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Endocannabinoid system as a potential mechanism for *n*-3 long-chain polyunsaturated fatty acid mediated cardiovascular protection

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The presence of an active and functioning endocannabinoid (EC) system within cardiovascular tissues implies that this system has either a physiological or pathophysiological role (or both), and there is a substantial literature to support the notion that, in the main, they are protective in the setting of various CVD states. Moreover, there is an equally extensive literature to demonstrate the cardio- and vasculo-protective effects of *n*-3 long-chain (LC)-PUFA. It is now becoming evident that there appears to be a close relationship between dietary intervention with *n*-3 LC-PUFA and changes in tissue levels of EC, raising the question as to whether or not EC may, at least in part, play a role in mediating the cardio-and vasculo-protective effects of *n*-3 LC-PUFA. This brief review summarises the current understanding of how both EC and *n*-3 LC-PUFA exert their protective effects in three major cardiovascular disorders (hypertension, atherosclerosis and acute myocardial infarction) and attempts to identify the similarities and differences that may indicate common or integrated mechanisms. From the data available, it is unlikely that in hypertension EC mediate any beneficial effects of *n*-3 LC-PUFA, since they do not share common mechanisms of blood pressure reduction. However, inhibition of inflammation is an effect shared by EC and *n*-3 LC-PUFA in the setting of both atherosclerosis and myocardial reperfusion injury, while blockade of L-type Ca^{2+} channels is one of the possible common mechanisms for their antiarrhythmic effects. Although both EC and *n*-3 LC-PUFA demonstrate vasculo- and cardio-protection, the literature overwhelmingly shows that *n*-3 LC-PUFA decrease tissue levels of EC through formation of EC–*n*-3 LC-PUFA conjugates, which is counter-intuitive to an argument that EC may mediate the effects of *n*-3 LC-PUFA. However, the discovery that these conjugates have a greater affinity for cannabinoid receptors than the native EC provides a fascinating avenue for further research into novel approaches for the treatment and prevention of atherosclerosis and myocardial injury following ischaemia/reperfusion.

Endocannabinoids: *n*-3 LC-PUFA: Atherosclerosis: Hypertension: Cardioprotection: Arrhythmia

There is now a substantial literature demonstrating that there is an active and functioning endocannabinoid (EC) system within cardiovascular tissues. Moreover, there is an equally extensive literature to demonstrate the cardio- and vasculo-protective effects of *n*-3 long-chain (LC)-PUFA. Since both of these topics have been subject to recent detailed published reviews, this article is not intended to present a comprehensive review of topics that have previously been well covered. However, as is now becoming apparent, there appears to be a close relationship between dietary intervention with *n*-3 LC-PUFA and changes in tissue levels of EC. The aim of this review is therefore to attempt to draw comparisons between the effects of the EC and *n*-3

Abbreviations: 2-AG, 2-arachidonyl glycerol; AEA, anandamide; AMI, acute myocardial infarction; CB, cannabinoid; EC, endocannabinoid; I/R, ischaemia/reperfusion; LC, long chain; NAPE, N-arachidonyl-phosphatidyl ethanolamine; NO, nitric oxide.

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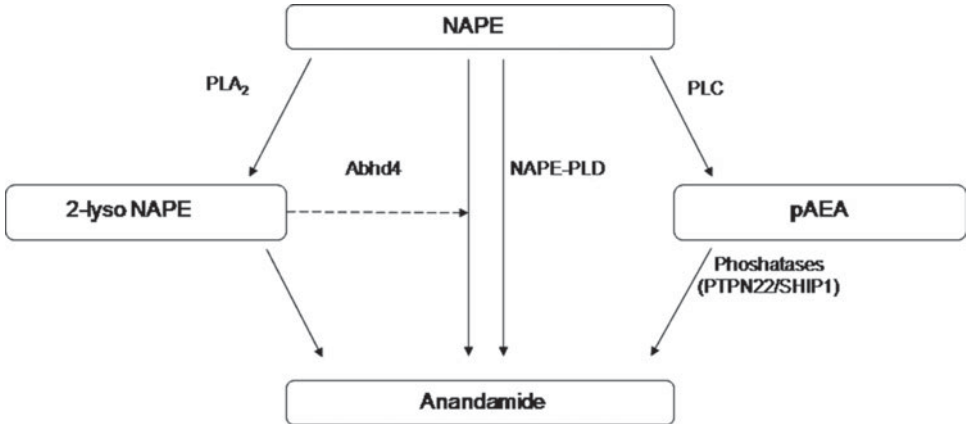

Fig. 1. Synthetic pathways for anandamide (AEA). NAPE, *N*-arachidonyl-phosphatidyl ethanolamine; pAEA, phospho-anandamide; NAPE-PLD, NAPE-selective phospholipase D; PLA_2, phospholipase A2; PLC, phospholipase C.

LC-PUFA in the setting of common CVD, and to weigh up the evidence around whether or not EC may play a role in the protective effects of LC-PUFA.

The endocannabinoid system

The EC system constitutes an endogenous signalling system that plays a pivotal role in a variety of centrally and peripherally regulated physiological processes. The system comprises G-protein-coupled cannabinoid (CB) receptors, their endogenous CB (EC) ligands and the associated enzymatic apparatus that controls their synthesis and degradation (as reviewed in[1]). The two recognised G-protein coupled receptors are the CB_1 and CB_2 receptors[2,3]; CB_1 receptors are predominantly expressed in the central nervous system, but are also located in numerous peripheral tissues including cardiac[4,5] vascular[6,7] and adipose[8] tissue. CB_2 receptors, while expressed primarily in the periphery, particularly by immune cells[3], have also been reported to be present in both the myocardium[9] and endothelial cells[10]. However, evidence from functional studies also suggests the existence of non-CB receptor targets of the EC, including transient receptor potential vanilloid type-1 channels, PPAR[5], the vascular 'anandamide' receptor[11] and the recently de-orphanised receptor GPR55[12].

The very presence of these receptors in cardiovascular tissue implies that they are there to mediate responses to endogenous ligands, either as part of cardiovascular homoeostasis, or to participate in pathological processes either as a stress response or as a causative factor. Indeed, endogenous ligands do exist that activate these receptors, the most studied of which are anandamide (AEA) and 2-arachidonyl glycerol (2-AG) and will therefore be the main focus of this review. However, it is worthy of note that various additional endogenous CB receptor ligands have been identified, including docosatetraenyl-ethanolamide, N-arachidonoyl dopamine, virodhamine and noladin (reviewed in[13]).

Endocannabinoid synthesis and sites of action

AEA was first shown to be synthesised from the phospholipid precursor *N*-arachidonyl-phosphatidyl ethanolamine (NAPE), via hydrolysis of NAPE by a calcium (Ca^{2+}) sensitive, NAPE-selective phospholipase D[14]. However, alternate routes (Fig. 1) of AEA biosynthesis have since been identified, including conversion of NAPE to 2-lyso-NAPE (via phospholipase A_2) and subsequently to AEA through a Ca^{2+} independent mechanism[15], and also hydrolysis of NAPE by phospholipase C to yield the phospho-AEA, which is further hydrolysed to produce AEA. The synthesis of 2-AG (Fig. 2), which is generated from arachidonic acid-containing phospholipids, can be synthesised via (i) the production of diacylglycerol from phosphatidylinositol via phospholipase Cβ and subsequent hydrolysis by *sn*1-diacylglycerol lipase to yield 2-AG[16] and (ii) through generation (via phospholipase A_1) of lyso-phosphatidylinositol from phosphatidylinositol and subsequent hydrolysis by a lyso-phosphatidylinositol selective phospholipase C to yield 2-AG (reviewed in[17]). In terms of receptor activation by these endogenous ligands (reviewed in[18]), AEA is a partial agonist at CB receptors, with a marginally higher affinity and markedly higher efficacy for the CB_1 receptor compared with the CB_2 receptor. 2-AG, on the other hand, has a similar affinity for both CB receptors but has a higher efficacy at the CB_2 receptor compared to AEA.

There are now numerous reports that both circulating and tissue EC levels are raised in a variety of cardiovascular-related pathologies, including cerebral[19], hepatic[20] and myocardial[21] ischaemia/reperfusion (I/R) injury, heart failure[22], diabetic cardiomyopathy[23], advanced atherosclerosis[24] and obesity-related cardiovascular dysfunction[25]. However, the nature of the specific EC, and its precise role (i.e. whether it is protective or detrimental) varies widely between pathological conditions. For the purposes of this review, the discussion will focus on the potential role of the EC in the setting of three cardiovascular disorders,

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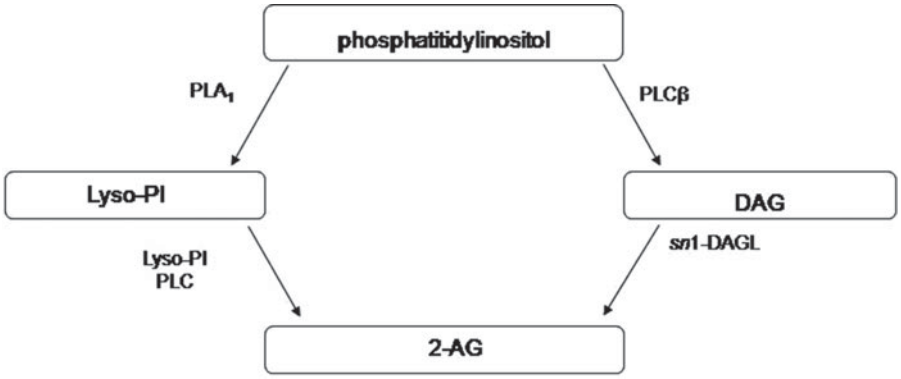

Fig. 2. Synthetic pathways for 2-arachidonyl glycerol (2-AG). Lyso-PI, lyso-phosphatidylinositol; DAG, diacylglycerol; PLA_1, phospholipase A_1; PLCβ, phospholipase C-beta; *sn*-1-DAGL, *sn*-1 specific diacylglycerol lipase.

namely hypertension, atherosclerosis and acute myocardial I/R.

Interactions between long-chain-PUFA and endocannabinoids

There is a significant literature concerning the cardio- and vasculo-protective effects of *n*-3 LC-PUFA and various underlying mechanisms have been proposed including: anti-inflammatory and anti-oxidant effects; modulation of cardiac ion channels; reduction of TAG; influence on membrane microdomains and downstream cell signalling pathways; improved cardiac mitochondrial function; antithrombotic and antiarrhythmic effects (reviewed in[26–29]). What is interesting is that the beneficial effects of *n*-3 LC-PUFA are observed in very similar settings to those in which changes in levels of EC are observed, which begs the question as to whether or not there are shared or integrated mechanisms through which both the *n*-3 LC-PUFA and the EC exert their effects. Indeed, there is building evidence of interactions between the *n*-3 LC-PUFA and activation of the EC system (recently reviewed in[30,31], although the findings are variable in terms of whether or not *n*-3 LC-PUFA raise or lower EC levels. Berger *et al*[32] were one of the first groups to demonstrate a definitive effect of *n*-3 LC-PUFA ingestion on raising brain levels of AEA in piglets, and this has since been extended in studies in mice where DHA supplementation significantly altered EC-related metabolites in plasma and brain[33]. In contrast, other studies have shown that *n*-3 LC-PUFA deficiency increases, while supplementation decreases, 2-AG levels in the brains of mice[34]. Thus the impact of *n*-3 LC-PUFA on EC levels appears to be dependent upon the EC in question. In terms of the effects of *n*-3 LC-PUFA on EC levels in peripheral tissues the picture appears to be largely opposite to the effects on central EC levels, as dietary *n*-3-PUFA supplementation, given as either fish oil[35] or krill oil[36] in models of either high-fat feeding or metabolic syndrome, decreases both AEA and 2-AG levels in adipose and heart tissue, effects that are associated with an anti-obesogenic effect and an improvement in glucose tolerance[37]. These findings raise the possibility that modulation of the EC system by *n*-3-PUFA may be an important part of their protective mechanisms of action. The remainder of this review will therefore attempt to synthesise the evidence for the protective mechanisms of ECs and *n*-3-PUFA to identify where similarities exist and where they do not.

Could the endocannabinoid system be a mechanism for the effects of *n*-3 long-chain-PUFA in hypertension?

Both AEA[38–40] and 2-AG[41] elicit complex vasodilatory[42] and cardio-depressive responses *in vivo* that are sensitive to inhibition by CB_1 receptor antagonists[43] and are absent in CB_1-knockout mice[41]. Together this implies that the CB_1 receptor is responsible for mediating the cardiovascular responses to the EC. The simple fact, however, that CB receptor antagonists do not elicit significant blood pressure responses[4,13,39,43,44], and that blood pressure in CB_1 knockout mice is comparable with wild-type controls[41], suggests that EC do not play a tonic role in blood pressure maintenance. However, in hypertensive rats CB_1 receptor antagonists increase blood pressure, whereas inhibitors of AEA metabolism normalise blood pressure[4], suggesting that under situations of pathophysiological (i.e. hypertensive) stress the EC are produced as a compensatory mechanism. While, as far as the authors are aware, no determination of EC levels has been made in either hypertensive animal models or in clinical samples from hypertensive patients, the increased expression of CB_1 receptors in both the heart and endothelium of hypertensive rats[4] is suggestive of an up-regulation of the EC system aimed at redressing the balance. The blood pressure lowering effects of EC in hypertension have been attributed to a combination of a tonic suppression of cardiac contractility (and thus cardiac output) along with a direct vasodilator effect.

The effect of *n*-3 LC-PUFA on blood pressure has long been an issue of controversy. Recent analysis of clinical and epidemiological studies[45] shows that, while very high (≥3 g/d) doses of *n*-3 LC-PUFA do

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produce a small but significant decrease in blood pressure (especially systolic blood pressure), this is evident only in certain groups such as older hypertensive subjects and individuals with hypertriglyceridaemia[46]. Moreover, lower doses of *n*-3 LC-PUFA as a single treatment are ineffective in lowering blood pressure in mild essential hypertensive patients[47] or individuals with metabolic syndrome[46]. Thus, like the EC system, *n*-3 LC-PUFA only appear to modify blood pressure in the setting of hypertension.

The blood pressure lowering effects of EC are thought to be mediated through an endothelial mechanism involving the release of nitric oxide (NO)[37], via both CB_1[48] and non-CB_1/CB_2 (possibly vanilloid) receptors[48,49]. Conversely, the proposed mechanism(s) by which *n*-3 LC-PUFA reduce blood pressure are numerous (reviewed in[50]) and include effects on sodium excretion, interference with the renin-angiotensin system and enhancement of endothelial NO production, although with respect to the latter there is a degree of ambiguity between experimental findings. *In vitro* studies on isolated bovine and ovine blood vessel preparations show that EPA relaxes blood vessels through endothelial NO release[51,52]. However, studies in human subjects are inconsistent with this and have reported no effect of long-term *n*-3 LC-PUFA supplementation (0·45–3·4 g/d EPA +DHA) on flow-mediated dilatation and/or arterial stiffness (surrogate markers of endothelial function) in either normal subjects[53,54], hypertensives[55] or patients with peripheral vascular disease[56], although microvascular endothelial function is improved in individuals with type II diabetes[57].

At present there is no data around whether or not *n*-3 LC-PUFA influence EC levels in the vasculature in hypertension. However, the profiles of EC and *n*-3 LC-PUFA in the setting of hypertension differ substantially: *n*-3 LC-PUFA are not particularly effective as either anti-hypertensive or NO-releasing agents, while EC are potent endothelium (and presumably NO) dependent vasodilators. On this basis, current evidence suggests that there is no correlation between *n*-3 LC-PUFA and EC in hypertension.

Could the endocannabinoid system contribute to the anti-atherosclerotic effects of *n*-3 long-chain-PUFA?

There is a growing evidence base for a role of the EC system in atherosclerotic lesion progression. CB_2 activation has been associated with an anti-atherogenic effect on the basis of the following: (i) the phytocannabinoid Δ^9-tetrahydrocannabinol reduces atherosclerotic lesion progression and suppresses leucocyte adhesion to the vascular wall in high-fat fed $ApoE^{-/-}$ mice, an effect that is abolished by co-administration with a CB_2 selective antagonist (SR144528)[58]; (ii) the CB_2 agonist JWH-133 attenuates smooth muscle cell proliferation and migration[59] and (iii) the suppressant effect of the CB_1/CB_2 receptor agonist WIN 55,212-2 on TNFα and superoxide production in human peripheral blood mononuclear cells is sensitive to CB_2 receptor blockade[60]. However, more recent data have shown that neither CB_2 receptor activation with a selective agonist (JWH-133) nor CB_2 receptor gene deletion modulate atherogenesis in high-fat fed $LDLR^{-/-}$ mice[61]. In contrast to the picture with CB_2 receptors, evidence that CB_1 activation results in atherogenesis is derived from the findings that patients with advanced coronary artery disease exhibit elevated circulating levels of both AEA and 2-AG along with increased CB_1 receptor expression in coronary plaques[24]. Taken together with the findings that the CB_1 selective antagonist rimonabant (i) attenuates lesion development in a murine model of atherosclerosis via mechanisms involving suppression of proinflammatory gene expression and macrophage recruitment[62] and (ii) inhibits vascular smooth muscle cell proliferation and migration[63], this has led to the proposal that EC exert a pro-atherogenic effect, signalling through CB_1 receptors. However, it should be borne in mind that all of the afore-mentioned studies employed phyto- or synthetic CB ligands, which may behave differently from the endogenous EC, AEA and 2-AG. In fact, AEA attenuates TNFα-induced expression of inter-cellular adhesion molecule 1 and vascular cell adhesion molecule 1 in human coronary artery endothelial cells and attenuates TNFα-stimulated human acute monocytic leukaemia cells monocyte adhesion, both actions being sensitive to CB_1 and CB_2 receptor blockade[64], suggesting that AEA at least exhibits effects that are more in line with an anti-, rather than pro-, atherogenic effect. With regard to 2-AG, there is in fact a dearth of literature about its effects on either atherogenesis or the cellular events involved in the atherogenic process.

The impact of *n*-3 LC-PUFA intervention in atherosclerosis progression has long been a subject of contention, but there is now sufficient evidence from several large-scale randomised trials (DART Trial[65], GISSI-Prevenzione Study[66], JELIS Study[67]) to demonstrate the effectiveness of *n*-3 LC-PUFA supplementation in the primary and secondary prevention of CHD. The main effects of *n*-3 LC-PUFA appear to involve modulation of processes key to atherosclerosis progression and plaque stablisation[68,69] via both direct (i.e. at the level of the plaque) and indirect (i.e. through alterations in lipid metabolism) mechanisms. Since the aim of this article is to identify similarities between the actions of *n*-3 LC-PUFA and EC, and since EC influences in atherogenesis are related to direct effects on the developing plaque, this discussion will be confined to the direct effects of *n*-3 LC-PUFA on plaque stability, which are predominantly of an anti-inflammatory and antioxidant nature.

Dietary fish-oil intake has been documented to lower chemoattractant (platelet-derived growth factor and monocyte chemoattractant protein 1) production in mononuclear cell fractions[70] and decrease surface expression of the adhesion molecules inter-cellular adhesion molecule 1 and vascular cell adhesion molecule 1 in cultured human aortic endothelial cells[71], both of which actions would serve to reduce the inflammatory response and subsequent leucocyte infiltration that fuels

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plaque progression. *n*-3 LC-PUFA also directly alter plaque morphology by inducing structural changes consistent with increased stability, characterised by an increased fibrous cap thickness and reduced macrophage infiltration[72], reduced foam cell infiltration and mRNA expression of matrix metalloproteinase-7, -9 and -12[73], and increased collagen content of the plaques[74].

These positive effects of *n*-3 LC-PUFA on atherosclerotic plaque resemble very closely those seen with agents that modulate CB_2-mediated effects in the EC system. Although there is evidence of increased CB receptor expression in atherosclerotic plaques, there are no definitive data to show that EC levels (as opposed to the receptors that mediate their effects) are similarly elevated. However, *n*-3 LC-PUFA do reduce cardiac EC levels in high-fat fed mice[36] and unpublished results from our own laboratory have shown that both heart and vascular tissue levels of both AEA and 2-AG are markedly elevated in high-fat fed ApoE-/- and that these are normalised by dietary intervention with EPA. At face value it could therefore be argued that the anti-atherogenic effect of *n*-3 LC-PUFA could be explained by removal of EC, and thus their pro-atherogenic influence mediated by CB_1 receptors; however, this would not take account of the possible protective role of the EC exerted via CB_2.

Could the endocannabinoid system contribute to the beneficial effects of long-chain-PUFA in IHD?

I/R injury represents the principal cause of tissue damage following acute myocardial infarction (AMI), while the electrical disturbances that occur during an AMI pose a threat to life through sudden arrhythmic death.

Myocardial injury

Elevated levels of tissue and circulating EC have been reported following myocardial I/R injury in both experimental animals[9] and, more recently, in patients with recent AMI[75]. The cellular source and exact role of the EC remains a point of contention and has been the subject of a number of comprehensive reviews (e.g.[76,77]). AEA has been shown in some studies to reduce myocardial infarct size in isolated rat hearts[78], an effect that is sensitive to both CB_1 and CB_2 receptor antagonists, whereas in others it does not[79]. Moreover, suppression of AEA metabolism by either pharmacological inhibition or genetic deletion of fatty acid amine hydrolase exacerbates oxidative/nitrative stress-dependent doxorubicin-induced myocardial injury, an effect that is reversed by CB_1 antagonism, and increases AEA-induced cardiomyocyte cell death[80], further supporting a role for AEA-induced myocardial injury via CB_1.

In contrast, reports of a cardioprotective effect of 2-AG are more consistent[79,81], although the evidence is similarly conflicting around whether or not selective CB_1 and CB_2 receptor agonists do[79] or do not[78] mimic 2-AG-induced cardioprotection, suggesting that a site distinct from the classical CB receptors might be involved. 2-AG has also been implicated in myocardial preconditioning (a potent endogenous form of protection against I/R injury[78]) triggered by ischaemic[82], remote[83] and pharmacological[81] stimuli, effects largely attributed to activation of CB_2, rather than CB_1, receptors[83,84].

The potential of *n*-3 LC-PUFA to act as direct cardioprotective agents in the setting of AMI (as opposed to preventing cardiac events) has been evident for well over 20 years. Clinically, patients with a high level of fish-oil derived *n*-3 LC-PUFA consumption have been reported to have smaller infarcts following an AMI and to demonstrate an improved response to coronary thrombolysis[85]. Experimentally, prolonged (>2 weeks duration) dietary intervention with fish-oil derived *n*-3 LC-PUFA has been shown to reduce the extent of tissue injury (infarct size) in numerous studies via a broad range of proposed mechanisms including attenuation of platelet function[86], opening of cardiac K^+-activated Ca^{2+} channels[87], increased expression of protein kinase Cδ[88] and altered fatty acid composition of mitochondrial phospholipids, in particular the mitochondrial antioxidant phospholipid cardiolipin[89]. Interestingly, *n*-3 LC-PUFA have also been described to induce cardioprotection similar to ischaemic preconditioning[90], either when given as a 'pre-emptive' infusion into the coronary circulation immediately prior to the onset of I/R[91], or following prolonged dietary intervention[90], possibly through a reduction of reperfusion-induced oxidative stress and induction of heat shock protein 72[92]. Moreover, a positive effect on post-infarction ventricular remodelling has also been demonstrated[93].

The proposed cellular mechanisms for EC-induced cardioprotection are plentiful, and some similarities can be drawn to the cardioprotective effects of *n*-3 LC-PUFA. In particular, as with the *n*-3 LC-PUFA, inhibition of inflammation and oxidative/nitrosative stress, are believed to be major targets for EC,[75,76]. However, activation of the myocardial protein kinase C and p38 mitogen-activated protein kinase pro-survival pathways[90], induction of heat shock protein 72[94] and generation of NO have all also been implicated in EC-induced cardioprotection. Although there are no reports of *n*-3 LC-PUFA affecting kinase pathways in the heart, they are known to influence mitogen-activated protein kinase activity in the brain[95], endothelial cells[96] and human T-cells[97] and therefore whether or not EC play a role in these effects remains to be explored. In terms of NO production, *n*-3 LC-PUFA suppress myocardial NO synthase activity in hypertensive hearts[98] ; whether or not *n*-3 LC-PUFA influence NO production in the setting of myocardial I/R remains to be explored.

Arrhythmias

Endogenously released EC are implicated as being anti-arrhythmic[83,99,100] against both ischaemia and reperfusion-induced arrhythmias through an action at CB_2 receptors. Likewise, a clear beneficial effect of the *n*-3 LC-PUFA is seen against I/R-induced arrhythmias; a meta-analysis of twenty-seven experimental studies

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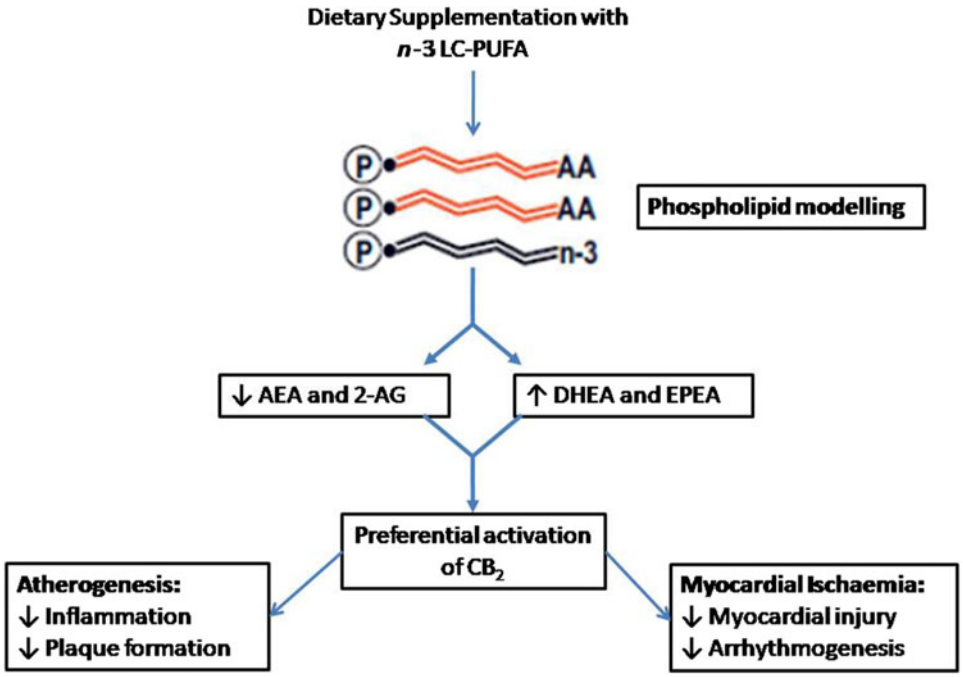

Fig. 3. (colour online) Proposed mechanism of *n*-3 LC-PUFA–endocannabinoid interaction in atherogenesis and myocardial ischaemia. AEA, anandamide; 2-AG, 2-arachidonyl glycerol; DHEA, docosahexaenoyl ethanolamide; EPEA, eicosapentaenoyl ethanolamine; CB_2, cannabinoid receptor type 2.

into the antiarrhythmic effects of *n*-3 LC-PUFA revealed that they afford significant protection particularly against ventricular tachycardia and ventricular fibrillation[101]. The mechanisms responsible for these anti-arrhythmic effects have been thoroughly reviewed[102] and are believed to be largely due to direct effects of *n*-3 LC-PUFA on cardiomyocyte transmembrane currents, resulting in electrophysiological changes such as (i) slowing of the Na^+ current (reducing excitability and slowing ventricular conduction), (ii) reduced opening of L-type Ca^{2+} channels (thus reducing early after-depolarisations), (iii) an effect on the Na^+/Ca^{2+} exchanger (to reduced delayed after-depolarisations) and (iv) reduced spontaneous release of Ca^{2+} from the sarcoplasmic reticulum (reduced triggered activity).

In terms of the cellular mechanisms underlying the antiarrhythmic effects of EC, there is a paucity of data on this, although studies on the direct electrophysiological effects, at least of AEA, give some insight as to these. Like the *n*-3 LC-PUFA, AEA suppresses action potential duration and blockade of L-type Ca^{2+} channels in cardiac myocytes[103], but although there are no data concerning the effect of AEA on Ca^{2+} release from the sarcoplasmic reticulum (a known action of *n*-3 LC-PUFA), the intriguing observation that AEA inhibits IP_3-induced Ca^{2+} release from the cardiomyocyte nucleus[104] suggests that this may be worth pursuing as a common mechanism. However, AEA also exerts electrophysiological effects that are not shared with the *n*-3 LC-PUFA, such as suppression of the cardiac transient outward potassium current I(to) through a non-CB_1/CB_2receptor-mediated pathway and augmentation of the ATP-sensitive potassium current I(KATP) through a CB_2-dependent mechanism[105], both of which would contribute to an antiarrhythmic effect. Moreover, AEA suppresses noradrenaline release from sympathetic nerves innervating the heart[106], which would potentially reduce catecholamine-related arrhythmias during early ischaemia; this is in contrast with *n*-3 LC-PUFA, which do not influence cardiac sympathetic tone[107].

Can the beneficial effects of *n*-3 long-chain-PUFA in CVD be linked to those of the endocannabinoid system?

From the afore-mentioned discussion, while it is evident that a link between *n*-3 LC-PUFA and EC is unlikely to exist in the setting of hypertension, there are sufficient similarities regarding the effects of both in atherosclerosis and IHD to consider that a connection exists. Considering the cardinal role that inflammatory cells play in many CVD states, and the similar influence of both *n*-3 LC-PUFA and EC on inflammatory processes in atherogenesis, this raises the intriguing possibility that EC may be the 'missing link' in understanding the mechanisms underlying the vasculo-protective effects of *n*-3 LC-PUFA. Similarly, common mechanisms exist for both the tissue-sparing and antiarrhythmic effects of both *n*-3 LC-PUFA and EC, again raising the notion that modulation of the EC system by *n*-3 LC-PUFA may play a part in the underlying mechanisms. However, counterintuitive to this hypothesis is that while EC levels are consistently seen to be up-regulated in these pathological conditions, *n*-3 LC-PUFA tend to suppress

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EC levels. While this may be interpreted as an overall reduction of activity of the EC system by *n*-3 LC-PUFA, it is probably more probably that these changes are a consequence of a shift in the *n*-3/*n*-6 balance of membrane lipids, resulting in compensatory increases in the *n*-3 LC-PUFA-derived acyl conjugates docosahexaenoyl ethanolamide and eicosapentaenoyl ethanolamine[33]. Indeed, it is plausible that these *n*-3 LC-PUFA–EC conjugates may in part be responsible for some of the beneficial effects of *n*-3 LC-PUFA, since docosahexaenoyl ethanolamide and eicosapentaenoyl ethanolamine both bind to CB_1 and CB_2 receptors in human and mouse leucocytes[108,109] and docosahexaenoyl ethanolamide has been shown to exert anti-inflammatory effects in mouse peritoneal and RAW264·7 macrophages[110]. Moreover, in contrast to AEA, docosahexaenoyl ethanolamide appears to have a greater affinity for CB_2 than for CB_1, in human inflammatory cells[108] ; what this likely means at a cellular level is that, in the presence of high *n*-3 LC-PUFA concentrations, EC are converted to *n*-3 LC-PUFA–EC conjugates, which then act as 'surrogate' CB_2 agonists and thus alter the balance between activation of CB_1 *v.* CB_2 receptors in favour of CB_2 (Fig. 3). However, it must not be overlooked that the EC are known to act at sites other than the classical CB_1/CB_2 receptors and therefore the *n*-3 LC-PUFA–EC conjugates may similarly exert actions at sites distinct from CB receptors, although as far as we are aware this has not yet been tested.

Conclusions

There is no doubt that *n*-3 LC-PUFA and the EC AEA and 2-AG each demonstrate protective effects in the setting of CVD. Mechanistically speaking, at least in the setting of atherosclerosis and AMI, there are sufficient similarities to suggest that some (but by no means all) relationships exist between the two in these effects. The fact that *n*-3 LC-PUFA influence endogenous EC levels, possibly resulting in the generation of conjugates that act preferentially on the CB receptors linked to the beneficial effects of the EC, is an attractive explanation for this, but further studies are warranted before this hypothesis can be proven or refuted.

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Conflicts of interest

None.

Authorship

Both authors made an equal contribution to gathering the information from the literature for this review. C. W. drafted the final version of the review.

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