When Orexins Meet Cannabinoids: Bidirectional Functional Interactions

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Abstract

A growing body of evidence suggests the existence of biochemical and functional interactions between the endocannabinoid and orexin systems. Cannabinoid and orexin receptors have been shown to form heterodimers in agreement with the overlapping distribution of both receptors in several brain areas, and the activation of common intracellular signaling pathways, such as the MAP kinase cascade. The activation of orexin receptors induces the synthesis of the endocannabinoid 2-arachidonoyl glycerol suggesting that the endocannabinoid system participates in some physiological functions of orexins. Indeed, functional interactions between these two systems have been demonstrated in several behavioral responses including nociception, reward and food intake. The present review is focused on the latest developments in cannabinoid-orexin cross-modulation and the implications of this interesting interaction.

Keywords: cannabinoid, orexin, heterodimers, reward, nociception, feeding

1. The endocannabinoid and orexin systems

The endocannabinoid system (ECS) consists of two main endogenous ligands: anandamide (AEA) and 2-arachidonoylglycerol (2-AG) called endocannabinoids, the synthetizing enzymes: diacylglycerol lipase-α (DAGL) and N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD), the degradation enzymes: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), and two major G protein-coupled receptors (GPCR): the cannabinoid type 1 (CB1R) and type 2 (CB2R) receptors. Other putative cannabinoid receptors have been described including the vanilloid type 1 receptor (TRPV1) [1], and the G protein-coupled receptor 55 (GPR55) [2]. Endocannabinoids are lipid signaling molecules that display distinct pharmacological profiles, as well as regional diversity [3]. Different to most neurotransmitters, they are not stored in presynaptic vesicles, but are synthetized and released on demand in the postsynaptic terminals in response to elevations of intracellular Ca2+ [4]. These compounds act as retrograde synaptic messengers travelling across synapses, where they bind to presynaptic cannabinoid receptors located in both neurons [5] and glia [6] to modulate neurotransmitter release. CB1R are the most abundant GPCR in the central nervous system (CNS) [7] and are widely distributed in the brain in areas such as the cortex, hippocampal formation, basal ganglia, and cerebellum [8]. In all these structures, CB1R are localized presynaptically on serotonergic, noradrenergic, dopaminergic, GABAergic, and glutamatergic nerve terminals [9–11]. However, there is also data demonstrating a postsynaptic localization for CB1R [12,13] in cortical dendrites where they can modulate self-inhibition processes [14,15], and in dendrites of a subset of hippocampal pyramidal neurons that upon activation recruit c-Jun-N-terminal kinases and nitric oxide [16]. In addition, CB1R are present intracellularly in mitochondria, at both presynaptic terminals and at somatodendritic compartments of glutamatergic and

GABAergic hippocampal neurons [17]. Both cannabinoid CB1R and CB2R are mainly coupled to Gi/o resulting in the inhibition of the adenylate cyclase/cAMP cascade and voltage-gated Ca2+ channels, as well as the stimulation of inwardly rectifying K+ currents, and of mitogen-activated protein kinase (MAPK) activity [18]. Interestingly, a growing amount of evidence shows that CB1R can couple with different subunits of the classic inhibitory Gi/o proteins, but also with Gαz, Gαq/11, and Gα12/13 [19,20], and that under specific circumstances, CB1R can shift toward activation of Gs and potentiation of neurotransmission [21,22]. In addition, activation of CB1R in astrocytes increases intracellular calcium probably mediated by Gq proteins [23], and in the cardiovascular system, CB1R can bind to Gs or Gq/11 to modulate vasoconstriction and hypertension [24]. The ECS is involved in a wide variety of brain processes including brain plasticity, learning and memory, nociception, inflammation, appetite regulation, metabolism, energy balance, sleep–wake cycle, regulation of stress, emotions, reward and addiction [25–27]. Fundamentally, this neuroregulatory system is highly complex and a great amount of research is still being carried out to understand more about the intricate ways in which the ECS functions in normal and pathological states.

The orexin/hypocretin (OX) system is composed of two endogenous ligands: OX-A/ hypocretin-1 and OX-B/ hypocretin-2, and two GPCRs: the OX-1/ hypocretin-1 (OX1R) and the OX-2/ hypocretin-2 (OX2R) receptors. OX-A and OX-B are neuropeptides produced from a common precursor peptide by a cascade of enzymatic reactions [28,29]. OX-A is a 33 amino acid peptide activating both OX1R and OX2R with similar potencies, whereas OX-B is a 28 amino acid peptide that is modestly selective for the OX2R [28]. OX-expressing cell bodies are specifically localized in the lateral and perifornical areas of the hypothalamus, but their projections are widely distributed throughout the CNS [28–31]. The OX receptors are abundantly expressed in the cerebral cortex, basal ganglia,

ventral tegmental area (VTA), nucleus accumbens, hippocampus, hypothalamic and thalamic nuclei, dorsal and medial raphe, locus coeruleus (LC), preoptic area, periaqueductal gray (PAG) and reticular formation [32]. OX1R activation triggers signaling through Gq/11 proteins resulting in activation of phospholipase C with subsequent initiation of the phosphatidylinositol cascade. However, in Chinese hamster ovary (CHO) cells expressing OX1R, other types of signaling have been described such as coupling to phospholipase A2 and DAGL, with the subsequent production of 2-AG [33], or Gi-protein coupling with inhibition of AC [34]. In addition, in primary neuronal cell cultures from rat cerebral cortex [35] and in primary cultures of rat astrocytes [36], OX1R stimulate cAMP production. OX2R have also been shown to couple with multiple G proteins including both Gq/11 and inhibitory Gi proteins [37]. Stimulation of both receptors activates the MAP kinase pathway leading to ERK1/2 and p38 kinase phosphorylation [38,39]. The OX system is involved in the control of numerous physiological functions and behaviors including attention, alertness, locomotion, regulation of sleep, reward and motivational processes, food intake, energy balance and metabolism [40]. OXs also have antinociceptive effects at spinal and supraspinal levels in several pain models [41], and are involved in stress-induced analgesia [42].

The ECS and OX system have common anatomical distributions in the CNS and thus share physiological functions [8,32,43]. OX1R, OX2R and CB1R are widely expressed in the hypothalamus where they regulate energy homeostasis and central neuroendocrine and autonomic functions [40,44]. In the mesocorticolimbic system, the septal nuclei and the amygdaloid nuclei, both OX receptors and CB1R co-exist and regulate natural reward and addiction processes [45–47]. Moreover, these receptors are located in brain stem areas such as the raphe nuclei, the LC, the reticular formation and the PAG, where they

are involved in the regulation of anxiety-like responses, sleep/wake cycle and nociception [48–50].

This review is not set out to be an exhaustive account of the functions of the ECS and OX system, but rather an update of the bidirectional interactions between these systems that have been reported in the last years, specifically in the areas of appetite regulation and energy balance, nociception and reward processes.

2. Biochemical interactions between orexins and cannabinoids

A growing body of biochemical and functional evidence suggests that GPCRs, historically considered monomers, form and function as homo- and heterodimers, or even higher-ordered oligomers. These dimers/oligomers often display unique ligand binding, distinct phenotypic trafficking, and specific signaling properties in comparison with their individual monomers [51,52]. Congruent with an overlapping distribution of CB1R and OX1R in certain brain areas, the first evidence of functional cross-talk between these receptors was demonstrated by co-expression of CB1R and OX1R in CHO cells [53]. Thus, a major CB1R-dependent enhancement of OX-A's potency to activate the MAPK pathway was shown, and this effect required functional CB1R since the specific CB1R antagonist rimonabant blocked this response. Moreover, by electron microscopy experiments, the authors concluded that CB1R and OX1R were close enough to form hetero-oligomers [53]. The possible existence of CB1R-OX1R heteromerization was further assessed by co-expressing both receptors in HEK293 cells [54]. Treatment with rimonabant resulted in decreased potency of OX-A to activate the MAPK ERK1/2 only in cells co-expressing the two receptors. The OX1R antagonist SB-674042 also reduced the potency of a CB1R receptor agonist to phosphorylate ERK1/2 only when the two

receptors were co-expressed [54]. Moreover, single cell fluorescence resonance energy transfer (FRET) imaging indicated that both CB1R and OX1R were present as heterodimers/oligomers in intracellular vesicles [54]. More recently, the same group provided further evidence of such heteromerization based on covalent labeling of extracellular domains of CB1R and OX1R with "SNAP" and "CLIP" tags. These are polypeptides which can be labeled covalently with a variety of reagents such as cell-impermeant fluorophores, allowing reliable monitoring of these heteromers at the cell surface [55]. Interestingly, a higher potency of OX-A to regulate the CB1R-OXR1 heteromer compared with the OX1R-OX1R homomer was found in this study, suggesting a functional interplay between these two systems [55]. The formation of CB1R-OX1R heteromeric complexes has been recently demonstrated in embryonic mouse hypothalamic neurons [56]. These heteromers were found to affect intracellular calcium levels, 2-AG biosynthesis and ERK phosphorylation [56]. In addition, by using a bioluminescence energy transfer (BRET) assay, both OX1R and OX2R subtypes were shown to be capable of forming constitutive homo- and heteromeric complexes with one-another and with CB1R [57]. However, although all these data provide unequivocal identification of CB1R-OX1R heteromerization, whether these complexes are functional under physiological conditions, and whether they are dynamically regulated remains to be elucidated [58]. The development of bivalent ligands, which preferentially interact with the receptor heterodimers, will represent a possible approach of determining their physiological significance [59].

Apart from heteromerization, some studies suggest that the most important part of the interaction between CB1R and OX1R results from 2-AG production in response to OX stimulation [60]. Release of 2-AG following the administration of OX-A has been demonstrated in CHO cells [33]. In these cells, the activation of PLC was shown to be

responsible for DAG production, which in turn is used by DAGL as a substrate for 2-AG formation [33]. Therefore, it is possible that the activation of CB1R by 2-AG could contribute to increase the potency of OX signaling observed in CHO cells. Moreover, as it will be described later in this review, this OX-induced 2-AG release has been demonstrated to be of great relevance in several studies. Thus, 2-AG modulates the effects of OXs on nociception, addiction and food intake.

3. Involvement of endocannabinoids in the effects of orexins on food intake and energy balance

The OX system is a key modulator of both food intake and energy expenditure. Under physiological conditions, hormonal control of food intake is mainly mediated by the action of leptin and ghrelin in the hypothalamus, where they exert opposing effects on the activity of anorexigenc pro-opiomelanocortin (POMC)/cocaine-amphetamine-related transcripts (CART), and orexigenic agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons in the arcuate nucleus. Leptin, produced by adipocytes, indirectly controls the activity of OX neurons via long form leptin receptors (LepRb) in neurotensin-expressing cells in the lateral hypothalamus (LH) [61,62], and through the action of neuropeptides released from the POMC or AgRP/NPY neurons [63,64]. On the other hand, ghrelin released during fasting directly activates OX neurons by binding to ghrelin receptors (GHSR) located in these neurons [65–67]. Correspondingly, OX stimulates orexigenic NPY-containing neurons and decreases the activity of anorexigenic POMC-containing neurons, via OX1R [68,69]. Thus, the control of feeding by OX involves local bidirectional interactions in the hypothalamus between OX neurons and NPY and POMC cells [70–73], their modulation by peripheral hormones, and their output connections to limbic, locomotor, autonomic and neuroendocrine systems [28,29,74].

Furthermore, the control of the pathways involved in homeostatic food intake is preponderantly modulated by the ECS. Thus, the levels of 2-AG increase in the hypothalamus in normal animals during fasting, decrease during refeeding, and return to normal when rats are satiated [75], while in rodent models of obesity, elevated levels of endocannabinoids within hypothalamic nuclei have been observed [76,77]. In addition, a wide distribution of CB1R has been demonstrated in the hypothalamus, with a presynaptic localization on GABAergic terminals innervating NPY/AgRP and melanin-concentrating hormone (MCH) neurons and on glutamatergic projections to OX neurons [78], and positioned post-synaptically on POMC/CART [69] and corticotrophin-releasing hormone [25] neurons. Under physiological conditions, the orexigenic action of cannabinoids has been attributed mainly to their excitatory effect on MCH cells mediated by inhibition of inhibitory GABAergic input to these neurons. In contrast, cannabinoids reduce the activity of OX cells due to the presynaptic attenuation of glutamate release, leading to a decrease in arousal [78]. In addition, it has been shown that the peripheral administration of sub-threshold doses of the CB1R antagonist rimonabant blocks the orexigenic effect of OX-A administered by intracerebroventricular route [79]. The ECS may influence food intake by regulating the expression and/or action of OXs. Thus, intracerebroventricular injection of the CB1R inverse agonist AM251 produced a significant decrease in the number of neurons expressing OX-A in the hypothalamus [80].

In conditions where hormone levels are dysregulated, such as in obesity, OX neurons may contribute to hyperphagia and obesity [81]. Indeed, in leptin-knockout (ob/ob), and in diet-induced obese mice the balance between GABA/Glutamatergic innervation to OX cells is shifted to predominantly inhibitory CB1R-expressing inputs. An overexpression of the biosynthetic enzyme for the endocannabinoid 2-AG, DAGL, was also observed in these animals. These changes were reversed by leptin administration. Notably, these

alterations lead to a retrograde inhibition of GABAergic terminals by 2-AG, increased neuronal activity of OX cells, and enhanced signaling activity in projection areas mediating reward, motivation and food intake, such as the VTA, nucleus accumbens and arcuate nucleus [81]. Subsequent electrophysiological studies in slices from the LH of ob/ob mice confirmed that the upregulation of 2-AG by OX neurons leads mainly to a depression of inhibition, and consequently the excitatory drive on OX neurons becomes functionally predominant [82]. More recently, a role for the interaction between OX-A release in the arcuate nucleus and CB1R located in POMC neurons has been put forward in promoting hyperphagia [69]. Thus, the activation of OX1R by OX-A induces the synthesis of 2-AG, which binds to postsynaptic CB1R in POMC cells leading to a decrease in POMC and α–melanocyte-stimulating hormone (α-MSH) synthesis in the paraventricular nucleus of the hypothalamus, and to hyperphagia (Figure 1). During obesity, characterized by leptin signaling deficiency, this system is hyperactivated and high levels of OX-A and 2-AG correlate with decreased α-MSH levels and increased body mass index [69].

Interestingly, OX neurons have also been shown to protect the body from obesity by promoting energy expenditure [83]. Thus, the overexpression of OXs induces resistance to high fat diet-induced obesity and insulin insensitivity by promoting energy expenditure and reducing food consumption. This action of OX cells is partly mediated through OX2R and an increase in leptin sensitivity [84]. More recently, using *in vivo* calcium imaging it has been demonstrated that OX neurons are activated by hunger signals, but are rapidly inactivated by eating irrespective of the nature of the food, reducing food foraging and promoting storage of energy [85,86]. On the other hand, the complete selective inactivation of the OX system in the brain can promote overeating and increases body weight in free-feeding mice, a condition that is reversed by dieting [85]. These studies

indicate that the OX system is more involved in food seeking than in food consumption, and put forward the interesting notion that in pathological conditions where the activity of OX neurons is dysregulated, adjustments in food intake could play a normalizing role [85].

The ECS also modulates body weight and fat mass, probably through peripheral lipogenesis [87]. In addition, chronic blockade of CB1R improves peripheral metabolic parameters. Notably, an interaction between the OX system and the ECS on feeding behavior and on the activity of glucose sensitive neurons in the arcuate nucleus has been recently suggested. Thus, the local administration of the CB1R antagonist AM251 into the arcuate nucleus modulates food intake induced by OX-A, and blunts its effects on glucose responsive neurons in this structure [88]. However, it is still not known whether the OX system and the ECS interact in periphery and in the brain to modulate energy balance.

4. Involvement of endocannabinoids in the effects of orexins on nociception

OX neurons project to several regions of the CNS involved in the regulation of pain, including the ventrolateral periaqueductal gray (vlPAG) and spinal dorsal horn, where OX receptors are densely distributed [30,32]. The descending antinociceptive pathway controls pain perception at the spinal level by the activation of PAG neurons leading to the excitation of cells in the rostroventral medulla, that send inhibitory projections to the dorsal horn of the spinal cord via the dorsolateral funiculus [89]. The antinociceptive effects of OXs have been widely demonstrated (see [41] for review), and one mechanism that has been proposed to explain this role is the interaction with the ECS at the level of the PAG. Hence, Ho et al. [90] have demonstrated that activation of OX1R by OX-A in PAG slices increases the synthesis of 2-AG via the phospholipase C-DAGLα route, and

inhibits GABAergic tone leading to an increase in PAG neuronal activity (Figure 1). This mechanism of action has also been postulated to contribute to stress-induced analgesia [91]. In addition, recent studies show that although both OX-A and an OX2R selective agonist injected in the vlPAG induce antinociception, only OX1R-mediated antinociception is blocked by the CB1R receptor antagonist AM 251 [42]. Moreover, the antinociceptive effect of intra-LH administration of carbachol is mediated through the activation of OX1R and OX2R in the vlPAG [92,93]. CB1R activation in this brain area also contributes to the modulation of carbachol-induced antinociception [92,93]. In addition, the OX system and the ECS interact at other brain areas involved in nociception, such as the LC since local administration of OX-A in this area induces analgesia through activation of OX1R and CB1R [94]. Finally, since eating disorders/obesity are often associated to altered pain perception [95], the relationship between the OX system and the ECS has been investigated in this case. Obese ob/ob mice exhibiting total leptin deficiency show hypoalgesic behavior associated with an increase in OX-A and 2-AG levels in the vlPAG, leading to the disinhibition of the vlPAG neurons and activation of the descending antinociceptive pathway [95]. Furthermore, these authors found that high fat diet-induced obese mice showing leptin signaling deficiency only in the arcuate nucleus, also present high levels of OX-A and 2-AG in the vlPAG, but analgesia is only unmasked following systemic leptin receptor antagonism. Therefore, they concluded that the activation of the descending antinociceptive pathway mediated by the interaction between the OX and ECS partly underlies the increased pain thresholds in conditions associated with impaired leptin signaling [95].

5. Involvement of endocannabinoids in the modulation of the brain reward system by orexins

The OX system regulates the mesocorticolimbic dopaminergic pathway, which is the circuit responsible for the pleasure feelings associated with natural and drug rewards. Numerous studies have demonstrated a role for the OX system in the addictive properties of drugs of abuse [47,96], consistent with the existence of reciprocal connections between OX-rich nuclei and brain areas involved in reward processing [30], including the VTA and the nucleus accumbens. OXs seem to regulate reward seeking by modulating VTA dopaminergic transmission. In agreement, intra-VTA injection of OX-A increased extracellular dopamine levels in both the prefrontal cortex and the nucleus accumbens [97]. OXs influence VTA dopamine cell firing not only via direct depolarization [98], but also by interacting with glutamate and GABA transmission. Activation of GABAergic neurons in the VTA constitutes an inhibitory input on dopaminergic neurons, while increase of glutamate transmission is an excitatory influence on VTA dopaminergic activity. In brain slices, OXs were shown to increase dopaminergic activity in the VTA via long-term potentiation of glutamatergic transmission [99], and also by directly increasing the firing of GABAergic neurons [98]. Although activation of GABAergic cells is predicted to decrease VTA dopaminergic activity, OXs could facilitate a switch in the balance between excitatory and inhibitory synaptic inputs to VTA dopaminergic neurons in some conditions, such as the exposure to drugs of abuse. Indeed, the ability of OX to increase glutamate transmission in parallel with a decrease in GABA transmission in the VTA has been recently described in brain slices of animals treated with morphine by using electrophysiology [100]. On the other hand, it is well established that endocannabinoids and CB1R participate in the rewarding properties of natural rewards, and also in those induced by different drugs of abuse [45,101]. Acting as retrograde

messengers, endocannabinoids produce inhibition of neurotransmitter release in the VTA. The activation of CB1R located on axon terminals of GABAergic neurons in the VTA inhibits GABA transmission, thus removing this inhibitory input on dopaminergic neurons [102]. Glutamatergic transmission in the VTA, arising mainly from neurons of the prefrontal cortex, is similarly inhibited by CB1R activation [103]. The final effect of endocannabinoids on the modulation of VTA dopamine neurons, which depends on the balance between excitatory and inhibitory inputs, is predominantly excitatory [45].

A recent study has revealed an interesting interaction between OXs and endocannabinoids within the VTA in the regulation of cocaine relapse induced by stress [104]. In a sequence of elegant experiments, these authors demonstrated that acute restraint stress activates OX neurons in the LH, increases OX-A levels in the VTA, and reinstates extinguished conditioned place preference induced by cocaine. Notably, the activation of postsynaptic OX1R on VTA dopaminergic neurons by OXs leads to the production of 2-AG through the PLC and DAGL enzymatic cascade, which then retrogradely inhibits GABA release through CB1R, followed by the disinhibition of VTA dopaminergic neurons [104]. Moreover, stress-induced reinstatement of cocaine place preference was prevented by either systemic or intra-VTA injection of an OX1R or CB1R antagonist, and by a DAGL inhibitor [104]. This study provides a novel mechanism for stress-induced cocaine relapse based on this OX1R-PLC-DAGL-2-AG cascade in charge of the final disinhibition of VTA dopaminergic neurons (Figure 1). In agreement with this mechanism, a previous behavioral study showed that intra-VTA injection of CB1R receptor or OX1R antagonists produced comparable and non-additive reductions in the conditioned place preference induced by chemical stimulation of the LH with the cholinergic agonist carbachol [105]. This result suggests that CB1R and OX1R in the VTA regulate this behavioral response by a common mechanism, rather than activating two parallel pathways. In addition, an

interaction between CB1R and both OX1R and OX2R in the nucleus accumbens has also been shown to be involved in the conditioned place preference induced by LH stimulation [106,107]. Recently, it has been described that OX2R and CB1R located in the VTA play a role in the rewarding properties of nicotine as revealed by using a conditioned place preference paradigm [108]. Future investigation at molecular, cellular and behavioral levels will be required to better understand the exact mechanisms and potential therapeutic implications of the interaction between these systems in the regulation of the reward circuit. The formation of CB1R-OX1R heteromers with specific signaling properties could be important for the interaction of these systems in the regulation of reward, as has been previously described for CB1R and dopamine D2 receptors [21].

6. Involvement of orexins in the pharmacological effects of cannabinoids

As described in the previous sections of this review, the contribution of the ECS in several physiological functions of OX has been clearly established. However, so far few studies have evaluated whether the opposite is also occurring, that is, does the OX system participate in the pharmacological effects of cannabinoids? A relevant aspect of OX-cannabinoid interplay is observed in the addictive properties of cannabinoids. Cannabis is the most frequently used illicit drug worldwide, and the number of people seeking treatment for cannabis use disorder has dramatically increased in the last decades [109]. Therefore, the identification of new therapeutic targets to improve treatment outcomes for cannabis dependence is imperative considering that currently there are no effective pharmacotherapeutic approaches for this disorder [110].

The first evidence supporting the modulation of OXs on cannabis dependence was based on modifications in the expression of OX-A in peripheral blood cells in cannabis-

dependent smokers when compared to nicotine-dependent smokers and non-smokers [111]. However, peripheral OX mRNA levels do not necessarily reflect the situation in the CNS; hence these differences are probably related to the peripheral actions of Δ^9-tetrahydrocannabinol (THC), and not to its central effects involved in the development of dependence. Nevertheless, subsequent behavioral studies have demonstrated that the OX system modulates cannabinoid reward. Thus, the intravenous self-administration of the synthetic cannabinoid WIN55,212-2 was reduced by both genetic deletion and pharmacological blockade of OX1R [112]. In contrast, OX2R were not involved in this response. Also, the increase in dopamine extracellular levels in the nucleus accumbens induced by THC was blocked in mice lacking the OX1R, suggesting that cannabinoids require orexinergic transmission to modulate the dopaminergic mesolimbic pathway. Moreover, contingent WIN55,212-2 self-administration, but not passive exposure to this cannabinoid, increased the activation of OX neurons in the LH [112], indicating that the recruitment of OX cells within this structure is mainly due to operant seeking for the reinforcing effects of this drug, and not to its pharmacological actions [112]. As a whole, these data show that OX1R antagonists could represent an interesting pharmacological tool for cannabis dependence in humans.

In a more general sense, a recent study evaluated the involvement of the OX system in several acute pharmacological effects of THC. The hypothermia, supraspinal antinociception and anxiolytic-like effects induced by THC were modulated by OX through OX2R signaling [113]. In contrast, OXs were not shown to be involved in the hypolocomotion, spinal antinociception, amnesic- or anxiogenic-like effects produced by this cannabinoid. Interestingly, OX1R did not participate in any acute pharmacological effects of THC [113]. Therefore, the blockade of OX1R would specifically abolish the reinforcing properties of cannabinoids without affecting other pharmacological responses

of these compounds [112,113] (Figure 2). This is important since one of the major challenges in cannabinoid research consists in identifying possible mechanisms to dissociate the therapeutic action of cannabis from its detrimental consequences, among which cannabis addiction represents a pivotal concern.

Concluding remarks

Anatomical, biochemical and behavioral studies support the existence of bidirectional interactions between the ECS and the OX system. There is an increasing amount of data indicating that endocannabinoids exert a crucial modulation of the effects of OXs on food seeking and antinociception through the release of 2-AG in hypothalamic and brain stem areas. Moreover, new data is pointing towards a relevant role of OXs on stress-induced cocaine relapse also via a 2-AG-mediated mechanism in brain reward structures. This intriguing evidence emphasizes the prominent role of the endocannabinoid-mediated retrograde inhibition of GABAergic neurons in several major physiological brain functions: motivation for food and drug reward, as well as, a pain perception. The involvement of OX1R in the rewarding properties of cannabinoids, but not in other pharmacological effects, is of special interest since OX1R antagonists could be potentially useful to treat cannabis dependence without affecting other beneficial properties of these compounds. Although the mechanisms underlying this interaction are poorly understood, the existence of CB1R-OX1R heteromers with specific functional signaling properties could be the basis for some of these effects. The development of new tools to further probe this functional interaction may lead to novel therapeutic approaches to neurological disorders mediated by these receptors. This field, where OXs meet cannabinoids will certainly expand with time as research develops on their molecular and behavioral interactions, providing deeper insight into its relevance in physiological and

pathological conditions, and a better understanding of their potential therapeutic implications.

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Conflict of interest

The authors declare no competing interests.

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Figure 1. Orexins repress neuronal activity through endocannabinoid signaling. Upon orexin release, activation of postsynaptic OX1R leads to biosynthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG) through a Gq-protein-phospholipase C-diacylglycerol lipase (PLC-DAGL) cascade. 2-AG diffuses to the extracellular space and activates CB1R present in surrounding cells, producing hyperpolarization of the neuronal membrane and inhibiting subsequent neurotransmitter release at the axon terminal. This mechanism has functional consequences at diverse orexinergic target sites: 1. Within the ventral tegmental area (VTA), OX1R-induced 2-AG release leads to inhibition of GABAergic interneurons and subsequent disinhibition of dopaminergic neurons, contributing to cocaine relapse; 2. In the arcuate nucleus (ARC), POMC neurons are retrogradely inhibited by their own OX1R-induced 2-AG generation, this leads to diminished satiety signaling, producing hyperphagia and contributing to obesity; 3. Within the periaqueductal gray (PAG) GABAergic interneurons are also inhibited by 2-AG release, resulting in disinhibition of non-determined antinociceptive pathways. PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; NT, neurotransmitter; OX, orexin neurons; LH, lateral hypothalamus.

Figure 2. Orexinergic modulation of cannabinoid-induced behavioral effects. Exogenous cannabinoids, such as THC or WIN55,212-2, produce a series of behavioral responses, presumably through CB1R. Some of these responses are independent of orexin transmission, including hypolocomotion, anxiogenic-like and amnesic-like effects. However, antinociception, hypothermia and anxiolytic-like responses are modulated by OX2R signaling. Besides, OX1R stimulation contributes to cannabinoid-induced reinforcement. This means that selective OX1R antagonists could be employed as a therapeutic tool to prevent the potential abuse liability associated to cannabinoid-based therapies while preserving their beneficial effects.