

Endocannabinoids and the Heart

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Abstract: Endocannabinoids, such as anandamide and 2-arachidonoylglycerol, are synthesized from membrane phospholipids in the heart and other cardiovascular tissues. They activate cannabinoid CB₁ and CB₂ receptors, transient receptor potential V1 (TRPV1), peroxisome proliferator-activated receptors, and perhaps a novel vascular G-protein-coupled receptor. Inactivation is by cellular uptake and fatty acid amide hydrolase. Endocannabinoids relax coronary and other arteries and decrease cardiac work but seem not to be involved in tonic regulation of cardiovascular function. They act as a stress response system, which is activated, for example, in myocardial infarction and circulatory shock. Endocannabinoids are largely protective; they decrease tissue damage and arrhythmia in myocardial infarction and may reduce progression of atherosclerosis (CB₂ receptor stimulation inhibits lesion progression), and fatty acid amide hydrolase knockout mice (which have enhanced endocannabinoid levels) show decreased cardiac dysfunction with age compared with wild types. However, endocannabinoids may mediate doxorubicin-induced cardiac dysfunction. Their signaling pathways are not fully elucidated but they can lead to changed expression of a variety of genes, including those involved in inflammatory responses. There is potential for therapeutic targeting of endocannabinoids and their receptors, but their apparent involvement in both protective and deleterious actions on the heart means that careful risk assessment is needed before any treatment can be introduced.

Key Words: endocannabinoid system, anandamide, cardioprotection, cannabinoid receptors, rimonabant, atherosclerosis

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INTRODUCTION

Endocannabinoids are substances occurring naturally within the body that mimic the activity of Δ^9 -tetrahydrocannabinol, which was shown in the 1960s to be the primary psychoactive ingredient of cannabis.¹ Most early interest in cannabinoids centered on their roles in the nervous system, and the two most actively pursued clinical applications, multiple sclerosis² and

obesity,³ quite possibly arise from actions on nerves. Studies of Δ^9 -tetrahydrocannabinol in the cardiovascular system as in the brain, were at first held back by the absence of tools to identify the receptors, and signaling mechanisms, mediating the observed responses.⁴ Increased availability of receptor agonists, antagonists, and inhibitors of endocannabinoid inactivation has enabled wider study of the cannabinoid system.⁵ In particular, the introduction of rimonabant, a cannabinoid receptor antagonist, as an antiobesity agent has focused attention on the potential for reducing cardiovascular risk.³ At the time of its discovery, first reported in 1994, rimonabant (then called SR141716A) was seen as an antagonist at central cannabinoid receptors,⁶ but it is now clear that its target receptor, the CB₁ receptor, occurs outside the brain. Indeed, although its actions as an antiobesity agent can be ascribed to the principle of antagonism of the central orexigenic actions of endocannabinoids, it is now clear that its beneficial effects may also involve, to a greater or lesser extent, modification of endocrine activity through actions on peripheral CB₁ receptors in the liver and adipose tissue.⁷ The years between the discovery of rimonabant and its introduction into the clinic have been exciting ones in the story of the cannabinoids, and one outcome has been a great expansion of our knowledge of their actions on the heart and circulation.

Endocannabinoids and Their Receptors

Less than 20 years ago, Matsuda et al⁸ cloned a receptor for Δ^9 -tetrahydrocannabinol from a rat cerebral cortex complementary DNA library. This started the search for the neurotransmitter system with which the cannabinoids interact and, very soon, arachidonylethanolamide was identified in pig brain as a high-affinity ligand for this receptor.⁹ This agonist (Fig. 1) is more commonly called anandamide (from the Sanskrit “a-nanda” for happiness, joy, or enjoyment), and this first receptor is referred to as the CB₁ receptor because a second receptor, designated CB₂ and largely found in peripheral tissues, was later cloned from the HL60 human leukemia cell line.¹⁰ Both receptors couple through G_{i/o} proteins (for discussion of receptor signaling pathways, see reviews by Howlett et al¹¹ and Hiley and Ford⁵). CB₁ receptors have been suggested to evoke the effects of cannabinoids in the myocardium of human,¹² rat,¹³ and mouse,¹⁴ and stimulation evokes a negative inotropic effect. However, some evidence from isolated rat hearts is inconsistent with this simple picture of cardiac effects being solely due to CB₁ receptors (see under “Cardioprotection” below).¹⁵

Anandamide was reported to have a lower affinity for the CB₂ than the CB₁ receptor.¹⁰ A second naturally occurring arachidonic acid derivative, 2-arachidonoylglycerol (2-AG), binds to cells transfected with either CB₁ or CB₂ receptor

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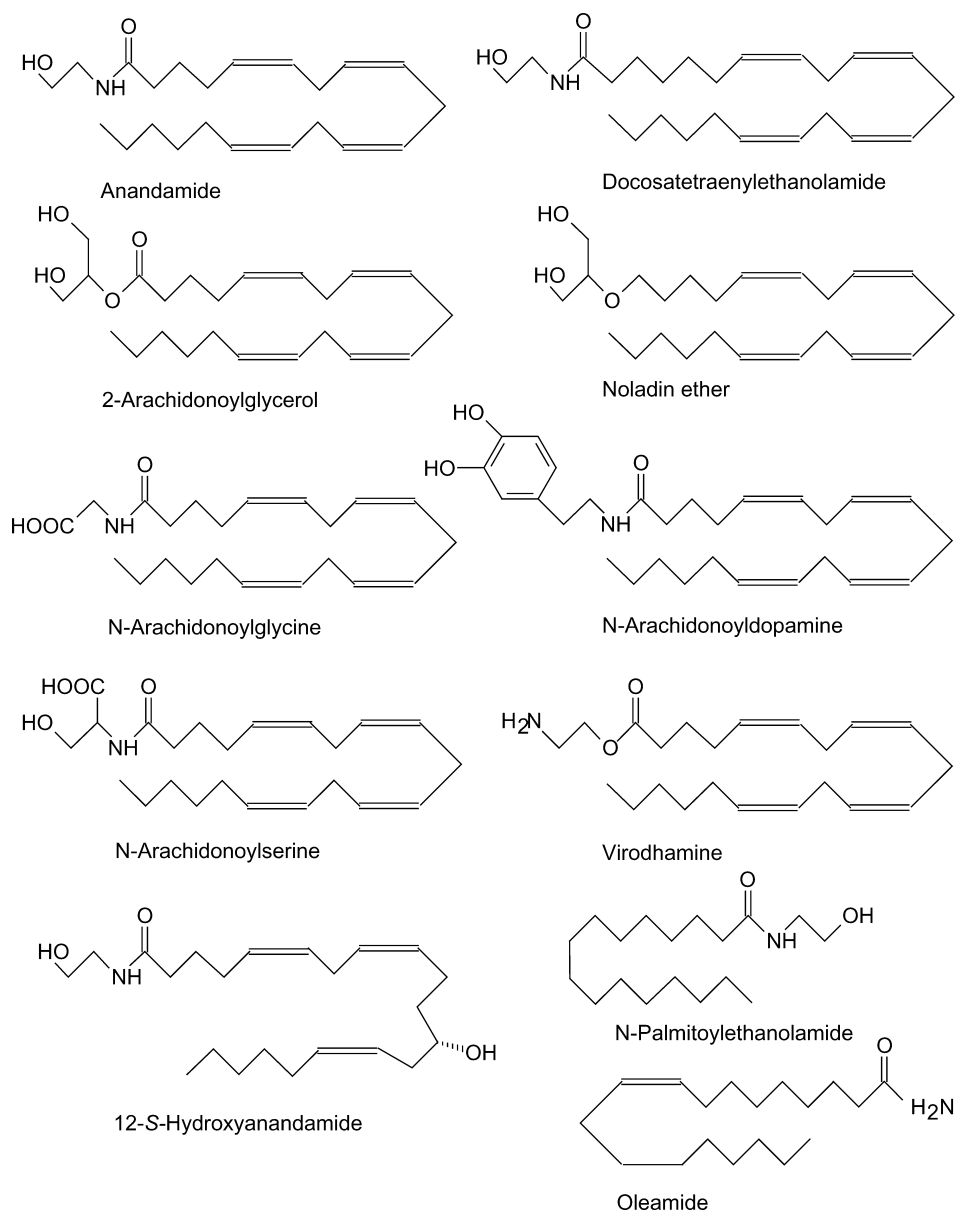


FIGURE 1. Structures of the major endocannabinoids identified to date; most are based on the 20-carbon fatty acid, arachidonic acid, although palmitoylethanolamide (the activity of which as an endocannabinoid is controversial) and the CB₁ cannabinoid and TRPV1 agonist, oleamide, are exceptions with shorter chain lengths. The most frequently studied is anandamide (an agonist at CB₁ and CB₂ receptors and TRPV1 channels), which with 2-AG (an agonist at both CB₁ and CB₂ receptors with generally higher efficacy than anandamide¹¹) is found to occur in many tissues of the cardiovascular system, including the heart and blood cells (especially platelets and monocytes). 12-*S*-hydroxyanandamide, which activates CB₁ receptors, is synthesized by 12-lipoxygenase from anandamide taken up by platelets.¹⁶ Because 12-*S*-hydroxyanandamide is not a substrate for FAAH, conversion of anandamide to this oxygenated derivative might have the effect of prolonging signaling due to anandamide synthesis.

complementary DNA but generally shows somewhat a higher activity at CB₂ receptors.¹⁷ Anandamide and 2-AG were the first identified members of an expanding family of putative endocannabinoids. These now include docosatetraenylethanolamide (CB₁ receptor agonist),¹⁸ *N*-arachidonoyl dopamine (CB₁ receptor-selective ligand),¹⁹ and virodhamine (*O*-arachidonoyl ethanolamine; CB₁ receptor partial agonist/CB₂ receptor agonist; see Fig. 1 for structures).²⁰ Noladin ether (2-arachidonoylglycerylether), which is structurally related to 2-AG, is a potent ligand at the CB₁ receptor but only weakly associates with CB₂ receptors,²¹ and the anandamide derivative produced by 12-lipoxygenase, 12-*S*-hydroxyanandamide, is also a CB₁ receptor agonist.¹⁶ *N*-palmitoylethanolamide has been suggested to be a selective CB₂ receptor agonist,²² but this is a controversial assertion because it has not been confirmed by other studies. For example, Griffin et al²³

reported that it was only weakly active at rat cloned CB₂ receptors expressed in HEK293 cells, whereas its stimulation of GTPγS binding to rat cerebellar membranes was sensitive to rimonabant and so is not likely to be due to activation of CB₂ receptors. Certainly, *N*-palmitoylethanolamide can act at peroxisome proliferator-activated receptors (PPARs), namely, PPAR-α,²⁴ and at another G-protein-coupled receptor, GPR55.²⁵ Both of these types of receptors are discussed later in respect of the actions of anandamide.

It has been suggested that there is at least one other G-protein-coupled cannabinoid receptor in the cardiovascular system on the basis of the actions of abnormal cannabidiol. This compound is inactive at CB₁ and CB₂ receptors but lowers blood pressure, even in CB₁ and CB₁/CB₂ receptor-knockout mice.²⁶ Furthermore, responses to anandamide in rat isolated mesenteric arteries have a pharmacology that is not

consistent with actions at the cloned cannabinoid receptors.²⁷ For example, even though the anandamide-induced relaxation of rat coronary arteries is sensitive to rimonabant (at the high concentration of 1 μ M), it is not antagonized by another CB₁ receptor antagonist, AM251.²⁸ Therefore, a new vascular “anandamide receptor” has been proposed,²⁹ which could also mediate the actions of abnormal cannabidiol.²⁶ Studies on this novel site have been facilitated by O-1918, a derivative of abnormal cannabidiol; it antagonizes the actions of anandamide and abnormal cannabidiol but has no activity at the “classical” cannabinoid receptors.³⁰ *N*-arachidonoylserine, a weak ligand at both CB₁ and CB₂ receptors, has been suggested to be an endogenous ligand for this novel receptor. This, too, dilates the rat mesenteric artery and, like anandamide, stimulates phosphorylation of p44/42 mitogen-activated protein kinase and protein kinase B/Akt in cultured endothelial cells.³¹

The presumption of G-protein coupling for the new receptor, like that of the CB₁ and CB₂ receptors, arises from the actions it is thought to mediate being pertussis toxin sensitive.^{27,30} Its identity is unclear, although the orphan receptor GPR55 is a possible candidate.³² Application of the synthetic cannabinoid, CP55,940, or the endocannabinoids, anandamide and virodhamine, to cells expressing GPR55 increases GTP γ S incorporation.²⁵ There is still considerable doubt that GPR55 is the vascular receptor for anandamide: (a) it does not couple through G_{i/o}, as would be expected for a pertussis toxin-sensitive system, but through G_{α13}.^{25,33}; (b) the vasorelaxant response to abnormal cannabidiol in the mesenteric artery is still present in GPR55-null mice³⁴; and (c) other work suggests not only that the endogenous ligand is lysophosphatidylinositol but also that anandamide is inactive when using phosphorylation of extracellular signal-regulated kinase (ERK) as the assay.³⁵

Anandamide can also activate transient receptor potential V1 (TRPV1) vanilloid receptors on sensory nerve endings,³⁶ a property it shares with Δ^9 -tetrahydrocannabinol and cannabidiol.³⁷ Activation of TRPV1 channels releases calcitonin gene-related peptide and evokes vasodilatation, although the effect is clearly regional, because in contrast to the actions of anandamide on rat mesenteric resistance artery, rat hepatic artery, and guinea pig basilar artery,³⁶ relaxation of rat coronary artery does not involve TRPV1.²⁸ There are no reports of TRPV1 on myocardial cells, but there is evidence that, on cardiac afferent nerves, it plays a role in signaling in ischemia (although the role of anandamide in this has not been investigated).³⁸ Very high plasma levels of anandamide in mice are associated with TRPV1 and the Bezold–Jarisch reflex.³⁹

The final targets for anandamide are the PPAR, although this has not yet been shown in the heart. Anandamide, noladin ether, virodhamine, and the endocannabinoid-like oleoylethanolamine bind to PPAR- α ⁴⁰ (a mechanism that might be important in cytoprotection in cerebral infarction), and anandamide induces 3T3-L1 fibroblasts to differentiate into adipocytes by a process sensitive to the PPAR- γ antagonist, GW9662.⁴¹ Δ^9 -tetrahydrocannabinol has also been shown to initiate PPAR- γ activity.⁴² Actions on any of these different types of receptors are often linked to signaling pathways that enable endocannabinoids to regulate gene expression. This

gives them considerable potential to act as regulators of long-term processes such as inflammation and apoptosis.

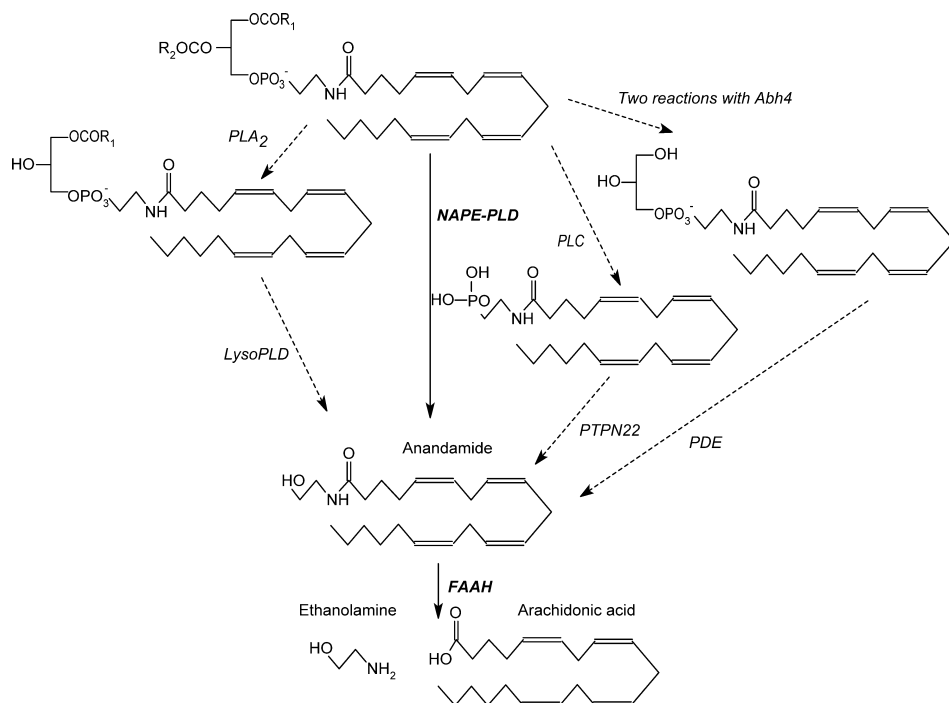
Synthetic and Degradative Pathways

The synthetic pathways for anandamide and 2-AG are mainly thought to involve breakdown of preformed arachidonoylphospholipids in which glycerol is esterified with the fatty acid at the *sn*-1 position rather than the much more usual *sn*-2 position.⁴³ 2-AG is formed by the action of an *sn*-1-specific diacylglycerol lipase.⁴⁴ The primary route for anandamide synthesis is the formation of the *N*-acylethanolamine phospholipid (NAPE) precursor by a Ca²⁺-activated *N*-acyltransferase. The NAPE is then broken down by an *N*-acylethanolamine-specific phospholipase D (NAPE-PLD).⁴⁵ NAPE-PLD has been isolated from rat heart,⁴⁶ and both synthetic processes are found in myocardial microsomal fractions of many species. However, their activity ratio varies widely between species (from 0.002 to 15) with high NAPE-PLD activity in rats and guinea pigs, contrasting with very low *N*-acyltransferase activity.⁴⁷ Production of anandamide exhibits redundancy,^{48,49} but the other anandamide-generating pathways (shown in Fig. 2) do not necessarily occur in the heart. In one of these synthetic routes (found in mouse brain and RAW264.7 macrophages), phospholipase C hydrolyzes NAPE to phosphoanandamide which is then acted upon by phosphatases, including the protein tyrosine phosphatase, PTPN22, to give the endocannabinoid.⁵⁰ Alternatively, in mouse brain and some peripheral tissues, NAPE is first subjected to sequential deacylations (possibly by the enzyme α/β -hydrolase 4) to give a glycerophospho-*N*-acylethanolamine which is then acted upon by a metal-dependent phosphodiesterase to liberate anandamide.⁵¹ High levels of activity of NAPE lipase activity are found in brain and testis, but somewhat less of this enzyme occurs in the heart (approximately 12% of that in the brain).

Inactivation of anandamide is probably by cellular uptake by an anandamide membrane transporter⁵² (although this is not universally accepted)⁵³ followed by hydrolysis into ethanolamide and arachidonic acid by fatty acid amide hydrolase (FAAH).⁵⁴ 2-AG is broken down by FAAH and a specific monoacylglycerol lipase.⁵⁵ Messenger RNA for the lipase is expressed in the heart,⁵⁶ but there has been no direct visualization of FAAH or its expression in myocardial cells. There is indirect evidence for FAAH in the heart; levels of anandamide, but not 2-AG, are elevated in the hearts of FAAH^{-/-} mice.¹⁴ Interestingly, these mice also show decreased deterioration of cardiac function with age relative to their FAAH^{+/+} littermates (see later under “Cardiac Dysfunction”),⁵⁷ which might be due to enhancement of the effects of endogenous anandamide. The finding of measurable levels of anandamide in mouse heart contrasts with an earlier report that anandamide was undetectable in lipid extracts of normal rat heart, although 2-AG was present.⁵⁸ Nevertheless, it seems that both synthetic and metabolic capacities for endocannabinoids are present in the heart, but the capacity for producing the NAPE precursor is very low in several species, including humans,⁴⁷ and with the exception of mice, the evidence for anandamide synthesis and activity is generally circumstantial.

Other cells of the cardiovascular system can synthesize endocannabinoids. Anandamide and its associated enzymes

FIGURE 2. Synthetic pathways for anandamide. The primary pathway involves the hydrolysis of an arachidonate-containing phospholipid, in which the arachidonic acid has already been converted to the ethanolamine derivative, by NAPE-PLD. Anandamide is then inactivated by hydrolysis, mediated by FAAH. Alternative pathways proposed include sequential action of a phospholipase A2 (PLA2) and lysophospholipase D; breakdown of the glycerophospholipid by phospholipase C (PLC) and then dephosphorylation by a protein tyrosine phosphatase (PTPN22); and deesterification of the precursor by the action of α/β -hydrolase 4 (Abh4) with further processing by a phosphodiesterase (PDE).⁴⁹ Anandamide might reach the FAAH site by means of a high-affinity transporter.



are found in endothelial cells from rat kidney⁵⁹ but it is not synthesized by bovine coronary artery endothelial cells.⁶⁰ 2-AG is produced by human umbilical vein endothelial cells stimulated by A23187, a calcium ionophore, or thrombin.⁶¹ Enhanced intracellular Ca^{2+} also causes release of anandamide and 2-AG from macrophage-derived cell lines.⁶² In platelets and neutrophils, anandamide is taken up and converted by lipoxygenase to 12-*S*-hydroxyanandamide.¹⁶ Thus, cardiac function and coronary perfusion might be modulated by endocannabinoids derived not only from cardiac tissues but also from the circulating cells.

Inhibition of the membrane transporter and FAAH provides the means for enhancing the activity of endocannabinoids produced by the tissues of the body. The transport system can be inhibited by several fatty acid analogues, including arvanil, olvanil, *N*-(4-hydroxyphenyl)arachidonoylamide (AM404), and *N*-(3-furanylmethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (UCM707).^{41,63} Many of these agents also inhibit FAAH,⁵³ but there are high-affinity and relatively selective inhibitors available for the enzyme and an endogenous inhibitor, 2-octyl- γ -bromoacetoacetate, originally isolated from human cerebrospinal fluid.⁶⁴ A detailed structure/activity study of fatty acid α -keto heterocycles produced some very potent compounds, including OL-92, which has a subnanomolar inhibition constant.⁶⁵ URB532 and URB597, which have IC₅₀ values around 1 μ M, are carbamates and lack the characteristic long-chain fatty acid moiety of both the endocannabinoids and the other FAAH inhibitors.⁶⁶ In addition to hydrolyzing anandamide, FAAH acts upon other fatty acid amides including the primary amide, oleamide, which is active at CB₁ receptors and TRPV1 and might have a role in cardiovascular signaling.⁶⁷

Endocannabinoids in Cardiovascular Syndromes

Administration of anandamide, Δ^9 -tetrahydrocannabinol, or synthetic cannabinoids causes hemodynamic changes that are complex, involving phases of both increased and decreased blood pressure and changes in heart rate. These effects are mainly mediated by CB₁ receptors and include actions on the nervous system, both central and peripheral.⁶⁸ Interestingly, in view of its actions on TRPV1 receptors, anandamide does not affect blood pressure in CB₁ receptor-knockout mice, even though it evoked vanilloid receptor-dependent relaxation in isolated mesenteric beds from these animals.²⁶ This suggests that regulatory effects of endocannabinoids in at least some beds may be purely local. Smoking cannabis in humans is associated with a small increase in the risk of heart attack, lasting 1–2 hours, which is probably due to increased sympathetic outflow.⁶⁹ On the other hand, there is little evidence that the endocannabinoid system is normally activated in the healthy cardiovascular system. Administration of rimonabant, a CB₁ receptor antagonist, does not affect heart rate or blood pressure in anesthetized rats,⁷⁰ and the Rimonabant in Obesity clinical trials show only a tiny (<1 mm Hg), albeit significant, decrease in blood pressure in obese but normotensive patients over 1 year of treatment.⁷¹ This contrasts with the greater fall in blood pressure in obese patients with hypertension and in those with type 2 diabetes.

In spontaneously hypertensive rats (SHRs), CB₁ receptor blockade increases blood pressure and left ventricular activity, suggesting that the endocannabinoid system modulates the effects of the condition.¹³ CB₁ receptor expression was increased in the heart in SHRs, relative to Wistar-Kyoto normotensive animals, which translated into increased hypotensive effects in response to exogenous cannabinoids

in the SHR. Further evidence for activation of the endocannabinoid system in hypertension came from the observation that inhibition of FAAH, with URB597, reduced blood pressure and cardiac function to the levels seen in the normotensive animals. These results present the intriguing possibility that the endocannabinoid system is one that is activated in the cardiovascular system only in disease states.

Shock

Just over 10 years ago, Kunos's laboratory reported that rimonabant increased blood pressure in rats subjected to hemorrhagic shock even though, as noted above, the cannabinoid receptor antagonist had no effect in controls.⁷⁰ It was concluded not only that the endocannabinoid system was activated by hypovolemia but also that endocannabinoids, generated by monocytes and platelets, were protective because rimonabant shortened survival time despite the pressor effect. Endocannabinoids are also generated by circulating cells in both the endotoxic shock and the cardiogenic shock occurring after experimentally induced myocardial infarction.⁷² The CB₁ receptor does not seem to be involved in the deep depression of blood pressure seen after exposure to bacterial lipopolysaccharide because, although it can be attenuated by rimonabant, it cannot be reversed by AM251, another selective CB₁ receptor antagonist.⁷³ This separation of effects of the two antagonists is characteristic of the vasodilator responses to anandamide in mesenteric blood vessels from rats,⁷⁴ although both CB₁ blockers antagonize anandamide in rat coronary artery.¹⁵ As discussed above, when considering the receptors for endocannabinoids, the identity of the receptor mediating the non-CB₁, but rimonabant-sensitive vascular responses, has yet to be elucidated, but it is clear that endocannabinoid production can be induced when the cardiovascular system is functioning under deleterious conditions.

Cardioprotection: Infarction and Arrhythmia

Endotoxin administration not only produces cardiovascular shock but can also protect the heart against ischemia/reperfusion injury.⁷⁵ At least some of the protective effect is due to endocannabinoids because, although rimonabant had no effect, the highly selective CB₂ receptor antagonist, SR144528, did block the response. Increased expression of the inducible and Ca²⁺-independent isoform of nitric oxide synthase (NOS type II) is the response most usually associated with endotoxic shock,^{76,77} and therefore, the question arises of whether or not there is an association between the endocannabinoid system and nitric oxide (NO) in this phenomenon. Lagneux and Lamontagne⁷⁵ reported that the cardioprotection in their study was both sensitive to the NOS inhibitor, N^o-nitro-L-arginine, and could be mimicked by administering sodium nitroprusside. What is particularly interesting is that SR144528 reduced the protective effect of the NO donor, suggesting that NO worked through the endocannabinoids rather than the cannabinoids through NO. This contrasts with the results of experiments using cultured cardiomyocytes from neonatal rats in which Δ⁹-tetrahydrocannabinol, acting through CB₂ receptors (because SR144528 blocked the response but not rimonabant), induced expression of NOS type II and elevated NO production.⁷⁸

These results rule out a role for the postulated vascular cannabinoid receptor because rimonabant was used at concentrations (1 and 10 μM) sufficient to block actions at this site.⁷⁹

CB₂ receptors are also involved in heat stress preconditioning, which can be attenuated by both blockade of these receptors and NOS inhibition.⁸⁰ Activation of the CB₂ receptor also reduces leukocyte activation in experimentally induced myocardial infarction in mice.⁸¹ In this case, the protection was induced by a synthetic cannabinoid, WIN55212-2, rather than an endocannabinoid, and it was blocked by the CB₂ antagonist, AM630, whereas CB₁ blockade, with AM251, was ineffective. WIN55212-2 treatment reduced levels of myeloperoxidase, interleukin-1β, and the CXC chemokine ligand 8 in the damaged tissue.

More recently, Wagner et al⁸² showed that endocannabinoids were involved in preconditioning by NO. They reported that delayed preconditioning, which occurs 24 hours after pretreatment with NO derived from transdermal nitroglycerin, is mediated by CB₁ receptors because it was sensitive to AM251 but not AM630. It will be noted that the effect of AM251, and therefore the likely involvement of the CB₁ receptor, is a major contrast with the results with endotoxic shock and heat stress where this antagonist was ineffective. Wagner et al also showed that tissue levels of 2-AG in the heart, but not of anandamide, were elevated 2.6-fold by the NO preconditioning protocol and that pretreatment with either 2-AG or the metabolically stable noladin ether reduced infarct size when either was given 30 minutes before no-flow ischemia.

The mechanisms involved are not yet worked out. Although Kola et al⁸³ used neither endocannabinoids nor cannabinoid receptor antagonists in their study, they proposed that cannabinoids (in their case Δ⁹-tetrahydrocannabinol) acting on cannabinoid receptors might act by stimulating adenosine monophosphate-activated protein kinase (AMPK) activity, which causes a shift in metabolism to anaerobic glycolysis and fatty acid oxidation in reperfusion. In this context, it has to be noted that Δ⁹-tetrahydrocannabinol can act at noncannabinoid receptors because O'Sullivan et al⁴² have shown that it acts as an antagonist at PPAR-γ receptors in blood vessels, and activation of these nuclear receptors can lead to AMPK activation.⁸⁴ As noted previously, anandamide can activate PPAR-γ, although this has not been shown in the heart.⁴¹

Because Wagner et al⁸² found that anandamide levels were not elevated, a role for endogenous anandamide has yet to be proven. However, inclusion of it or its metabolically stable analogue, methanandamide, in the perfusion fluid reduced the area of infarction (but did not affect recovery of cardiac function) when administered from 5 minutes before induction of ischemia until the end of a 60-minute reperfusion period.⁸⁵ The effect was blocked by rimonabant and SR144528, suggesting that CB₁, CB₂, and/or the novel rimonabant-sensitive non-CB₁ receptors mediate the response. Both CB₁^{12,13,39} and CB₂ receptors⁸⁶ have been found in the myocardium, but neither arachidonoylcyclopropylamide (a CB₁-selective agonist) nor JWH133 (a CB₂-selective agonist), alone or together, reduced the extent of infarction.

This unusual pharmacology was very close to that we had previously found for the coronary vasodilator and negative

inotropic effects of anandamide, methanandamide, and other cannabinoids.¹⁵ Therefore, although the actions of endogenous endocannabinoids can often be inferred to be at the known cannabinoid receptors by blockade with selective antagonists, it seems that the effects of exogenous anandamide in the heart and vasculature can rarely be simply attributed to actions at one or both of these classical cannabinoid receptors. Furthermore, the endocannabinoids can evoke protective effects by several signaling pathways, and Howlett et al⁸⁷ have recently discussed these in relation to the regulation of the production of reactive oxygen and nitrogen species during damage to the central nervous system.

Further evidence for a cardioprotective role for CB₂ receptors, in particular, comes from work on “remote preconditioning.” This is the phenomenon whereby ischemia in one vascular bed confers protection in another. In one model, a period of 15-minute occlusion of the mesenteric artery, followed by a 15-minute reperfusion, is imposed on anesthetized rats before 30-minute occlusion of the left coronary artery and 2-hour reperfusion. This preconditioning reduced arrhythmia and infarct size relative to hearts from sham-operated rats, and the protective effect was blocked by CB₂ receptor blockade (with AM630) but not by CB₁ receptor antagonism (with AM251).⁸⁸ In experiments with isolated hearts, it will naturally be assumed that the endocannabinoids conferring the protection originate in the cells of the heart and its tissues but, in the case of remote preconditioning, the active agents could arise from circulating cells (eg, platelets and monocytes) activated to collect or produce endocannabinoids when passing through the postischemic bed. The source of cardioprotective endocannabinoids is therefore an open question. It should be noted that, in this study, CB₂ receptor blockade reduced the arrhythmias induced by the cardiac ischemia/reperfusion protocol. This accords with the data of Krylatov et al⁸⁹ that a nonselective cannabinoid receptor agonist, HU-210, reduces ischemia-induced arrhythmia by activation of the CB₂ receptor. Also, some of the beneficial effects might be on nonmyocardial cells. CB₂ receptor activation (with synthetic cannabinoid agonists) of endothelial cells from human coronary artery decreases the proinflammatory activation of these cells by tumor necrosis factor- α (TNF- α), and this is manifested by reduced production of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein.⁹⁰

It is not only in the heart that endocannabinoids might have a role in regulation of postischemic injury. Readers are referred to the recent review by Pacher and Haskó⁹¹ for a wider discussion of their possible roles in modulating damage, including after stroke and hepatic ischemia.

Cardiac Dysfunction

From the above, it seems that endocannabinoids act as “good guys” throughout cardiovascular pathology. However, activation of CB₁ receptors can be proarrhythmic as shown by Motobe et al⁹² in their study of the increased risk of arrhythmia and sudden cardiac death associated with the use of bone cement in hip replacement surgery. A small clinical trial showed that plasma levels of anandamide and 2-AG were elevated in patients on whom the cement was used compared

with those where the procedure took place without its use; this was interpreted as showing that the increased risk arose as a result of the presence of the endocannabinoids. It should be noted that the study did not exclude the possibility that the endocannabinoids are a protective response to a threat induced by the presence of the cement.

However, the authors’ assumption that the endocannabinoids were detrimental to cardiac function has received support from results with experimental models in which cannabinoid antagonists can be used. For example, the antitumor agent, doxorubicin, impairs heart performance in mice, and the dysfunction can be alleviated by CB₁ receptor blockade with rimonabant or AM251. Doxorubicin treatment is associated with elevated myocardial anandamide levels but not with changes in CB₁ or CB₂ receptor expression.⁸⁶ When incubated with a myocardial cell line (H9c2), doxorubicin induced apoptosis, which also was reduced by CB₁ receptor blockade; but the effect was not sensitive to a CB₂ blocker or CB₁ and CB₂ receptor agonists. Similarly, work on cardiac dysfunction suggests that endocannabinoids mediate the cardiac dysfunction associated with cirrhosis. Administration of AM251, to block CB₁ receptors, improved cardiac function in carbon tetrachloride-induced cirrhosis in rats, and it was also shown that anandamide levels were increased in the hearts of rats with cirrhosis compared with the controls.⁹³

In contrast, aging-associated cardiac dysfunction is reduced in FAAH-null mice, which could be interpreted as showing a need for increased endocannabinoid activity in the heart.⁵⁷ The homozygous knockout mice exhibited decreased levels of markers for oxidative stress and inflammation because the increased expression of gp91phox, TNF- α , and NOS type II associated with age in wild-type mice was largely absent in the knockouts. The apparent benefit of increased endocannabinoid activity was ascribed to the inhibitory effects of cannabinoids on inflammatory conditions, which include, among others, atherosclerosis (see below), colitis, and arthritis.⁹⁴

Cannabinoids and Cardiac Remodeling

Endocannabinoids do seem to be beneficial after myocardial infarction, a severe consequence of which is remodeling of the heart. This results in increased risk of heart failure and arrhythmia, and the usual approach to minimizing this is to treat patients with angiotensin-converting enzyme inhibitors.⁹⁵ Wagner et al⁹⁶ sought to determine whether the elevated levels of endocannabinoids after experimentally induced infarction led to deleterious or beneficial changes in heart function. Administration of AM251 for 12 weeks after the insult exacerbated the decline in left ventricular capability, whereas giving the nonselective cannabinoid agonist, HU-210, enhanced left ventricular performance. However, the beneficial effects of HU-210 were greatest in cases of restricted degrees of infarction, which may reduce the potential utility of this approach.⁹⁷

It has been considered for some time that cannabinoid receptors have the potential to regulate apoptosis because they can signal through both proapoptotic and antiapoptotic pathways.⁵ Their lipophilicity also means that they have the capability to penetrate cells and act intracellularly, even without the assistance of a membrane transporter. Recently, it

has been shown that a number of cannabinoid agents, including HU-210, Δ^9 -tetrahydrocannabinol, and anandamide, decrease rat heart mitochondrial O_2 consumption⁹⁸ and that mitochondria are involved in marijuana-induced cell death.⁹⁹ Furthermore, vanilloid receptor agonists, which overlap with endocannabinoids at least in the form of anandamide and oleamide, have proapoptotic actions on mitochondria.¹⁰⁰ Coupled with the observations on apoptosis in FAAH-null mice,⁵⁷ it is clear that endogenously produced cannabinoids have strong potential for the regulation of apoptosis, and hence remodeling, in the heart.

Coronary Blood Vessels, Atherosclerosis, and Endothelium

In discussing the receptors mediating the responses of endocannabinoids, it has already been stated that anandamide and other cannabinoids relax the blood vessels of the heart.¹⁵ These responses in the rat are sensitive to CB_1 receptor blockade but not to CB_2 receptor or TRPV1 antagonism. Their physiological relevance remains unclear because there is no substantial evidence for spontaneous activity of the endocannabinoid system in the heart or its vessels under non-pathological conditions. However, the CB_2 receptor is widely recognized as being associated with cells that mediate immune responses and inflammation,^{10,22,94} and so it is not surprising that cannabinoids have been shown to reduce lesion progression in the apolipoprotein E knockout mouse model of atherosclerosis.¹⁰¹ In these experiments, the mice were given a low oral dose of Δ^9 -tetrahydrocannabinol and the response was inhibited by the CB_2 receptor antagonist, SR144528. The target seems to be CB_2 receptors on macrophages and T lymphocytes within the lesion, and the treatment also reduced leukocyte adhesion in the mesenteric vasculature. Interestingly, increasing the dose beyond an optimal dose of 1 mg·kg⁻¹·d⁻¹ resulted in a decreased effectiveness, which suggests either that Δ^9 -tetrahydrocannabinol is acting as a partial agonist or that there are opposing effects that can be activated by the cannabinoid. No data were provided for the effects of the CB_2 antagonist alone, so it is not possible to state explicitly that the endocannabinoid system is, or is not, activated within the lesion. However, this seems unlikely because the antagonist did not increase lesion progression beyond the control level although it completely reversed the effects of Δ^9 -tetrahydrocannabinol. A potential effect for endocannabinoids on the progression of vascular lesions is indirectly indicated by the observation that estrogen increases anandamide release from endothelial cells, and this inhibits platelet activation; this might be one of the mechanisms by which hormone replacement therapy and natural estrogens confer protection on the cardiovascular system.¹⁰²

One mechanism by which endocannabinoids could affect the progression of atherosclerosis, or restenosis after treatment of coronary artery obstructions, is by modulation of the actions of inflammatory factors. It has already been pointed out (see under "Cardioprotection") that stimulation of CB_2 receptors reduces the inflammatory activation of endothelial cells by TNF- α .⁹⁰ This cytokine also induces proliferation and migration of cultured human coronary artery vascular smooth muscle cells together with activation of

signaling molecules such as Ras, mitogen-activated protein kinase, ERK1/ERK2, and Akt.¹⁰³ Interestingly, these actions are associated with greatly increased expression not only of CB_2 receptors but also of CB_2 receptor agonists, such as JWH133 and HU-308, bring about a concentration-dependent reduction in the effects of TNF- α . As noted already, there is as yet no substantive evidence for local modulation of the progression of atherosclerotic lesions by endocannabinoids, but the difficulties in constructing suitable studies are such that it is not currently possible to discount such a hypothesis.

CONCLUSIONS: ENDOCANNABINOIDS AS TARGETS IN CARDIAC THERAPY

The clinical use of rimonabant for the treatment of obesity³ marks the first manipulation of the endocannabinoid system for therapeutic purposes, but it has not been a run-away success as concerns have already been expressed about its use with respect to side effects arising from actions on the brain.¹⁰⁴ Indeed, this review of endocannabinoid effects on the heart shows that it is not possible yet to define them simply as protective or injurious to the heart and its blood vessels, although the balance does seem to favor a protective role. Therefore, the use of cannabinoid receptor ligands as therapeutic agents needs careful consideration, not only with regard to potential central side effects. Because the endocannabinoid system seems to be activated in response to threatening pathophysiological circumstances, its actions can be enhanced not only by the deployment of agonists but also by inhibitors of FAAH or cellular uptake. This approach has the potential advantage that the increased agonist activity would be largely confined to the sites at which the endogenous processes have already been activated to meet the threat.

The consideration of the field of cannabinoid therapies has moved on even since the appearance in 2006 of the major review by Pacher et al⁹⁴ of the prospects of the endocannabinoid system as a successful therapeutic target. Doxorubicin cardiotoxicity, for example, is newly identified as a condition in which endocannabinoid activity might be implicated.⁸⁶ Therefore, use of a CB_1 receptor antagonist, preferably without central effects, might be beneficial for those patients undergoing chemotherapy with this cytotoxic agent because its mechanisms of attack on the tumor and on the heart are different.¹⁰⁵ Such use would be limited to patients with cancer and therefore small numbers of individuals; but manipulation of endocannabinoid activity in more prevalent pathologies such as myocardial infarction, circulatory shock, and perhaps atherosclerosis will need careful balancing of actions. The circumstances of the condition, relating to the endocannabinoids produced, their cellular source, and the profile of gene expression of the receptors and enzymes involved in their production, action, and inactivation will need to be more fully understood before the system can be satisfactorily targeted for therapeutic purposes.

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