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

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 CB1 and CB2-receptors, which mediate the numerous effects of ∆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, arachidonylethanolamide (anandamide) and sn-2 arachidonoylglycerol, the first two endocannabinoids identified, are formed from plasma membrane phospholipids and act as CB1 and/or CB2 agonists. The presence of both CB1 and CB2-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 CB2-receptor antagonist. Endocannabinoids and synthetic cannabinoids, the latter through either CB1 or CB2-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 CB2-receptors. Thus, the endogenous cardiac cannabinoid system, through activation of CB2-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 CB1 et CB2, responsables des effets du ∆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’arachidonylethanolamide (anandamide) et le sn-2 arachidonoylglycerol, produits à partir de phospholipides membranaires et possédant des propriétés agonistes au niveau des récepteurs CB1 et/ou CB2, ont été les deux premiers endocannabinoïdes découverts. La présence de récepteurs CB1 et CB2 dans le cœur de rat a été confirmée par notre laboratoire.

THE ENDOGENOUS CANNABINOID SYSTEM

Cannabis has been used for centuries, for both medicinal and recreational purposes. Delta-9-tetrahydrocannabinol (δ9-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 δ9-THC has remained elusive for years. Early studies have shown that cannabinoids inhibit adenylyl 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 a new receptor that, in the presence of δ9-THC, could inhibit forskolin-induced cyclic AMP accumulation [2]. This discovery of the first cannabinoid-receptor, namely CB1-receptor, was followed by the characterization of a second cannabinoid-receptor, CB2, in cells of immune origin [3]. Although CB1 and CB2-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]. Arachidonoylthanolamide (AEA or anandamide) was thus the first endocannabinoid identified. A second compound with the ability to preferably bind to CB2-receptors, 2-arachidonoylglycerol (2-AG) has been recently 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-arachidonoylglycerol ether (noladin ether), which shows high affinity for CB1-receptors [8], and O-arachidonyl ethanolamine (virodhamine), which is a weak activator of CB1-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 CB2-receptors in vitro [10].

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 CB2-receptors [11], but its analgesic and anti-inflammatory effects are reversed by a selective CB2-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 type 1 (TRPV1) in cells, although activation of TRPV1 by anandamide may be involved in some pathological situations, the contribution of this channel to 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 Ca2+), 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 than the 2-AG isomer [15]. As for the fatty acid ethanolamides, 2-AG can be formed on demand by several neurotransmitters coupled to either Ca2+ 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 G\(_i\), 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 immunomodulatory effect 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 sympathoinhibitory 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 \(\rightarrow\) 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 \(\rightarrow\) and conduit \(\rightarrow\) 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\(^{2+}\)-activa-

**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 PEA, 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 \([47]\). The mechanism is 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-
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 CB1-receptor antagonist, but was significantly reduced by a CB2-receptor antagonist. We have also observed a CB2-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).

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 CB1-receptor or CB2-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 CB1-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 Δ9-THC can inhibit the progression of atherosclerosis in the apolipoprotein E knockout mouse model, through immunomodulatory effects mediated by CB2-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 CB1-receptor antagonist, SR141716A, which is in fact an inverse agonist [52]. Interestingly, the cardioprotective effect of the CB1-receptor agonist, ACEA, but not of the CB2-receptor agonist, JWH015, was blocked by a NO synthase inhibitor in rat hearts, which coincides with the presence of CB1-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 CB1-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 CB1-receptor agonists can reduce infarct size, the effects of LPS, heat stress, and non-selective cannabinoid agonists are mediated mainly by CB2-receptors. Thus, the endogenous cardiac cannabinoid system, through activation of CB2-receptors, appears to be an important mechanism of protection against myocardial ischemia.

KEY-WORDS: cannabinoids, myocardial infarction, type-2 cannabinoid receptors, endothelium, nitric oxide

References

References