The endogenous cardiac cannabinoid system: a new protective mechanism against myocardial ischemia

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Summary

The pharmacological (and recreational) effects of cannabis have been known for centuries. However, it is only recently that one has identified two subtypes of G-protein-coupled receptors, namely CB_1 and CB_2-receptors, which mediate the numerous effects of Δ^9-tetrahydrocannabinol and other cannabinoids. Logically, the existence of cannabinoid-receptors implies that endogenous ligands for these receptors (endocannabinoids) exist and exert a physiological role. Hence, arachidonoylethanolamide (anandamide) and *sn*-2 arachidonoylglycerol, the first two endocannabinoids identified, are formed from plasma membrane phospholipids and act as CB_1 and/or CB_2 agonists. The presence of both CB_1 and CB_2-receptors in the rat heart is noteworthy.

This endogenous cardiac cannabinoid system is involved in several phenomena associated with cardioprotective effects. The reduction in infarct size following myocardial ischemia, observed in rats exposed to either LPS or heat stress 24 hours before, is abolished in the presence of a CB_2-receptor antagonist. Endocannabinoids and synthetic cannabinoids, the latter through either CB_1 or CB_2-receptors, exert direct cardioprotective effects in rat isolated hearts. The ability of cannabinoids to reduce infarct size has been confirmed in vivo in anesthetized mice and rats. This latter effect appears to be mediated through CB_2-receptors. Thus, the endogenous cardiac cannabinoid system, through activation of CB_2-receptors, appears to be an important mechanism of protection against myocardial ischemia. Arch Mal Cœur 2006 ; 99 :242-6.

Résumé

Le système cannabique cardiaque : une nouvelle protection contre les effets délétères de l'ischémie du myocarde

L'emploi du cannabis à des fins thérapeutiques (et récréatives) remonte à plusieurs siècles. Ce n'est toutefois que très récemment que des récepteurs spécifiques aux cannabinoïdes, nommément les récepteurs CB_1 et CB_2, responsables des effets du Δ^9-tétrahydrocannabinol et autres cannabinoïdes, ont été identifiés. L'existence même de ces récepteurs implique logiquement la présence d'agonistes naturels (les endocannabinoïdes) ainsi que des fonctions physiologiques propres. L'arachidonyléthanolamide (anandamide) et le *sn*-2 arachidonylglycérol, produits à partir de phospholipides membranaires et possédant des propriétés agonistes au niveau des récepteurs CB_1 et/ou CB_2, ont été les deux premiers endocannabinoïdes découverts. La présence de récepteurs CB_1 et CB_2 dans le cœur de rat a été confirmée par notre laboratoire.

Ce système cannabique cardiaque est impliqué dans de nombreux phénomènes induisant une protection naturelle contre les effets délétères de l'ischémie. Par exemple, la diminution de la taille de l'infarctus observée chez des rats préalablement exposés à des LPS ou à un stress thermique est abolie en présence d'un antagoniste des récepteurs CB_2. Les endocannabinoïdes ainsi que les agonistes cannabinoïdes de synthèse sélectifs pour les récepteurs CB_1 ou CB_2 exercent un effet cardioprotecteur direct dans le cœur isolé de rat. Ces effets bénéfiques sur la taille de l'infarctus ont été confirmés in vivo chez la souris et le rat anesthésiés, et semblent impliquer davantage les récepteurs CB_2. Le système cannabique cardiaque apparaît donc comme un mécanisme de protection naturelle contre les effets délétères de l'ischémie du myocarde. Arch Mal Cœur 2006 ; 99 : 242-6.

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THE ENDOGENOUS CANNABINOID SYSTEM

Cannabis has been used for centuries, for both medicinal and recreational purposes. Delta^9-tetrahydrocannabinol (Δ^9-THC), which is the major component of cannabis extract, exerts effects similar to that of cannabis in the central nervous system. The mechanism of action of Δ^9-THC has remained elusive for years. Early studies have shown that cannabinoids inhibit adenylate cyclase (AC) in the nervous system. Furthermore, AC inhibition was observed only in certain cell types, ruling out a direct inhibitory effect of cannabinoids on the enzyme itself, or a non-specific effect through changes in membrane fluidity. In the late 1980's, specific binding sites for cannabinoids were described in a rat brain membrane preparation [1]. Two years later, Matsuda, et al. isolated and cloned from a rat cerebral cortex cDNA library a new receptor that, in the presence of Δ^9-THC, could inhibit forskolin-induced cyclic AMP accumulation [2]. This discovery of the first cannabinoid-receptor, namely CB_1-receptor, was followed by the characterization of a second cannabinoid-receptor, CB_2, in cells of immune origin [3]. Although CB_1 and CB_2-receptors remain the only two identified by molecular cloning thus far, some pharmacological data suggest the existence of other receptors [4].

The presence of specific cannabinoid-receptors implies the existence of natural ligands, and an arachidonic acid derivative isolated from a porcine brain extract, with the ability to displace a radio-labeled cannabinoid analogue from its binding sites, was soon discovered [5]. Arachidonoylethanolamide (AEA or anandamide) was thus the first endocannabinoid identified. A second compound with the ability to preferably bind to CB_2-receptors, 2-arachidonoylglycerol (2-AG) has been originally isolated from canine gut and later found in brain neurons [6]. These two endocannabinoids (fig. 1) are certainly the most characterized, especially in the brain [7]. Two additional endocannabinoids have been recently identified: 2-arachydonoyl glyceryl ether (noladin ether), which shows high affinity for CB_1-receptors [8], and O-arachidonoyl ethanolamine (virodhamine), which is a weak activator of CB_1-receptors [9]. The latter may be due to the chemical instability of virodhamine, which is rapidly converted into anandamide in an aqueous milieu [7]. Most recently, oleamide, the primary amide of oleic acid, has been found to bind selectively to CB_1-receptors in vitro [10].

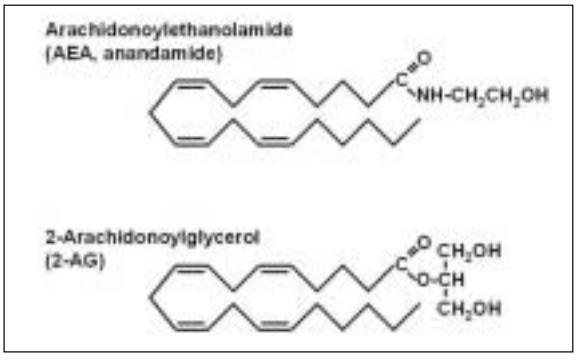

FIG. 1 – Chemical structure of the first endocannabinoids identified.
FIG. 1 – Structure chimique des premiers endocannabinoïdes découverts.

The first endocannabinoid identified, anandamide, was not the first fatty acid ethanolamide to be discovered. Palmitoylethanolamide (PEA) was isolated in 1957 from soybeans and shown to possess anti-inflammatory properties [6]. The pharmacological status of PEA is unclear: it weakly binds to cells transfected with CB_2-receptors [11], but its analgesic and anti-inflammatory effects are reversed by a selective CB_2-receptor antagonist [12]. Another fatty acid ethanolamide, oleoylethanolamide (OEA), despite structural similarities with anandamide and PEA, shows weak analgesic properties but exerts potent appetite-suppressing effects in rats [7].

Anandamide, unlike the other endocannabinoids, has also been shown to act as an agonist of transient receptor potential vanilloid type1 (TRPV1) [13, 14]. Although activation of TRPV1 by anandamide may be involved in some pathological situations, the contribution of this cation channel in the cardiovascular effects of anandamide appears to be limited.

The biosynthesis of endocannabinoids involves simple enzymatic reactions using membrane phospholipids as substrate [6, 15]. The fatty acid ethanolamides (anandamide, PEA, OEA) are formed through the transfer, by the enzyme N-acyl-transferase, of an acyl group from a donor phospholipid to form an amide bond to the ethanolamine portion of a receiving phosphatidylethanolamine. The N-acyl-phosphatidylethanolamide thus formed remains trapped in the plasma membrane, serving as a «stock» of readily available cannabinoids. Upon stimulation of the cannabinoid-producing cell (through an increase in cellular free Ca^{2+}), the acylethanolamide is cleaved by phospholipase D, allowing the release of the corresponding endocannabinoid. 2-AG is formed from a single phospholipid molecule, *sn*-1-acyl,2-arachidonoyl-phosphatidylinositol, through the action of phospholipase C (PLC), yielding DAG, and *sn*-1-DAG-lipase [6, 15]. 2-AG can spontaneously convert through a non-enzymatic acyl migration to 1-AG, which is either equipotent or slightly less potent then the 2-AG isomer [15]. As for the fatty acid ethanolamides, 2-AG can be formed on demand by several neurotransmitters coupled to either Ca^{2+} channels or PLC [15].

Once formed, signal transduction is terminated by transport into cells, followed by enzymatic degradation. A selective, temperature-sensitive, saturable, and Na^+-independent carrier has been partially characterized and could contribute, through facilitated diffusion, to the uptake of anandamide [15, 16] and 2-AG [7]. Cannabinoids with a saturated fatty acid, like PEA, are not taken up by this transporter [7]. Anandamide can then be hydrolyzed into ethanolamine and arachidonic acid by the fatty acid amide hydrolase (FAAH) [17, 18]. Although FAAH can also hydrolyze 2-AG, another enzyme, corresponding to monoglyceride lipase, contributes as well [7]. Recently, an enzyme selective for PEA and involved in its hydrolysis has been identified [19].

Both subtypes of cannabinoid receptors are coupled, through Gi, to AC and inhibit the formation of cyclic AMP [6, 20]. In addition, CB_1-receptors can inhibit N- and Q-type calcium channels and increase membrane potassium conductance [6]. Also, cannabinoids have been shown to increase PKC activity [21] and produce a concentration-related increase in the activity of MAP kinases [22]. The expression of the two receptor subtypes in human tissues varies: CB_1-receptors are abundant in the central nervous system, and are expressed to a lower extent in several peripheral tissues including the adrenal gland, the heart, and lungs [23]. In contrast, the CB_2 gene, which appears to be weakly expressed in the brain, is particularly abundant in immune tissues [23]. The pharmacological effects of cannabinoids coincide with receptor distribution: central effects like analgesia, antiemetic effect, psychotropic effect, sedation, and increased appetite, are mediated through CB_1-receptors, whereas the immunosuppression action is mediated by CB_2-receptors [6]. The CB_2 gene expression is supposedly low in human cardiac tissue [23], which contributed to the belief that the heart is lacking CB_2-receptors. However, we found that both CB_1 and CB_2-receptors are expressed to a similar degree in the rat heart [24].

CARDIOVASCULAR EFFECTS OF CANNABINOIDS

Cannabinoids exert different cardiovascular effects in vivo, the most often described being hypotension and bradycardia [25, 26]. Part of these effects may be secondary to CB_1-mediated sympathetic inhibition [26]. However, cannabinoids exert direct vascular effects as well. Anandamide, PEA, and synthetic cannabinoids induce an endothelium-independent relaxation in preconstricted rat mesenteric arteries [27]. The dilatory effect of anandamide, but not of PEA, is blocked by a CB_1-receptor antagonist [27], confirming that these two endocannabinoids have a different selectivity toward their receptors. In contrast, Wagner et al. [28] reported that the vasodilatation of the perfused rat mesenteric bed to anandamide is partially endothelium-dependent, and that the endothelium-independent component is not blocked by a CB_1-receptor antagonist. A recent study has compared the vasorelaxant actions of anandamide in resistance (G3) and conduit (G0) mesenteric arteries of the rat [29]. In small resistance vessels, the vasorelaxation occurred through stimulation of TRPV1, CB_1-receptors, and an endothelial receptor coupled to endothelium-derived hyperpolarizing factor (EDHF) release. By contrast, in the larger mesenteric artery, vasorelaxation was endothelium-independent and was almost entirely due to stimulation of TRPV1 and CB_1-receptors. It should be noted that the hypotensive effect of anandamide remained unchanged in TRPV1 knockout mice [30], but disappeared completely in CB_1-receptor knockout mice [31]. Surprisingly, anandamide was still able to produce a vasodilatation when injected into the mesenteric circulation in these CB_1-receptor knockout mice [31]. Cannabinoids can induce hyperpolarization of vascular smooth muscle [32], through activation of Ca^{2+}-activated potassium channels of strong (BK_{Ca}) and small (SK_{Ca}) conductance [33, 34], and some have suggested that the EDHF in rat mesenteric [35] and coronary [36] vascular beds might be an endocannabinoid. However, several studies reported different pharmacological profiles between EDHF and endocannabinoids [32, 33]. An interesting study reported that the endothelium-dependent relaxation of bovine coronary arteries to anandamide results from its hydrolysis by the FAAH, yielding ethanolamine and arachidonic acid, the latter being reincorporated into prostaglandins and epoxyeicosatrienoic acids (EETs, an EDHF contender) [37].

The physiological role of endocannabinoids in blood pressure regulation is unknown, but some evidences suggest a pathological one. LPS markedly increases platelet 2-AG and macrophage anandamide levels [38], and contribution of these endocannabinoids in the hypotension during hemorrhagic and septic shock [39, 40] as well as during acute myocardial infarction [41] has been suggested. In addition, in experimental models of hypertension, endocannabinoids and inhibitors of FAAH reduce blood pressure, whereas antagonism of CB_1-receptors enhances it [42].

CARDIOPROTECTIVE EFFECTS OF CANNABINOIDS

In ischemic hearts, cannabinoids exert clear cardioprotective effects. We were the first to report that the infarct size-limiting effect of LPS, administered 24 hours before myocardial ischemia in rats, involved CB_2-receptor activation [43]. A few months later, a Russian team reported that a cannabinoid agonist, HU-210, exerted an antiarrhythmic effect during ischemia-reperfusion in rats in vivo, effect blocked by a CB_2-receptor antagonist [44]. One year later, Marie Joyeux et al., from the Université Joseph Fourier, Grenoble, have reported that the infarct size-reducing effect conferred by exposing rats to a heat stress 24 hours prior to myocardial ischemia was not altered by a CB_1-receptor antagonist but was completely abolished by a CB_2-receptor antagonist [45].

We recently evaluated the direct protective effect of several cannabinoids, endogenous and synthetic, in rat isolated hearts exposed to low-flow ischemia and reperfusion [46]. Both PEA and 2-AG, but not anandamide, exert a marked cardioprotective effect, improving post-ischemic ventricular recovery, preventing LDH and CK leakage, and limiting infarct size. The effect of 2-AG involved both receptor subtypes. On the other hand, the effect of PEA was insensitive to the CB_1-antagonist, but blocked completely by the CB_2-antagonist. We also observed that ACEA and JWH015, two synthetic cannabinoids selective for CB_1 and CB_2-receptors, respectively, reduced infarct size [46]. A recent study confirmed the ability of anandamide to reduce infarct size in rat isolated hearts [47]. They did not, however, observe any reduction in infarct size with ACPA and JWH133, two synthetic cannabinoids selective for CB_1 and CB_2-receptors, respectively. The reason of such a discrepancy remains unknown, but diffe-

rences in agonists, concentrations and solvents used, as well as duration of administration, may play a role.

The cardioprotective effects of cannabinoids have been confirmed in vivo as well. Di Filippo et al. have observed a reduction in infarct size with the cannabinoid agonist, WIN55212-2, administered 30 min prior to left coronary artery ligation in anesthetized mice [48]. This effect was still observable in the presence of a CB_1-receptor antagonist, but was significantly reduced by a CB_2-receptor antagonist. We have also observed a CB_2-receptor-mediated, significant reduction in infarct size with the cannabinoid agonist, CP55940, following left anterior descending coronary artery ligation and reperfusion in anesthetized rats (fig 2).

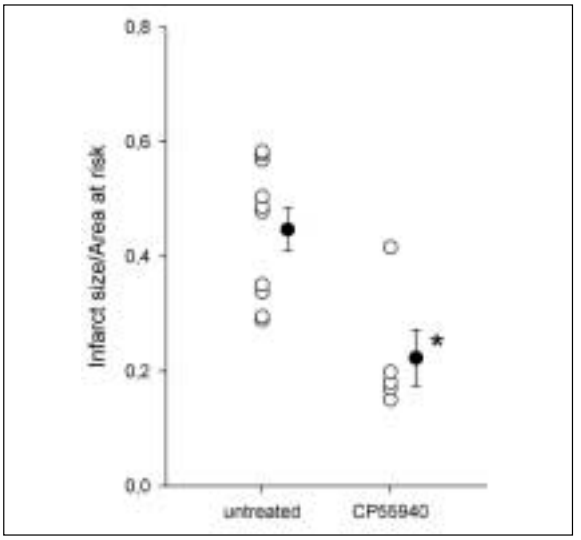

FIG. 2 – Effect of the cannabinoid agonist, CP55940 (0,1 mg/kg, i.v.) on infarct size (measured with TTC staining), expressed as a ratio of the area at risk (measured with Evans Blue perfusion), after 30 min of left anterior descending coronary occlusion and 60 min of reperfusion in anesthetized rats. *P<0,05 compared with untreated rats.
FIG. 2 – Effet de l'agoniste cannabique CP55940 (0,1 mg/kg, i.v.) sur la taille de l'infarctus (évaluée par coloration au TTC), exprimée par un ratio de la zone à risque (estimée par une perfusion de bleu d'Evans), après 30 min de ligature de l'artère interventriculaire gauche et 60 min de reperfusion chez le rat anesthésié. *P < 0,05 comparativement aux rats témoins (untreated).

Cannabinoids can also protect the endothelial function against the short-term and long-term deleterious effects of ischemia-reperfusion in rats. We have reported that the protective effect of ischemic preconditioning on the endothelial function can be abolished by either CB_1-receptor or CB_2-receptor antagonists [24]. Furthermore, perfusion with either PEA or 2-AG, 15 min before and throughout the ischemic period, mimicked ischemic preconditioning inasmuch as it protected the endothelium in a similar fashion. Wagner et al. have observed that pretreatment with a specific CB_1-receptor antagonist restored blood pressure but impaired endothelial function and increased early mortality after myocardial infarction in rats [41]. Conversely, a 12-week treatment with the cannabinoid agonist, HU-210, prevented endothelial dysfunction in a similar model of myocardial infarction [49]. Interestingly, oral treatment with Δ^9-THC can inhibit the progression of atherosclerosis in the apolipoprotein E knockout mouse model, through immunomodulatory effects mediated by CB_2-receptors [50].

There may be a link between the endothelium and the ability of cannabinoids to reduce infarct size. We have found that kinins can reduce infarct size through the release of endothelium-derived autacoids in rat isolated hearts [51]. Surprisingly, the cardioprotective effect of kinins was also blocked by the CB_1-receptor antagonist, SR141716A, which is in fact an inverse agonist [52]. Interestingly, the cardioprotective effect of the CB_1-receptor agonist, ACEA, but not of the CB_2-receptor agonist, JWH015, was blocked by a NO synthase inhibitor in rat hearts, which coincides with the presence of CB_1-receptors on the endothelium of rat coronary arteriolar and capillary endothelial cells [53]. Although it remains highly speculative at this point, one may imagine that endothelial CB_1-receptors can, besides mediating the effects of cannabinoids, exert a facilitatory effect on other endothelium-dependent autacoids.

CONCLUSIONS

The endogenous cardiac cannabinoid system is involved in several phenomena associated with cardioprotective effects, such as LPS exposure and heat stress. Furthermore, endocannabinoids and synthetic cannabinoids exert cardioprotective effects in isolated hearts and in anesthetized mice and rats as well. Although selective CB_1-receptor agonists can reduce infarct size, the effects of LPS, heat stress, and non-selective cannabinoid agonists are mediated mainly by CB_2-receptors. Thus, the endogenous cardiac cannabinoid system, through activation of CB_2-receptors, appears to be an important mechanism of protection against myocardial ischemia.

KEY-WORDS : Cannabinoids, Myocardial infarction, Type-2 cannabinoid-receptors, Endothelium, Nitric oxide

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