American Heart Association | American Stroke Association

Cannabinoids, the Heart of the Matter

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Cardiovascular disease (CVD) is a global epidemic representing the leading cause of death in some Western countries. According to the American Heart Association, a total of 92.1 million US citizens currently have ≥1 forms of CVD, with numbers expected to grow reaching up to 43.9% of the US population by 2030. In 2013, 17.3 million people (≈31% of all deaths) died due to CVD, and the number is expected to rise to at least 23.6 million in the next 15 years and cause an estimated economic burden of 1.0 trillion US dollars by 2030.[1]

Given its pathological and economic burden, there is a striking need for both mechanistic insights into CVD and the development of avenues for therapy. This review highlights the potential of cannabinoids and their receptors as targets for intervention. The endocannabinoid system (ECS) is upregulated in cardiovascular disease states, and cannabinoids in general influence disease progression.[2] Moreover, there are paradoxical indications as to whether therapies directed at the ECS, or exogenous drugs derived from marijuana, could have therapeutic impact in CVD.

The recent changes in the legalization of cannabis for both medical and recreational use has made cannabis consumption almost as conventional as tobacco use (Hall et al). This makes understanding the long-term effects of cannabis, whether harmful or beneficial, imperative. While acute adverse effects (impairment of memory, coordination and judgement) are widely accepted, most studies on long-term use and effects have been inconclusive.[3–7] These inconclusive results could possibly be due to the barriers in conducting cannabis research such as: access to the quality and quantity of cannabis needed for research as well as cannabis being classified as a Schedule 1 substance.[7] Other difficulties of long-term population studies also include separating tobacco users from marijuana users, as these two usually coincide. For example, recent publications from the CARDIA (Coronary Artery Risk Development in Young Adults) cohort study concluded no significant association with neither marijuana use and CVD or atherosclerosis risk.[5,6] Furthermore, in the CARDIA study looking at the association of smoking marijuana to atherosclerosis, only the subjects that smoked both marijuana and were tobacco users showed an associated risk with subclinical atherosclerosis.[6] Even with current challenges and knowledge gaps in cannabis research there has been some strong evidence for cannabis having therapeutic roles in treating everything from pain, and muscle spasms in multiple-sclerosis patients, to reducing nausea in chemotherapy patients.[7] This evidence, along with the knowledge of the ECS being upregulated in CVD, makes it an attractive therapeutic target for CVD.

This literature begins by reviewing CVD, including the contributing pathologies within CVD, and current therapeutic approaches. That is followed by the review of the ECS and its physiological and pathological roles, and details the receptors involved in the ECS. Finally, we discuss the connection of CVD to the ECS and delve into the possible manipulations of these pathways that could be employed for future therapeutic approaches.

Contributing Pathologies Within the CVD Spectrum

Coronary Artery Disease

Coronary arteries are crucial in supporting the myocardium with oxygen-rich blood. When low-density lipoproteins (LDL),

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macrophages, and lymphocytes accumulate within the artery wall (triggered by different environmental, behavioral, and genetic risk-factors) they form lesions. Over decades, necrotic cells and cholesterol accumulate to form hardened plaques, which leads to a complex, chronic inflammatory disease known as coronary artery disease (CAD) or atherosclerosis.[8] When the thrombogenic plaque material ruptures and blood clots form on the plaque, it eventually leads to spontaneous temporary loss of blood supply, a condition known as unstable angina. Contrasting with stable angina, these periods are spontaneous and not exercise-induced. Symptoms include chest pain, a feeling of pressure or tightness in the chest, and shortness of breath. When the thrombus completely blocks the vessel (thrombosis), or a part breaks off (embolization) blocking off a smaller downstream blood vessel, a myocardial infarction occurs which permanently damages heart muscle tissue and can lead to death.

Ischemia/Reperfusion Injury

Myocardial ischemia-reperfusion injury (IRI) is the result of restricted blood supply to tissues which causes a shortage of oxygen (ischemia) and the resulting injury of the myocardium caused by the restoration of coronary blood flow (reperfusion). It is an acute complication of atherosclerosis[9] that can also be caused by, or associated with, hypertension, hyperlipidemia, diabetes mellitus and insulin resistance, and aging. The condition results in a pattern of structural and metabolic changes, and leads to irreversible injury of the myocardium.[10] Myocardial IRI may lead to myocardial infarction, cardiac arrhythmias, and contractile dysfunction.[11] The risk for developing myocardial IRI is higher for males, and also includes: diabetes mellitus, dyslipidemia, hypertension, insulin resistance, nutritional deficits, and physical inactivity; as well as smoking, high total cholesterol and low HDL, and high blood pressure.[1,12]

Hypertension

A person is defined to be hypertensive when blood pressure is 140 mm Hg systolic, or exceeds this value, and/or when it is 90 mm Hg diastolic or higher.[13,14] Most people having their first stroke have a blood pressure higher than 140/90 mm Hg.[13] A condition commonly caused by hypertension, as well as heart valve stenosis, is cardiac hypertrophy. An estimated 85.7 million (34%) of the American population aged ≥20 years is hypertensive. The prevalence of high blood pressure increases with age and is highest among African Americans (45.0% of males, and 46.3% of females are hypertensive, compared with 34.5% of white males and 32.3% of white females). By 2030, an estimated 41.1% of American adults will be hypertensive.[1] While 76% of hypertensive patients in the United States use antihypertensive medication, only 54.4% can control the condition.[1]

Hypertrophy

Cardiac hypertrophy is an adaptive and compensatory response of the heart to maintain cardiac function, although the cardiac muscle cells must work harder.[15] It is characterized by a higher rate of protein synthesis, a decrease in size of the heart chamber, and thickening of left and right ventricular walls; as a result, poses as a major risk factor of developing arrhythmia and heart failure.[1,16]

Endothelial Dysfunction

Coronary vascular tone is regulated by endothelial cells releasing multiple vasoactive compounds that act on smooth muscle cells,[17] including nitric oxide, prostacyclin , substance P, and cytochrome P450 monooxygenase (CYP450) metabolites of arachidonic acid. These autacoids are responsible for vasorelaxation of the endothelium in the vasculature.[17,18] Early atherosclerosis is marked by endothelial dysfunction caused by biochemical and physical injuries, as well as immune-mediated damages and oxidative stress; resulting in altered platelet and leukocyte adhesion to the vascular surface, initiating an inflammatory response in the artery wall.[9,19] The activated endothelial cells locally produce chemokines, chemokine receptors, and adhesion molecules, eg, Vascular cell adhesion protein 1 (V-CAM-1), Intercellular Adhesion Molecule 1 (ICAM-1).[20] Inflammatory cells cause leukocyte rolling and cell adhesion along the vascular surface, which secrete chemokines and cytokines, resulting in chronic vascular inflammation.[9]

Oxidative Stress

Oxidative stress is a common factor across all contributing pathologies in CVD.[21] Oxidative stress arises when the production of reactive oxygen species exceeds the total antioxidant capacity of the body. Reactive oxygen species act as second messengers in signaling cascades in the cardiovascular system and are produced by cardiac mitochondria.[22,23] When the concentration of reactive oxygen species exceeds total antioxidant capacity, damage can be caused to biomolecules, eventually leading to loss-of-function and cell death. There are several cardiovascular risk factors that aid in promoting oxidative stress: poor diet, diabetes mellitus, obesity, smoking; as well as excessive environmental pollution.[23]

Current Therapeutic Approaches to CVD

Prevention

Epidemiologic studies show that there is a 2-fold increased risk for development of CAD and hypertension associated with a physically inactive lifestyle.[12] Patients exercising on a regular basis after having a myocardial infarction lower the

risk of death by 20% to 25% compared with controls. Patients with established CAD can improve symptoms of angina and congestive heart failure, as well as reducing the severity of exercise-induced ischemia. Regular exercise reduces systolic and diastolic blood pressure, decreases total and LDL cholesterol, and increases HDL cholesterol.[12,24]

A plant-based diet can help prevent and even reverse CAD. The Lifestyle Heart Trial found a reduction in regression of atherosclerosis, reduction in frequency of angina episodes (91% of patients), and a reduction in LDL (37.2%) similar to that accomplished by LDL-lowering medication.[12,25] Coronary events are reduced by 73%, and 70% decrease in all-cause mortality in the plant-based group compared with the control group that were given the recommended American Heart Association diet; 53% of the control group had progression of atherosclerosis.[12,25]

Management and Treatment

There are different options that patients can consider for treating CAD, and hypertension. Natural treatments include lifestyle changes: increase physical activity, changing to a healthy diet, losing excess weight, cessation of smoking, and stress reduction. Drug regimens center around antihypertensive agents (angiotensin-converting-enzyme inhibitors angiotensin receptor 2 blockers), 3-hydroxy-3-methylglutaryl-coenzyme A inhibitors or statins, beta-blockers, or blood-thinning medication.[12,25,26] Statins have been the most prescribed drug class in the United States and are used to treat hypercholesterolemia by lowering LDL cholesterol, apolipoprotein B, very low-density lipoprotein (VLDL), and plasma triglycerides. This is done by inhibiting the enzyme responsible for cholesterol synthesis catalysis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase.[26,27] Statins have proven to significantly decrease the number of cardiovascular events due to their anti-inflammatory and immunomodulatory effects.[9] Moreover, they are well-tolerated by most patients, but can have dose-dependent muscle-related adverse effects (myopathy) where the levels of plasma creatine kinase are 10 times higher than normal.[28] This condition goes along with pain, tenderness, weakness, and restriction in mobility.[28,29] The human monoclonal antibody, alirocumab, inhibiting proprotein convertase subtilisin-kexin type 9 (PCSK9), was found to decrease LDL levels in patients by 62% when added to a statin therapy.[30]

Ischemic preconditioning uses the transient induction of controlled and non-lethal myocardial low-flow ischemia and reperfusion to achieve cardio-protection. The ECS is activated by heat or bacterial lipopolysaccharides (LPS), and 2-arachidonoglycerol (2-AG) levels are increased.[31] 2-AG, but not anandamide, provides protection in isolated perfused rat hearts by reducing infarct size and myocardial damage.[31] The activation of CB2 receptors confers immediate protection against a subsequent prolonged and injurious period of ischemia.[31,32] Furthermore, ischemic preconditioning has a protective effect on diabetic and aging hearts.[33]

Angiotensin-converting-enzyme inhibitors and angiotensin receptor 2 blockers, such as ramipril and telmisartan, are used to treat hypertension by either competitive inhibition of ACE—which prevents the conversion of AT1 to AT2, or by blocking type 1 angiotensin 2 receptors on blood vessels to avoid binding of angiotensin 2. Both are powerful vasoconstrictors, however most of the patients do not achieve recommended blood pressure targets and remain at high cardiovascular risk and experience adverse effects like coughing.

The Endocannabinoid System

Physiological and Pathophysiological Roles of the ECS

The ECS is implicated in a great number of physiological processes. At the cellular level, the ECS controls cell proliferation, differentiation, cell survival, and apoptosis in different tissues such as adipose tissue,[34] epithelial cells, bone, blood, gonads, as well as the brain.[35,36] Endocannabinoids are synthesized in the central nervous system and regulate pain perception, motor functions, control of tremor and spasticity, learning, memory, thermogenesis, regulation of weak/sleep cycles, axonal pathfinding, synaptic plasticity, emotional behavior, stress response, feeding and appetite, reproductive function, and sex behavior. Besides the brain, endocannabinoids can be synthesized and function in peripheral tissues eg, the heart, GI tract, blood cells, adipose tissue, muscle, liver and pancreas; where they influence inflammatory responses, platelet aggregation, blood pressure, heart rate, vasodilatation, and energy balance.[36–38] Consequently, the endocannabinoid system is involved in many pathologies and can be correlated to neurodegenerative disorders, obesity, diabetes mellitus, cardiovascular disorders, cancer, and inflammatory processes.[35,37,38]

Endocannabinoids and Their Receptors

The endocannabinoid signaling system emerged as a potential therapeutic target over the past years. In focus are the Δ9-THC mimicking hydrophobic polyunsaturated fatty acid derivatives, anandamide and 2-AG, that bind and functionally activate one or both cannabinoid receptor subtypes (CB1 and CB2), as well as other receptors. Endocannabinoids are synthesized on demand in response to increased intracellular calcium concentrations or stimulated by metabotropic glutamate receptors located on postsynaptic neurons.

Anandamide and 2-AG

Anandamide was the first endogenously produced cannabinoid to be discovered in 1992.[31,39] The precursor for anandamide synthesis is the phospholipid N-arachidonoyl phosphatidylethanolamine, and the process is mediated by hydrolytic release of polyunsaturated acids by a phospholipase.[31,40,41] Generally, anandamide has a low overall efficiency and acts as partial agonist for CB1 and vanilloid TRP receptors and it also binds weakly to CB2 receptors.[42] Metabolism of anandamide happens rapidly by fatty acid amide hydrolase (FAAH).[31,43] 2-AG was discovered shortly after anandamide in 1995.[44,45] It is synthesized by the enzyme diacylglycerol lipase from arachidonic acid-containing diacylglycerol and metabolized by soluble monoacylglycerol lipase.[31,46] Compared with anandamide, 2-AG is produced in much higher numbers and binds potently to CB1 and CB2, but not to vanilloid receptor.[47] Binding results in retrograde (post to pre-synaptic) signaling and an inhibition of adenylate cyclase, as well as blocking of voltage-gated N-type calcium channels, which have important functions in the central nervous system and chronic and neuropathic pain perception.

Metabotropic Cannabinoid Receptors

The cannabinoid (CB) receptors CB1 and CB2 are both members of the superfamily of metabotropic G-protein-coupled receptors (GPCRs), and have been cloned and identified in the human, rat, and mouse myocardium.[40,43,48] The cannabinoid receptors are present in high abundance throughout the body where they, and their ligands, are involved in many important physiological functions and interactions with other neurotransmitters.[31,46]

CB1 receptors are abundant metabotropic G-protein-coupled receptors found predominantly in neurons of the brain at regions associated with higher cognitive functions, movement control, motor and sensory functions of the autonomic nervous system, and neurotransmission modulation.[31,49] In addition, CB1 receptors also function in the peripheral nervous system: in vascular and cardiac tissue, adipocytes, liver, GI tract and uterus to regulate basic physiological mechanisms such as energy balance and reproduction.[48,50,51] The CB1 receptor also has influence on memory and learning behavior, plays a role in addiction processes, and mediation of the psychoactive effect of Tetrahydrocannabinol (THC).[52,53] Signal transduction happens through the interaction with G proteins to inhibit adenylyl cyclase, activate mitogen-activated protein kinases, inhibit voltage-gated Ca^{2+} channels, and activate K^+ currents; as well as to influence nitric oxide signaling.[49,53] CB1 agonists include: Δ-9-THC, endogenous anandamide, 2-AG, 2-Arachidonoyl dopamine; and highly potent HU210, CP55940 and CP55244. Antagonists include: rimonabant, and structurally similar antagonists like AM241, taranabant, ACHSR, and AM4113.[53] CB1 receptors can be co-expressed with CB2 receptors.

CB2 receptors were first identified on macrophages and are also expressed on mast cells, B cells, some blood cells, and in the peripheral nervous system like the tonsils and thymus, and mediate cannabinoid-induced immune modulation.[9,40,49] Like CB1 receptors, CB2 receptors belong to class A serpentine receptors that are coupled to G proteins, and modulate pathways of adenylyl cyclase, mitogen-activated protein kinases, extracellular signal-regulated kinases 1/2 (ERK1/2), some Ca^{2+} and K^+ ion channels, and nuclear factors of activated T-cells and B-cells.[52] 2-AG is considered a primary agonist of CB2 receptors. Exogenous cannabinoids like THC and cannabinol and synthetic cannabinoids including WIN-55212-2 and CP55940 have also been described to bind to CB2.[54]

Ionotropic Cannabinoid Receptors (TRPV1)

The transient receptor potential cation channel subfamily V, member 1 (TRPV1) is an ionotropic non-selective cation channel that is predominantly expressed in peripheral sensory neurons and widespread in the cardiovascular system. TRPV1 ion channels have important functions as cellular sensors, and are involved in nociception, taste perception, thermosensation, mechano- and osmolarity sensing, and regulation of signal transmission.[15,55,56] In addition to ECS and physicochemical activators,[15,33,55–58] TRPV1 is activated by tetrahydrocannabinol, cannabinol, cannabigerol and some propyl homologs of THC and cannabigerol.[59–67] Cannabichromene (CBC), cannabidiol, and cannabinol are strong TRPA1 agonists and desensitizers, and THCV (from a botanical extract) is a potent regulator of TRPA1.[62]

The ECS in CVD Pathology

The endocannabinoid system plays a major role in the cardiovascular system, especially in disease states. Under normal conditions in healthy animals, modulation of the ECS has minor consequences and does not result in tonic changes.[43] In disease, however, the ECS is dysregulated, or dysregulation results in disease.[68] There are reports ranging from patient-reported and anecdotal evidence through in vitro and in vivo studies and some clinical/epidemiological studies that suggest modulation of the ECS occurs during the development of CVD pathology. This, in turn, suggests a therapeutic potential in the modulation of the ECS. Endocannabinoids and their synthetic analogs have important effects on the cardiovascular system, including complex mechanisms affecting the vasculature and the myocardium, but also autonomic outflow regulation through sites of action in the central and peripheral nervous system.[43,66,69,70] Modulation of the ECS has been suggested as a therapeutic

avenue for various disorders of the cardiovascular system ranging from atherosclerosis and restenosis, hypertension, cirrhotic cardiomyopathy; to myocardial infarction, and chronic heart failure.[42]

Myocardial Infarction (Ischemia/Reperfusion Injury)

In the myocardium in human adipose tissue of ischemic hearts, CB1 receptor expression is upregulated, accompanied by CB2 receptor and FAAH downregulation, as well as reduced cell survival signaling.[9,71,72] This goes along with an increase in circulating immune cells in obese patients with adverse cardiovascular events. Coronary dysfunction is associated with elevated endocannabinoid plasma levels in obese people, due to CB1 receptor upregulation, and results in cellular dysfunction and cell death in endothelial cells and cardiomyocytes.[73] The role of CB2 receptors is to decrease leukocyte infiltration and enhance pathways that contribute to survival.[9,71,72,74] TRP channels account for the sensing mechanisms of cardiac pain caused by myocardial ischemia.[75] TRPV1-positive sensory nerves react to multiple ischemic metabolites and sequelae (substance P, Calcitonin gene-related peptide (CGRP), pH) and cause chest pain.[33,76,77]

Heart Failure and Cardiomyopathy

In heart failure and cardiomyopathies, expression of cardiomyocytes and endothelial cells change; as well as the number of circulating immune cells and platelets. In cardiomyocytes and endothelial cells CB2 receptors initiate inflammation, and CB1 receptors promote cardiac dysfunction and cell death.[42]

Atherosclerosis

Atherosclerosis is an inflammatory condition in which the concentration of immune cells is elevated, and vascular smooth muscle cell expression is changed. CB2 receptors promote monocyte chemotaxis, infiltration and activation; leading to vascular inflammation, plaque development, and vascular smooth muscle cell proliferation.[9,71,72,74] Furthermore, CB1 receptor expression in macrophages and endocannabinoid concentration of anandamide and 2-AG in the blood is meaningfully higher in patients with unstable angina compared with patients with stable angina[47] indicating that receptor expression, and thereby theoretical endocannabinoid-receptor interaction, is directly linked to disease severity in patients with atherosclerosis. CB1 agonism reduces the blood pressure significantly more in spontaneously hypertensive rats than in normotensive rats, and CB1 receptor expression is higher in the heart and aortic endothelium in spontaneously hypertensive rats compared with normotensive rats.[43,50] In addition, Δ-9-THC-stimulated human T-cells show less proliferation and inhibition of interferon-gamma production, as well as downregulation of T-helper 1 cells[78] that are present in atherosclerotic lesions and which contribute to the inflammatory state of the lesions. The endocannabinoid system (ECS) and cannabinoid receptors (both CB1 and CB2) are highly active in cells found in atherosclerotic plaques such as macrophages and vascular smooth muscle cells.[2,79] Macrophages are activated by oxidized LDL through increased (anandamide) and 2-arachidonoylglycerol (2-AG) concentration, as well as upregulation of CB1 and CB2, thus initiating cholesterol accumulation in immune cells, affecting endothelial cells, and vascular smooth muscle cells.[2,79,80]

Pro-, and Anti-Atherogenic Effects of Cannabinoids

The active psychotropic component of marijuana (Δ-9-THC), along with other CB1 and CB2 ligands, have been suggested to have an anti-atherogenic effect on coronary artery disease. However, there are also reports that CB1 agonism is pro-atherogenic, where activation of CB1 results in activation of mitogen-activated protein kinases and increased AT1 receptor expression which elevates the concentration of reactive oxygen species, promoting endothelial cell injury, and lipid accumulation in macrophages.[81] CB1 antagonism and CB2 agonism have been suggested to be anti-atherogenic, where decreased Platelet-derived growth factor (PDGF) dependent proliferation and migration of vascular smooth muscle cells, repressed cytokine gene expression, less macrophage recruitment (2) Δ-9-THC agonism reduced levels of intracellular adhesion molecules, leading to less migration of monocytes, decreased inflammatory response and size-reduction of arterial plaque and inhibited action of oxidized LDL.[47,78,82] Finally, rimonabant, a selective CB1 antagonist used to treat obesity, reduces progression of atherosclerosis by lowering gene expression of proinflammatory cytokines in macrophages.[47] Interestingly, rimonabant also decreases progression of CAD at a low concentration (30 mg/kg instead of 50 mg/kg) while not affecting serum cholesterol and associated weight loss; but through decreasing the expression and number of macrophages, and associated proinflammatory cytokines in the aorta.[83] Administration of rimonabant decreases vascular smooth muscle cell proliferation and migration, and decreases inflammatory response in human coronary arteries.[82]

Exogenous Cannabinoids and CVD: Paradoxes and Therapeutic Potential

Exogenous cannabinoids refer to cannabinoids that are obtained from the plant *Cannabis sativa* (phytocannabinoids) or synthesized analogs. The most studied phytocannabinoids include Δ-9-tetrahydrocannabinol (Δ-9-THC), cannabinol, and

cannabidiol.[84] These compounds, as well as native mixtures from marijuana, are the subject of intense interest due to their therapeutic potential. Medical marijuana users report (anecdotally and outside of the peer-review system) effects such as stabilization of angina episodes, increased cardiovascular well-being and health. However, the heart-racing effects of acute marijuana exposure are well-documented, specifically after smoking marijuana, where both heart rate and left ventricular function significantly elevate for at least 1 hour. Direct use of marijuana in humans induced an amplified risk (up to 4.8 times) for myocardial infarction (MI) in the first hour of consumption; however, the risk rapidly declines after 1 hour.[4,7,85,86] In a study that evaluated data that spanned 15 years concluded that marijuana consumption is not independently associated with cardiovascular risk factors.[85] Nonetheless, there is a possible increased risk of mortality for patients who have already had a past myocardial infarction compared with marijuana smokers that did not.[87] Some early studies concluded that marijuana users do not have a significantly increased risk of stroke and heart failure later showed that those risks were significant upon longer follow up with multivariable analysis showing greater relative risk of myocardial infarction the younger group.[3,88] The CARDIA cohort study which spanned 25 years showed no association of marijuana use with CVD or atherosclerosis risk.[5,6] Furthermore, many studies have been inconclusive and suggest that further research on a larger scale with repeated measures are needed.[3,4,7] Despite the possible CVD risks associated with marijuana use, there is rational potential for modulation of the ECS using exogenous cannabinoids derived from plants or synthetic sources, as single drugs or complex mixtures, for treatment of disease in the CVD space. Potential therapeutic avenues are explored below:

Modulation of FAAH

Modulation of FAAH is one of the main gatekeepers in the control of many cardiac functions: FAAH-knockout mice are less susceptible to age-associated decline in cardiac function compared with wild-type mice, notably without disruption to CB1-regulated functions like body temperature and locomotion.[89] FAAH−/− mice have a higher endogenous concentration of anandamide (and related fatty acid amides) in brain, heart, liver, and other organs.[90,91] Moreover, endocannabinoids tonically suppress contractility in hypertension. By increasing the concentration of endogenous anandamide (by inhibition of FAAH), reduced blood pressure, cardiac contractility, and vascular resistance can be achieved.[50]

Modulation of CB1

Recent studies suggest that CB1 receptors play a major role in cardiovascular regulation. This has been implicated in ischemic preconditioning and ischemia-reperfusion injury of the myocardium.[43,50] CB1 receptors that are in the myocardium mediate negative ionotropy, both in vivo and in vitro,[42] making the heart less contractile. In vascular tissues, CB1 activation results in vasodilation. Both effects are assumed to accompany the pro-hypotensive effect of anandamide in anesthetized rodents. Moreover, CB1 antagonism reverses the effect in spontaneously hypertensive rats resulting in increased blood pressure and left ventricular contractile activity. Conversely, CB1 agonism with anandamide tonically suppresses cardiac contractility in hypertension, leading to normalized blood pressure, reduced cardiac contractility and vascular resistance when blocking the degradation of anandamide;[50] representing a good approach for therapeutic intervention to decrease cardiovascular risk factors such as hypertension.[9]

Modulation of CB2

There is experimental evidence implying CB2 receptors are involved in the progression of atherosclerosis. The effects of oral administration of Δ-9-THC in a murine model of established atherosclerosis was determined, and results showed a significant inhibition of disease progression with increased expression of CB2 receptors on atherosclerotic plaques in both humans and mice.[92] CB2 agonism results in less proliferation capacity of lymphoid cells and decreased interferon-gamma secretion; as well as inhibition of macrophage chemotaxis.[92] This protective effect could be reversed by CB2 antagonist SR144528.[9]

Modulation of Ionotropic Cannabinoid Receptors

Vanilloid receptors mediate the response of cardiac spinal afferent nerves after an ischemic period and act as molecular sensors to detect myocardial and tissue ischemia and activating cardiac nociceptors.[75,93] Pretreatment with capsaicin results in cardiac dysfunction with increased left ventricular end-diastolic pressure and leads to altered expression of a variety of neuronal and non-neuronal genes in the heart.[94] TRPV1 receptors increase SP release from capsaicin sensory neurons to protect the heart from injury, and regulate normal cardiac function and development of cardiac adaptation to ischemic stress.[95,96]

Additionally, 12-lipoxygenase-derived eicosanoids are found to protect against myocardial I/R injury by activation of TRPV1 in Langendorff rat hearts.[97] A mixture of 12(S)-HpETE (12-LOX arachidonic acid metabolite and endogenous ligand for TRPV1) and arachidonic acid was used. Infarct size was reduced by 40%, an effect that could be reversed by the 12-LOX inhibitor baicalein, or by desensitization with capsaicin on local sensory C-fiber afferent nerves. HpETE and arachidonic acid both caused dose-dependent coronary

vasodilation that was suppressed by TRPV1 antagonist capsazepine, or with a CGRP antagonist, clearly showing the upregulation of TRPV1 during I/R injury.[96,97]

In vivo, mice lacking functional TRPV1 were protected from pressure overload cardiac hypertrophy compared with wild-types, showing that TRPV1 may be involved in the progression of cardiac hypertrophy.[15] Recent studies also show that pre-clinical administration of the TRPV1 antagonist BCTC (4-(3-Chloro-2-pyridinyl)-N-[4-(1,1-dimethylethyl)phenyl]-1-piperazinecarboxamide) resulted in prevention of loss of heart function in a mouse model of cardiac hypertrophy and heart failure; indicating that TRPV1 antagonism offers novel treatment options.[98]

Comparison between TRPV1 KO mice and their wild-type littermates show that the genetically modified mice have no pain response, less swelling after subcutaneous injection with vanilloid compounds, and an attenuated response to acidified environments or heat (43°C). TRPV1-/- mice had no reduction in body temperature after subcutaneous injection with capsaicin, and homozygous mice show no aversion of drinking capsaicin-supplemented drinking water.[99] Furthermore, TRPV1 knockouts are hypometabolic (less oxygen consumption), hypervasoconstricted (lower-tail skin temperature), preferred lower ambient temperature, and showed a higher locomotor activity compared with wild-types.[93] Administration of the TRPV1 endogenous agonist anandamide and exogenous agonist Resiniferatoxin (RTX) both induced stress-related hyperactivity in TRPV1+/+ mice, but not in TRPV1 knockouts.[93]

Summary: Promise and Challenges for Cannabinoid Therapeutics and CVD

Endocannabinoids and cannabinoid-related compounds may be a promising approach as therapeutic agents for cardiovascular diseases. However, there are several challenges that arise when considering these targets.

Likely Side Effect Profiles of Drugs Targeting Broadly Expressed Metabotropic and Ionotropic Cannabinoid Receptors

Putting the striking progress aside when it comes to understanding the functions of the TRP channel family, some problems persist. TRPV1 channel ligation can be beneficial in one organ and induce unacceptable adverse side effects in another.[56] For example, in clinical trials, small molecule TRPV1 (TRPV3 and TRPA1) antagonists were used to treat inflammatory, neuropathic, and visceral pain, but studies were terminated because of adverse effects induced by TRPV1 antagonists.[100] Hyperthermia and impaired noxious heat sensation placed patients at risk for scalding injury. Second generation TRP antagonists that do not induce an increase in core body temperature has been reported, nevertheless the therapeutic utility is not yet known.[100] Administration of CB1 agonists in vivo causes complex hemodynamic changes, including changes of heart rate as well as increased and decreased blood pressure.[101]

Regulatory and Formulation Issues Arising From Use of Plant-Derived Compounds or Native Mixtures

Safety and efficacy of cannabis-derived medicines are a major issue. If therapeutic potential resides in a single molecule component or a derivative, then production and regulation of the therapy are straightforward. If the efficacious agent is a complex mixture that reflects some or all of the secondary metabolome complexity of *C. sativa*, then safe and consistent production become challenging. In this case, controlled preparation of synthetic mixtures is likely to be more desirable than attempting to produce native extracts to the level of consistency required for FDA approval.

In summary, the endocannabinoid system is highly active in cardiovascular disease states. Modulation of the ECS, CB1, and TRPV1 antagonism, as well as CB2 agonism, have proven to modulate disease state and severity in CVD. Studies are underway to develop drugs to change the course of cardiovascular diseases. Areas of promise include: CB1 antagonism which has an anti-inflammatory effect and reduces smooth muscle cell proliferation, as well as CB2 agonism which results in decreased expression of adhesion molecules, reduced inflammatory response, reduced plaque size in atherosclerosis and inhibits the action of oxidized LDL. Of particular interest is the fact that TRPV1 antagonism protects the heart from heart failure-associated remodeling, especially given the plethora of available TRPV1 antagonists tested in Phase II clinical trials for pain, and other indications.

Further research and clinical trials are necessary to develop a safe agonists and antagonists for the treatment of cardiovascular diseases, and ligands for CB1, CB2 and TRPV1 that are found in cannabis may yet play a role in either guiding rational design, the demonstration of effective systems to target, or as single or combinatorial therapies in their own rights.

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