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The role of the CB_1 receptor in the regulation of sleep

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ABSTRACT

During the 1990s, transmembranal proteins in the central nervous system (CNS) that recognize the principal compound of marijuana, the delta-9-tetrahydrocannabinol (Δ^9-THC) were described. The receptors were classified as central or peripheral, CB_1 and CB_2, respectively. To this date, it has been documented the presence in the CNS of specific lipids that bind naturally to the CB_1/CB_2 receptors.
The family of endogenous cannabinoids or endocannabinoids comprises oleamide, arachidonoylethanolamine, 2-arachidonylglycerol, virodhamine, noladin ether and *N*-arachidonyldopamine. Pharmacological experiments have shown that those compounds induce cannabimimetic effects. Endocannabinoids are fatty acid derivates that have a variety of biological actions, most notably via activation of the cannabinoid receptors. The endocannabinoids have an active role modulating diverse neurobiological functions, such as learning and memory, feeding, pain perception and sleep generation. Experimental evidence shows that the administration of Δ^9-THC promotes sleep. The activation of the CB_1 receptor leads to an induction of sleep, this effect is blocked via the selective antagonist. Since the system of the endogenous cannabinoids is present in several species, including humans, this leads to the speculation of the neurobiological role of the endocannabinoid system on diverse functions such as sleep modulation. This review discusses the evidence of the system of the endocannabinoids as well as their physiological role in diverse behaviours, including the modulation of sleep.

1. Introduction

1.1. Exogenous cannabinoids

Marijuana is a name given referring to the plant *Cannabis sativa* and it has been used for diverse cultures for many purposes during centuries. For example, mystical ceremonies, social interaction as well as for therapeutically aims have been the uses for this plant (Ameri, 1999; Hollister, 1986, 1995; Kalant, 2001; Pertwee, 2006; Robson, 2001; Zias et al., 1993). Among the multiple compounds present in the marijuana, it has been demonstrated that the principal active compound is delta-9-tetrahydrocannabinol (Δ^9-THC; Gaoni and Mechoulam, 1964; Iversen, 2003; Fig. 1).

There are interesting reports showing the use of this plant with medical purposes. For instance, smoking marijuana decreases the intraocular pression in patients with glaucoma and it reduces the pain and the nausea caused by the chemotherapy in patients with terminal cancer (Burns and Ineck, 2006). Smoking marijuana also induces a significant improvement in the muscle dysfunction in patients with multiple sclerosis whereas it diminishes the nausea caused by the retroviral treatment in patients with AIDS (Arias-Horcajadas, 2007; de Jong et al., 2005).

Although there is experimental evidence showing the positive effects induced by marijuana, paradoxically, there are also reports that demonstrate that Δ^9-THC displays negative effects since it induces molecular changes, including fragmentation of DNA as well as apoptosis (Ameri, 1999; Hall and Solowji, 1998; Hollister, 1986; Martin, 1986; Pertwee, 2005; Sarne and Mechoulam, 2005; Scallet et al., 1987; Whitlow et al., 2003).

The negative effects caused by Δ^9-THC have been also observed in several behavioural tests. For instance, the administration of this compound in rats induces hypomotility, hypothermia, and antinociception among other effects (Ameri, 1999; Hall and Solowji, 1998; Hollister, 1986; Iversen, 2003; Molina-Holgado et al., 1995). At this date, it is a widely accepted that most of the cellular and behavioural effects caused by the cannabis are due to the activation of the cannabinoid receptors.

2. Cannabinoid receptors

The family of the cannabinoid receptors comprise the central (CB_1) and the peripheral (CB_2) receptor. In the rat brain, Herkenham et al. (1990) described the distribution of the CB_1 receptor using quantitative radiography whereas Matsuda et al. (1990) reported the mRNA

Abbreviations: 5-HT, serotonin; 2-AG, 2-arachidonylglycerol; AC, adenililcyclase; ACh, acetylcholine; AD, adenosine; AMT, anandamide membrane transporter; ANA, anandamide; Ca^{2+}, calcium; CB_1 and CB_2, cannabinoid receptors; CBD, cannabidiol; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopamine; FAAH, fatty acid amide hydrolase; HI, histamine; Hypocretin, HCRT; K^+, potassium; NADA, n-arachidonyldopamine; NA, noradrenaline; NE, noladin ether; OLE, oleamide; PLC, phospholipase C; PPTg, pedunculopontine tegmental nucleus; REMS, rapid eye movement sleep; SWS, slow wave sleep; VIR, virodhamine; W, wakefulness; Δ^9-THC, delta-9-tetrahydrocannabinol.

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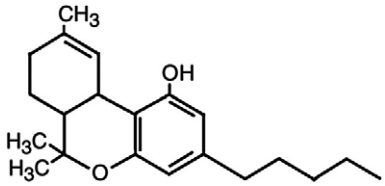

Fig. 1. Molecular structure of the principal compound of marijuana, delta-9 tetrahydrocannabinol (Δ9-THC).

localization using *in situ* hybridization in the rat brain. In both experiments, the presence of the CB_1 receptor was shown in specific areas of the central nervous system (CNS) such as cortex, hippocampus, striatum, limbic system, cerebellum, and brainstem (Glass et al., 1997; Hurley et al., 2003; Mackie, 2005; McPartland and Glass, 2003; Moldrich and Wenger, 2000; Ong and Mackie, 1999; Salio et al., 2002).

2.1. The CB_1 receptor

This protein possesses 7 transmembranal domains and inhibits AMPc formation through G_i alpha subunit protein (Axelrod and Felder, 1998; Guo and Ikeda, 2004; Howlett 2005).The pioneer studies showed that this receptor was present in the pre-synaptic terminals. However, later experiments have demonstrated its presence in the post-synaptic axons as well (Salio et al., 2002). This result has lead to the idea that the CB_1 receptor could be either increasing and/or inhibiting the neurotransmitter release.

Extracellular field potential recordings and patch-clamp experiments have shown that exogenous agonist for the CB_1 receptor reduces synaptic transmission and pharmacologically isolated AMPA receptor- and $GABA_A$ receptor-mediated post-synaptic currents in mice (Azad et al., 2003), diminishes the glutamatergic neurotransmission (Azad et al., 2003; Gerdeman and Lovinger, 2000; Hampson et al., 1998) whereas it enhances the release of acetylcholine (ACh; Acquas et al., 2000; Verrico et al., 2003). Additionally, it has been demonstrated that cannabinoid agonists dose-dependently increase the firing rate of locus coeruleus noradrenergic neurons (Muntoni et al., 2006) and the activation of the CB_1 receptor enhances the activity of the serotonergic (5-HT) system (Devlin and Christopoulos, 2002; Fan, 1995; Gobbi et al., 2005). For instance, Bambico et al. (2007) reported that doses of exogenous cannabinoids promoted the 5-HT activity. Finally, it has been also documented that the activation of the CB_1 receptor induces an inhibition of gap junctions (Howlett and Murkhopadhay, 2000; Venance et al., 1995).

It has been suggested that the activation of the CB_1 receptor modulates the neurotransmitter release due to the inhibition of calcium (Ca^{2+}) or activation of the potassium (K^+) channels. This idea has been supported by the evidence showing that the CB_1 receptor inhibits the Ca^{2+} channels types P, Q and N, and activates the K^+ channels (Daniel et al., 2004; Hashimotodani et al., 2007; Mackie et al., 1993, 1995; Twitchell et al., 1997). Fig. 2 shows the intracellular pathway triggered by the activation of the CB_1 receptor. Once the ligand binds and activates the receptor, a PG_i activates a phospholipase C (PLC), as well as the K^+ channels. On opposite, the PG_i inhibits the adenililcyclase (AC) as well as the Ca^{2+} channels.

2.1.1. Distribution of the CB_1 receptor

The localization of the CB_1 receptor has been documented in rat brain (Moldrich and Wegner, 2000; Oropeza et al., 2007), whereas the neuroanatomical distribution and density of this protein in the human brain has brought new perspectives about the role that the endocannabinoid system might be playing in cognitive processes. In healthy human brains, the density of the CB_1 receptor is notable in areas of the CNS such as thalamus, hypothalamus, cortex, hippocampus, limbic system, basal ganglia (Glass et al., 1997; Hurley et al., 2003; Mackie 2005) suggesting an important physiological role in the modulation of diverse behaviours such as sleep.

Glass et al. (1993) have described a diminution in density of the CB_1 receptor in brains of patients that had presented Huntington's disease (97%), compared to healthy controls. This loss was localized in substantia nigra. Authors concluded that the diminution in the number of the CB_1 receptor could be related to the generation of this degenerative disease, specifically with the deficit in the motor control. These findings have been supported from other observations (Benarroch, 2007; Hurley et. al., 2003).

2.2. The CB_2 receptor

This receptor was cloned by Munro et al. (1993) and its localization was restricted to cells of the immune system. The mRNA was present in macrophages as well as in the monocites but apparently absent in

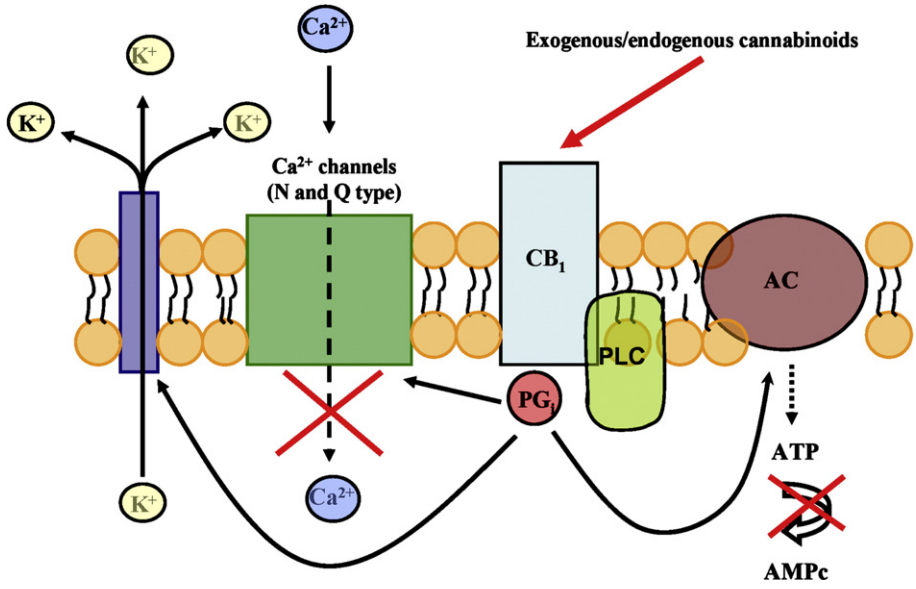

Fig. 2. Activation of the CB1 cannabinoid receptor leads to the blockade of calcium (Ca^{2+}, N and Q type) and activates potassium (K^+) channels. Exogenous or endogenous cannabinoids induce an inhibition of the activity of the adenylyl cyclase (AC) decreasing the synthesis of the cAMP whereas activating a PLC as well. This might be the molecular basis of the behavioral effects induced by exogenous/endogenous cannabinoids. Abbreviations: AC, adenylate cyclase; CB1, CB1 cannabinoid receptor; PGi, Gi coupled protein; PLC, phospholipase C.

the CNS. Brown et al. (2002b) have confirmed the mRNA localization of the CB_2 receptor in rats using *in situ* hybridization showing its presence in liver, lung, and testicles but complete absence in the CNS. However, recently Van Sickle et al. (2005) reported the presence of the CB_2 receptor in the brainstem. This evidence definitely will raise new perspectives about the presence of both cannabinoid receptors in the CNS as well as their neurobiological role modulating diverse functions.

The CB_2 receptor shares some intracellular elements that are activated by the CB_1 receptor. For example, it involves a PG_i and inhibits the AC (Glass et al., 1999; Howlett, 2005). The intracellular pathways that are activated by this receptor deserve to be explored in the future.

3. The endogenous ligands for the cannabinoid receptors

N-arachidonoylethanolamine (anandamide [ANA]) was discovered by Raphael Mechoulam's group and it was the first lipid present in the brain that binds to the CB_1/CB_2 receptors but most importantly, ANA displayed cannabinoid-like properties (Devane et al., 1992). The same group that described ANA discovered the second endocannabinoid named 2-arachidonoylglycerol (2-AG), which mimicked similar pharmacological properties with Δ^9-THC and ANA (Mechoulam et al., 1995). To this date, ANA and 2-AG bind and activate the cannabinoid receptors described so far (Howlett and Mukhopadhyay, 2000; Sugiura and Waku, 2000).

Oleamide (OLE) is a fatty acid amide that was detected in the cerebrospinal fluid (CSF) of sleep-deprived cats and rats (Cravatt et al., 1995; Lerner et al., 1994). This lipid has been reported to have effects on a wide range of receptors and neurotransmitter systems (Coyne et al., 2002; Dougalis et al., 2004; Fedorova et al., 2001) as well as on diverse behaviours, such as learning and memory, pain perception (Akanmu et al., 2007; Martínez-González et al., 2004; Murillo-Rodríguez et al., 2001b; Varvel et al., 2006) and sleep (Basile et al., 1999; Cravatt et al., 1995; Mendelson and Basile, 1999). OLE has been suggested as a member of the endocannabinoid family (Fowler, 2004; Leggett et al., 2004) since it binds to the CB_1 receptor (Fowler, 2004; Legget et al., 2004; Mendelson and Basile, 1999).

New members of the endocannabinoid family have been added. A new molecule with cannabinoid-like properties was reported by Hanus et al. (2001). This compound was named noladin ether (NE) which binds to the CB_1/CB_2 receptor (Shoemaker et al., 2005). Among the effects caused by NE, it has been reported that this lipid is able to increase [(3)H]-GABA uptake (Venderova et al., 2005). Moreover, the administration of NE (0.001 mg/kg) significantly increased food consumption whereas a higher dose (0.1 mg/kg) did not affect food consumption, but increased activity (Avraham et al., 2005).

On the other hand, Porter et al. (2002), while developing an improvement in bioanalytical methods to the identification and quantification of ANA, discovered the presence of a new compound with similar molecular weight as ANA but this new compound possessed a different retention time, shorter than ANA. The authors concluded that this lipid was a composition of arachidonic acid with ethanolamine with an esther molecule bound (the opposite of ANA, since the link between arachidonic acid with ethanolamine is an amide bound). Having this in mind, the molecule was named *O*-arachidonoylethanolamine, also known as *Virodhamine* (VIR), from the Sanskrit word for opposite (Porter et al., 2002). Among the few physiological properties of VIR described so far, it has been described that VIR relaxes the rat's small mesenteric artery (Ho and Hiley, 2004) whereas Hayase et al. (2005) have shown recently that the anxiety-related behavioural symptoms are significantly attenuated by VIR.

N-arachidonyldopamine (NADA) is the most recent member of the endocannabinoid compounds discovered (Huang et al., 2000). An active and protective role of NADA for the management of AIDS has been suggested (Sancho et al 2005) whereas O'Sullivan et al. (2005) reported that NADA did not modify the vasorelasation in rat aorta. As a result of their recent discovery, there is no experimental evidence to suggest a solid physiological role of NE, VIR and NADA. Fig. 3 shows the similarity among the molecular structure of endocannabinoids ANA, 2-AG, NE, VIR and NADA.

The distribution of ANA in the CNS which includes regions such as cortex, hippocampus, striatum, cerebellum, and brainstem (Bisogno et al., 1999; Felder et al., 1996; Murillo-Rodríguez et al., 2006a) is similar with the 2-AG neuroanatomical distribution (Bisogno et al., 1999; Mechoulam et al., 1995). It is important to point out that the significant presence of ANA and 2-AG are at the same regions in the CNS where the CB_1 receptor is present.

Biochemically, the release of the endocannabinoids is different from classical neurotransmitters since they are not stored in synaptic vesicles. It has been hypothesized that endocannabinoids are released "on demand" (Cadas et al., 1997; Di Marzo et al., 1994; Hillard, 2000; Lovinger, 2007). Therefore, there is a biological mechanism that involves the activity of membrane phospholipids that are the precursors of those compounds. In this model, the biosynthesis of the endocannabinoids is followed by their immediate release (Lovinger, 2007).

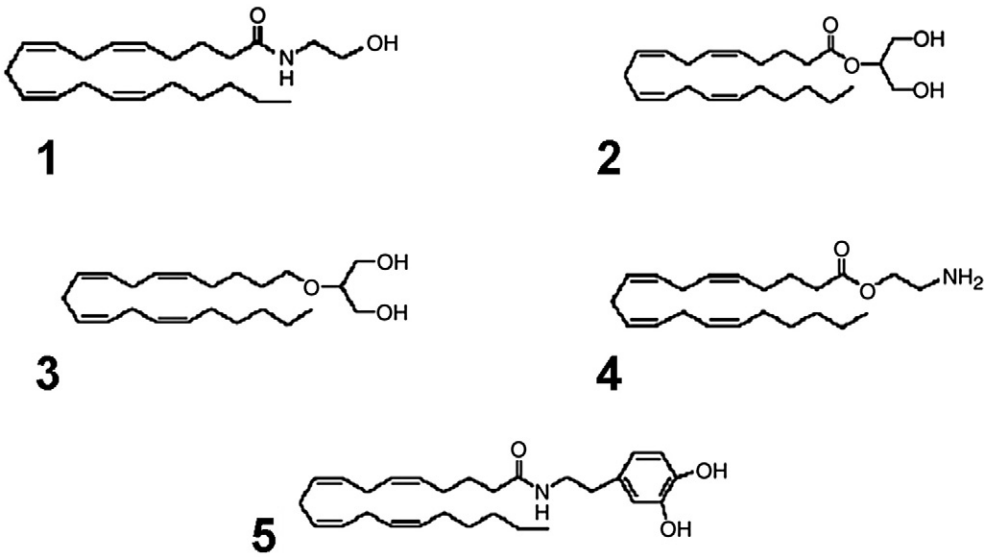

Fig. 3. Molecular structures of the endocannabinoids: arachidonylethanolamide (1), 2-arachidonylglycerol (2), noladin ether (3), virodhamine (4), and *N*-arachidonyldopamine (5).

The mechanism of degradation involves two different pathways. Briefly, in the first one, the compounds are transported to the interior of the cell via specific transporters. For ANA it has been suggested the existence of the anandamide membrane transporter (AMT). Once the lipid is inside the cell, a hydrolysis mechanism is turned on via the fatty acid amide hydrolase (FAAH; Ueda, 2002). This pathway is common for ANA whereas for the degradation of 2-AG, an enzyme named monoacyl glycerol lipase is apparently the responsible for its hydrolysis (Dinh et al., 2002; Hillard, 2000; Howlett and Mukhopadhyay, 2000; Ueda, 2002; Vandevoorde, 2008). Drugs that block the activity of the AMT (de Lago et al., 2004) or FAAH (Fegley et al., 2005) have shown that endogenous levels of ANA are modulated and these drugs improve several pathological conditions such as anxiety or tumor growing (Bifulco et al., 2004; Bortolato et al., 2007; Russo et al., 2007).This result represents a tentative therapeutical option to treat diverse disorders by enhancing endogenous tone of ANA.

3.1. The intracellular effects of the endocannabinoids

It is known that ANA inhibits the Ca^{2+} channels type N (Daniel et al., 2004; Guo and Ikeda, 2004; Howlett and Mukhopadhyay, 2000; Mackie et al., 1993) and activates K^+ channels (Mackie et al., 1995) whereas it facilitates the activity of the MAP Kinase (Valk et al., 2000; Wartmann et al., 1995). Furthermore, the systemic administration of ANA in rodents increases the c-Fos expression in CNS areas including cortex, thalamus, cerebellum and brainstem (McGregor et al., 1998; Patel et al., 1998).

3.2. The behavioural effects of the endocannabinoids

The pharmacological evidence provided to this date suggests that ANA mimics effects caused by Δ^9-THC, including antinociception, hypothermia, hypomotility, cataplexia, hyperphagia, and deteriorates learning and memory processes (Fride and Mechoulam, 1993; Hillard, 2000; Murillo-Rodríguez et al., 1998; Smith et al., 1994; Stein et al., 1996; Tallett et al., 2007; Wiley et al., 1995; Williams and Kirkham, 2002). For instance, Justinova et al. (in press) reported that Δ^9-THC plays an active role in the seeking behaviour paradigm; whereas the administration of this molecule induces catalepsy (Sano et al., 2008). Additionally, in mice, the injection of Δ^9-THC deteriorates the performance in the Morris water maze test (Senn et al., 2008). A similar result has been reported in rats, the spatial memory task was disrupted after injection of Δ^9-THC (5.0 mg/kg; Cha et al., 2007).

Sanofi developed a drug that act as an antagonist binding to the CB_1 receptor. This compound was named SR141716A (Rinaldi-Carmona et al., 1995). Several pharmacological effects caused by Δ^9-THC, ANA and 2-AG is blocked using this drug (Arias-Horcajadas, 2007; Mallet and Beninger, 1998; Murillo-Rodríguez et al., 2001a, 2003; Rodríguez de Fonseca et al., 2005; Tallet et al., 2007). These results suggest that exogenous cannabinoids and endocannabinoids are modulating diverse biological functions via the CB_1 receptor. The same pharmaceutical company also reported the first synthetic ligand for the CB_2 receptors named SR144528 (Rinaldi-Carmona et al., 1998).

4. Sleep aspects and the effects of endocannabinoids on sleep

It is worthy to describe some of the sleep aspects on the architecture of sleep. Regulation of the sleep–waking cycle is complex and involves brain circuits and molecules that regulate sleep and wakefulness (W). There is interplay among many neuroanatomical and neurochemical systems to maintain the waking state, such as acetylcholine (ACh), dopamine (DA), noradrenaline (NA), 5-HT, histamine (HI), and hypocretin (HCRT). On the other hand, the sleep-onset is governed by the interacting forces of the sleep drive, which steadily increases with duration of W, and circadian fluctuations in arousal level. Sleep is generated by the activity of sleep-promoting neurons placed in the anterior hypothalamus that utilize GABA to inhibit wake-promoting regions in the hypothalamus and brainstem. Then, brainstem regions inhibited during W and slow wave sleep (SWS) becomes active during rapid eye movement sleep (REMS). Ascending projections from cholinergic neurons in the brainstem activate the thalamus which in turn activates the cortex (Brown et al., 2002a; Jones, 2005; Siegel, 2006).

Sleep–wake cycle is maintained by different systems which use specific neurochemicals. For instance, to start our day, therefore, to generate waking, it requires to turn-on the glutamate, HI, HCRT, NA, and ACh systems. At the end of the day, when we feel sleepy, the sleep induction is related with the activity of brain areas such as the ventrolateral preoptic nucleus and the release molecules such as GABA, and adenosine (AD; Blanco-Centurión et al., 2006; Brown et al., 2001; Gottesmann, 2002; Saper et al., 2001).

4.1. Cannabinoids and sleep

It is widely known that marijuana and Δ^9-THC modulate the sleep–wake cycle. During the 1960s, 70s and 80s several experiments were carried out in order to evaluate the effects of the cannabinoids on sleep. The main conclusion of those experiments was that in humans, the dosage of 70 mg/day of cannabinoids increases sleep (Buonamici et al., 1982; Feinberg et al., 1975, 1976; Freemon, 1982; Pivik et al., 1972).

Although the effects on the sleep were evident after the administration of cannabis, the main concern was based on the fact that marijuana contains a mixture of several compounds. Therefore, it was difficult to differentiate if the effects observed in sleep were caused by a specific compound, such as Δ^9-THC, cannabidiol (CBD) or cannabinol. Just recently, it has been demonstrated that administration of CBD, non-psychotropic constituent of marijuana, increases W in humans (Nicholson et al., 2004). The treatments included Δ^9-THC (5 mg), combined with CBD (5 mg) and Δ^9-THC (15 mg) combined with CBD (15 mg). The concomitant administration of the cannabinoids decreased sleep and the higher dose combination produced a significant increase in alertness.

A similar result was reported by our group. Microinjections of CBD enhanced waking in rats (Murillo-Rodriguez et al., 2006b). Regarding this, CBD administered icv (10 μg/5 μL) at the beginning of the lights-on period increased W and decreased REMS whereas increased the extracellular levels of DA. Additionally, we observed that CBD induced an enhancement of c-Fos expression in waking-related brain areas, including hypothalamus and dorsal raphe nucleus. Thomas et al. (2007) reported that CBD is an antagonist for this receptor and then it displays affinity for the CB_1 receptor. However, the mechanism of action of CBD on sleep modulation in rats remains to be elicited. As we can notice, this effect is the opposite of the one caused by the Δ^9-THC. This raises the possibility that different compounds of marijuana modulate sleep in opposite directions (Mechoulam et al., 2007).

4.2. The endocannabinoid system and sleep

But what might be the physiological role of the endocannabinoids on sleep? The very first approach to answer this question was carried out by Santucci et al. in 1996. The authors injected systemically SR141716A (0.1, 0.3, 1, 3, and 10 mg/kg, ip) to rats and they found after 4 h of sleep-recordings, a dose-dependently increase in the time spent in W whereas SWS and REMS remained diminished. As a conclusion, they suggested the wake-inducing properties of SR141716A might be due to the blocking of the CB_1 receptor.

Later, in 1998, our laboratory showed that the endocannabinoid ANA modulated the sleep (Murillo-Rodríguez et al., 1998). Icv injections in rats of ANA during the lights-on period induced an opposite effect observed by Santucci and colleagues. A significant decrease in W and an enhancement in SWS and REMS were found. We also observed that the effects caused by ANA on sleep were more evident once this lipid was injected into the pedunculopontine tegmental nucleus (PPTg), a sleep-related nucleus.

If the changes observed in sleep after the administration of ANA were due to the activation of the CB_1 receptor, we had hypothesized that the administration of SR141716A before the injection of ANA either icv or into PPTg, might block the effects on sleep (Murillo-Rodríguez et al., 2001a). Administration of SR141716A, 15 min prior to ANA, readily prevent the ANA induced changes in sleep. This experimental manipulation showed that SR141716A efficiently blocked the effects induced by ANA on sleep.

On the other hand, proteins such phospholipases would be interacting in the sleep-inducing properties of ANA. For instance, the activity of the PLC, which is coupled to the CB_1 cannabinoid receptor (De Petrocellis et al., 2007; Ho et al., 1999), was evaluated. The selective PLC inhibitor U73122 (Lockhart and McNicol, 1999; Miyamoto and Ohshika, 2000) injected before ANA administration blocked the sleep-inducing effects caused by ANA. We concluded that ANA enhanced sleep activating the CB_1 receptor and it requires the activity of the PLC for its sleep-inducing effects (Murillo-Rodríguez et al., 2001a).

Could the sleep-inducing properties of ANA involve specific sleep-inducing molecules? It might be possible that ANA could induce sleep via the activation of one sleep-inducing molecule such as AD. Samples from microdialysis were collected from basal forebrain from rats in order to measure the extracellular levels of AD after the injection of ANA. The systemic administration of ANA (10 mg/kg, ip) induced an increase in the extracellular levels of AD during the first 3 h after administration of ANA. The administration of SR141716A significantly decreased AD levels. When SR141716A was injected before AEA, it blocked the increase in AD. Furthermore, we found that injection of ANA caused a decrease in W and a significant increase in SWS time. Additionally to this result, the enhancement in sleep was blocked by SR141716A (Murillo-Rodríguez et al., 2003). We confirmed that ANA modulates sleep and indeed, a sleep-inducing molecule such as AD was involved. All the evidence mentioned previously suggests that the endocannabinoid system modulates sleep (Murillo-Rodríguez et al., 1998, 2001a, 2003; Santucci et al., 1996).

5. Potential mechanism of action of the CB_1 receptor on sleep modulation

A possible link between the localization of the CB_1 receptor in sleep-inducing areas might be the potential mechanism of sleep promotion. I have hypothesized that the CB_1 receptor localized in neurons in pons and the basal forebrain, as demonstrated by others (Moldrich and Wenger, 2000; Ong et al., 1999), might be activating cholinergic neurons placed in the same regions (Harris, 2005; Jones, 2000; Sarter and Bruno, 2000). It is known that activation of CB_1 receptor enhances the release of ACh (Acquas et al., 2000). There is wide evidence showing that the release of ACh from the brainstem and the basal forebrain is higher during sleep (Fuller et al., 2006; Kodama et al., 1990; McCarley, 2007; Williams et al., 1994). Therefore, if the CB_1 receptor is expressed in cholinergic neurons (in the brainstem such as PPT/LDT complex and the basal forebrain), and these cells are activated by ANA, they might increase the release of ACh to induce sleep. This activation would trigger the thalamus neuron activity to enhance the cortical desynchronization (Jones, 2000; Szymuziak et al., 2007). There is solid evidence showing that the projections from the brainstem and the basal forebrain to the thalamus are important elements for sleep modulation (Fuentealba and Steriade, 2005; Fuller et al., 2006; McCarley 2007; Szymusiak et al., 2007).

The sleep–wake cycle could be under the influence of the diurnal variations of ANA. For instance, it is known that this endocannabinoid has been detected and quantified in several biological samples such as CSF, pons, hippocampus, and hypothalamus in the rat over 24 h. In CSF samples, ANA was enhanced during the lights-on period whereas its concentration diminished during the lights-off period. In the pons, this endocannabinoid showed its maximum values during the dark phase, and in the hypothalamus, it was observed that ANA rose during the lights-on period. It has been hypothesized that ANA is likely accumulated in parenchyma during the lights-off period (when the rodents are awake) and then, released into the CSF to reach out specific target regions in the CNS to modulate sleep (Murillo-Rodríguez et al., 2006a).

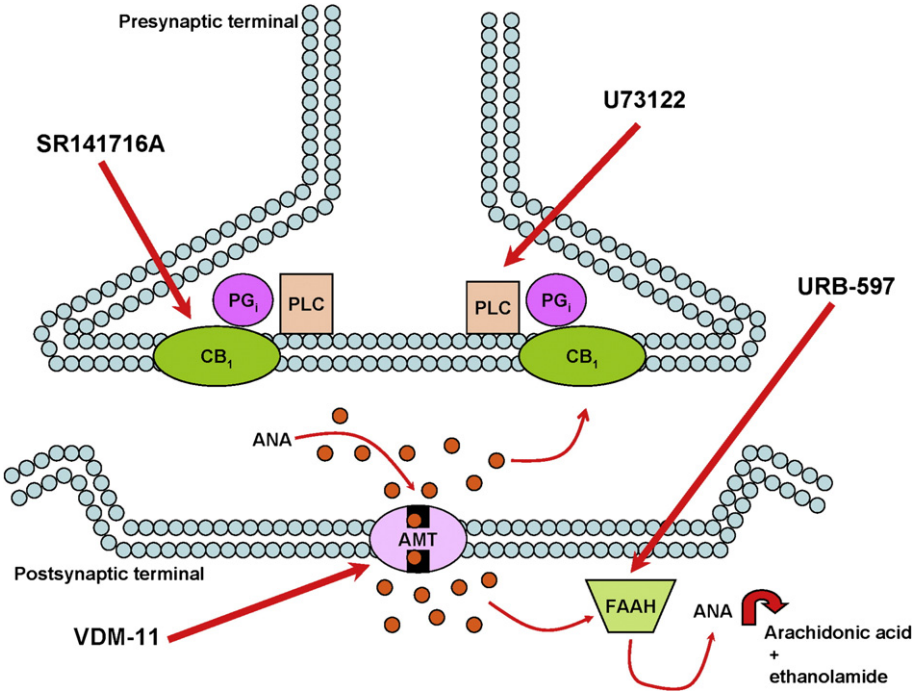

Fig. 4. Schematic representation of the hypothetical mechanism of action of the endocannabinoid system modulating sleep. Pharmacological blockade of the CB_1 receptor using SR141716A increases alertness whereas microinjection of the ligands either anandamide or cannabinoids enhances sleep. U73122, a selective PLC inhibitor, blocks the anandamide´s sleep-inducing effects. Similar results have been observed using VDM-11, an AMT blocker whereas inhibition of the activity of FAAH via URB-597 diminishes sleep. Abbreviations: AMT, anandamide membrane transporter; CB_1, CB_1 receptor; FAAH, fatty acid amide hydroalse; PGi, Gi coupled protein; PLC, phospholipase C.

5.1. Diurnal and homeostatic variations of the CB_1 receptor

Despite the lack of evidence of the neurophysiological role of the endocannabinoids on sleep modulation, there are significant reports about the pharmacological properties of ANA on sleep. However, what could be the role of the CB_1 receptor on sleep modulation? Importantly, it seems that this protein also participates in sleep promotion. It has been reported that the highest peak for the protein in the brainstem was at 13:00 h, whereas for the mRNA the zenith was found at 21:00 h. The lowest expression was detected at 01:00 and 09:00 h, for the protein and the mRNA, respectively (Martínez-Vargas et al., 2003). These results suggest that the expression of the CB_1 receptor is linked with a circadian component. One might think that the availability of this protein across the 24 h cycle would be related with modulation/maintenance of dependent-time functions such as sleep.

Additionally, the CB_1 receptor displays behavioural state-dependent variations (Navarro et al., 2003). During complete sleep deprivation the mRNA and protein of this protein is increased in rats compared to the animals that were not deprived of sleep. Thus, this result suggests that the CB_1 receptor might be modulating the sleep homeostasis mechanism through its availability. Further experiments will be needed to fully understand this biological process.

6. The endocannabinoid system. The potential therapeutical use

The pharmacological evidence of the role of the effects of the endocannabinoid system, which includes ANA, 2-AG, the receptors CB_1 and CB_2, the FAAH as well as AMT on different behaviours, offers attractive ideas to consider this system for therapeutical purposes as suggested by Ortega-Gutierrez (2005), including the treatment of diverse pathologies or to improve medical conditions (Bortolato et al., 2007; Burns and Ineck, 2006; Calignano et al., 1998; D'Argenio et al., 2006; Mechoulam et al., 2002; Mendizabal and Alder-Graschinky, 2003; Pazos et al., 2004; Porter and Felder, 2001; Robson, 2001).

To treat excessive somnolence, the development of drugs aimed to block the CB1 receptors (such as SR141716A) or to inhibit the activity of the PLC (using U73122) might be consider as new candidates in the near future as therapeutical options. Moreover, the use of drugs that block the AMT (including VDM-11) or to inhibit the FAAH activity (such as URB597) might represent an opportunity to explore their medical uses to treat insomnia by enhancing the endogenous levels of ANA (Fig. 4).

7. Discussion

Cannabinoids have been always identified as harmful drugs because of their negative effects on diverse neurobiological functions. The discovery of the endocannabinoid system, composed of endogenous lipids, receptors and metabolic enzymes, has brought information on the significance of endocannabinoid signalling in multiple neurophysiological processes. This system has been involved in molecular aspects such as cell survival, fertilization, and behavioural aspects including, learning and memory consolidation, pain perception, and sleep modulation.

The central and peripheral administration of ANA induces behavioural and cellular changes similar to those caused by Δ^9-THC. Most of the behavioural and molecular changes observed after the administration of ANA are the consequence of the activation of the CB_1 and CB_2 receptors and these effects are blocked using a specific antagonist. The CB_1 receptors have been found in CNS suggesting an active role in the regulation of homeostasis, such as sleep.

From the pharmacological and pharmaceutical perspective, the endocannabinoid system might be considered in the near future to treat diverse pathologies. Different and novelty strategies developing new drugs considering the elements of the endocannabinoid system could be useful as an effective approach to the prevention and management of sleep disturbances such as insomnia or excessive diurnal somnolence. The CB_1 antagonists, such as SR141716A, should be considered as pharmacological and pharmaceutical options for treating narcolepsy, whereas the endocannabinoid enhancers for managing insomnia.

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