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 receptors[1]. The existence of these receptors implies the presence of endogenous ligands and in 1992 the first endocannabinoid, anandamide (*N*-arachidonylethanolamide), which is the ethanolamide of arachidonic acid, was isolated from the porcine brain[2]; 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*-acylethanolamines (NAEs), and other polyunsaturated NAEs (Ref. 3) have similar effects via activation of G protein-coupled cannabinoid (CB_1 or CB_2) receptors. The CB_1 receptors are found predominantly in the brain and peripheral nervous system, and the CB_2 receptor appears to be exclusive to immune tissues. The two receptors activate similar transduction mechanisms, including inhibition of adenylate cyclase and N-type Ca^{2+} 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 CB_1 compared with CB_2 receptors[4] and might not act as a full agonist at CB_2 receptors, at least in model cell systems[5]. *N*-palmitoylethanolamide has been proposed to be a selective agonist at the CB_2-like mast cell receptor, but there are now some suggestions that this might be a subtype different from the CB_2 (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 reverse[9]. Second, and more likely in the majority of situations, the NAEs could be produced by the Ca^{2+}-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 Ca^{2+} 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 hydrolysis[12]. The enzyme, which is sensitive to inhibition by the serine protease inhibitor phenylmethylsulphonyl fluoride has a high substrate specificity for anandamide[13]. The fates of the arachidonate and ethanolamine produced by hydrolysis are uncertain although arachidonate is rapidly re-incorporated into membrane phospholipids[14] 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 reported[15]. 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 CB_1 receptor-dependent inhibition of sympathetic tone via a presynaptic 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

M. D. Randall, Lecturer in Pharmacology, and **D. A. Kendall,** Reader in Molecular Pharmacology, School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK NG7 2UH.

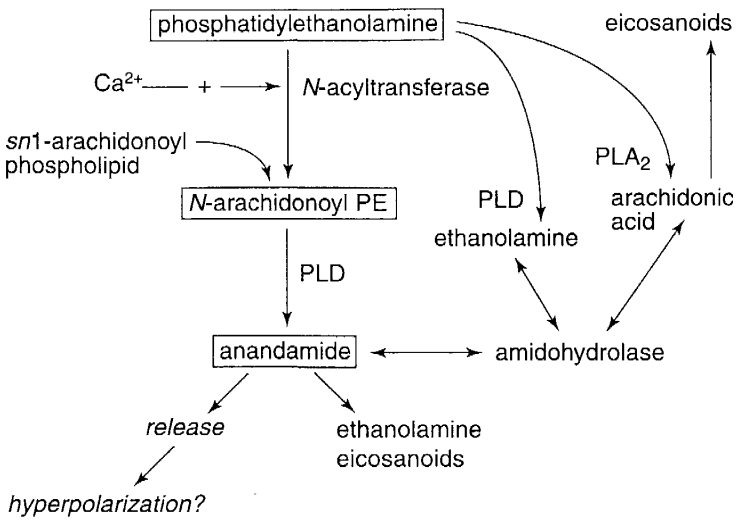

Fig. 1. Proposed routes for the formation and degradation of anandamide and other acylethanolamines. Increases in intracellular Ca^{2+} activate an *N*-acyltransferase which promotes the transfer of arachidonate from arachidonoyl phospholipids (e.g. phosphatidylcholine) to phosphatidylethanolamine (PE). The *N*-arachidonoyl phosphatidylethanolamine (*N*-arachidonoyl PE) formed serves as a substrate for Ca^{2+}-activated phospholipase D (PLD) yielding anandamide which could be liberated, possibly to cause hyperpolarization via K^+ channel activation. Anandamide is hydrolysed to ethanolamine and arachidonic acid by an amidohydrolase which might also catalyse the reverse reaction forming anandamide from ethanolamine and arachidonate themselves liberated from PE by PLD and PLA_2, respectively. Although arachidonate liberated from anandamide is rapidly re-incorporated into membrane phospholipids, exogenous anandamide could potentially act as a substrate for oxygenases to form eicosanoids.

sympathetic tone in the former[17]. The pressor component of the response to anandamide was not sensitive to cannabinoid receptor block, perhaps reflecting a non-CB_1 receptor-mediated response[17]. 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 metabolism[21].

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 colleagues[25] 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 metabolites[26]. By contrast, in mesenteric vessels[22,23,27,28] and the coronary vasculature[24] anandamide induces vasorelaxation in the presence of blockers of both NO synthase and cyclooxygenase and also in the absence of the endothelium[22], 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 agents[22]. 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 Hiley[28].

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 acid[32]. 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).

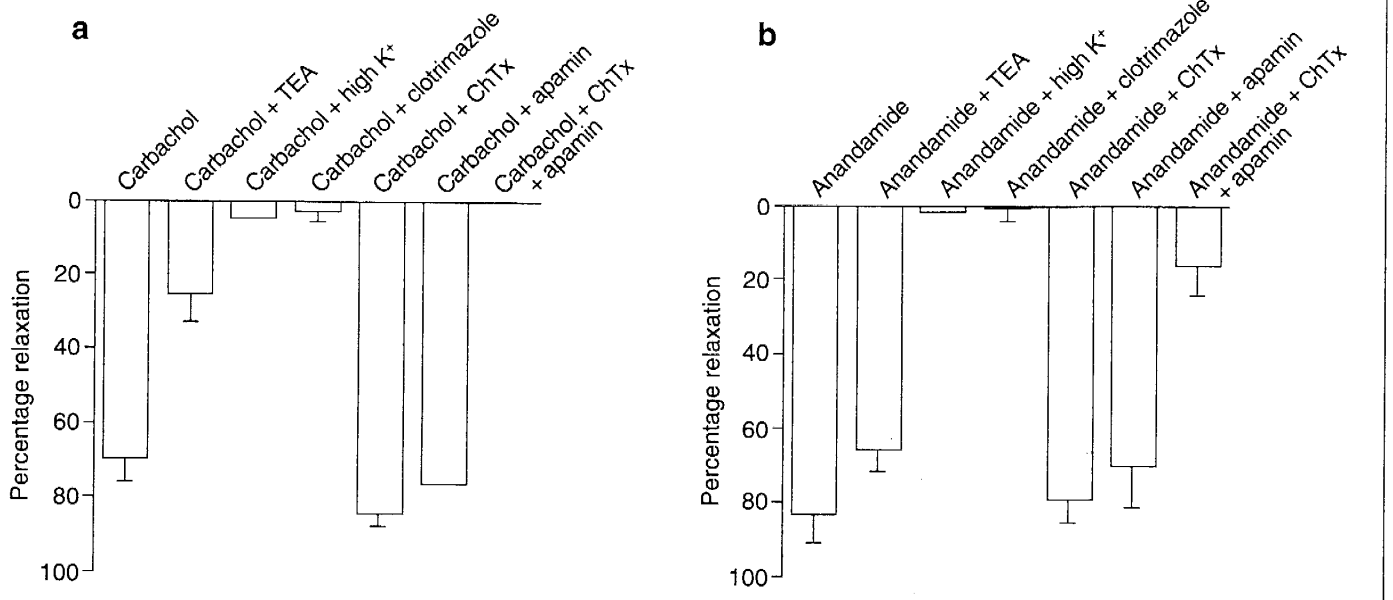

Fig. 2. Comparison of (**a**) EDHF-mediated and (**b**) anandamide-induced vasorelaxation in rat isolated mesentery. The vasorelaxant responses to the endothelium-dependent relaxant (a) carbachol (164 nmol) and (b) anandamide (1 μmol) have been compared in the presence of TEA (tetraethylammonium, 10 mM; a nonselective K^+ channel blocker); high extracellular K^+ (60 mM; to oppose hyperpolarization); the cytochrome P450 inhibitor, clotrimazole (10 μM); the inhibitors of large [ChTx (charybdotoxin) 100 nM] and small (apamin, 500 nM) conductance K^+ channels, alone and in combination. All experiments were performed in the presence of N^G-nitro-L-arginine methyl ester (100 μM); to inhibit NO synthesis and indomethacin (10 μM) to inhibit cyclooxygenase. Some of the data are derived from Ref. 23.

In the rat isolated perfused mesenteric arterial bed[22] and coronary vasculature[24] 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 Hiley[28], 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 inhibitors[37].

Regardless of the diverse effects of the cytochrome P450 inhibitors, these agents may also be viewed as EDHF inhibitors[37]. In the mesenteric (proadifen and clotrimazole[23]; Fig. 2) and coronary (clotrimazole[24]) 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 reponse 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)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-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