JPET Fast Forward. Published on September 22, 2023 as DOI: 10.1124/jpet.123.001665
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Role of cannabinoid signaling in cardiovascular function and ischemic injury

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Running Title: Cannabinoids in cardiovascular function and ischemic injury
Recommended Section: Cardiovascular Pharmacology

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Conflicts of Interest: None

Funding: National Institute on Drug Abuse (R01 DA044999) and National Heart, Lung, and Blood Institute (R15 HL145546).

Manuscript Statistics
Number of text pages: 23
Number of figures: 3
Number of references: 140
Number of words in the Abstract: 223
Number of words in the Significance Statement: 75

JPET Fast Forward. Published on September 22, 2023 as DOI: 10.1124/jpet.123.001665
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Abbreviations: AA: arachidonic acid; AEA: anandamide; 2-AG: 2-arachidonylglycerol, FAAH: fatty acid amide hydrolase, MAGL: monoacylglycerol lipase; CB_1R: cannabinoid type 1 receptor, CB_2R: cannabinoid type 2 receptor, Δ^9-THC: delta-9-tetrahydrocannabinol,

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Abstract

Cardiovascular disease represents a leading cause of death, morbidity, and societal economic burden. The prevalence of cannabis use has significantly increased due to legalization and an increased societal acceptance of cannabis. Therefore, it is critically important that we gain a greater understanding of the effects and risks of cannabinoid use on cardiovascular diseases as well as the potential for cannabinoid-directed drugs to be used as therapeutics for the treatment of cardiovascular disease. This review summarizes our current understanding of the role of cannabinoid receptors in the pathophysiology of atherosclerosis and myocardial ischemia and explores their use as therapeutic targets in the treatment of ischemic heart disease. Endocannabinoids are elevated in patients with atherosclerosis, and activation of cannabinoid type 1 receptors (CB_1R) generally leads to an enhancement of plaque formation and atherosclerosis. In contrast, selective activation of cannabinoid type 2 receptors (CB_2R) appears to exert protective effects against atherosclerosis. Endocannabinoid signaling is also activated by myocardial ischemia. CB_2R signaling appears to protect the heart from ischemic injury while the role of CB_1R in ischemic injury is less clear. This narrative review serves to summarize current research on the role of cannabinoid signaling in cardiovascular function with the goal of identifying critical knowledge gaps and future studies to address those gaps in a way that facilitates the development of new treatments and better cardiovascular health.

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Significance Statement

Cardiovascular diseases including atherosclerosis and myocardial infarction are a leading cause of death. Cannabinoid drugs have well-known acute effects on cardiovascular function including tachycardia and orthostatic hypotension. The recent legalization of marijuana and cannabinoids for both medical and recreational use has dramatically increased their prevalence of use. This narrative review on the role of cannabinoid signaling in cardiovascular disease contributes to a better understanding of this topic by integrating current knowledge and identifying critical gaps.

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Introduction

Cardiovascular disease is the leading cause of death in the United States (US). More than 20 million people in the US have cardiovascular disease, and 697,000 people died (20% of all deaths) from cardiovascular diseases in 2020 (Tsao et al., 2022). Approximately 805,000 people in the US experience a heart attack each year (Tsao et al., 2022). Recent changes in state laws and increased societal acceptance of cannabis have significantly increased the prevalence of cannabis use in the US despite its schedule I classification by the Drug Enforcement Administration. However, the cardiovascular risks of cannabinoid use and the potential for cannabinoid receptors to be used as therapeutic targets for the treatment of atherosclerosis, myocardial ischemia, and other cardiovascular disorders remains unclear. This review summarizes our current understanding of the role of cannabinoid receptors in the pathophysiology of atherosclerosis and myocardial ischemia and explores their use as therapeutic targets in the treatment of ischemic heart disease.

Endocannabinoid Signaling System (ECS)

While the medicinal and recreational effects of cannabis have been known for thousands of years and were reported by Chinese Emperor Shen Nung in 2737 BC, a clear understanding of the mechanisms responsible for the effects of cannabis on the human body have only emerged over the last 50 years. In 1964, pioneering work by Gaoni and Mechoulam identified delta-9-tetrahydrocannabinol (Δ^9-THC) as the psychoactive component in hashish (Mechoulam and Gaoni, 1965). Two cannabinoid receptors were identified in subsequent years. These cloned receptors include the neuronal cannabinoid type 1 receptor (CB_1R) that is responsible for mediating the psychoactive effects of Δ^9-THC and is highly expressed in the brain (Matsuda et al., 1990). A second cannabinoid type 2 receptor (CB_2R) was cloned and is highly expressed in

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cells of the immune system (Munro et al., 1993). Both CB_1R and CB_2R are expressed in cardiovascular tissues including the heart, smooth muscle, and endothelial cells of the vasculature (Liu et al., 2000; Liu et al., 2003; Pacher et al., 2005; Weis et al., 2010). These receptors are typically coupled to $G\alpha_{i/o}$ proteins in neurons where activation of pre-synaptic CB_1R results in the activation of mitogen-activated kinases (MAPK) and G protein-coupled inward rectifying potassium channels (GIRKs) (Mackie et al., 1995) as well as inhibition of adenylyl cyclase (Howlett and Fleming, 1984) and voltage-gated calcium channels (VGCCs) (Mackie and Hille, 1992). Similarly, cannabinoid agonists have been shown to activate MAPK signaling pathways including p38 and c-Jun N-terminal kinases (JNK) in cardiomyocytes and vascular endothelial cells (Mukhopadhyay et al., 2010; Rajesh et al., 2010) and decrease cAMP accumulation in the isolated rat heart (Krylatov et al., 2005).

Two primary endocannabinoids, N-arachidonoylethanolamine (AEA; anandamide) (Devane et al., 1992) and 2-arachidonoyl-glycerol (2-AG) (Sugiura et al., 1995; Stella et al., 1997) have been identified and exert effects on cardiac function through CB_1R and CB_2R (Pacher et al., 2005). Endocannabinoids are produced from plasma membrane phospholipids by endocannabinoid synthesizing enzymes, while endocannabinoid signaling is terminated by endocannabinoid hydrolysis enzymes (Piomelli, 2003). Synthesis of anandamide can occur through at least three separate biosynthetic pathways while the majority of 2-AG is produced through the conversion of diaglycerol to 2-AG by sn-1-diaglyerol lipase alpha and beta (DAGLα/β) (Bisogno et al., 2003). Breakdown of AEA is mediated by fatty-acid amide hydrolase (FAAH) (Cravatt et al., 1996) while 2-AG is hydrolyzed predominately through the activity of monoacylglycerol lipase (MAGL) (Dinh et al., 2002). These biosynthetic and metabolic pathways may provide opportunities for the development of therapeutic targets.

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Cannabinoid signaling in cells of the cardiovascular system.

The adult myocardium is composed of approximately 56% cardiomyocytes, 27% fibroblasts, 10% vascular smooth muscle cells, and 7% endothelial cells (Banerjee et al., 2007). Likewise, the vasculature is also composed of multiple cell types including vascular smooth muscle cells, endothelial cells, fibroblasts, and perivascular adipocytes. In addition, macrophages can be found throughout the cardiovascular system. CB_1R and CB_2R are found in many of these cell types where they mediate the effects of both endogenous and exogenous cannabinoids.

Cardiomyocytes: CB_1R and CB_2R are expressed at very low levels in the mouse and human myocardium (Rajesh et al., 2012; Valenta et al., 2018; Rajesh et al., 2022). However, expression of these receptors is significantly upregulated under some pathological conditions including obesity (Valenta et al., 2018), heart failure (van Esbroeck et al., 2020) cardiomyopathy (Matyas et al., 2020), and type I diabetes (Rajesh et al., 2022). Under conditions of doxorubicin-induced cardiomyopathy (Mukhopadhyay et al., 2010) or diabetic cardiomyopathy (Rajesh et al., 2012), CB_1R signaling promotes increased oxidative/nitrosative stress, activation of p38 and JNK kinases, and increased apoptosis and cardiomyocyte death. CB_1R receptor signaling also decreases isoproterenol and forskolin-induced cAMP levels (Liao et al., 2013) and suppresses the activation of L type calcium channels in isolated cardiomyocytes (Li et al., 2009). CB_1R signaling results in suppression of cardiac contractile function (Bonz et al., 2003). CB_2R has been reported to protect isolated cardiomyocytes from oxidative stress and to protect the heart from ischemia / reperfusion injury (Defer et al., 2009). However, CB_2R has little or no impact on cardiomyocyte function under basal conditions.

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Vascular smooth muscle cells: CB_1R and CB_2R have opposing roles in regulating vascular smooth muscle cells. CB_1R activation promotes smooth muscle cell proliferation and migration in vitro (Rajesh et al., 2008b) and also increases smooth muscle cell proliferation in vivo following carotid balloon injury (Molica et al., 2013). In contrast, CB_2R activation suppresses smooth muscle cell proliferation following balloon injury and also suppresses pro-inflammatory signaling pathways (Ras, p38 MAPK, ERK, JNK, and Akt) in smooth muscle cells (Rajesh et al., 2008a). These data suggest that CB_1R blockade or CB_2R stimulation may provide novel therapeutic strategies to suppress vascular smooth muscle cell proliferation, stenosis, and vascular remodeling.

CB_1R located in vascular smooth muscle do not play a major role in regulating blood pressure. Wang et al. recently reported that mice in which CNR1 was deleted from vascular smooth muscle exhibit no changes in basal blood pressure. However, these animals had significantly larger infarct volumes following ischemic stroke (Wang et al., 2022). These data indicate that CB_1R expression in vascular smooth muscle is protective (perhaps via CB_1R-mediated vasodilation) under conditions of cerebral ischemia. Previous work demonstrated that CB_1R on cerebral vascular smooth muscle cells regulates the tone of cerebral arteries by decreasing calcium influx and promoting cerebral vasodilation (Gebremedhin et al., 1999). Others have suggested that cannabinoid receptors may play a role in cerebral vascular dysfunction associated with subarachnoid hemorrhage and traumatic brain injury (Benyó et al., 2016). The cerebrovascular actions of endocannabinoids and cannabinoid receptors was recently the topic of a detailed review (Benyó et al., 2016).

Endothelial cells: Multiple studies have demonstrated that CB_1R stimulation promotes inflammation in the vasculature. CB_1R signaling in coronary artery endothelial cells is coupled to

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pro-inflammatory signaling pathways (p38, JNK NF-KB), increased generation of reactive oxygen species, apoptosis, and cell death (Rajesh et al., 2007; Rajesh et al., 2010). More recent work demonstrated that Δ^9-THC concentration-dependently decreases cell viability in cultured endothelial cells and increases the synthesis of pro-inflammatory cytokines and production of reactive oxygen species (nitric oxide synthase-2 and NADPH oxidase) (Wei et al., 2022). These effects of CB_1R signaling lead to dysfunction of vascular endothelial cells in vivo. These Δ^9-THC-induced changes were blocked by CRISPR-based suppression of CB_1R expression and by CB_1R blockade with genistein (Wei et al., 2022), demonstrating that CB_1R plays an important role in Δ^9-THC -induced endothelial inflammation. This is consistent with endothelial dysfunction that occurs following exposure to marijuana smoke (Wang et al., 2016; Wei et al., 2022). CB_1R signaling also enhances neointima formation following balloon-induced injury and promotes the formation of atherosclerotic lesions in apoprotein E knockout mice (Molica et al., 2013). These pro-inflammatory and pro-atherosclerotic effects of CB_1R signaling are consistent with the identification of cannabis use as a risk factor for the cardiovascular disease (Skipina et al., 2022b).

In contrast to CB_1R, CB_2R signaling suppresses atherosclerosis by attenuating the endothelial expression of cell adhesion molecules that enable monocytes to migrate through the endothelium (Zhao et al., 2010b); (Rajesh et al., 2007). CB_2R signaling also inhibits endothelial production of monocyte chemoattractant protein-1 and monocyte binding to cultured human coronary artery endothelial cells (Rajesh et al., 2007). CB_2R agonists also suppress TNF-α-induced activation of a variety of pro-inflammatory signaling proteins (Ras, p38, ERK, JNK, and Akt) in endothelial cells (Rajesh et al., 2008a). Thus, CB_2R suppresses inflammatory processes in endothelial cells that contribute to cardiovascular disease.

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Fibroblasts: CB_1R signaling enhances interstitial fibrosis in the heart and worsens cardiac dysfunction under a variety of pathological conditions including diabetes (Rajesh et al., 2012), doxorubicin-induced cardiomyopathy (Mukhopadhyay et al., 2010), and following myocardial infarction (Slavic et al., 2013). Fibrosis is alleviated under these conditions by genetic deletion or pharmacological blockade of CB_1R (Rajesh et al., 2007); (Rajesh et al., 2012); (Slavic et al., 2013). Rimonabant blocks interleukin-1-induced upregulation of matrixmetalloprotease-9 in isolated cardiac fibroblasts and decreases hydroxyproline and collagen content in the heart following ischemic injury (Slavic et al., 2013). These CB_1R signaling events occurring in fibroblasts are associated with detrimental cardiac remodeling.

CB_2R signaling in fibroblasts plays a protective role in the heart. Diabetes-induced cardiac fibrosis is attenuated by CB_2R agonists and enhanced by genetic deletion of CB_2R (Rajesh et al., 2022). CB_2R also decreases collagen and fibronectin synthesis in the heart following an ischemic insult (Li et al., 2016). CB_2R also suppressed collagen secretion, Akt phosphorylation, and oxidative stress in isolated cardiac fibroblasts following hypoxic injury (Li et al., 2016). Thus, CB_1R and CB_2R have opposing roles in the regulation of cardiac fibroblast function.

Macrophages: Macrophages express both CB_1R and CB_2R. CB_1R activation on macrophages potentiates NLRP3-mediated inflammation (Jourdan et al., 2013), increases the production of pro-inflammatory cytokines (TNF-α, interleukin-6, MCP-1) (Mai et al., 2015), and promotes macrophage migration (Mai et al., 2015). In contrast, CB_2R signaling suppresses inflammation (Denaës et al., 2016; Kumawat and Kaur, 2023) and may protect the vasculature against the formation of atherosclerotic lesions. Oxidized low density lipoprotein stimulates the production of endocannabinoids and the expression of both CB_1R and CB_2R in macrophages

(Jiang et al., 2009), and CB_1R promotes the accumulation of intracellular cholesterol in macrophages (Jiang et al., 2009). Thus, inflammatory processes are enhanced by CB_1R signaling and attenuated by CB_2R signaling in macrophages.

Acute cardiovascular effects of cannabinoids

Studies have demonstrated that Δ^9-THC acts as a partial agonist at CB_1R and CB_2R while endocannabinoids and many synthetic cannabinoids such as CP55,940 and WIN55,212-2 are full agonists at these receptors (Govaerts et al., 2004). Research done in the 1970s demonstrated that oral and inhaled administration of Δ^9-THC or cannabis produces a number of acute cardiovascular effects including dose-dependent tachycardia (Renault et al., 1971), reduced peripheral vascular resistance (Benowitz et al., 1979), and episodes of orthostatic hypotension (Benowitz and Jones, 1975) and syncope (Benowitz and Jones, 1975). Since these early studies, the Δ^9-THC content in cannabis has increased dramatically from ~3-6% in the 1970s to greater than 30% in cannabis strains that are available for recreational and medical use today (ElSohly et al., 2016; Pennypacker et al., 2022). Additional research from more recent studies also demonstrates robust acute effects of oral Δ^9-THC or inhaled cannabis containing Δ^9-THC on cardiovascular function including blood pressure and heart rate (Martin-Santos et al., 2012; Kayser et al., 2020). These effects of Δ^9-THC on heart rate that have been observed in adults have also been found to occur in adolescents (Murray et al., 2022). Pharmacokinetic and pharmacodynamic modeling found that the half-life for the effect of Δ^9-THC on elevated heart rate is ~8 minutes (Strougo et al., 2008). These studies demonstrate that Δ^9-THC rapidly increases heart rate and can decrease blood pressure in adults and adolescents.

Early work suggested that 18-20 days of oral Δ^9-THC administration results in a decrease in the magnitude of orthostatic hypotension, possibly due to the development of tolerance

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(Benowitz and Jones, 1975). However, more recent work in a carefully controlled inpatient setting found that tolerance developed to the subjective "high" effects of oral Δ^9-THC but tolerance was not observed for the cardiovascular effects (Gorelick et al., 2013). Multiple studies have unequivocally demonstrated that CB_1R antagonists such rimonabant, surinabant, or AVE1625 block the cardiovascular effects of Δ^9-THC (Klumpers et al., 2013); (Zuurman et al., 2010); (Gorelick et al., 2006); (Huestis et al., 2007). It is unclear why tolerance develops to the psychoactive effects produced by CB_1R signaling but does not develop to the CB_1R-mediated cardiovascular effects.

Cannabinoid-induced tachycardia increases myocardial oxygen demand, and exercise tolerance is decreased in patients with angina following inhalation of a single marijuana cigarette (Aronow and Cassidy, 1974). A case crossover study of 124 patients admitted for myocardial infarction found that inhaled cannabis transiently increases the relative risk of myocardial infarction by 4.8 fold for one hour following cannabis consumption (Mittleman et al., 2001). Cannabis consumption has also been found to cause transient changes in the electrocardiogram including ST segment elevation, T wave flattening and inversions, increased P-wave width, and decreased P-wave amplitude (Kochar and Hosko, 1973); (Beaconsfield et al., 1972). These electrophysiological changes are consistent with case reports of cardiac arrhythmias associated with cannabis smoking (Singh, 2000).

Since the actions of CB_1R agonists (including Δ^9-THC) in the brain cause psychoactive effects that are associated with substance use, the development of peripherally restricted agonists represents a drug discovery approach that might circumvent abuse liability associated with direct-acting CB_1R agonists. AZD1940 and AZD1704 are two peripherally restricted CB_1R agonists that were developed by Astra Zeneca as novel pain therapeutics. However, the use of

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these compounds failed in clinical trials due to serious cardiovascular and metabolic side effects including heart rate changes, hypotension, weight gain, and liver toxicity (Pacher et al., 2018); (Kalliomäki et al., 2013). Some synthetic cannabinoids are 100 times more potent than Δ^9-THC at CB_1R, suggesting that the adverse cardiovascular effects of these synthetic cannabinoids are likely to be much more severe than those of Δ^9-THC (Marusich et al., 2022). These cardiometabolic effects represent a significant challenge to the therapeutic use of CB_1R agonists.

Under pathological conditions involving shock (such as myocardial infarction, endotoxin exposure, and liver cirrhosis), endocannabinoids acting at vascular CB_1R have been shown to contribute to vasodilation and hypotension in rodent models (Wagner et al., 1997; Wagner et al., 2001; Varga et al., 1998). As described earlier in this review, both AEA and 2-AG are rapidly degraded by hydrolytic enzymes to terminate signaling. Degradation of AEA by FAAH produces ethanolamine and arachidonic acid (AA), an important precursor in the production of eicosanoids involved in inflammation, vasodilation, and vasoconstriction including prostaglandins, epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, and leukotrienes (Maccarrone, 2017; Marusich et al., 2022). AA can also be generated by degradation of 2-AG by MAGL which causes the production of AA and glycerol (Maccarrone, 2017). These AA-derived signals can exert anti- or pro-inflammatory effects on the cardiovascular system that are not mediated by CB_1R or CB_2R and can be either cardioprotective or damaging depending on the context and the specific AA-derived signaling molecule produced (Pacher et al., 2018; Beccacece et al., 2023).

Atherosclerosis.

Role of the endocannabinoid system in atherosclerosis. The process by which atherosclerosis develops was the subject of a recent review (Jebari-Benslaiman et al., 2022).

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Atherosclerotic plaque formation is initiated when low density lipoproteins (LDL) that are trapped beneath the endothelium become oxidized. Oxidized LDL stimulates the synthesis of cell adhesion molecules on the overlying endothelial cells which subsequently attract monocytes and macrophages. These immune cells move through the endothelium into the sub-endothelial space where they engulf oxidized LDL, resulting in the formation of lipid-filled "foam cells". The attraction of additional macrophages and neutrophils results in the formation of a "necrotic core" which becomes covered by a fibrous cap that is composed primarily of smooth muscle cells and collagen. The resulting atheroma is prone to rupture, resulting in platelet aggregation, activation of blood clotting proteins, and potential occlusion of the vessel.

Endocannabinoids have been implicated in multiple steps of atherogenesis **(Figure 1**). Jehle et al. (Jehle et al., 2016) reported that deletion of DAGLα (the enzyme that produces 2-AG in macrophages) decreased the formation of atherosclerotic plaque and infiltration of macrophages into arterial walls. Consistent with this observation, increasing endogenous 2-AG concentrations in vascular tissues, either by genetic deletion or pharmacological inhibition of MAGL, resulted in increased plaque formation and an increase in monocyte and macrophage infiltration into the vessel wall (Vujic et al., 2016; Jehle et al., 2018). Enhancement of anandamide concentrations in mice, either by genetic deletion of FAAH (Lenglet et al., 2013) or by FAAH inhibition using URB597 (Hoyer et al., 2014) resulted in the formation of plaques with increased neutrophil infiltration, increased matrix metalloproteinase 9 expression, and decreased collagen content. These changes in plaque composition resulted in plaques that were more vulnerable to rupture compared to plaques that developed in mice with normal anandamide concentrations. Finally, elevated levels of circulating anandamide and 2-AG in patients with

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coronary artery disease (compared to patients without coronary artery disease) suggests a role for endocannabinoids in atherosclerotic plaque formation (Sugamura et al., 2009).

Role of CB_1R.

CB_1R expression is upregulated in monocytes during their differentiation into macrophages, and activation of CB_1R promotes the release of pro-inflammatory cytokines including interleukin 1-β, interleukin-8, and tumor necrosis factor-α (Sugamura et al., 2009). Recent work assessing blood samples from recreational marijuana smokers confirmed a role for CB_1R in marijuana-induced increases in pro-inflammatory cytokines (Wei et al., 2022). Δ^9-THC exposure also induces endothelial cell dysfunction, oxidative stress, inflammation, and the formation of atherosclerotic lesions in the vasculature (Wei et al., 2022). These effects of Δ^9-THC were attenuated by CB_1R blockade with genestein, siRNA-induced knockdown of CB_1R, and by CRISPR-mediated deletion of CB_1R expression (Wei et al., 2022), implicating CB_1R in these Δ^9-THC-induced vascular changes.

Studies using atherosclerotic prone mouse models also point to a role of CB_1R in atherogenesis. Rimonabant inhibits the formation of atherosclerotic lesions and attenuates the production of pro-inflammatory cytokines in LDL receptor knockout (KO) mice fed a western diet (Dol-Gleizes et al., 2009). Rimonabant also decreased atherosclerotic lesion development and produced favorable changes in the serum lipid profile (decreased plasma triglycerides, increased HDL cholesterol) in dyslipidemic ApoE3-Leiden-cholesteryl ester transfer protein (CETP) mice fed a western diet (van Eenige et al., 2021).

Human studies provide further evidence for the involvement of CB_1R in atherosclerosis. CB_1R expression is upregulated in human coronary atheromas isolated from coronary arteries of

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patients with unstable angina when compared to atheromas from patients with stable angina (Sugamura et al., 2009). This is consistent with the finding that CB_1R expression was increased in unstable lipid rich plaques that are more prone to rupture compared to stable fibrous plaques (Sugamura et al., 2009). A randomized, double-blind clinical trial found that rimonabant improved cardiometabolic risk factors such as body weight and waste circumference in obese patients while producing favorable changes in lipid profile (increased HDL and decreased triglyceride levels) and decreased C reactive protein levels (Nissen et al., 2008). These data suggest a facilitative role for CB_1R in vascular inflammation and atherosclerosis and raise the possibility that peripherally-restricted CB_1R antagonists, without psychiatric side effects associated with action at CB_1R in the brain, might be a possible therapeutic option for slowing the progression of atherosclerosis.

Role of CB_2R.

In contrast to the pro-atherogenic effects of CB_1R signaling (Figure 1), activation of CB_2R suppresses atherogenesis (**Figure 2**). Zhao et al. reported that activation of CB_2R suppresses the expression of cell adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule 1, and P selectin in endothelial cells. This decreased the infiltration of macrophages into the vascular wall and significantly reduced atherosclerotic plaque formation in the mouse aorta (Zhao et al., 2010b). Other work found that treatment with WIN55212-2, a mixed CB_1R/CB_2R agonist decreased the number of macrophages, decreased NF-KB signaling, and decreased the expression of pro-inflammatory genes in atherosclerotic plaques from ApoE-/- mice. Importantly, these effects of WIN55,212-2 on plaque formation and macrophage infiltration were blocked by AM630, suggesting that they were mediated through CB_2R (Zhao et al., 2010a). Furthermore, genetic deletion of CB_2R in atherosclerosis-prone

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ApoE-/- mice resulted in increased infiltration of leukocytes into atherosclerotic lesions and increased the production of reactive oxygen species in the arterial wall of mice fed a high cholesterol diet (Hoyer et al., 2011). In addition, CB_2R in circulating immune cells suppresses immune cell infiltration into atherosclerotic lesions (Hoyer et al., 2011). In addition, Steffens et al. identified CB_2R in macrophages and T lymphocytes in atherosclerotic plaques from apoprotein E knockout mice (Steffens et al., 2005). They found that orally administered Δ^9-THC suppressed the progression of atherosclerotic plaques in these animals and that this effect was blocked by a CB_2R-selective antagonist (SR144528). Finally, CB_2R signaling alters the extracellular matrix composition of atherosclerotic plaques such that they have greater collagen content, decreased smooth muscle content, and increased structural stability (Netherland et al., 2010). Taken together, these studies suggest that CB_2R signaling exerts protective effects on atherosclerosis by suppressing the recruitment of immune cells in the vascular wall and by promoting changes in plaque composition that increase stability and decreases the likelihood of atherosclerotic lesion rupture. This raises the possibility that CB_2R selective agonists might be useful therapeutic agents to slow the progression of atherosclerosis. This possibility is intriguing since CB_2R agonists do not exert negative neuropsychiatric side effects due to limited expression of this receptor in the brain. To date, no human clinical trials have assessed the effect of CB_2R selective agonists on atherosclerosis. Human clinical trials should be performed to address this critical gap in our understanding surrounding a therapeutic role for CB_2R signaling in the development of atheroslerosis in humans.

Impact of recreational cannabinoids on atherosclerosis. The impact of recreational cannabis use on atherosclerosis-related cardiovascular disease was recently the topic of a detailed review (Pacher et al., 2018). Some investigators have found that cannabis use is associated with

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an increase in atherosclerotic cardiovascular risk score (Skipina et al., 2022a) and an elevated risk for atherosclerosis-related cardiovascular disorders including myocardial infarction (Chami and Kim, 2019; Wei et al., 2022), acute coronary syndrome (Richards et al., 2019) stroke (Hemachandra et al., 2016; Zhao et al., 2021), transient ischemic attack (Hemachandra et al., 2016), and chronic cardiovascular diseases (Richards et al., 2019). In contrast, other work has found no association between cannabis use and clinical markers of atherosclerosis such as carotid intima-media thickness (Jakob et al., 2021) and coronary or abdominal aorta calcium scores (Auer et al., 2018) and no association between marijuana use and stroke, transient ischemic attacks, coronary artery disease, or cardiovascular mortality (Reis et al., 2017; Dutta et al., 2021). Mahtta reported that recreational use of alcohol, tobacco, amphetamine, and cannabis were each independently associated with early onset atherosclerotic cardiovascular disease (Mahtta et al., 2021), suggesting that the increased risk of cardiovascular disease may result from other lifestyle factors associated with recreational drug use. Indeed, the American Heart Association recently published a position statement that current evidence on cardiovascular outcomes associated with recreational cannabis use is inconclusive. This report cited confounding limitations in the analysis and interpretation of current clinical research on the cardiovascular impact of cannabis use including concurrent use of cannabis with tobacco and other recreational drugs, variations in cannabinoid content in cannabis, and biases associated with the use of hospitalized cannabis users (and hospitalized non-user control patients) (Page et al., 2020). The scheduling of cannabis as a Schedule I controlled substance has made it difficult for researchers to conduct appropriately controlled and sufficiently powered clinical studies. Most federally funded research using cannabis in the US has used strains containing between 2-10% Δ^9-THC cultivated at the University of Mississippi National Center for Natural Products

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Research. These strains are not necessarily representative of strains containing 20-30% Δ^9-THC that are commonly used by recreational cannabis users. The cardiovascular impact of the consumption of cannabis strains that have high Δ^9-THC concentrations represent a critical gap in our understanding and is an area requiring further research.

In summary, CB_1R and CB_2R signaling play important (and often opposing) roles in atherogenesis by modulating pro-inflammatory cytokine production, pro-atherosclerotic lipid profiles, infiltration of immune cells into the atherosclerotic wall, adhesion of monocytes to the endothelium, lipid accumulation in foam cells, and by altering the composition and structural stability of atheromas. While CB_1R activation tends to enhance the development of atherosclerosis (Figure 1), signaling by CB_2R exerts a protective effect (Figure 2) that could potentially be exploited therapeutically using treatment with CB_2R selective agonists.

Myocardial ischemia.

Endocannabinoid signaling in the ischemic heart. Endocannabinoid signaling has been shown to play a role in protecting the heart from ischemic injury (Figure 3). 2-AG and anandamide concentrations are elevated in plasma from patients with coronary artery disease compared to healthy control patients (Sugamura et al., 2009). Anandamide concentrations in blood collected directly from the coronary arteries of patients with acute coronary syndrome are 10 times higher than anandamide concentrations in blood collected from systemic circulation, suggesting that anandamide is released locally from the ischemic heart (Maeda et al., 2009). Similarly, 2-AG concentrations were found to be significantly elevated in blood isolated from coronary arteries in myocardial infarction patients compared to blood collected from the coronary arteries of patients without coronary blockage (Wang et al., 2012). Consistent with these findings in the human heart, ischemia induced by ligation of the left anterior descending

coronary artery elevated 2-AG concentrations in the mouse heart and plasma (Schloss et al., 2019). The increased 2-AG detected in infarcted hearts from these mice resulted from increased cardiac expression of DAGL, the biosynthetic enzyme responsible for 2-AG production, and decreased expression of MAGL, the hydrolytic enzyme that metabolizes 2-AG (Schloss et al., 2019). The increased expression of 2-AG leads to an increase in the number of neutrophils and monocytes in the blood and infarcted heart. An opposite pattern of increased DAGL mRNA expression and decreased MAGL gene expression was observed in bone marrow suggesting that myocardial infarction might lead to a 2-AG gradient driving the recruitment of neutrophils and monocytes from bone marrow into the bloodstream and to the infarcted heart. Interestingly, the effects of 2-AG on neutrophil and monocyte recruitment were blunted in CB_2R KO mice suggesting that these protective effects of 2-AG are CB_2R-mediated. These human and animal studies suggest that endocannabinoids are released in response to a cardiac ischemic insult. Other work has found that anandamide is released from the ischemic brain, (Muthian et al., 2004) and 2-AG is released from the ischemic kidney, suggesting that activation of the endocannabinoid system might be a conserved and universal protective response to ischemic injury in both cardiac and non-cardiac tissues.

Impact of CB_1R and CB_2R signaling on the ischemic heart. Evidence from rodent models indicates that CB_2R signaling protects the heart from ischemic injury. This is supported by work demonstrating that ischemic cardiac injury is worsened by genetic deletion of CB_2R (Defer et al., 2009; Duerr et al., 2015; Hu et al., 2019) or pharmacological inhibition of CB_2R using selective antagonists (Hajrasouliha et al., 2008; González et al., 2011; Yu et al., 2019). CB_2R KO mice exhibit attenuated expression of antioxidant enzymes and increased infiltration of macrophages into the myocardium following an ischemic insult, indicating that endocannabinoid signaling

through CB_2R attenuates ischemia-induced inflammation (Duerr et al., 2015). Administration of CB_2R agonists prior to ischemia or at the onset of reperfusion decreased infarct size, apoptosis, release of enzymatic markers of myocardial injury (lactate dehydrogenase and troponin), fibrosis, and inflammation following an ischemic insult (Di Filippo et al., 2004; Montecucco et al., 2009; Wang et al., 2012; Li et al., 2013b; Li et al., 2016; Yu et al., 2019; Liu et al., 2021). CB_2R is coupled to an Akt – phosphatidylinositol-3-kinase-dependent signaling pathway that has been shown to inhibit opening of the mitochondrial permeability transition pore. This preserves mitochondrial integrity and reduces apoptosis and infarction size (Li et al., 2013b; Li et al., 2014). CB_2R signaling also protects cardiomyocytes from calcium overload-induced injury by suppressing the influx of calcium that occurs during an ischemic insult (Li et al., 2013a). In contrast to cardioprotective effects of CB_2R on the myocardium, 2-AG released from the ischemic heart acts at CB_2R on nearby myeloid tissues to recruit neutrophils and monocytes from bone marrow to the site of ischemic injury. Disruption of normal 2-AG signaling through CB_2R in mice using systemic treatment with JZL-184, a MAGL inhibitor, exacerbated myocardial fibrosis and myocardial contractile function (Schloss et al., 2019). When considered collectively, the currently available data indicate that endocannabinoids act through CB_2R-mediated mechanisms that are protective against myocardial ischemic injury.

Studies investigating the impact of CB_1R have generated mixed results. *In vivo* studies with rimonabant demonstrated that CB_1R blockade enhances the post-ischemic recovery of contractile function by decreasing cardiac fibrosis and facilitating cardiac remodeling following myocardial infarction (Lim et al., 2009; Slavic et al., 2013). The protective effect of rimonabant was absent in CB_1R KO mice, confirming that this effect of rimonabant was mediated by CB_1R. However, genetic deletion of CB_1R had no impact on infarct size, suggesting that acute versus

chronic disruption of CB_1R signaling might have different effects on cardiac ischemic injury. Others have suggested that CB_1R signaling within the heart may be cardioprotective while stimulation of CB_1R located in unidentified tissue/s outside of the heart might worsen myocardial ischemic injury (Lim et al., 2009). Thus, the impact of CB_1R signaling on myocardial ischemic injury remains unclear. Several avenues of future research could provide additional clarity. For example, experimental approaches that allow inducible deletion of CB_1R in mice would allow investigators to parse out the temporal effects of CB_1R signaling on myocardial ischemic injury, and studies with cardiac-specific CB_1R KO animals would help to differentiate the roles of cardiac versus non-cardiac CB_1R signaling following a cardiac ischemic insult. Furthermore, a great deal of progress has been made generating positive and negative allosteric modulators of CB_1R that have yet to be studied in the context of ischemic injury.

Impact of recreational cannabis on myocardial infarction. The impact of recreational cannabis on the risk of myocardial infarction was recently the topic of a detailed review (Pacher et al., 2018). Several large clinical studies suggest that cannabis use is associated with an increased risk of myocardial infarction, especially in the first hour after cannabis use when the well-known tachycardic effects of cannabinoids occur (Mittleman et al., 2001; Patel et al., 2020; Ladha et al., 2021; Ma et al., 2021; Wei et al., 2022). In addition, numerous case reports have documented elevated ST segment and elevated troponin levels indicative of myocardial infarction in young healthy individuals using cannabis despite the absence of coronary atherosclerosis or other risk factors for ischemic heart disease. The use of synthetic cannabinoids, with potencies that are often more than 100 times greater than naturally occurring phytocannabinoids in cannabis, pose unique risks for cardiac events such as chest pain, dyspnea, and myocardial infarction (Armenian et al., 2018). Nevertheless, an unequivocal cause-effect

JPET Fast Forward. Published on September 22, 2023 as DOI: 10.1124/jpet.123.001665
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relationship between cannabis use and the risk of myocardial infarction remains controversial due to concurrent tobacco use, obesity, and other cardiovascular risk factors (Mittleman et al., 2001) and also bias that is inherent to studies using hospitalized patients to study the impact of cannabis on the risk of experiencing a myocardial infarction (Page et al., 2020).

A recently published review on the mechanisms by which cannabinoids might induce myocardial infarction posited that Δ^9-THC increases myocardial oxygen demand by increasing cardiac contractile force and heart rate while simultaneously decreasing the coronary flow rate and limiting blood flow to the myocardium (Weresa et al., 2022). Smoked cannabis, but not vaporized or oral cannabinoids, leads to the formation of carboxyhemoglobin which limits the capacity of red blood cells to transport oxygen to tissues including the heart. Thus, cannabinoids could act through multiple mechanisms to increase the risk of myocardial ischemia.

Cannabinoid system as a therapeutic target for cardiovascular diseases.

Correlation between plasma endocannabinoid levels and cardiovascular risk. Plasma 2-AG concentrations in obese men are positively correlated with cardiometabolic risk factors including abdominal obesity, body mass index, waist girth, plasma triglyceride and insulin levels and negatively correlated with plasma high density lipoprotein levels (Côté et al., 2007), In addition, AEA concentrations are significantly elevated in obese patients and correlate with coronary endothelial and circulatory dysfunction (Quercioli et al., 2011; Quercioli et al., 2012). These data suggest that increased production of endocannabinoids may provide a mechanistic link between obesity and coronary circulatory dysfunction (Al Suwaidi et al., 2001; Schindler et al., 2006).

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Role of endocannabinoids in cardiomyopathy. Doxorucibin is a topoisomerase inhibitor that is commonly used for cancer chemotherapy. Therapeutic use of this agent is often limited by doxorubicin-induced toxicity which is characterized by oxidative / nitrative stress, apoptosis, and declining cardiac function. Genetic deletion of FAAH, the enzyme that metabolizes AEA, enhances doxorubicin-induced oxidative / nitrative stress, increases apoptosis and cell death, and increases doxorubicin-induced cardiac dysfunction and mortality in mice (Mukhopadhyay et al., 2011). Doxorubicin-induced toxicity is attenuated by CB_1R blockade in both human cardiomyocytes (Mukhopadhyay et al., 2010) and in mice (Mukhopadhyay et al., 2007). Similarly, myocardial AEA levels, CB_1R expression, oxidative / nitrative stress, and apoptosis are increased in the heart under conditions of diabetic cardiomyopathy (Rajesh et al., 2012). Pharmacological blockade or genetic deletion of the CB_1R suppresses diabetes-induced oxidative stress, apoptosis, and inflammation in the heart and preserves myocardial contractile function. These data provide evidence that endogenous cannabinoids acting on CB_1R worsens doxorubicin- and diabetes-induced cardiomyopathy. In contrast, deletion of the CB_2R worsens oxidative / nitrative stress, inflammation, apoptosis, and contractile dysfunction associated with diabetic cardiomyopathy, indicating that endocannabinoid signaling via CB_2R has a cardioprotective effect under these pathological conditions (Rajesh et al., 2022).

Clinical impact of CB_1R blockade on cardiovascular risk. Dysfunction of the endocannabinoid system plays an important role in obesity. Plasma 2-AG concentrations are significantly greater in obese individuals than lean subjects and are positively correlated with visceral fat mass (Blüher et al., 2006). In addition, the number of mRNA transcripts encoding CB_1R and FAAH in visceral adipose tissue is significantly lower in obese individuals compared to lean subjects (Blüher et al., 2006). Variations in genes involved in endocannabinoid signaling

may predispose individuals to weight gain. A single nucleotide polymorphism in the gene encoding fatty acid amide hydrolase (P129T) results in decreased FAAH expression and activity (Chiang et al., 2004). Sipe et al. reported that people who are homozygous for this polymorphism exhibit a significantly greater body mass index than those who are heterozygotes or inherit the wildtype version of this gene, suggesting that this heritable polymorphism may play a role in obesity (Sipe et al., 2005).

Rimonabant was approved in Europe in 2006 for the treatment of obesity. Clinical trials on rimonabant found significant improvements in cardiovascular risk factors including decreased body weight, improved dyslipidemias, and decreased fasting glucose and insulin levels in overweight patients (Hollander, 2007; Nissen et al., 2008; Van Gaal et al., 2008b). The Rimonabant in Obesity (RIO) study found that obese patients who were prescribed rimonabant in combination with a modestly hypocaloric diet (600 kcal/day deficit) lost significantly more weight after 1 year than patients taking a placebo in concert with the hypocaloric diet. Rimonabant produced significant improvements (compared to placebo) in several cardiometabolic risk factors including waist circumference, insulin resistance, prevalence of metabolic syndrome, A1C, HDL-cholesterol, and triglycerides (Van Gaal et al., 2005; Van Gaal et al., 2008a). These benefits were sustained following a second year of rimonabant treatment, indicating that chronic rimonabant therapy could produce long term improvements in these cardiometabolic risk factors (Van Gaal et al., 2008b). The Comprehensive Rimonabant Evaluation Study of Cardiovascular Endpoints and Outcomes (CRESCENDO) trial was established to determine whether long term rimonabant treatment decreased the risk of myocardial infarction, stroke, and other cardiovascular causes of death. This study was a double-blind placebo-controlled clinical trial involving 974 hospitals in 42 countries and was intended to

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follow patients taking rimonabant or placebo for a minimum of 33 months. However, due to an increase in psychiatric side effects, including suicidal ideation, this study was terminated prematurely after 14 months with no significant cardiovascular findings (Topol et al., 2010). The prevalence and severity of these adverse psychiatric effects led to rimonabant's removal from the European market in 2008. Two other clinical studies found no impact on clinical indicators of the progression of atherosclerosis including atheroma volume in the coronary vasculature (Nissen et al., 2008) or carotid intima-media thickness (O'Leary et al., 2011) in patients taking rimonabant for 12 – 30 months. The investigators suggested that the benefits of rimonabant may have been masked by the fact that most patients had lipid profiles that were well controlled by statins prior to initiation of the study (Nissen et al., 2008). Similar to the CRESCENDO trial, both of these studies found adverse psychiatric effects in patients using rimonabant.

In light of the finding that adverse psychiatric effects are significantly increased by rimonabant, peripherally acting CB_1R antagonists or monoclonal antibodies that do not enter the CNS may be more therapeutically useful. Wei et al. (Wei et al., 2022) recently identified genistein as a CB_1R antagonist with minimal penetration into the CNS. Genistein reduced Δ^9-THC-induced atherogenesis *in vivo*, providing evidence that this ligand may provide an alternative approach to targeting peripheral CB_1Rs without inducing adverse effects in the CNS. Another enticing therapeutic possibility involving CB_1R is the use of negative allosteric modulators that might dampen endocannabinoid signaling tone in the context of cardiovascular disease. The use of CB_1R negative allosteric modulators to reduce endogenous CB1R signaling (as opposed to antagonists directed to the orthosteric binding site) is an approach that might reduce the likelihood of adverse psychiatric side effects.

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Current data indicates that CB_2R signaling exerts a protective effect in the context of atherosclerosis and myocardial ischemia. Therefore, the prophylactic use of selective CB_2R agonists in patients with cardiovascular risk factors is a therapeutic option that warrants more study. Interestingly, CB_2R is virtually non-existent in the uninjured brain under normal conditions and the use of CB_2R agonists has not been linked to psychoactivity or adverse psychiatric effects in either rodent models or human studies. Another therapeutic possibility warranting further study is the use of inhibitors of endocannabinoid hydrolysis enzymes to enhance levels of anandamide and 2-AG. Since chronic genetic and pharmacological blockade of MAGL has been shown to desensitize and downregulate CB_1R in the brain, MAGL inhibitors may be useful for treating human diseases including cardiovascular conditions (Schlosburg et al., 2010). Similar to the use of allosteric modulators, therapeutic approaches to increase basal endocannabinoid signaling tone are less likely to produce CB_1R-associated negative side effects compared to direct acting orthosteric agonists. Finally, to fully realize the potential of different cannabinoid-directed therapeutics, it is important that we gain a clear understanding of the temporal requirements for cannabinoid signaling in cardiovascular disease. For example, it is important to understand the capacity of each cannabinoid-directed therapeutic compound to prevent the establishment of cardiovascular disease as opposed to their abilities to reverse disease once it has occurred. This knowledge will shed insight on whether treatments need to be given prophylactically to patients at risk for cardiovascular disease or whether they might also be useful to treat established disease.

Acknowledgements

This work was supported by funding from the National Institute on Drug Abuse (R01 DA044999) (JG and DM) and the National Heart, Lung, and Blood Institute (R15 HL145546) (BR). We would also like to acknowledge and thank the reviewers for extremely detailed and helpful suggestions that helped to substantively improve this manuscript.

Data Availability Statement

No data was presented in this manuscript.

Author Contributions

Wrote or contributed to the writing of the manuscript: Rorabaugh, Guindon, and Morgan.

JPET Fast Forward. Published on September 22, 2023 as DOI: 10.1124/jpet.123.001665
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Figure Legends

Figure 1. Role of endocannabinoids and CB_1R signaling in atherosclerotic disease.

Increasing levels of 2-AG or AEA by inhibiting their hydrolytic enzymes resulted in increased atherosclerotic plaque formation and infiltration of macrophages into arterial walls. Δ^9-THC exposure induces the formation of atherosclerotic lesions in the vasculature. While inhibition or genetic deletion of CB_1R has protective effects on the severity of atherosclerotic plaque formation.

Figure 2. Role of CB_2R in atherosclerotic disease.

Activation of CB_2R signaling exerts protective effects on atherogenesis by suppressing the infiltration of macrophages into the vascular wall. Genetic deletion or pharmacological inhibition of CB_2R in atherosclerosis-prone ApoE-/- mice increased infiltration of leukocytes into atherosclerotic lesions.

Figure 3. Endocannabinoid signaling in cardiac ischemia

Endocannabinoid signaling exerts a protective effect on ischemic injury in the heart and both 2-AG and anandamide concentrations are elevated in plasma from patients with coronary artery disease. Cardiac ischemic injury is worsened by genetic deletion or pharmacological inhibition of CB_2R. CB_2R selective agonists cause a reduction in the size of myocardial infarction. Ischemic injury elevated 2-AG concentrations in the mouse heart and plasma leading to an increase in the number of neutrophils and monocytes in the blood and infarcted heart. CB_1R blockade can enhance the post-ischemic recovery of contractile function following myocardial infarction. Several clinical studies suggest that cannabis use is associated with an increased risk of myocardial infarction.

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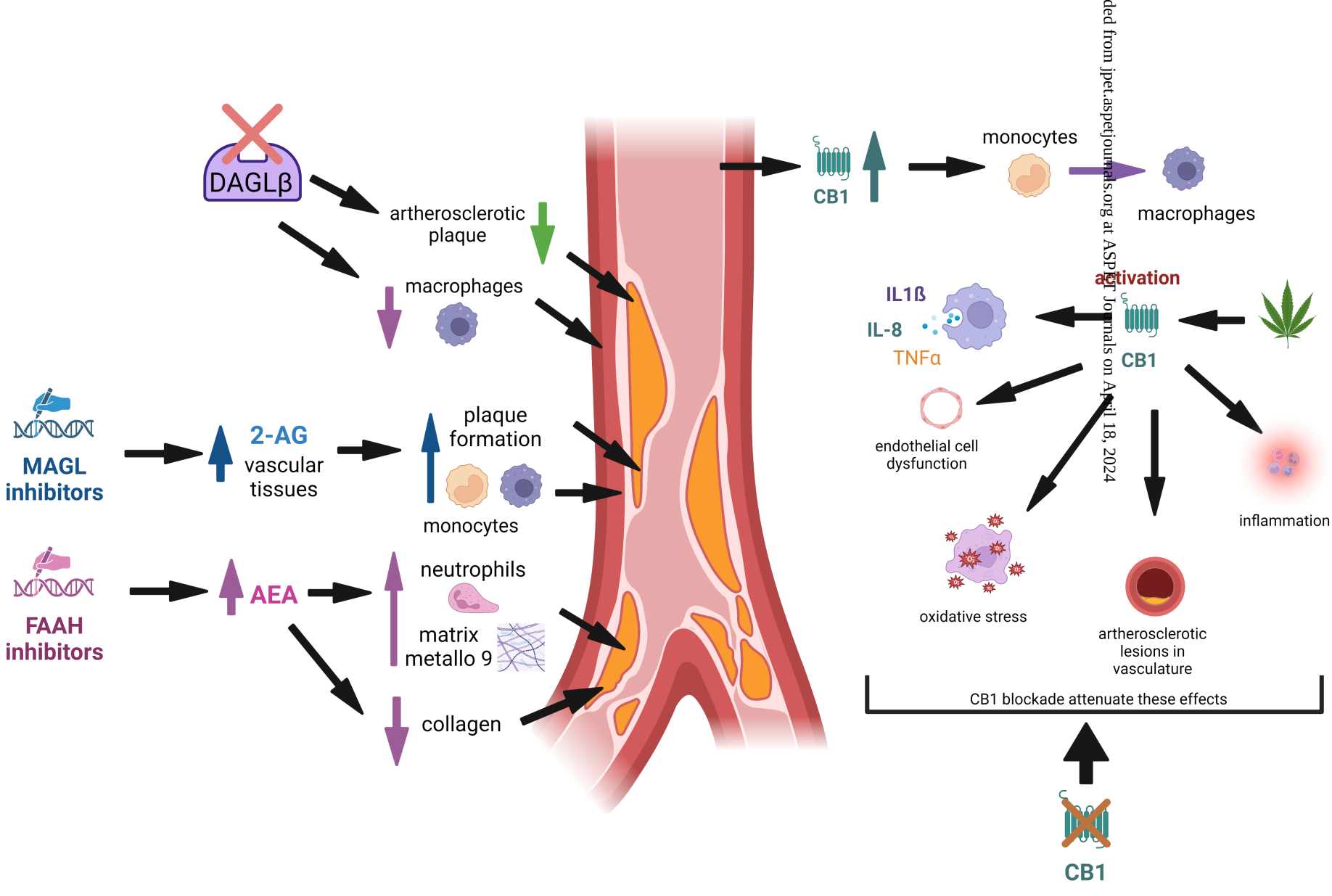

Figure 1

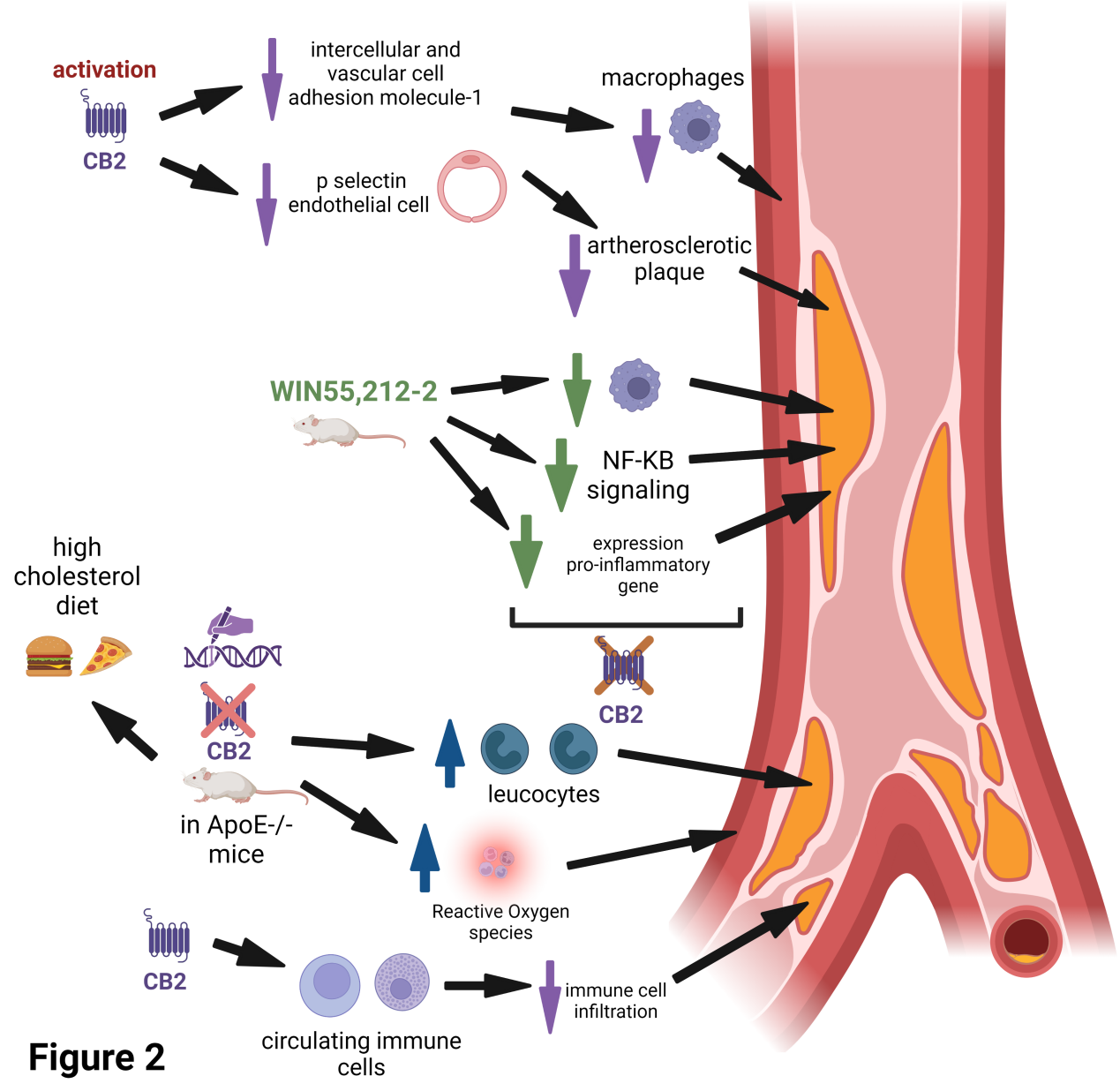

Figure 2

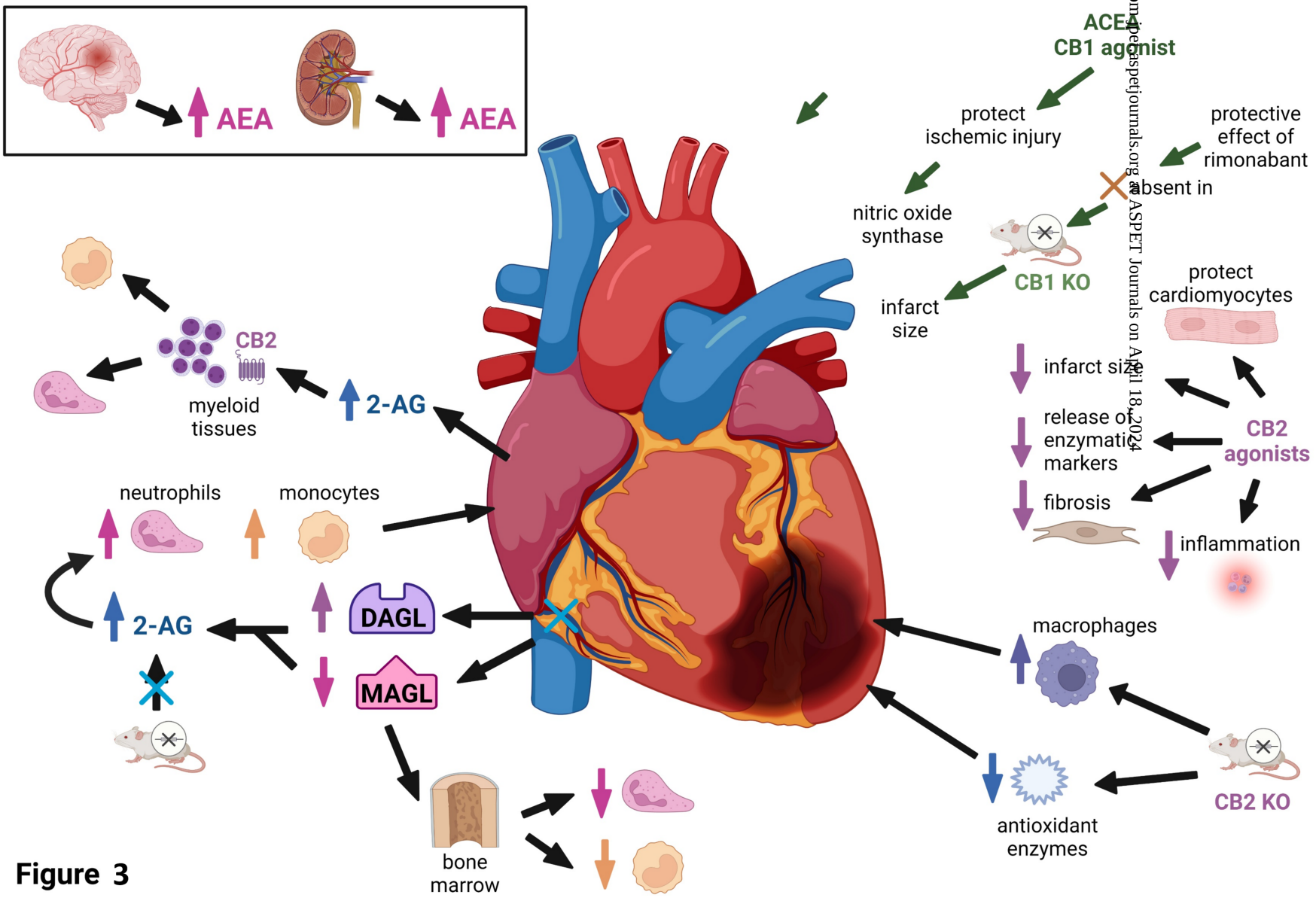

Figure 3