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Anandamide and endocannabinoid system: an attractive therapeutic approach for cardiovascular disease

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Abstract: Cardiovascular disease is currently not adequately managed and has become one of the main causes of morbidity and mortality worldwide. Current therapies are inadequate in terms of preventing its progression. There are several limitations, such as poor oral bioavailability, side effects, low adherence to treatment, and high dosage frequency of formulations due to the short half-life of the active ingredients used, among others. This review aims to highlight the most relevant aspects of the relationship between the cardiovascular system and the endocannabinoid system, with special attention to the possible translational effect of the use of anandamide in cardiovascular health. The deep and detailed knowledge of this interaction, not always beneficial, and that for years has gone unnoticed, is essential for the development of new therapies. We discuss the most recent and representative results obtained in the field of basic research, referring to the aforementioned subject, emphasizing fundamentally the main role of nitric oxide, renal physiology and its deregulation in pathological processes.

Keywords: anandamide, cardiovascular disease, CB_1 receptors, endocannabinoid system, nitric oxide

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Introduction

Hypertension, one of the most frequent cardiovascular diseases, remains the most important risk factor for the development of associated cardiovascular pathologies and it affects millions of people around the world. Although there are a large number of antihypertensive therapies, these are insufficient to properly control blood pressure. The failures and disadvantages of conventional therapies have stimulated the research for the development of new types of antihypertensive alternatives. These new agents would possess distinct mechanisms of action that would allow a better blood pressure control and more effective prevention of cardiovascular disease, and others related, such as myocardial infarction and stroke.[1]

Cannabinoids (CBs) and their endogenous and synthetic analogs induce important hypotensive effects by complex mechanisms. It has been found recently that the endogenous CB system is involved in the mechanism of hypotension associated with hemorrhagic, endotoxic, and cardiogenic shock, as well as with advanced liver cirrhosis. In this sense, it has also been proposed that the pharmacological manipulation of the endocannabinoid system (ECS) may offer a new approach to antihypertensive therapy.[2] For this reason, we decided to focus our review on the cardiovascular effects of endocannabinoids (eCBs) and its action on blood pressure.

The central interest of our present review is to highlight the effects of the ECS, which is less known that of the phytocannabinoids. The eCB mediators have additional objectives, not shared with phytocannabinoids. Therefore, the superposition of mechanisms between eCBs and phytocannabinoids is only partial and difficult to analyze simultaneously.[3]

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The ECS consists of arachidonic-acid-derived mediators such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), receptors [CB receptor 1 (CB_1) CB_2, CB_1/CB_2, GPR55, TRPV1, among others], and enzymes that are involved in the hydrolysis of these mediators. Among the enzymes implicated are fatty acid amide hydrolase (FAAH), which catalyzes the *in vivo* catabolism of AEA to arachidonic acid and ethanolamine,[4–7] monoacylglycerol lipase for the metabolism of 2-AG, and cyclooxygenase 1 and 2 (COX-1/2) implicated in the metabolism of AEA and 2-AG.[8] Thereby, AEA and 2-AG are endogenous ligands for CB receptors.[4] There is, strong evidence that the ECS participates in the adjustment of the cardiovascular system, especially in arterial hypertension (AHT).[9] In investigations previously performed on anesthetized spontaneously hypertensive rats (SHRs), the use of CB_1 receptor antagonists has been shown to increase blood pressure with values higher than usual for this strain, and administration or inhibition of AEA degradation products causes hypotension. These discoveries indicate that the ECS could be a possible therapeutic target of relevance in AHT.[10] In addition, further trials were performed in rats under the effects of anesthesia where the hypotensive effect of 2-AG remained over time, even with the use of rimonabant (selective CB_1 receptor antagonist), however, the effect was blocked by indomethacin indicating the participation of a cyclooxygenase (COX) enzyme metabolite.[11] Therefore, it is inferred that endogenous ligands of the ECS and its metabolites and derivatives are involved in the physiological actions of the cardiovascular system. It has also been reported that in addition to the aforementioned enzymes, several isoforms of the cytochrome P450 (CYP450) enzyme complex exert their action on endogenous arachidonic acid to generate epoxyeicosatrienoic and hydroxyeicosatetraenoic acids that function as powerful intermediaries of signposting and have a relevant action in the regulation of blood pressure and also in other physiological processes. Because of the structural similarity that exists between AEA and lipid compounds, it is probable that some CYP450s may be implicated in the metabolism of AEA.[7] In addition, CB receptors such as the transient receptor potential cation channel subfamily V type 1 (TRPV1) are located predominantly in nerve fibers that innervate the cardiovascular system, the activation of these receptors stimulates the delivery of a series of sensory neuropeptides, including gene-related peptide of calcitonin (CGRP) and substance P, mighty vasodilators that exert their action in multiple vascular beds. Previous studies have shown that TRPV1 blockade increases blood pressure in Wistar rats with hypertension induced by the deoxycorticosterone (DOCA)-salt model, in addition, it was observed that a diet rich in sodium chloride could activate TRPV1 receptors, generating an antioxidant protective and anti-inflammatory effect.[12] Other investigations report the involvement of the AEA transporter in the CGRP production and blood pressure regulation. In the first series of experiment, BPs of patients with essential hypertension was monitored, and blood samples were collected for determining plasma levels of AEA and CGRP. Lymphocytes of peripheral blood were separated to measure the activity of AEA transporter. In the second series of experiment, hypertensive rats (SHR) were used to seek the explanations for the phenomena observed in hypertensive patients. In addition to measuring plasma levels of AEA and CGRP and the activity of AEA transporter, the dorsal root ganglia (DRG), the major site of CGRP synthesis, were saved for CGRP mRNA expression analysis. In the third series of experiment, plasma levels of ADMA and nitric oxide in the above-mentioned hypertensive rats were determined, and in where it was observed that in both groups the plasma level of AEA was high but the activity of the AEA transporter was simultaneously reduced with a decrease of CGRP synthesis.[13] To understand the impact of AEA on the relaxation of thoracic aortas in renal vascular hypertensive rats, studies have proved that AEA stimulated a remarkable relaxation in the rat aortas, which was dependent on the endothelium by activation of the receptors CB_1 and CB_2, and the oxidative phosphorylation of endothelial nitric oxide synthase (eNOS) for the nitric oxide (NO) production.[14]

Other research groups have shown that the ECS is not only involved in the modulation of responses during hypertensive pathology but also has a number of beneficial effects in other types of cardiovascular diseases.[15,16] Moreover, it has been shown that the action on CB_2 receptors is implicated in the adhesion, migration, proliferation, and function of immune cells during the process of atherosclerotic plaque formation.[11]

Recent studies have demonstrated other endogenous lipids with structural similarities to AEA may also produce vascular actions. This set of AEA analogs includes the N-acylamino acids which have been identified in various mammalian tissues. An example of the aforementioned compounds is N-arachidonoyl dopamine, a CB_1 receptor and TRPV1 agonist that causes mesenteric

vasorelaxation. Furthermore, it has also been shown that N-arachidonoyl serine, which does not exert activity on the classical receptors type CB or TRPV1, is also a vasorelaxant.[17]

Several reports show that particularly high levels of AEA and the enzymes that metabolize this eCB are found in the kidneys. Likewise, the renin–angiotensin–aldosterone system (RAAS) has been characterized at the renal level as the main prohypertensive hormonal system. On the other hand, the kidney also contains an endocrine antihypertensive system that is responsible for three specific biological properties: vasodilation, inhibition of sympathetic activation and renal excretion of ions and water. The AEA and COX-2 metabolites located in the renal medulla stand for a significant antihypertensive system implicated in the long-range regulation of blood pressure. Thus, the inducible isoform of the enzyme cyclooxygenase (COX-2) is known to be constitutively expressed in the kidney, particularly in the medulla.[6] COX-2 was shown to metabolize AEA to prostaglandin ethanolamide analogs called prostamides.[18] When AEA was administered by infusion into the renal medulla, there was a significant increase in urinary flow with elevation of natriuresis. This finding indicates that an AEA metabolized by COX-2 action would indirectly produce the renal excretory effects of this eCB.[6] The AEA and its metabolites are also involved as modulators and mediators of signaling in the inflammatory process, so they could be involved in chronic kidney disease processes associated with inflammation and cardiovascular disease. Contemporaneous cognizance of the AEA and its derivative roles indicates the growing need for major investigation to determine and study the potential action of ECS in the kidney.[19] In addition, the presence of functional CB_1 receptor at the renal level, has also been documented, but there is still disagreement about the expression of CB_2 receptors in this organ. Moreover, the renal medulla was shown to have high levels of AEA relative to the cortex, and that AEA intramedullary administration incremented urine volume along with sodium and potassium elimination, with little effect on mean arterial pressure (MAP).[6] A well-known feature of AEA is that, when administered exogenously, it has the ability to stimulate NO release by renal endothelial cells, suggesting a fundamental activity of ECS in the regulation of renal hemodynamics. Other studies have reported that AEA increases renal blood flow and reduces the glomerular filtration rate by activation of CB_1 receptors present in afferent and efferent arterioles. These findings are independent of their effects on blood pressure and sodium excretion rate.[20] A recent study showed that AEA regulates sodium transport at the loop of Henle ascending thick limb level. In this segment, AEA stimulates the production of NO, blocks the apical transporter Na+/H+ and the cotransporter Na+/K+/2Cl^-, through the CB_1 receptor,[21] suggesting an additional AEA activity as a diuretic agent. Another important sodium transporter that maintains the volume and composition of the extracellular fluid is the Na+/K+-adenosine triphosphatase (ATPase) pump situated in the cells of the proximal tubule, which can also increase diuresis when blocked by NO.[20]

It is also noted that eCBs influence the behavioral responses associated with their lipophilic nature, which allow them to easily cross the blood–brain barrier. In anesthetized rats, microinjection of AEA into the periaqueductal gray matter of the central nervous system (CNS) caused an increment in renal sympathetic nervous activity with increased blood pressure. The response was diminished by previous microinjection of the CB_1 receptor antagonist, AM-281, at the same site.[22] These data suggest that eCBs can regulate the sympathetic and cardiovascular constituents through CB_1 receptors causing opposite effects, depending on where they act. Related to this subject, recent review articles published by our laboratory describe the importance of the use of new therapeutic alternatives with pharmacological vectorization for the treatment of various cardiovascular diseases, especially when it comes to the use of complex active principles such as CBs.[23–25]

Effect of anandamide on the cardiovascular system

Previous knowledge indicates that one of the most well-known actions of AEA on the cardiovascular system is its ability to induce vasorelaxation in a large number of vascular beds by various mechanisms. Likewise, this eCB is able to elicit multiple additional responses, such as those reported in recent studies, where injecting an endovenous bolus of AEA into anesthetized SHRs produced a three-phase response similar to that observed with tetrahydrocannabinols. The initial phase of this reaction consisted of a dramatic drop in heart rate and blood pressure that continued for a few seconds. This vagal component is followed by a short hypertensive replay not mediated through the sympathetic nervous system, nor by the participation of CB_1 receptors, and it is suggested

that it consequently stems from vasoconstriction of certain vascular territories such as the spleen. The third and most relevant response induced by AEA is related to hypotension and modest bradycardia, which persists for 2–10 min. This phase is absent in normotensive conscious rats, but is present and has a long duration in awake SHRs. Evidence indicates that hypotension of this third stage produced by CBs is mediated by CB_1 receptors. One study has shown the marked positive correlation between the concentrations of diverse CB agonists causing hypotensive and bradycardic effects and their affinity constants for binding to CB_1 receptors in the brain.[10] Confirming this, is the absence of hypotension and bradycardia produced by CBs in mice lacking CB_1 receptors.[10,11]

Furthermore, it has been suggested that AEA relaxes blood vessels *in vitro* through a yet unidentified 'non-CB_1/non-CB_2' vascular CB receptor that is responsive to O-1918 {1,3-dimethoxy-5-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-benzene}, a synthetic analog of cannabidiol without psychoactive effects.[26] In addition, other studies manifest that part of the reduction in blood pressure observed in the course of the third phase of the cardiovascular effect of AEA (described in the previous paragraph) could be related to the activation of the 'non-CB_1/non-CB_2' receptor.[27] This new 'non-CB_1/non CB_2' endothelial receptor, called CBe, would participate in the vasodilator effects of a significant amount of CBs and in a diversity of vascular beds. The precise identity of this receptor is still unrevealed, and a potential candidate would be the orphan GPR18 receptor.[28] A metabolically stable analog of AEA, R-methanandamide has also been shown to produce hypotension and bradycardia in animal prototypes, as well as vasorelaxation in several vascular beds. This vasodilation is also independent of CB_1 or CB_2 receptors, and stems from the activation of a putative AEA 'non- CB_1/non-CB_2' receptor. In rabbit aortic ring preparations, rimonabant did not block the R-methanandamide-mediated vessel relaxation dependent on the endothelium, which reinforces the hypothesis about the possible existence of the CBe receptor.[29]

Another study in conscious rats whose acute hypertension was brought about by administration of angiotensin II (Ang II) and arginine–vasopressin demonstrated that CBs can induce a decrease in blood pressure and massive vasodilation. Under these conditions, the hemodynamic effects of AEA would not be produced through the CB_1 receptor; instead, they would be modulated by the inhibition of the FAAH enzyme.[30] There are data indicating that TRPV1 receptors also promote the cardiovascular effects of AEA in some models of hypertension, including SHRs and rats fed a high salt diet, probably due to the high production of AEA in response to increased arterial pressure induced by sodium chloride, and at the same time leading to a greater release of CGRP.[31] These effects would possibly imply an impairment of peripheral vascular reactivity and renal function and may have a regulatory action on the increase of salt-induced blood pressure. The synthetic CB, WIN55212-2, also produced depressant and vasodilating effects on acute hypertension, mediated by CB_1 receptors. These results clearly demonstrate that the hypotensive mechanisms of action rely on the type of CB involved and the type of hypertension being treated in each particular case.[30,32]

In other investigations, use of FAAH inhibitors, such as URB597 and AM3506, have been observed to normalize elevated blood pressure and heart rate, as well as cardiac contractility of SHRs, without modifying those variables in normotensive rats. The AM3506 manages to exert its effects through the inhibition of FAAH activity, which correlates with the increase in levels of tissue AEA and the consequent activation of CB_1 receptors. The most intense response observed in hypertensive animals compared with normotensive animals would be associated with an increment in the binding of CB_1 receptors to G proteins at the CNS level in the group of SHR animals. In addition, chronic blockade of FAAH not only allows blood pressure to normalize, but also limits the evolution of associated cardiac hypertrophy, a major risk factor in cardiovascular incidents and death (Figure 1).[33] However, a study of precontracted human pulmonary arteries (hPA) showed that AEA decomposition products, COX pathways, NO, potassium channels and CBe receptors, play an important role in induced relaxation by AEA in the hPA endothelium.[5] It is inferred that FAAH metabolism products are involved in such relaxation, and a concomitantly high expression of FAAH was demonstrated in lungs and human pulmonary arteries. Also, the use of the FAAH inhibitor, URB597, reduced the AEA-induced relaxation in hPA. In this regard, it is known that arachidonic acid derived from AEA can be converted by the COX-1 or COX-2 enzymes into vasoactive eicosanoids, such as prostaglandins and prostacyclins. In this study, the nonselective COX inhibitor (indomethacin) and the selective COX-2 antagonist (nimesulide)

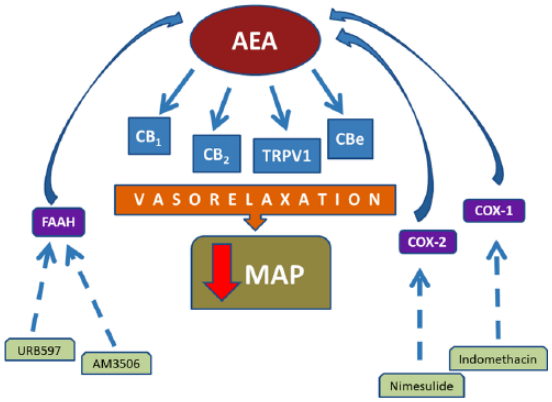

Figure 1. Anandamide's signaling pathways linked to vasorelaxation.
This effect would mainly occur through the activation of CB_1/CB_2, TRPV1, and CBe receptors. Inhibition of the enzymes COX-1/COX-2 and FAAH, responsible for the metabolism of AEA, would increase the bioavailability of AEA, increasing its effects on the cardiovascular system. Continuous lines indicate 'activation' and 'inhibition or blocking' dashed lines. CB, cannabinoid; TRPV1, transient receptor potential cation channel subfamily V type 1; CBe, non-CB_1/non CB_2 endothelial receptor; COX, cyclooxygenase; FAAH, fatty acid amide hydrolase; AEA, anandamide; MAP, mean arterial pressure.

decreased AEA-induced relaxation, confirmed by a decrease (of 20% with indomethacin, and of 55–80% with nimesulide) in the relaxant effect provoked by the highest concentration of anandamide used. A similar investigation determined that AEA produced relaxation of the rat pulmonary arteries previously contracted with U-46619. The CB_1, CB_2 and TRPV1 receptor antagonists (AM251, AM630 and capsaicin, respectively) did not modify the response induced by AEA, whereas AEA was shown to produce endothelium-dependent relaxation of the rat pulmonary arteries by stimulation of the O-1918-sensitive CBe receptor and prostacyclin type A vasoactive derivatives.[34]

Regarding the cardioprotective effect of AEA and ECS, one study investigated the cardioprotective role of propofol mediated by ECS in an *in vivo* model of ischemia/reperfusion injury and an *in vitro* model of hypoxia/reoxygenation injury. The results showed that administration of propofol in both models produced an increase in the values of AEA and 2-AG. It was also observed that in the *in vivo* model, the infarct size, and in the *in vitro* model, the cardiomyocyte apoptosis, both decreased. These effects were mediated by an increase in the concentration of antioxidant species and decrease in pro-oxidant substances. Additionally, responses similar to those of propofol were obtained when using URB597 (inhibitor of eCB degradation) and VDM11 (inhibitor of eCB reuptake).[35]

Lu and colleagues[36] determined that eCBs play a fundamental role in diminishing endothelin-1-induced hypertrophy and explored the signaling pathways involved. Hypertrophic indicators such as cardiomyocyte enlargement and fetal gene activation were silenced by anandamide and its metabolic stable analog, R-methanandamide. The capacity of R-methanandamide to inhibit the hypertrophic indicators was mediated by CB_2 and CB_1 receptors, respectively.

Another study conducted in rats showed that AEA administered intravenously would exert a cardioprotective effect against ischemia-reperfusion injury through the generation of heat shock protein 72, whose effects would be mediated by CB_2 receptor activation.[37]

It has also been shown that AEA would function as a cardioprotective against cardiac dysfunction induced by doxorubicin treatment. It was observed that the left ventricles of those animals that were treated with AEA prior to the administration of doxorubicin did not show a decrease in fractional shortening, ventricular wall thickness and developed pressure in the left ventricle in contrast to those animals that had received pre-treatment with AEA.[38]

Other results indicate that during the first phases of myocardial infarction, the activation of presynaptic CB_1 receptors by eCBs would inhibit the neurotoxic effects that cause an increase in heart rate and vasoconstriction. Therefore, the inhibition of the exacerbated release of noradrenaline by the sympathetic nerves that innervate the heart and blood vessels mediated by CB_1 receptors, would act as a cardioprotective in ischemia.[39]

It is concluded that AEA and its synthetic analogs produce vasodilation in a wide variety of vascular beds by various types of receptors such as CB_1, TRPV1 and CBe. The participation of one or the other receptors depends on their location, the type of vascular bed and the conditions under which these vascular beds are located. Additionally, the greater bioavailability of AEA mediated by FAAH inhibition increases its vasorelaxant action. It should be noted that in the particular case of the pulmonary arteries, the AEA exerts its vasorelaxing effect together with the collaboration of metabolites derived from its

own degradation by the COX enzyme. In addition, and of particular interest for the present review, numerous investigations support an important cardioprotective role of AEA and its analogs during the development of multiple heart-level effects under different circumstances.

Active compounds related to anandamide and its cardiovascular effects

Although one of the most studied eCBs is the AEA, there are also other active AEA-derived compounds with structures and pharmacodynamic behavior similar to arachidonic acid that turn these substances into active ingredients relevant for use with scientific and therapeutic purposes. Related to this, it is known that the cardiovascular effects of AEA are potentiated during hypertension, which is why some studies have inquired how hypertension influences the responses to oleamide, an eCB-type fatty acid amide structurally similar to the AEA.[40] The vasorelaxant responses of oleamide in SHR aortic rings were significantly elevated, similar to AEA, compared with Wistar Kyoto normotensive controls (WKY), which were unaltered by the inhibition of the NOS enzyme with N(ω)-nitro-L-arginine methyl ester (L-NAME) of the FAAH with URB597, being in turn independent of the endothelium CB_1 receptors. However, inhibition of COX with indomethacin increased vasorelaxant reactions in the WKY aortas, so that they were equivalent to those of SHRs. These results indicate that the COX pathway has a key role in regulating oleamide-induced vasodilation in the aortas of normotensive rats, and the effect disappears during hypertension, probably as a process of adjustment to the augmentation in blood pressure.[41]

Parmar and colleagues[17] carried out a study to determine whether N-arachidonoyl glycine (NAGly), an endogenous lipid with structural characteristics similar to AEA that does not activate CB receptors, could modulate vascular tone; from the results, the authors concluded that NAGly would produce vasorelaxation in small mesenteric arteries of rats by calcium-activated voltage-dependent potassium channels (BKCa). In this case, it is suggested that NAGly would bind to an unknown receptor coupled to G protein by stimulating the NO endothelial release and consequently activating BKCa in the vascular smooth muscle.

Moreover, it is known that estrogens and phytoestrogens are stimulants of AEA effects on the vascular wall by the activation of estrogen receptors. They promote the AEA release from human endothelial cells and potentiate AEA-induced vasorelaxation, increasing the bioavailability of CGRP in rat mesenteric vessels. The CGRP is delivered, at least in part, as an outcome of activation of the AEA-induced TRPV1 receptor.[42]

There is an eCB molecule known as N-oleoylethanolamine (OEA) that was originally found mainly in the small intestine and was related to regulation of food intake and weight loss. However, recent reports in rat mesenteric arteries noted that OEA levels are approximately 100-fold greater than AEA levels. OEA, besides being a substance that promotes satiety, is also a vasorelaxant of the mesenteric arteries, and it has been found less potent than AEA.[43] In a study where the main objective was to assess the vascular effects of OEA, production of a dose-dependent vasorelaxation was observed in insulated mesenteric artery beds and in thoracic aortic rings of rats, with a considerable response in mesenteric vessels. The vasorelaxation induced by OEA was blocked by a CB_1 receptor antagonist only in aortic rings. The vasorelaxant effects of OEA depend in part on the sensory nerve activity mediated through TRPV1 receptors and a functional endothelium. Additionally, vasorelaxation induced by OEA increases after inhibition of COX. The OEA may also impede the release of intracellular calcium into arterial preparations, which also leads to smooth muscle relaxation of vascular walls.[44] In conclusion, several structurally similar compounds to AEA also produce vasorelaxant effects like those of AEA, although through different mechanisms. Of special interest, estrogenic compounds produce an enhancement of the effect of AEA by increasing their release, which is why they are also considered to be related to AEA compounds despite the absence of a structural kinship between them (Table 1).

Side effects of anandamide and other endocannabinoid at vascular level

The involvement of the inflammatory process and reactive oxygen species in atherogenesis is well known, and it has been reported that ECS also plays a fundamental role in the development of this pathology.[45] Specifically, links between ECS, reactive nitrogen species, oxidative stress, and atherosclerosis have been postulated. Signaling triggered by the CB_2 receptor has been

Table 1. Active compounds related to anandamide and its cardiovascular effects.

Compound	Action site	Mechanism of action	Structural relation with AEA
Oleamide	Aortic rings (rats)	COX signaling pathway	Yes
N-arachidonoyl glycine	Small mesenteric arteries (rats)	Unknown receptor coupled to G protein induction–NO release and BKCa activation	Yes
Estrogens and phytoestrogens	Endothelial cells (human) Mesenteric vessels (rats)	AEA release–TRPV1 activation–CGRP delivery	No
N-oleoylethanolamine	Mesenteric arteries and thoracic aortic rings (rats)	CB_1 activation of COX signaling pathway–interference of intracellular calcium release	Yes

AEA, anandamide; BKCa, calcium-activated voltage-dependent potassium channels; CB_1, cannabinoid receptor 1; CGRP, gene-related peptide of calcitonin; COX, cyclooxygenase; NO, nitric oxide; TRPV1, transient receptor potential cation channel subfamily V type 1.

shown to increase the regulation of anti-inflammatory and antioxidative pathways, and that CB_1 signaling provokes opposite effects. The eCB levels and its receptors are elevated in the vessels' pathological processes, suggesting that ECS is activated in the wall of the vasculature in these pathologies.[46] In similar studies, the effects of CB_1 receptor activation with AEA or the synthetic agonist HU210 on cell death and signal transduction pathways interrelating with human coronary artery endothelial cells were investigated.[47] It was shown that CB_1 receptor activation in endothelial cells augments the cell death process in pathological states, when the eCB synthesis or metabolic pathways are deregulated by exaggerated inflammation or oxidative/nitrosative stress, thus collaborating to the apparition and progress of endothelial dysfunction and the pathophysiology of various cardiovascular effects. Moreover, inhibition of CB_1 receptors could produce beneficial results in kidney diseases, and probably in other diseases associated with inflammation, oxidative/nitrosative stress, and cell death.[48] Likewise, it has been reported in several models of obesity and diabetes mellitus type 1 and 2 that the eCBs produced in renal cells activate the CB_1 receptors and help development of oxidative stress, inflammation, and renal fibrosis. These effects can be modulated chronically by CB_1 receptor antagonists. On the contrary, the activation of renal CB_2 receptors decreases the injurious effects of these chronic pathologies,[20] while their antagonism with selective CB_2 ligands such as SR144528 would result in blocking the anti-inflammatory effects elicited by the activation of these receptors (Figure 2).[49] However, some studies carried out at the CNS and digestive level have reported antioxidant effects of the CB_1 receptor activation,[50–52] which could be explained by the existence of probable antagonistic effects of these receptors according to their anatomical location, which also reinforces the search of pharmacological vectorization strategies. In this sense, the healing potential of CB_1 receptor antagonists and agonists (depending on its localization) is restricted because of their side effects on CNS, such as impairment of cognition and learning, among others.[5] Therefore, the development of these receptors antagonists and agonists with action on specific peripheral sites, could mean an innovative therapeutic focusing for the treatment of various types of diseases, in which the ECS would be involved.

In addition, there have been studies on the ECS involvement in the development of human aortic aneurysms, in which the levels of messenger RNA (mRNA) of CB receptors (CB_1, CB_2, TRPV1, and GRP55) have been reported to be considerably more in those patients with aneurysms than in controls. The concentration of 2-AG was significantly higher, and the levels of AEA and its metabolite palmitoylethanolamide were significantly lower in subjects with aneurysms. The mentioned data provide evidence on the activation of ECS in human aortic aneurysms

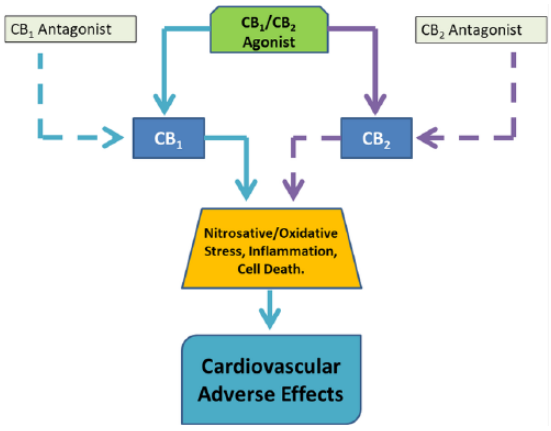

Figure 2. Cardiovascular adverse effects mediated by cannabinoid 1/cannabinoid 2 receptors under pathological conditions.
Activation of CB_1 receptors or antagonism of CB_2 receptors leads to an increase in oxidative/nitrosative stress in some cell types, which triggers an exacerbated inflammatory process often culminating in cell death. In contrast, activation of CB_2 receptors or antagonism of CB_1 receptors produces cytoprotective effects that prevent the occurrence of adverse effects at the cardiovascular level. Continuous lines indicate 'activation' and 'inhibition or blocking' dashed lines.
CB, cannabinoid.

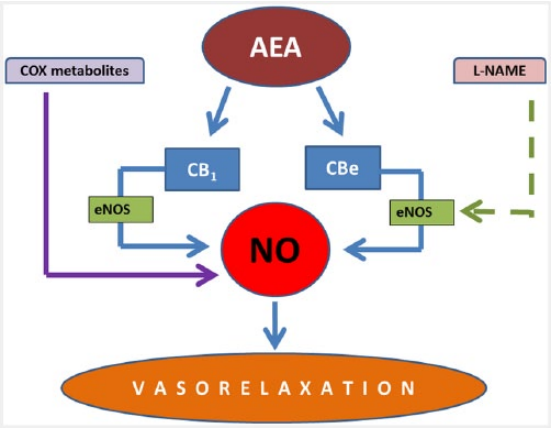

Figure 3. Central role of nitric oxide as mediator of vasorelaxation induced by anandamide.
The agonism of the CB_1 and CBe receptors with AEA provokes the activation of the enzyme eNOS responsible for NO synthesis, stimulating a drastic increase in the concentration of this vasodilator factor at the vascular endothelium level. The production of prostacyclin-like metabolites derived from the action of COX on AEA also boosts NO synthesis, whereas inhibitors of eNOS such as L-NAME prevent it. Continuous lines indicate 'activation' and 'inhibition or blocking' dashed lines.
CB, cannabinoid; AEA, anandamide; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; COX, cyclooxygenase; L-NAME, N(ω)-nitro-L-arginine methyl ester; CBe, non-CB_1/non CB_2 endothelial receptor.

associated with chronic inflammation and vascular remodeling.[53]

Recent evidence shows there are interactions between the RAAS system and the ECS, suggesting AEA and 2-AG can regulate vascular contraction caused by Ang II, which can occur independently of the CB_1 receptor. This process is of great importance since, for example, increasing vascular sensitivity to Ang II is a marker of eclampsia.[4] In this way, it can be concluded that despite its beneficial effects on the vasculature and related organs, AEA and other eCBs can also produce side effects associated with inflammation and oxidative stress. Once again, we emphasize that the observed effect is influenced by the location of the receptors that participate in these processes, as well as by situations of exaggerated inflammation or oxidative/nitrosative stress.

Outstanding role of nitric oxide in the mechanisms mediated by anandamide and other endocannabinoids

At present, several published articles have demonstrated the participation of NO as a key regulator of processes such as atherogenesis and renal damage.[54–57] Moreover, NO plays a leading role as the key intermediary in most of the physiological processes induced by eCBs and it is fundamentally involved in the regulation of cardiovascular homeostasis. Related to this subject, the effects of AEA on human arteries have been characterized, and it is reported that vasorelaxation induced by eCBs was inhibited by eNOS blockers, it was concluded that AEA produces NO-dependent vasorelaxation in mesenteric arteries, with less efficacy in the animal models, and similar to what was observed in other reports where dependence of CB_1 and CBe receptor activation was verified.[28] It was also reported that in hPA precontracted with the prostanoid receptor agonist TP, U-46619, the vascular response to AEA instigates the opening of potassium channels and the stimulation of NO release. This event could be a consequence of CBe receptor activation, sensitive to O-1918, or to the production of COX-derived vasoactive substances of prostacyclin type. In addition, it was demonstrated in the same study that the NOS inhibitor, L-NAME, reduced AEA-induced relaxation (Figure 3).[5] Similarly, another investigation reported that NO levels decreased the secondary pulmonary hypertension related to heart failure, suggesting a vasoprotective action of NO.[58]

Additionally, in studies on mesenteric arteries of obese Zucker rats (OZR) and nonobese rats (LZR), a deteriorated vascular function was observed particularly in the obese, which was manifested by an alteration in the capacity of endothelium to deliver relaxation and contraction factors; consequently, the vascular relaxation of AEA is decreased in obese rats. Likewise, the incubation of mesenteric arteries with AEA produced eNOS phosphorylation in LZR rats and decreased the phosphorylation of this protein in OZR rats.[59]

Rat aortas have recently been studied in order to examine whether eCB produces vascular actions mediated by peroxisome proliferator-activated factor gamma receptor (PPARγ). Both AEA and N-arachidonoyl-dopamine were reported to produce significant PPARγ-mediated vasodilation for 2 h by a NO-dependent mechanism. The PPARγ agonists have a lot of positive cardiovascular effects including improved NO bioavailability, decreased blood pressure, *in vivo*, and attenuation of atherosclerosis.[15]

Several reports describe the new discovery of VSN16, an unprecedented water-soluble agonist that acts as a vasorelaxant agent through the activation of 'non-CB_1/non-CB_2' receptors in the vasculature, where VSN16 relaxes the mesenteric arteries in an endothelium-dependent mode. This vessel relaxation was inhibited by large concentrations of rimonabant and AM251, as well as by O-1918. VSN16 acts on the endothelium, releasing NO and activating calcium-dependent potassium ion channels and TRPV1. The water solubility of this new agonist might be effective in inducing peripheral CB-like effects without accompanying serious central cardiovascular responses.[60]

In addition, Herradón and colleagues[61] demonstrated the participation of the CBe receptor and an AEA-derived metabolite produced by COX-2; this metabolite acts on prostaglandin E2 receptors type 4 (PGE_2-EP4) in the endothelial relaxation vessel in the rat aorta generated by AEA. The vasorelaxant effect of AEA may be mediated mainly by two mechanisms: (a) by acting on the CBe receptor that promotes NO production and consequent vasorelaxation; (b) by FAAH metabolism, that results in the formation of arachidonic acid which then acts as a COX-2 substrate and leads to the generation of a similar product, PGE_2, which binds to the EP4 receptor in vascular smooth muscle and provokes vasorelaxation. In addition, this study indicates that the two described endothelial vasorelaxant pathways are not reciprocally excluding. In fact, there is proof for the interrelationship of the endothelial pathways of NO and COX. It does not regulate COX activity in normal and inflamed tissues, and it has been shown that NO can modulate PGE_2 synthesis by the activation of COX enzymes.[62] Such interactions explain the similarity between the vasorelaxant effects of AEA when L-NAME or indomethacin was used in this study.[61] Likewise, findings from other research teams showed that aortic ring preparations, AEA or R-methanandamide produced vasorelaxation, which was blocked by NOS inhibitors.[29] Additionally, it has been observed that vasorelaxant potency of AEA through activation of potassium channels and TRPV1 receptors increases in the mesenteric vascular beds of rats with hypertension induced by chronic NOS inhibition (trough administration of L-NAME). However, the existence of this *in vitro* evidence does not coincide with the results of *in vivo* studies, as they provide no evidence for the positive regulation of eCB or its receptors in this hypertensive model.[63] Further studies also demonstrated that AEA induces vasodilation of the renal afferent arterioles by the endothelial release of NO.[11] In this sense, this section highlights the participation of NO as an essential mediating factor of the beneficial vascular effects of diverse members of the eCB family, especially AEA, through multiple signaling pathways.

Anandamide and renal function linked to cardiovascular disease

The kidney is one of the most important regulators of blood pressure and is widely influenced by ECS. Therefore, there is a great amount of research that relates the action of eCBs on the renal physiology and its alteration in the hypertensive state. A study by Wang and colleagues[12] demonstrated that TRPV1 gene deletion aggravates renal injury resulting from hypertension induced by the DOCA-salt model distinguished by reduced creatinine clearance, albuminuria, tubulointerstitial lesion and interstitial infiltration of monocytes/macrophages. These findings suggest that TRPV1 and its agonists, such as AEA, may have a protective role in the prevention of kidney damage, possibly by inhibiting the inflammatory response during hypertension. Likewise, recent studies have shown that AEA and prostaglandin E_2-ethanolamide, a product of AEA metabolism synthesized by COX-2, have an

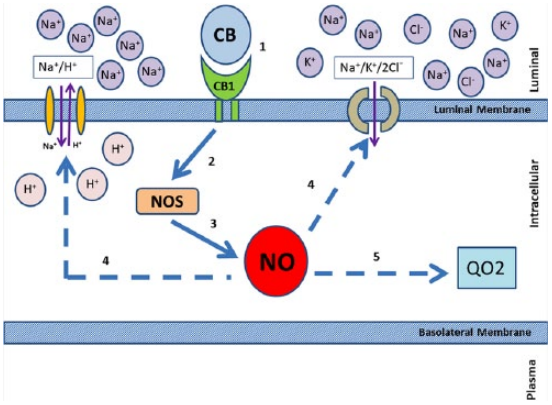

Figure 4. Diuretic/natriuretic effect mediated by cannabinoid 1 receptors at the level of the renal medulla (cells of the ascending thick limb of the loop of Henle).
Activation of CB_1 receptors on the apical membrane of these cells caused by various cannabinoids (CBs) (a) induces the activation of the enzyme NOS; (b) this results in increased production of NO; (c) which acts by inhibiting sodium reabsorption into the cell by blocking the Na+/H+ pump and the Na+/K+/2Cl$^-$ cotransporter; (d) this blockage of ionic transporters, in turn, leads to a decrease in cellular QO2; (e) continuous lines indicate 'activation' and 'inhibition or blocking' dashed lines.
CB, cannabinoid; NO, nitric oxide; NOS, nitric oxide synthase; QO2, oxygen consumption rate.

inhibitory effect on L-homocysteine-induced inflammatory injury on cultured podocytes, which ultimately leads to glomerular damage *in vivo*.[64]

In terms of ion transport at the renal level, it is known that the required energy (ATP) for the active resorption of sodium chloride in the cells of the ascending thick limb of the loop of Henle (TALs) relies on the oxygen consumption (QO2) and the oxidative phosphorylation processes taking place in these cells.[65] In view of this, Silva and colleagues demonstrated that AEA inhibits QO2 related to Na+ transport in TALs by CB_1 receptor activation and NOS in an NO-mediated mechanism which blocks apical Na+ (Figure 4). The results of such research also indicate that CB_1 receptor activation by AEA does not impede oxidative phosphorylation. Differences between various studies could be associated with the kind of cell studied, the concentration of AEA used and the reactivity of the tissue to specific eCBs. Therefore, the development of new specific CB_1 agonists could be proposed as precursors of an unprecedented class of diuretics.[21] Additionally, some publications have reported that renal excretive effects of AEA are antagonized by endovenous infusion of celecoxib, a selective COX-2 inhibitor, indicating the participation of an intermediate prostamide as a precursor of such effects. Specifically, intravenous prostamide E_2 decreased MAP and increased renal blood flow, opposite actions to those of Ang II. These results indicate that AEA and its metabolites in the renal medulla present antihypertensive properties as a consequence of its vasodilatory, sympatholytic and diuretic/natriuretic effects. PGE_2, the main renal vasodilator factor, was excluded from this mechanism because of its limited time (a matter of seconds) in plasma.[6]

Some years ago, research by Jenkin and colleagues[66] reported the involvement of CB_1 receptors in apoptosis of human proximal tubule epithelial cells (HK-2) during diabetic nephropathy. It was shown that mRNA and protein for CB_1, CB_2, TRPV1 and GPR55 receptors are expressed in these cells, and the activation of these receptors with AEA considerably i ncrements the hypertrophy in HK-2 cells. Consequently, it is probable that in the human proximal tubule, these receptors can modulate cellular function by activating distinct cell signaling pathways. Feizi and colleagues[67] evaluated the effect of different dosage of AEA analogs and CB-agonists: arachidonylcyclopropylamide (ACPA, CB_1 agonist) and JWH133 (CB_2 agonist) in ischemia-reperfusion injury of the mouse kidney. The results showed that different doses of ACPA or JWH133 prevent ischemia-reperfusion injury, which is also an indication of the participation of the ECS in the mouse kidney. Throughout the analysis of this section, a marked influence of the ECS on the kidney is concluded, due to its active participation in processes such as diuresis, natriuresis, and vasodilation to prevent kidney damage of different origins.

Conclusions and prospects

Following the review and analysis carried out in this article, we conclude that there are numerous advances in the study of the interactions and interconnections between the ECS and the cardiovascular system. However, there are still many aspects that need a deeper understanding since it is a theme that has begun to be explored relatively recently and whose results are often contradictory. It also shows the multiplicity and variability of responses that ESC is able to evoke on the cardiovascular system, according to the modifications made to the different types of

variables that influence the interaction between both systems. Some of these variables include vascular bed under study, type of hypertension analyzed, type of eCB used or of the stimulated receptor, the form of administration, among others. Moreover, due to the affinity of these lipid mediators for the CNS, it is of essential importance to create new cardiovascular therapies that achieve the location and the address of the pharmacological action of the mentioned active compounds. This aspect allows obtaining beneficial results, simultaneously minimizing their adverse effects. In this sense, the use of AEA and other related eCBs as active principles are proposed by using novel pharmacological vectorization strategies, such as the development of nanotechnology. The design and implementation of these new therapies could contribute significantly to improve the quality of life of patients affected by cardiovascular diseases. Of particular importance, the number of translational applications modulated by ECS that are already used in preclinical and clinical stages is marked. This has been achieved due to the multiplicity of therapeutic targets (receptors, enzymatic inhibitors/activators, metabolites, among others) that can be manipulated to obtain the desired effect (mainly at the vascular, renal and cardiac level). Finally, we need to mention that better management of these diseases would also result in a decrease in public health expenditures.

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