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MINI REVIEW

The complexities of the cardiovascular actions of cannabinoids

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The cardiovascular actions of cannbinoids are complex. In general they cause vasorelaxation in isolated blood vessels, while in anaesthetised animals they cause multiphasic responses which involve an early bradycardia and long-lasting hypotension. However, in conscious animals, the picture is one of bradycardia followed by pressor responses. Clearly, the responses to cannabinoids are dependent on the experimental conditions and synthetic cannabinoids and endocannabinoids exhibit different pharmacologies. In terms of mechanisms involved in the vascular responses to cannabinoids, the following have been implicated: the involvement of 'classical' cannabinoid receptors, the involvement of a novel endothelial cannabinoid receptor, the release of nitric oxide, the release of endothelium-derived hyperpolarising factor (EDHF), the activation of vanilloid receptors, metabolism of endocannabinoids to vasoactive molecules, and both peripheral inhibition and central excitation of the sympathetic nervous system.
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Abbreviations: 2-AG, 2-arachidonoyl glycerol; AM 251, *N*-piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; CB, cannabinoid; EDHF, endothelium-derived hyperpolarising factor; HU210, ((6aR)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol); SR141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide-hydrochloride; TASK, TWIK (tandem of P domains in a weak inward rectifying K^+ channel)-related acid-sensitive potassium channel; THC, Δ^9-tretrahydrocannabinol; VR, vanilloid receptor; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone

Introduction

The cardiovascular effects of both synthetic and endogenous cannabinoids have been extensively examined and reviewed (see Hillard, 2000; Kunos *et al*., 2000; Ralevic *et al*., 2002; Randall *et al*., 2002). What is clear is that the cardiovascular actions of the cannabinoids are complex and appear to be complicated by differences in experimental approach and prevailing conditions. The overwhelming findings from studies on isolated blood vessels are that both endogenous and exogenous cannabinoids are vasodilators. This is mirrored by studies on anaesthetised animals that report hypotensive effects (Varga *et al*., 1995). However, findings in conscious animals are more complex and do not support the notion that cannabinoids are hypotensive agents (Stein *et al*., 1996; Lake *et al*., 1997; Gardiner *et al*., 2001; Gardiner *et al*., 2002a; Gardiner *et al*., 2002b). Similarly, there is no general consensus regarding the molecular target(s) for cannabinoids. Indeed, the field might have become blurred by the assumption that synthetic cannabinoids and endogenous cannabinoids share common pharmacology and that *in vitro* findings translate to the *in vivo* situation. The purpose of this review is to summarise the key findings and to attempt to resolve the issues raised by *in vitro* and *in vivo* comparisons.

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The vascular effects of cannabinoids in isolated arteries (see Figure 1)

In vitro studies have identified that the prototypic anandamide is a potent vasodilator in a number of isolated vascular preparations. A more detailed overview of *in vitro* effects of anandamide can be found in the following reviews (Kunos *et al*., 2000; Högestatt & Zygmunt, 2002; Randall *et al*., 2002). Some studies have implicated the endothelium in relaxant responses to anandamide (Pratt *et al*., 1998; Chaytor *et al*., 1999; Wagner *et al*., 1999), with the release of prostanoids (Ellis *et al*., 1995; Fleming *et al*., 1999), nitric oxide (Deutsch *et al*., 1997) or endothelial-derived hyperpolarising factor (EDHF) (Chaytor *et al*., 1999). Some, but not all, studies have reported that anandamide acts through the stimulation of cannabinoid CB_1 receptors, although the intracellular pathway(s) coupling to vasodilatation have not been clearly identified (White & Hiley, 1997). More recently, a novel 'anandamide receptor' has been proposed to exist on the vascular endothelium (Járai *et al*., 1999; Wagner *et al*., 1999; Offertáler *et al*., 2003) and may be coupled to the release of EDHF.

An important step came in 1999 when Zygmunt and co-workers reported that anandamide stimulates vanilloid receptors on sensory nerves, leading to vasorelaxation *via* the release of the vasoactive neurotransmitter calcitonin gene-related peptide (CGRP), and this has since been widely confirmed (Ralevic *et al*., 2000; White *et al*., 2001; Ho & Hiley, 2003).

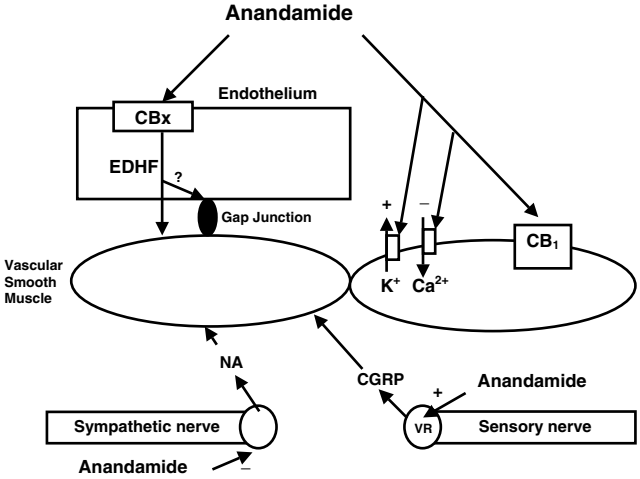

Figure 1 Schematic diagram showing possible mechanisms of vasorelaxation to anandamide. Putative mechanisms include (**a**) endothelium-dependent relaxation coupled to a novel endothelial cannabinoid receptor (CBx) coupled to EDHF (endothelium-derived hyperpolarizing factor), which may involve myoendothelial gap junctions; (**b**) activation (+) of potassium channels on vascular smooth muscle; (**c**) inhibition (−) of calcium channels on vascular smooth muscle; (**d**) the participation of cannabinoid (CB) receptors on vascular smooth muscle; (**e**) the release of CGRP from sensory nerves coupled to vanilloid receptors (VR); (**f**) presynaptic inhibition of sympathetic nerves leading to reduced noradrenaline (NA) release.

Subsequent work, however, has suggested that the participation of sensory nerves might depend on the prevailing or experimental conditions and is less important in the absence of a functional nitric oxide system (Harris *et al*., 2002).

In other studies, anandamide has been shown to inhibit calcium channels (Gebremedhin *et al*., 1999) and activate various K^+ channels (Randall & Kendall, 1997; Randall & Kendall, 1998; White *et al*., 2001), and these may also account for its vasorelaxant actions.

What is clear from the above is the lack of overall consensus or perhaps diverse mechanisms of action of cannabinoids; the following account will explore potential reasons for mechanistic differences.

Regional differences The first documentation of the vasorelaxant effects of anandamide was in rabbit cerebral vessels (Ellis *et al*., 1995), and since then many other blood vessels from different species have been examined.

It is clear that the magnitude of relaxant responses to anandamide differs between preparations. For example, small resistance mesenteric vessels show 100% vasorelaxation to anandamide, while the larger superior mesenteric artery has a maximal relaxation of around 40% (O'Sullivan *et al*., unpublished observations). Cerebral vessels show a maximum relaxation of 25–50% to anandamide (Ellis *et al*., 1995; Gebremedhin *et al*., 1999; Wagner *et al*., 2001 and, similarly, coronary vessels on an average relax by around 50% (Pratt *et al*., 1998; White *et al*., 2001). In rat aortae, the maximum relaxation to anandamide is approximately 20% (O'Sullivan *et al*., 2004a). In contrast, rat and rabbit carotid arteries (Holland *et al*., 1999; Fleming *et al*., 1999) do not relax to anandamide. Such differences between vessels may be due to differences in receptor populations or the prevailing mechanisms. For example, it has been suggested that cannabinoid CB_1 receptor expression is associated with mesenteric vessels, but not the thoracic aorta (Darker *et al*., 1998). There is also evidence that the putative endothelial cannabinoid receptor contributes to vasorelaxation in small mesenteric resistance vessels, but not the main superior mesenteric artery (O'Sullivan *et al*., unpublished observations).

Although vanilloid receptors on sensory nerves may play an important role in vasorelaxation to anandamide in mesenteric vessels (Zygmunt *et al*., 1999; Ralevic *et al*., 2000), this is not the case under all conditions (Harris *et al*., 2002) and in all vessels. In respect of the latter, it has been shown that sensory nerves do not play a role in coronary vessels from several species (Grainger & Boachie-Ansah, 2001; White *et al*., 2001; Ford *et al*., 2002). This indicates that the actions of anandamide may be dependent on vanilloid receptor density and/or density of perivascular nerve in a given blood vessel. This was emphasised in a study by Andersson *et al*. (2002) who showed that, while anandamide is a full agonist at the vanilloid receptor in mesenteric arteries, it is a weak agonist of this receptor in main bronchi. The authors attributed this to possible differences in receptor reserve and/or cellular uptake between the different tissues. Similarly, Vanheel & Van De Voorde (2001) reported that anandamide produces capsazepine-sensitive hyperpolarisations of daughter branches of the mesenteric artery, but not of the superior mesenteric artery, and suggested that this might relate to the relative density of perivascular sensory nerves or to regional differences in the distribution of vanilloid receptors. Such differences may explain some of the variation in vasorelaxant responses to anandamide between vascular beds, or indeed between different vessels within the same bed.

Some mechanisms of vasorelaxation may be specific to certain tissues, for instance nitric oxide has only been shown to mediate responses to anandamide in renal arteries (Deutsch *et al*., 1997) but not other vascular beds (Harris *et al*., 2002).

Species differences The vascular responsiveness towards anandamide varies between species. In the rat aorta, anandamide causes approximately a 20% maximal relaxation (O'Sullivan *et al*., 2004a), while in the rabbit aorta, this has been reported to be 80% (Mukhopadhyay *et al*., 2002). Similarly, in the rat coronary vessels anandamide causes about 30–40% relaxation (White *et al*., 2001), 50% relaxation in bovine vessels (Pratt *et al*., 1998) but 80% relaxation in ovine vessels (Grainger & Boachie-Ansah, 2001). However, anandamide does not cause vasorelaxation in porcine coronary vessels (Fleming *et al*., 1999). Interestingly, the only work so far in human vessels has shown that anandamide is not a vasorelaxant in myometrial arteries from pregnant women (Kenny *et al*., 2002).

Metabolism One important difference to emerge is the involvement of metabolism to arachidonic acid metabolites. Studies are divided into those where anandamide acts directly and those where its actions are dependent on metabolism. For example, there is evidence from bovine and ovine coronary vessels (Pratt *et al*., 1998; Grainger & Boachie-Ansah, 2001) that vasorelaxation to anandamide is dependent on metabolism *via* epoxygenase or cyclooxygenase pathways. However, in rat mesenteric vessels it is universally reported that vasorelaxation to anandamide is unaffected by cyclooxygenase

inhibition (White & Hiley, 1997). Despite this, in the rabbit mesenteric vessels, Fleming *et al*. (1999) reported that responses to anandamide were abolished by the cyclooxygenase inhibitor diclofenac, although additional actions of diclofenac cannot be excluded.

The metabolically stable analogue of anandamide, methanandamide, also exhibits vasorelaxant activities (Ralevic *et al*., 2000) and this would mitigate against metabolism being central to the actions of cannabinoids. Having said that, it is possible that methanandamide (Ralevic *et al*., 2000) has a greater dependence on sensory nerve activation than anandamide (Harris *et al*., 2002), presumably due to differences in their relative efficacies at vanilloid and cannabinoid receptors.

Methodological differences The various studies on the vascular actions of ananamide have been carried out under many different conditions, for example in both isolated arterial segments and intact perfused vascular beds, in the absence or presence of cyclooxygenase inhibitors, and against different spasmogens. These different approaches inevitably mean that straightforward comparisons between studies may be difficult.

The archetypal CB_1 receptor antagonist SR141716A has been widely used to investigate the involvement of CB_1 receptors in vasorelaxation to anandamide but this is confounded by the wide-ranging actions of SR141716A at various concentrations. For example, SR141716A also inhibits myoendothelial gap junctions (Chaytor *et al*., 1999), which themselves have been implicated in the actions of anandamide *via* endothelial-derived hyperpolarizing factor (EDHF) (Chaytor *et al*., 1999; Harris *et al*., 2002). SR141716A may also antagonise the novel, non-CB_1 endothelial cannabinoid receptor proposed by Kunos and co-workers (Járai *et al*., 1999; Offertáler *et al*., 2003).

The pharmacological profile of SR141716A differs depending on the concentration used. For example, White & Hiley (1997) have shown SR141716A at 100 nM to be ineffective against vasorelaxation to anandamide in isolated mesenteric vessels, but 1 μM to be inhibitory. Similarly, Harris *et al*. (2002) have shown 3 μM, but not 1 μM, to be effective against anandamide-induced vasodilatation in the perfused mesenteric bed. Furthermore, in many studies where more that one CB_1 receptor antagonist has been used, SR141716A has been effective at inhibiting anandamide-mediated relaxation, while AM251 or LY320135 have not (Chaytor *et al*., 1999; White *et al*., 2001; Ford *et al*., 2002; Harris *et al*., 2002).

Other cannabinoids

In addition to anandamide, synthetic cannabinoid compounds and other recently identified endocannabinoids have been reported to have vascular effects *in vitro*. Many synthetic cannabinoid receptor agonists have been shown to have vasorelaxant effects. Δ^9-Tetrahydrocannabinol (THC) causes indomethacin-sensitive relaxation of rabbit cerebral arteries (Ellis *et al*., 1995) and endothelium-independent vasorelaxation of isolated rabbit mesenteric vessels, which are sensitive to SR141716A (Fleming *et al*., 1999). Zygmunt *et al*. (2002) have also recently reported that THC causes release of CGRP from mesenteric vessels, although interestingly, this was not through stimulation of the vanilloid receptor subtype 1, and may involve another novel receptor in the vasorelaxant pathway to cannabinoids. The CB_1 receptor agonist HU-210 has also been reported to cause dilatation in the rat-perfused mesenteric bed (Wagner *et al*., 1999) and SR141716A-sensitive vasorelaxation of coronary and cerebral vasculature (Wagner *et al*., 2001). In isolated rabbit mesenteric vessels, HU-210 causes endothelium-independent, SR141716A-sensitive vasorelaxation (Fleming *et al*., 1999). WIN55,212 ((R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone), another potent CB_1 receptor agonist, has been shown to produce vasorelaxtion of feline cerebral vessels and these responses are sensitive to SR141716A (Gebremedhin *et al*., 1999). However, in rat mesenteric vessels, WIN55,212 causes endothelium-independent vasorelaxation (White & Hiley, 1998), and this does not appear to be through stimulation of either CB_1 or CB_2 or vanilloid receptors (Ho & Hiley, 2003). Similarly, the cannabinoid analogue, abnormal cannabidiol, elicits an endothelium-dependent non-CB_1/CB_2/vanilloid relaxation of mesenteric vessels (Ho & Hiley, 2003), and this is thought to be mediated through the novel endothelial cannabinoid receptor (Járai *et al*., 1999; Offertáler *et al*., 2003).

The endocannabinoid, 2-arachidonoylglycerol (2-AG), has been shown to cause endothelium-independent vasorelaxation of rabbit mesenteric vessels, mediated by both CB_1 and CB_2 receptors (Kagota *et al*., 2001), but does not have an effect in the perfused rat mesenteric arterial bed (Wagner *et al*., 1999), possibly due to its instability. We have recently shown that another endocannabinoid, *N*-arachidonoyl-dopamine (NADA), causes endothelium-dependent vasorelaxation of isolated rat mesenteric vessels that involves the novel endothelial receptor (coupled to EDHF release) and endothelium-independent relaxations *via* vanilloid receptors (O'Sullivan *et al*., 2004b).

The cardiovascular effects of cannabinoids in vivo

The *in vivo* cardiovascular effects of cannabinoids are complex, with both increases and decreases in blood pressure being reported (Stark and Dews, 1980; Dewey, 1986).

Studies in anaesthetised animals In parallel with studies on cannabinoids in isolated blood vessels, their *in vivo* cardiovascular actions have also been assessed. In 1995, Varga and co-workers reported that anandamide caused a triphasic response in anaesthetised rats. This included an initial vagally mediated bradycardia with secondary hypotension, a transient pressor effect followed by sustained hypotension, which was sensitive to both the cannabinoid CB_1 receptor antagonist, SR141716A and interference with sympathetic control. These early conclusions led to the suggestion that anandamide acted *via* CB_1 receptors to inhibit sympathetic control of blood pressure. A more extensive study in the following year confirmed the triphasic nature of the responses to anandamide, and it was concluded that the sustained depressor effect was due to presynaptic inhibition of sympathetic nerves (Varga *et al*., 1996). *In vitro* studies have also confirmed that cannabinoids inhibit sympathetic regulation (see Ralevic, 2003). Cannabinoid-induced sympathoinhibition by synthetic cannabinoids has also been reported in rabbits (Niederhoffer & Szabo, 1999) and rats (Niederhoffer *et al*., 2003). Significantly, Niederhoffer & Szabo (1999) reported that the synthetic cannabinoid, WIN55212-2, caused depressor effects in pithed rabbits with electrically stimulated, sympathetic tone

which were opposed by the CB_1 receptor antagonist, SR141716A, but that this was less marked in conscious animals. These findings clearly emphasise the influence of background sympathetic tone on responses to cannabinoids.

Haemodynamic studies in anaesthetised rats have also reported marked hypotension in response to anandamide, which appears to be due to reductions in peripheral resistance (Garcia *et al*., 2001). These responses were partly sensitive to SR141716A.

Malinowska *et al*. (2001) have reported that the initial bradycardia and depressor responses to anandamide in anaesthetised rats are due to activation of vanilloid receptors and that the long-lasting hypotensive phase was sensitive to SR141716A and thus presumed to be mediated *via* CB_1 receptors. However, as commented above, SR141716A has a range of actions independent of antagonism of CB_1 receptors and its inhibitory effects should be interpreted with caution. Further evidence for the potential participation of sensory nerves in the cardiovascular effects of anandamide comes from studies in anaesthetised rats which showed that intra-arterial injection of anandamide led to hypotension and increased ventilation (Smith & McQueen, 2001). These responses were mimicked by capsaicin, but inhibited by vanilloid receptor antagonists, desensitisation of vanilloid receptors and sectioning of the femoral and sciatic nerves, with the implication that they were due to sensory nerve reflexes evoked by anandamide.

In addition to studies on anandamide, the cardiovascular effects of 2-AG have also been investigated (Mechoulam *et al*., 1998; Járai *et al*., 2000). In this regard, 2-AG was shown to cause hypotension in anaesthetised rats (Mechoulam *et al*., 1998) and in anaesthetised mice, there was hypotension and tachycardia which did not appear to be mediated *via* CB_1 receptors but may have involved metabolism to arachidonic acid metabolites (Járai *et al*., 2000). By contrast, similiar cardiovascular effects were observed for a stable analogue of 2-AG, but these appear to have been mediated *via* CB_1 receptors.

In addition to endocannabinoids, a comparative study on the haemodynamic effects of HU210 and anandamide in anaesthetised rats reported that HU 210 caused a profound reduction in cardiac output leading to hypotension that was sensitive to SR141716A, while anandamide did not (Wagner *et al*., 2001). However, both agents were reported to cause cerebral and coronary vasodilatation, which were sensitive to SR141716A.

Studies in conscious animals Comparative work in conscious rats has also reported that anandamide caused a profound bradycardia, with a short lived depressor effect but this was followed by a longer lasting pressor effect (Stein *et al*., 1996). The bradycardic effect was sensitive to cyclooxygenase inhibition and ascribed to the production of arachidonic acid metabolites. Similarly in the conscious rat, Lake *et al*. (1997) reported that there was vagal activation but the prolonged hypotensive effects reported in anaesthetised animals were absent. Their explanation was that the depressor effect was masked by the pressor effect and since they also reported that the depressor effect was present in conscious hypertensive rats, they speculated that the depressor response was dependent on the level of sympathetic tone. Subsequent studies in conscious rats have underscored the complex nature of the *in vivo* responses. In this regard, Gardiner *et al*. (2002a) reported that intravenous anandamide caused a transient pressor effect that was accompanied by regional (hindquarters, mesenteric and renal) vasoconstriction in conscious rats. At higher doses, there was pronounced bradycardia and in some instances there was a depressor effect prior to sustained hypertension, with some degree of hindquarters vasodilatation following constriction. These complex cardiovascular effects were insensitive to the cannabinoid CB_1 receptor antagonist AM251. The bradycardia was opposed by atropine and the hindquarter vasodilatation appeared to be mediated *via* β_2-adrenoceptors, possibly due to adrenaline release. Furthermore, when the early bradycardia was blocked with atropine the initial hypotension was absent. Parallel studies on synthetic cannabinoids (WIN-55212-2 and HU 210) in conscious rats (Gardiner *et al*., 2002b) have reported that these agents caused pressor effects, accompanied by renal and mesenteric vasoconstriction but hindquarters vasodilatation. In contrast to the actions of anandamide, these cardiovascular effects were sensitive to the cannabinoid CB_1 receptor antagonist, AM251, but the hindquarters vasodilatation was also inhibited by a β_2-adrenoceptor antagonist. Studies on conscious normotensive and hypertensive rats have also demonstrated pressor effects with WIN 55,212-2, which were sensitive to ganglion blockade (Gardiner *et al*., 2001). From these findings it was concluded that the effects of synthetic cannabinoids were mediated *via* CB_1-receptors linked to increases in sympathetic activity. However, it should be noted that in *in vitro* studies synthetic cannabinoids reduce sympathetic activity (Ralevic, 2003). A further point to emerge from that study was that AM 251 alone did not affect blood pressure or regional haemodynamics, with the implication that endogenous cannabinoids do not influence cardiovascular control under resting conditions. A similar conclusion was also drawn from the CB_1 knockout mice (Ledent *et al*., 1999). Although the role, if any, of endocannabinoids in cardiovascular regulation remains to be established, Rademacher *et al*. (2003) reported that SR141716A injected into the nucleus tractus solitarius delayed baroreflex recovery in anaesthetised dogs, with the implication that endocannabinoids might play a role in this regulatory system. Furthermore, in the rat, injection of anandamide into the nucleus tractus solitarius increased baroreflex sensitivity in an SR147116A-sensitive manner, possibly *via* modulation of GABAergic or glutamergic neurotransmission (Seagard *et al*., 2004). It was also observed that pharmacologically induced increases in blood pressure were accompanied by increases in endogenous anandamide in the nucleus tractus solitarius, pointing to a possible modulatory role.

In conscious mice, Ledent *et al*. (1999) identified a biphasic response to anandamide with an initial pronounced depressor effect followed by more sustained hypotension and these changes were accompanied by bradycardia. More importantly, it was reported that the cardiovascular responses to anandamide were absent in CB_1-receptor knockout mice and, thus were assumed to be CB_1 receptor-mediated.

In vivo studies will inevitability involve both central and peripheral effects and in this respect intracisternal administration of various cannabinoid agonists in conscious rabbits caused both sympathoexcitation and increased vagal output, with bradycardia and at high doses pressor effects were observed (Niederhoffer & Szabo, 1999). In anaesthetised rats, administration of synthetic cannabinoids into the rostral ventrolateral medulla oblongata leads to increased sympathetic

activity and hypertension (Padley *et al*., 2003). Hence, central effects of cannabinoids may oppose their peripheral effects.

Studies in man Administration of cannabis-derived cannabinoids (including *via* smoking) in man is associated with pronounced tachycardia (as opposed to bradycardia reported in animals above) (see Dewey, 1986; Jones, 2002). This is accompanied by an increase in circulating noradenaline release but demonstrates rapid tolerance on repeated administration (see Jones, 2002). The tachycardia is also sensitive to SR141716A, implicating the involvement of cannabinoid CB_1 receptors (Huestis *et al*., 2001). According to Jones, the reasons for this difference in man compared to animal studies is not immediately clear but could be related to the high doses in animal studies and also differences in arousal between human volunteers and animals in the conscious and anaesthetised state.

Is there any consensus as to the in vivo *actions of cannabionids?* The above studies have highlighted clear differences in the actions of cannabinoids depending on the experimental conditions. Studies in anaesthetised animals report a multiphasic response with a clear initial bradycardia and a final long-lasting hypotensive phase, probably mediated *via* sympathoinhibition. The bradycardia is also seen in conscious animals but the long-lasting hypotensive phase is not. The lack of a hypotensive phase under 'physiological conditions' could reflect differences in sympathetic activity between the conscious and anaesthetised state. Another possibility is that anaesthetic agents directly influence the responses. In this regard, anandamide has been shown to inhibit the TASK-1 potassium channel that is anaesthetic sensitive (Maingret *et al*., 2001) and this might have bearing on the differences between the anaesthetised and conscious state. It is also possible that the central effects of cannabinoids might be more susceptible to inhibition by general anaesthetics.

Is there a correlation between in vivo *and* in vitro *effects?* The clear vasorelaxant effects reported from *in vitro* studies are largely postjunctional and will not be significantly influenced by *in vivo* control systems such as the autonomic nervous system, and this would certainly contribute towards differences between the two situations. Furthermore, central effects following *in vivo* administration may also complicate the peripheral effects.

Another consideration is the route of administration. *In vitro* studies are based on local application and this will lead to local effects, while *in vivo* studies usually involve systemic administration, with the potential for widespread effects and metabolism.

There is good evidence from *in vitro* studies that cannabinoids may exert dual effects on vascular control, for example action at vanilloid receptors may lead to sensory nerve-mediated vasodilatation but presynaptic cannabinoid receptors may oppose this (see Ralevic, 2003). Once again the predominant effect may be dependent on the prevailing conditions.

Some of the *in vitro* actions are uncovered when other systems are inhibited, for example, actions of anandamide *via* EDHF release are probably accentuated by removal of nitric oxide. However, in the *in vivo* situation, the physiological significance of EDHF has yet to be established. Once again, the actions of endocannabinoids may be dependent on the experimental conditions. Similarly, the balance between the endocannabinoid and endovanilloid actions of anandamide will also influence the overall effect.

Pathophysiological roles

The physiological significance of the cardiovascular effects of endocannabinoids are unclear and it may be that they are of more pathophysiological importance. In this regard, Wagner *et al*. (1997) demonstrated, in a rat model of haemorrhagic shock, that activated macrophages release anandamide which may contribute towards the hypotension. Similarly in endotoxic shock, the synthesis of 2-AG in platelets and anandamide in macrophages are increased (Varga *et al*., 1998). The release of anandamide by central neurones under hypoxic conditions, leading to improved blood flow and protection against ischaemia has also been advanced as a pathophysiological role for anandamide (Gebremedhin *et al*., 1999). In the context of cardiac ischaemia, Lagneux and Lamontagne (2001) reported that cardioprotection of the rat heart against ischaemia by pretreatment with lipopolysaccharide involved endocannabinoids. Subsequent work by that group also reported that palmitoylethanolamide and 2-arachidonoyl glycerol both caused cardioprotection *via* CB_2 receptor activation (Lepicier *et al*., 2003).

Concluding comments

The cardiovascular effects of cannabinoids and in particular the endocannabinoids are complex; their precise molecular targets are diverse and their relative contributions are uncertain. Furthermore, actions in isolated tissues do not necessarily translate to the whole animal situation. *In vivo*, the responses reported appear to be dependent on the experimental conditions, not least the use of general anaesthetics. However, much is to be gained by identifying the key targets; for example, can the vascular actions be best defined by considering novel cannabinoid receptors? Considering the cardiovascular actions of endocannabinoids, to what extent are they cannabinoids or vanilloids? These questions remain to be answered.

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