

Endocannabinoids: a new class of vasoactive substances

Michael D. Randall and David A. Kendall

Endogenous cannabinoids (endocannabinoids) have recently been identified in the CNS and attention has now turned to their cardiovascular actions. The prototypic endocannabinoid, anandamide, derived from arachidonic acid, has been shown to be a vasorelaxant, particularly in the resistance vasculature. This vasorelaxation has been shown to be both endothelium-independent and -dependent, depending on the vascular bed. It has been proposed that an endocannabinoid may mediate the nitric oxide- and prostanoid-independent component of endothelium-dependent relaxations, as these responses are sensitive to a cannabinoid receptor antagonist and show similarities to anandamide-induced relaxations. This hypothesis has generated much controversy and the emerging conflicts in the literature are discussed in this article by **Michael Randall and David Kendall**. Despite this controversy, it has recently been shown that anandamide is produced by endothelial cells. Clearly, much work is required to adequately define the physiological significance of endocannabinoids in the cardiovascular system.

A major advance in understanding the pharmacology of plant cannabinoids, the most potent of which is 9-tetrahydrocannabinol (THC), was prompted by the discovery of specific brain receptors¹. The existence of these receptors implies the presence of endogenous ligands and in 1992 the first endocannabinoid, anandamide (*N*-arachidonyl ethanolamide), which is the ethanolamide of arachidonic acid, was isolated from the porcine brain²; this was shown both to occupy cannabinoid receptors and to mimic the functional effects of THC. It now seems that anandamide is the prototype of a family of *N*-acyl ethanolamines (NAEs), and other polyunsaturated NAEs (Ref. 3) have similar effects via activation of G protein-coupled cannabinoid (CB₁ or CB₂) receptors. The CB₁ receptors are found predominantly in the brain and peripheral nervous system, and the CB₂ receptor appears to be exclusive to immune tissues. The two receptors activate similar transduction mechanisms, including inhibition of adenylate cyclase and *N*-type Ca²⁺ channels and activation of K⁺ channels. The pharmacology of the endocannabinoids has not been fully characterized but

they do appear to exhibit some receptor selectivity. For example, anandamide has a higher affinity for CB₁ compared with CB₂ receptors⁴ and might not act as a full agonist at CB₂ receptors, at least in model cell systems⁵. *N*-palmitoylethanolamide has been proposed to be a selective agonist at the CB₂-like mast cell receptor, but there are now some suggestions that this might be a subtype different from the CB₂ (Ref. 6).

The biosynthetic routes for the NAEs are beginning to be clarified and two mechanisms have been proposed (Fig. 1). First, the condensation of ethanolamine and arachidonic acid has been described^{7,8}, which requires high substrate concentrations, has an alkaline pH optimum and might be catalysed by the catabolic enzyme, anandamide amidohydrolase (amidase) working in reverse⁹. Second, and more likely in the majority of situations, the NAEs could be produced by the Ca²⁺-activated breakdown of a membrane phospholipid, *N*-acylphosphatidylethanolamine (NAPE), probably via a phospholipase D-type phosphodiesterase^{10,11}. There is no evidence for vesicular storage of the NAEs and it is likely that they are produced and released from membrane NAPE 'on demand' when intracellular Ca²⁺ is elevated.

With regard to the inactivation of endocannabinoids, there appears to be rapid uptake into cells, via a carrier, which delivers it to an amidase for rapid hydrolysis¹². The enzyme, which is sensitive to inhibition by the serine protease inhibitor phenylmethylsulphonyl fluoride has a high substrate specificity for anandamide¹³. The fates of the arachidonate and ethanolamine produced by hydrolysis are uncertain although arachidonate is rapidly re-incorporated into membrane phospholipids¹⁴ and is, therefore, an unlikely substrate for the synthesis of other biologically active agents (e.g. eicosanoids).

In vivo cardiovascular effects of cannabinoids

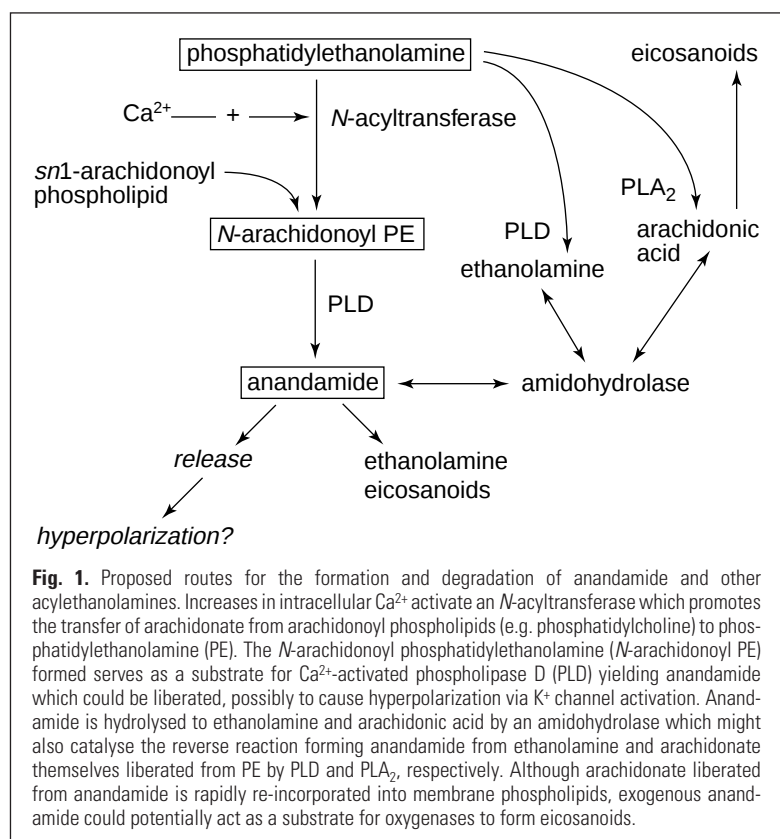
One of the potential therapeutic roles for cannabis has been as an antihypertensive, although developments in this direction have been stifled by the stigma associated with cannabis. The relatively small literature concerning the cardiovascular effects of exogenous cannabinoids contains variable observations with both vasodilator and vasoconstrictor actions being reported¹⁵. However, given the recent interest in endocannabinoids, attention is now turning towards the cardiovascular system. In this respect, exogenous anandamide causes bradycardia (with secondary hypotension) and a transient pressor effect which is followed by a longer lasting depressor effect in urethane-anaesthetized rats^{16,17}. This depressor effect is believed to be mediated by CB₁ receptor-dependent inhibition of sympathetic tone via a pre-synaptic mechanism, as the effect was independently attenuated by cervical spinal transection, α -adrenoceptor block and cannabinoid receptor block. This sympatholytic action has recently been reported to be greater in spontaneously hypertensive rats compared to normotensive controls, perhaps reflecting the higher level of

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sympathetic tone in the former¹⁷. The pressor component of the response to anandamide was not sensitive to cannabinoid receptor block, perhaps reflecting a non- CB_1 receptor-mediated response¹⁷. By contrast, Stein *et al.*¹⁸ reported that, although anandamide caused bradycardia in conscious rats, it caused a transient depressor response, followed by a longer pressor phase, and only at high doses was there delayed hypotension.

Recently, Calignano *et al.*¹⁹ have found that anandamide causes hypotension in guinea-pigs, which occurs independently of the autonomic nervous system but is mediated via CB_1 receptors. Furthermore, the hypotensive effect was potentiated by inhibition of anandamide reuptake, suggesting that this system terminates the cardiovascular actions of endocannabinoids. To circumvent the complication of metabolism, Vidrio *et al.*²⁰ used the stable cannabinoid agonist HU210, which, on administration to both conscious and anaesthetized rats, caused prolonged bradycardia and hypotension, which did not appear to be mediated through a sympatholytic action.

Anandamide and THC have both been shown to be vasorelaxants in the rat cerebral vasculature, but these effects were sensitive to indomethacin, suggesting that cannabinoids may cause relaxation through the stimulation of arachidonic acid metabolism²¹.

Vascular actions of endocannabinoids

In the rat isolated mesenteric and coronary vasculatures anandamide has been identified as a vasorelaxant, and it has been proposed that it may be an endothelium-derived agent^{22–24}. More recently, Deutsch and colleagues²⁵ have

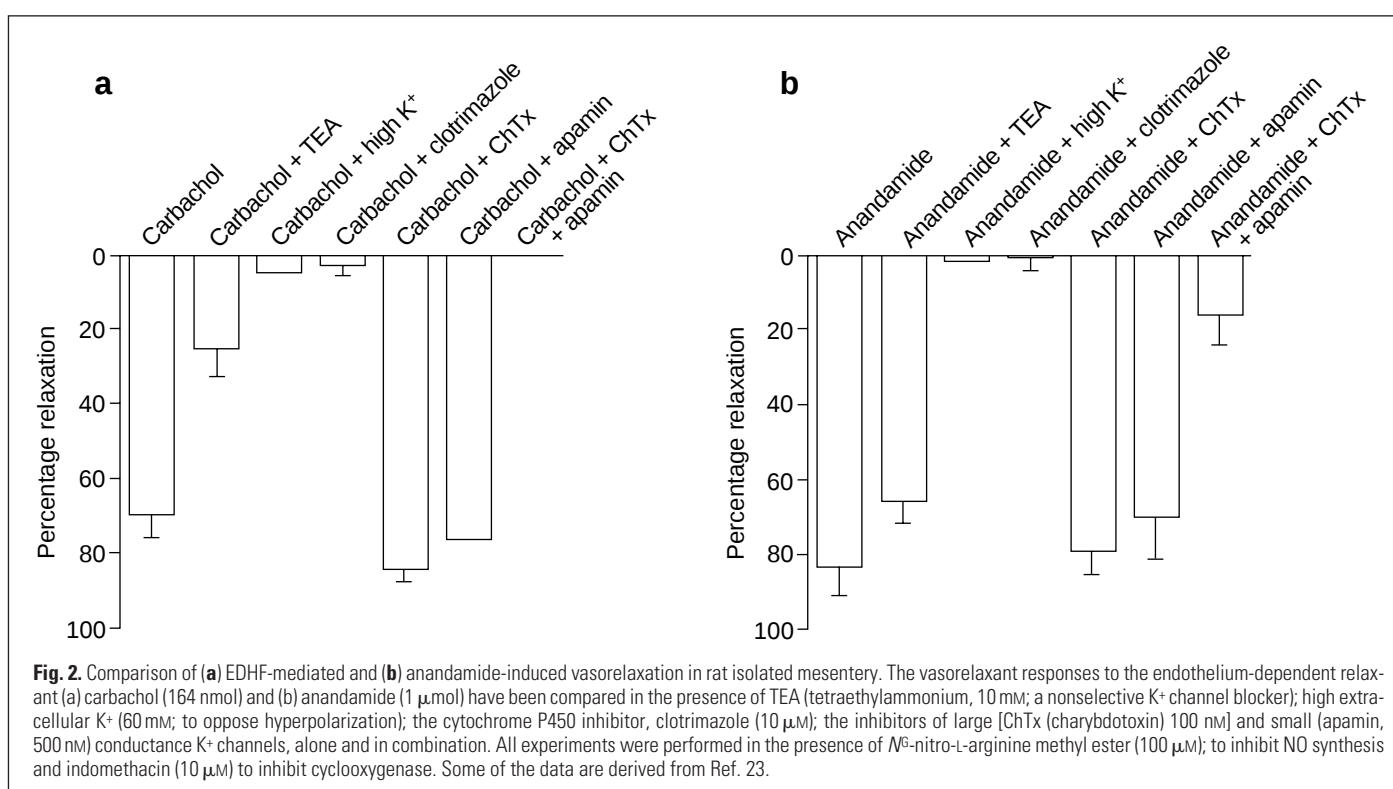
indeed reported that rat cultured renal endothelial cells contain anandamide together with synthase and amidase activities, thereby demonstrating that endocannabinoids may be endothelium-derived autacoids. In their study they confirmed that anandamide was a vasorelaxant in renal afferent arterioles, acting via endothelium-derived NO (Ref. 25). In bovine coronary arteries anandamide has been shown to cause endothelium-dependent relaxation via arachidonic acid metabolites²⁶. By contrast, in mesenteric vessels^{22,23,27,28} and the coronary vasculature²⁴ anandamide induces vasorelaxation in the presence of blockers of both NO synthase and cyclooxygenase and also in the absence of the endothelium²², and so acts independently of endothelial autacoids. Accordingly, the mechanisms underlying vasorelaxation may depend on the vascular bed.

In our early studies, we reported that responses to anandamide were abolished by high extracellular K^+ and proposed that cannabinoids might be hyperpolarizing agents²². This proposal was confirmed by Chataigneau *et al.*²⁹ and Plane *et al.*²⁷, who showed that anandamide caused vascular smooth muscle hyperpolarization or repolarization, but in both cases this effect was insensitive to cannabinoid CB_1 receptor block. The mechanism of hyperpolarization has been linked to K^+ channel activation, such that anandamide-induced relaxation is sensitive to nonspecific K^+ channel blockers^{22,24,28} (Fig. 2). In isolated mesenteric arterial segments Plane *et al.*²⁷ reported that vasorelaxation to anandamide was blocked by selective inhibitors of large conductance Ca^{2+} -activated K^+ channels, charybdotoxin and iberiotoxin, although this was not observed by White and Hiley²⁸.

Is an endocannabinoid an EDHF?

The central role of the endothelium in vascular control has been well established, with the identification of the vasodilators prostacyclin, and endothelium-derived relaxing factor (EDRF), identified as NO. However, in addition to NO there is a much neglected second EDRF, the endothelium-derived hyperpolarizing factor or factors (EDHF)^{30–32}. EDHF is known to be released in parallel with NO, and so contributes to endothelium-dependent relaxations through the activation of vascular smooth muscle K^+ channels, leading to hyperpolarization or repolarization. Cross-talk also appears to exist, whereby EDHF activity is upregulated on loss of NO (Ref. 33).

There is reasonably strong evidence that an EDHF is derived from arachidonic acid³². Given this evidence and the observation that the cannabinoid derived from arachidonic acid, anandamide, causes vasorelaxation via K^+ channel activation, we have proposed that an endogenous cannabinoid is an EDHF. To address this possibility, the effects of the highly selective cannabinoid antagonist, SR141716A (Ref. 34), have been investigated against NO- and prostanoid-independent, but endothelium-dependent, relaxations which are generally accepted to be mediated by EDHF(s).



In the rat isolated perfused mesenteric arterial bed²² and coronary vasculature²⁴ these EDHF-mediated relaxations to a variety of agonists were selectively antagonized by SR141716A (Refs 22, 24). This provided, for the first time, evidence that endocannabinoids could potentially mediate EDHF responses. These findings in the isolated perfused whole mesenteric arterial bed have been confirmed by White and Hiley²⁸, who found that SR141716A opposed both EDHF-mediated and anandamide-induced responses in mesenteric arterial segments; however, this was not observed by Plane *et al.*²⁷ using an identical preparation. Complementary to these *in vitro* studies, further investigations in conscious rats revealed that the endothelium-dependent vasodilator bradykinin caused substantial depressor effects, accompanied by regional vasodilatation, which were independent of NO activity but blocked by SR141716A (Ref. 22). Clearly, further characterization of the pharmacology of SR141716A is essential before it can be concluded that a cannabinoid(s) is an EDHF.

A comparison of EDHF and anandamide

The identity of EDHF(s) has remained elusive but it is an area of some considerable controversy. Indeed, the conflicts in the literature indicate that there may be more than one EDHF. Since the identification of EDHF there has been a series of reports implicating the involvement of cytochrome P450 monooxygenase metabolism of arachidonic acid (the epoxygenase pathway) leading to the production of epoxides of arachidonic acid, the epoxyeicosatrienoic acids (EETs) which represent a group of EDHFs (Refs 31, 35, 36). However, more recent

findings have cast doubt on this proposition as it is largely based on the use of nonselective cytochrome P450 inhibitors, which have recently been shown to act also as K⁺ channel inhibitors³⁷.

Regardless of the diverse effects of the cytochrome P450 inhibitors, these agents may also be viewed as EDHF inhibitors³⁷. In the mesenteric (proadifen and clotrimazole²³; Fig. 2) and coronary (clotrimazole²⁴) vasculatures, these inhibitors opposed EDHF-mediated responses and similarly antagonized relaxation to anandamide. These findings provide circumstantial evidence that EDHF and endocannabinoids share common pharmacological characteristics.

In order to compare the pharmacology of EDHF and endocannabinoids, recent studies have used more selective K⁺ channel inhibitors. Once again there are several important differences between the findings of various groups. Plane *et al.*²⁷ reported that EDHF and anandamide showed differential sensitivity to apamin (a blocker of small-conductance K⁺ channels), which blocked EDHF but not anandamide responses, whilst the inhibitory effects of apamin were found to be dependent on the agent used to release EDHF (Ref. 28). In our experiments, we have found that neither charybdotoxin nor apamin alone affected EDHF-mediated or anandamide-induced responses in the isolated mesentery, but when used in combination, charybdotoxin and apamin abolish both responses (Fig. 2). This has not, however, been observed in mesenteric arterial segments^{28,29}. Our findings are comparable to those observed by others in regard to EDHF (Ref. 38), and point to EDHF and endocannabinoids acting at a common site.

Concluding remarks

The endocannabinoids represent a novel class of vasoactive compounds, whose action on exogenous administration *in vivo* appears variable, perhaps due to rapid metabolism. In isolated vascular preparations endocannabinoids induce vasorelaxation, via K⁺ channel activation. This has led to the hypothesis that endocannabinoids may be an EDHF. There are now substantial conflicts in the literature, with evidence both for and against this proposal, which is perhaps a reflection of methodological and tissue-specific differences. Clearly, much work is required to define more adequately the vascular pharmacology of the endocannabinoids.

Note added in proof

Since the submission of this article Wagner and colleagues [Wagner, J. A. *et al.* (1997) *Nature* 390, 518–521] have shown, in a rat model of haemorrhagic shock, that macrophage-derived anandamide (and perhaps also anandamide released from the endothelium in response to macrophage activation) plays a substantial role in the accompanying hypotension. This accordingly identifies an important pathophysiological role for endocannabinoids in the cardiovascular system.

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Chemical names

HU210: (AR)-*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-1H-pyrazole-3-carboxamide hydrochloride

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