $CB_1$ receptors. Regarding the possible mechanism involved, it has been suggested that the upregulation of cardiac and vascular $CB_1$ receptors observed in SHR, compared to their normotensive controls may account for the increased sensitivity to the cardiovascular effects of anandamide (Bátkai *et al*., 2004b). Alternatively, a possible upregulation of $TRPV_1$ receptors in hypertension has been proposed on the basis of the finding that capsazepine partially inhibited the hypotensive effect of anandamide in hypertensive but not in normotensive rats (Li *et al*., 2003; Wang *et al*., 2005). Moreover, the potentiation of $TRPV_1$-dependent vasodilating actions of anandamide has been demonstrated in perfused mesenteric beds isolated from rats made hypertensive by long-term inhibition of NO synthase (NOS) when compared to their normotensive controls (Mendizábal *et al*., 2001). Experiments performed in $TRPV_1$ knockout mice have revealed that except for their involvement in mediating the cardiogenic sympathetic reflex, vanilloid receptors are not relevant in the regulation of blood pressure under normal conditions (Pacher *et al*., 2004). Nevertheless, it seems clear that changes in circulating or tissue anandamide levels under particular pathophysiological conditions may alter $TRPV_1$ function and thereby regulate blood pressure. The use of knockout mice in both *in vivo* and *in vitro* studies will further clarify the relevance of a putative-increased endocannabinergic tone in hypertension. For instance, are mice lacking $CB_1$ receptors more vulnerable to developing systemic hypertension? Does the lack of FAAH enzyme protect from development of hypertensive states? What are the consequences of TPRV1 ablation in the development and maintenance of hypertensive states in different models of hypertension?

Physical activity and exercise training have been related to beneficial effects in clinical disorders such as hypertension, heart failure, obesity and the decline of cognition associated with aging. The underlying mechanisms of these effects have been related to different observations such as a favourable influence in brain plasticity by facilitating neurogenerative, neuroadaptive and neuroprotective processes, or the attenuation of neural responses to stress in brain circuits

responsible for regulating peripheral sympathetic activity (for review see Dishman *et al.*, 2006). Interestingly, it has also been reported that exercise of moderate intensity increases serum concentrations of endocannabinoids in trained male college students running on a treadmill or cycling on a stationary bike for 50 min (Sparling *et al.*, 2003). This result not only suggests a new possible explanation for exercise-induced analgesia and sedation, but also for other physiological and psychological adaptations to exercise. In support of a role for the endocannabinoid system, it has recently been found that exercise can reduce adipose tissue via $CB_1$ receptors regulated by peroxisome proliferator-activated receptor-δ (Yan *et al.*, 2007). Further research will be necessary to characterize the precise nature of this endocannabinoid response to exercise, specifically the relative importance of factors such as sex and age as well as the nature of the activity, exercise duration and exercise intensity (for review relating endocannabinoids and exercise see Dietrich and McDaniel, 2004).

## Strategies for cannabinoid intervention: toward a balance between beneficial and adverse effects

Although cannabinoids have been used both recreationally and for medical purposes for more than 4000 years, they are still today the focus of strong social, legal and medical controversy over their therapeutic utility. The fact that most known cannabimimetics have very broad effects on organ systems, some of which are still unexplained, together with dose-limiting psychotropic side effects, are some of the reasons why the clinical application of these drugs has not yet reached its full potential.

Nevertheless, the use of the synthetic THC dronabinol (Marinol) and the synthetic THC analogue nabilone (Cesamet) has been approved in the US for the treatment of nausea and vomiting associated with chemotherapy as well as an appetite stimulant in AIDS. In spite of that, the efficacy of synthetic THC vs the totality of cannabis compounds is the subject of a contentious debate, mainly owing to pharmacokinetics (e.g., oral vs inhaled) and to the contribution of additional components of cannabis (e.g., cannabinol and cannabidiol) to therapeutic efficacy. Related to this question is the recent development of a sublingual spray, Sativex (GW Pharmaceuticals), that is a standardized cannabis extract containing approximately equal quantities of THC and cannabidiol, along with minor amounts of other cannabinoids. Sativex is licensed in Canada and has been submitted for approval regulation in several European countries as an adjuvant therapy for the symptom relief of neuropathic pain in multiple sclerosis.

After more than a decade of intensive effort by pharmaceutical companies to develop novel, potent and selective $CB_1$ and $CB_2$ receptor agonists and antagonists to be used as therapeutic agents, some potentially useful drugs have been developed. Regarding the treatment of cardiovascular disease, at least three strategies can be foreseen in the future clinical use of cannabinoid-based drugs (a) the use of $CB_1$ receptor antagonists, (b) the use of $CB_2$-selective agonists and (c) the use of inhibitors of endocannabinoid degradation.

Among the potential drugs affecting the endocannabinoid system, $CB_1$ receptor antagonists have received the most attention and are the farthest along in clinical studies. Rimonabant, also known as Acomplia, was the first $CB_1$ antagonist reported and has been approved for the treatment of cardiometabolic risk factors associated with obesity. It is still under study for other disorders that have a prominent craving component. Primarily based on the observation that cannabis preparations enhance appetite, an effect that is known to be mediated by $CB_1$ receptors, $CB_1$ blockers such as rimonabant were postulated as anti-obesity drugs. Although the proposal that $CB_1$ antagonists might lead to weight loss was confirmed in both preclinical (Ravinet Trillou *et al.*, 2003) and clinical studies (Van Gaal *et al.*, 2005), the underlying mechanisms of these observations were found to be quite different from the simple suppression of appetite. In contrast, increasing evidence seems to support the view that the rimonabant-induced decrease in body weight is rather a consequence of $CB_1$-mediated regulation of energy homoeostasis (for review Osei-Hyiaman *et al.*, 2006). Although the endocannabinoid system has been implicated in the regulation of central and peripheral mechanisms of energy balance control, preclinical studies suggest that $CB_1$ antagonists will have long-term efficacy for weight loss and improved lipid metabolism as a consequence of mechanisms that are primarily peripheral in origin. For instance, high-fat diet increases hepatic levels of the endocannabinoid anandamide, $CB_1$ density and basal rates of fatty acid synthesis, which is reduced by $CB_1$ blockade (Osei-Hyiaman *et al.*, 2005). Moreover, $CB_1$ activation appears to increase lipoprotein lipase activity in adipocytes (Cota *et al.*, 2003), suggesting that antagonism of this activation would increase lipolysis and favour a lean body phenotype. Thus, if a major peripheral site of action for $CB_1$ antagonists in obesity is definitely demonstrated, the development of a CNS-impermeant $CB_1$ antagonist might still be effective, while reducing the possibility of centrally mediated adverse effects.

Clinical studies with the $CB_1$ antagonist rimonabant are encouraging not only because it produced a significant weight loss and a reduction in waist circumference but also because it caused an improvement in lipid profile, insulin resistance and incidence of metabolic syndrome (reviewed by Cannon, 2005; Tonstad, 2006). Nevertheless, since the endocannabinoid system is likely to be involved in several pathways linked to anxiety and memory extinction, the possibility that chronic $CB_1$ blockade might be accompanied by psychiatric issues is a major concern for the therapeutic use of $CB_1$ antagonists. In this regard, although clinical studies have shown that anxiety and depression scale scores were similar between the rimonabant-treated and the placebo groups, among the patients that gave up treatment during the first year due to depression there were six subjects (1.0%) in the rimonabant and one subject (0.3%) in the placebo group (for review see Gelfand and Cannon, 2006).

Regarding the possible cardiovascular effects of long-term blockade of $CB_1$ receptors, preclinical studies seem to suggest that cardiovascular side effects are probably not to be expected during rimonabant treatment. For instance, the pharmacological blockade of $CB_1$ receptors did not affect sympathetic tone, blood vessel tone or heart rate in pithed

rats (Pfitzer *et al*., 2005), whereas the genetic ablation of these receptors resulted in normal basal blood pressure and heart rate in mice (Ledent *et al*., 1999). However, since it has been suggested that an increased endocannabinergic tone may be present in hypertensive states, the possibility that chronic blockade of $CB_1$ receptors may increase blood pressure in hypertensive patients should be taken into account. In this regard, the results obtained in the RIO-North America clinical trial suggest that rimonabant is not likely to induce significant changes in blood pressure in hypertensive states. No differences in either systolic or diastolic blood pressure were detected when the results obtained with hypertensive patients treated with rimonabant during 2 years were compared to those treated with placebo (Pi-Sunyer *et al*., 2006). Moreover, treatment with rimonabant is also associated with significant increases in high-density lipoprotein and decreases in C-reactive protein and triglyceride levels. Since these metabolic changes had been epidemiologically linked to the regression of coronary atherosclerosis and a reduction in the risk of myocardial infarction, stroke or cardiovascular death, it has been proposed that rimonabant may have additional potential applications apart from reducing body weight. Thus, the STRADIVARIUS (Strategy to Reduce Atherosclerosis Development Involving Administration of Rimonabant – the Intravascular Ultrasound Study) trial is designed to test whether improvements in cardiometabolic syndrome is accompanied by reductions in atherogenesis after long-term $CB_1$ blockade. In addition, the CRESCENDO trial (Comprehensive Rimonabant Evaluation Study of Cardiovascular ENDpoints and Outcomes) will assess whether rimonabant reduces the risk of a heart attack, stroke or death resulting from heart attack or stroke in patients with abdominal obesity plus other cardiovascular risk factors. Nevertheless, other studies have suggested that $CB_1$ receptor antagonism may have negative consequences on cardiac remodeling in an animal model of myocardial infarction (Wagner *et al*., 2003). Furthermore, since rimonabant can either block $CB_1$-mediated or the not yet identified non-$CB_1$/non-$CB_2$-binding sites, the possibility that cardiovascular side effects may appear during rimonabant treatment cannot be ruled out.

On the other hand, endocannabinoid-mediated cardiovascular effects appear to have survival value, as indicated by the increased mortality following blockade of $CB_1$ receptors in haemorrhagic (Wagner *et al*., 1997) and cardiogenic shock (Wagner *et al*., 2001b, 2003), despite the increase in blood pressure. In this regard, pretreatment with cannabinoid agonists, such as THC or HU-210 improved endothelial dysfunction and survival both in cardiogenic (Wagner *et al*., 2001b, 2003) and endotoxic shock (Varga *et al*., 1998), probably as the result of an improvement of tissue oxygenation. In addition, endocannabinoids may mediate important protective mechanisms against hypoxic damage in the heart and the vasculature, and also exert potent anti-inflammatory effects (for review see Hiley and Ford 2004; Walter and Stella 2004). In the context of septic shock, where an increased production of NO results as a consequence of NOS induction, cannabinoids could play a controversial role. Hence, although Ross *et al*. (2000) demonstrated that the cannabinoid agonist WIN 55212-2, acting via $CB_2$ receptors, actually inhibited LPS-induced NO release from macrophages, a recent observation shows that low concentrations of anandamide, devoid of relaxing effects, elicit an acute release of NO mediated predominantly by the activation of endothelial $TRPV_1$ receptors (Poblete *et al*., 2005). Moreover, since the dual role of cannabinoids observed in different kinds of shock could be reflecting the involvement of two SR141716-sensitive receptor subtypes, the proposed introduction of $CB_1$ antagonists as therapeutic agents for the management of shock needs further evaluation.

Another promising strategy in the clinical use of cannabinoid-based drugs is the development of $CB_2$ receptor agonists, such as AM1241, HU308 and JWH133, since they are devoid of known psychoactivity. Multiple animal studies suggest that agonists at this receptor may be clinically useful in the treatment of chronic pain, specifically neuropathic pain (Ibrahim *et al*., 2003), whereas some preliminary studies suggest beneficial effects of $CB_2$ receptors in the maintenance of bone density and the delay in the progression of atherosclerotic lesions. The involvement of $CB_2$ receptors in bone growth derives from the observation that $CB_2$-deficient mice have markedly decreased bone mass compared to their littermates expressing the receptor, whereas a $CB_2$-specific agonist attenuates ovariectomy-induced bone loss in mice (Ofek *et al*., 2006). Moreover, the finding that a particular silent single-nucleotide polymorphism in $CB_2$ gene correlates strongly with osteoporosis in a cohort of women (Karsak *et al*., 2005), gives further support to the view that $CB_2$ agonists might be useful as a new therapeutic approach to osteoporosis. Additionally, the finding that a low oral dose of THC inhibits atherosclerosis progression in the ApoE knockout mouse through $CB_2$-mediated immunomodulatory effects on lymphoid and myeloid cells (Steffens *et al*., 2005) may also lead to an entirely new application for $CB_2$ agonists. Whether this strategy would be more effective in reducing atherosclerosis progression than blocking $CB_1$ receptors with rimonabant as a consequence of improving cardiometabolic parameters remains to be established. We need not only a better understanding of the physiological role of $CB_2$ receptors in the immune system, but also a knowledge of the consequences of long-term $CB_2$ activation. For instance, human $CB_2$ receptors rapidly desensitize when expressed in the Chinese hamster ovary cell line (Bouaboula *et al*., 1999), suggesting that they might not maintain their efficacy if $CB_2$ agonists are chronically administered.

Finally, a third kind of approach deals with the possibility of regulating endocannabinoid levels by the inhibition of FAAH, the major degradative enzyme for anandamide and related amides. Enzymes for both anandamide and 2-AG degradation, FAAH and the monoacylglycerol lipase, respectively, have been cloned, although truly selective and/or potent inhibitors have been developed so far only for FAAH (for review see Cravatt and Lichtman, 2003). A number of studies have provided evidence that the enzyme inhibitors might be therapeutically useful in the treatment of pain (Cravatt and Lichtman, 2004) and neuropsychiatric disorders such as anxiety (Kathuria *et al*., 2003). Regarding cardiovascular pathology, their possible use as antihyperten-

sive agents is suggested by the observation that the FAAH inhibitor URB597 decreases arterial pressure to near-normotensive values in SHR, while it has no detectable haemodynamic effects in normotensive rats (Bátkai *et al*., 2004b).

Decreases in blood pressure similar to that caused by the FAAH inhibitors in SHR were observed after the treatment with the anandamide transport inhibitors, AM404 and OMDM-2 (Bátkai *et al*., 2004b). Nevertheless, the facts that the putative endocannabinoid transporter has not been cloned up to now and that several of the existing inhibitors, such as AM404, are also known to act at $CB_1$ and $TRPV_1$ receptors, make it difficult to interpret the above studies.

## Concluding remarks

There has been a significant progress in the understanding of the molecular mechanisms of cannabinoid action and this has led to the serious consideration of cannabinoids as possible therapeutic agents on the basis of scientific evidence. As in the case of the history of the therapeutic use of opioids, cannabinoid research is still the focus of legal and moral controversy, an issue that has powerfully contributed to the delay in the clinical application of these drugs. Nevertheless, in spite of the problems derived from their psychotropic side effects, which will probably be overcome by developing more specific agonists, the fact that most known cannabimimetics have very broad effects on organ systems, some of which are still unexplained, certainly emphasizes the need for caution. Moreover, $CB_1$ receptors are among the most abundant G-protein-coupled receptors in brain, present at nearly 10-fold higher levels than most other G-protein-coupled receptors (Devane *et al*., 1988; Herkenham *et al*., 1991). Hence, the consequences of the long-term inhibition of these receptors need further exploration. Regarding cardiovascular pathophysiology, although the understanding of cannabinoids action has largely improved, their effects are complex and cannot be explained by a single mechanism or a single site of action. Nevertheless, the endocannabinoid system is a promising target for the development of therapies for cardiovascular pathologies and this area of research will largely benefit from further experiments tending to give answers to several major issues. For instance, what is the role of non-$CB_1$/non-$CB_2$ receptors in cardiovascular pathophysiology? Will the identification of these novel receptors reconcile conflicting evidence in the literature? What are the long-term effects of $CB_1$ receptors blockade in terms of cardiovascular pathology? Hence, much more multidisciplinary work will be necessary to assess the exact function of cannabinoids in cardiovascular disease in order to develop new therapeutical approaches. This latter possibility will largely depend on the finding of appropriate strategies that could prevent the known side effects of cannabinoids as well as the chronic toxic effects of this novel group of compounds. Translating literature into science, and in words of the Hungarian writer Sandor Marai: 'the truth is precisely what I don't know' (Marai, 2001), there is a promising although controversial future in the field of endocannabinoid research.

## Acknowledgements

Work on this topic at the Instituto de Investigaciones Farmacológicas was supported by grants PICT 05-14107 from FONCYT and PIP 5695 from CONICET, Argentina.

## Conflict of interest

The authors state no conflict of interest.

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