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Role of the Endocannabinoid System in the Neuroendocrine Responses to Inflammation

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Abstract: A few years ago the endocannabinoid system has been recognized as a major neuromodulatory system whose main functions are to exert and maintain the body homeostasis. Several different endocannabinoids are synthesized in a broad class of cell types, including those in the brain and the immune system; they bind to cannabinoid G-protein-coupled receptors, having profound effects on a variety of behavioral, neuroendocrine and autonomic functions. The coordinated neural, immune, behavioral and endocrine responses to inflammation are orchestrated to provide an important defense against infections and help homeostasis restoration in the body. These responses are executed and controlled mainly by the hypothalamic-pituitary adrenal axis. Also, the hypothalamic-neurohypophyseal system is essential for survival and plays a role recovering the homeostasis under a variety of stress conditions, including inflammation and infection. Since the endocannabinoid system components are present at sites involved in the hypothalamic-pituitary axis regulation, several studies were performed in order to investigate the endocannabinoid-mediated neurotransmitters and hormones secretion under physiological and pathological conditions. In the present review we focused on the endocannabinoids actions on the neuroendocrine response to inflammation and infection. We provide a detailed overview of the current understanding of the role of the endocannabinoid system in the recovering of homeostasis as well as potential pharmacological therapies based on the manipulation of endocannabinoid system components that could provide novel treatments for a wide range of disorders.

Keywords: Anandamide, cannabinoid receptors, infection, cytokines, hormones, hypothalamus, pituitary, adrenal gland.

INTRODUCTION

Marijuana has been used for thousands of years for both medicinal and recreational purposes since the second millennium BC by the Assyrians through Arab, China, India and Egypt, arriving to Europe after Napoleonic crusade and yet only recently we began to understand the basic mechanisms and actions in the brain and periphery [1]. The isolation and structure elucidation of Δ^9-tetrahydrocannabinol (THC), the major psychotropic component of the plant *Cannabis sativa*, was achieved in 1964 by Raphael Mechoulam initiating the era of research on the mechanisms by which cannabis affects the body. Since the discovery of the cannabinoid type 1 (CB_1) receptor in 1988, the first cell membrane protein found within the brain that binds THC, a tremendous amount of research has been accumulated in the last two decades on this issue [1, 2]. The identification of the specific receptors for cannabinoids prompted the search for endogenous ligands. The isolation of these ligands and the characterization of components of an "endocannabinoid system" lead researchers toward its participation in multiple physiological and behavioral processes including metabolic functions, cognition, motor control, reproduction, pain, feeding behaviors, stress and immune responses. Moreover, a deregulation of the endocannabinoid system could be related to diverse and several pathologies, i.e. neuropsychiatric conditions such as depression, schizophrenia and anxiety, eating disorders and neuroinflammation [3]. The endocannabinoid system is a neuromodulatory system widespread distributed throughout the central nervous system (SNC) as well as in peripheral organs. Components of the endocannabinoid system are present throughout the hypothalamic-pituitary axis and several evidences suggest that both systems interact extensively. The current review summarized the most relevant findings relating to interactions between endocannabinoid and hypothalamic-pituitary systems, especially through the neuroendocrine response to inflammation.

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ENDOCANNABINOID SYSTEM

The endocannabinoid system is comprised by specific receptors, endogenous ligands and enzymes that synthesize, degrade and transport those ligands. The endogenous ligands called endocannabinoids are lipophilic arachidonic acid derivatives produced "on demand" in different organs and tissues. Currently, several endocannabinoids have been identified; the first discovered, anandamide (AEA) [4] is a polyunsaturated fatty acid amide formed by ethanolamine bound to a fatty acid with 20 carbons. It was surprising that anandamide exhibited the same potent pharmacological activities as those of exogenous cannabinoids, despite its chemical structure being completely different from those of plant-derived cannabinoids that are aromatic terpenoids. The second endocannabinoid to be discovered is 2-arachidonoylglycerol (2-AG) an ester between arachidonic acid and glycerol isolated from canine intestine that seems to be the most efficacious selective natural cannabinoid agonist [5, 6]. Other endocannabinoids also described are noladin ether, virodhamine, N-arachidonoyl dopamine and N-oleoildopamine which physiological relevance still needs to be investigated. We focused the present review on anandamide actions since they are the best characterized endocannabinoid at present.

Anandamide is a lipophilic compound produced from the remodeling of membrane phospholipid, and as such, is not stored into vesicles to be released following exocytosis. Instead, anandamide is made "on demand" from membrane-localized precursor, and immediately released via an as-yet-unidentified mechanism to act in an autocrine or paracrine manner. On this respect is similar to other modulators such as prostaglandins and leukotrienes, but differ from classic neurotransmitters. Since the enzymes that catalyze the formation of anandamide from its biosynthetic precursor are Ca^{2+}-sensitive, the trigger for endocannabinoid biosynthesis and release is the elevation of the intracellular Ca^{2+} concentration that follows either Ca^{2+} entry (such as after neuronal depolarization), or mobilization from intracellular stores (such as following stimulation of metabotropic receptors coupled to Gq/11 proteins), or both, indicating that anandamide is produced in moments of intense activity of

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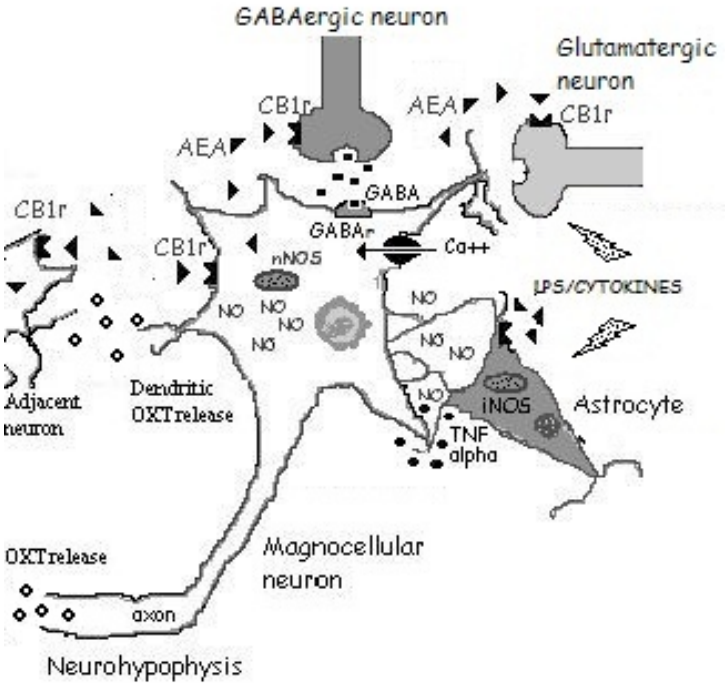

Fig. (1). Postulated mechanism for endocannabinoid system on the release of oxytocin (OXT) from magnocellular neurons after immune challenge. Cytokines from the periphery or bacterial lipopolisaccharide (LPS) increase the brain production of cytokines, between them TNF-alpha. There is also astrocytic inducible nitric oxide synthase (iNOS) activation with an increase in nitric oxide (NO) production. All these factors together with Ca++ depolarization induce the activation of magnocellular neurons increasing the release of oxytocin, from dendrites into different brain nucleus reaching adjacent neurons and axonal to general circulation via the neurohypophysis. Also, LPS increases the synthesis of anandamide (AEA) which stimulates neuronal nitric oxide synthase (nNOS) activity, increasing NO and therefore stimulating oxytocin release. Furthermore, anandamide acts as a retrograde messenger on cannabinoid receptors type 1 (CB_1r) present on presynaptic terminals of adjacent GABAergic and glutamatergic neurons. Since CB_1 are Gi/o coupled receptors, anandamide inhibition of GABA effect adds to the augmentation of oxytocin release. Moreover, anandamide also diminishes LPS-induced glutamate release.

the central nervous system [7, 8]. Since the phospholipid biosynthetic precursors for endocannabinoids seem to be ubiquitous in membranes, it is the pattern of expression of endocannabinoid biosynthesizing enzymes and cannabinoid receptors that determines the specificity of endocannabinoid action, whereas the localization of the degrading enzymes sets its duration [9].

Anandamide levels change in response to different stimulus, in different developmental stages and in diverse pathological conditions and usually acts restoring the physiological homeostasis by reducing excitotoxicity, inflammation and neuronal death and regulating hormones release. Those facts indicate the physiopathological relevance of this endocannabinoid and they also suggest a possible intervention on the control of its levels with therapeutic purposes.

There is no efficient mechanism of anandamide synthesis actually described. It was suggested that brain enzyme called fatty acid amide hydrolase (FAAH) catalyzed both the hydrolysis of anandamide and its synthesis from arachidonic acid and ethanolamine [10]. However this route seems to have not physiological importance since high levels of substrates are necessary. The principal pathway of anandamide biosynthesis is composed by two steps of enzyme reactions, starting from a membrane glycerophospholipid precursor. The first step is the transfer of an acyl chain of a glycerophospholipid to the amino group of the phosphatidylethanolamine (PE), catalyzed by calcium dependent N-acyltransferase (NAT). The second step is the hydrolysis of the generated N-acylphosphatidylethanolamine (NAPE) to N-acylethanolamine (anandamide belongs to this large family) and phosphatidic acid by a phosphodiesterase of the phospholipase D-type (NAPE-PLD) [11].

Endocannabinoids released into intercellular space binds to two well characterized cannabinoid receptors CB_1 and CB_2. Furthermore, other candidates such as the orphan G protein-coupled receptor 55 (GPR55), peroxisome proliferator-activated receptors (PPAR´s) and transient receptor potential vanilloid-1 (TRPV1) channels are either activated by cannabinoids and/or endocannabinoids. Both CB_1 and CB_2 belong to the seven-transmembrane G-protein-coupled receptor family with a glycosylated amino-terminus and an intracellular carboxyl-terminus. Their activation typically leads to inhibition of adenylate cyclase, attenuating the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and thereby can stimulate the mitogen-activated protein (MAP) kinase pathway. Another major action is the direct G protein-mediated closing of calcium channels, opening of potassium channels and the stimulation of protein kinase A (PKA) [12].

The CB_1 receptors are the most abundant receptors in the mammalian brain found in several areas including the olfactory bulbs, some cortical brain regions, hippocampus, amygdala, basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex and brainstem nuclei [13]. CB_1 receptors are also present at much lower concentrations in diverse peripheral tissues and cells such as immune cells, cardiovascular and reproductive tissues, adipocytes and gastrointestinal system [14]. In the central nervous system CB_1 receptors are mainly located on presynaptic nerve terminals and their activation brings about a decrease in neurotransmission; therefore, endocannabinoids act as "*retrograde messengers*" [15]. When anandamide is biosynthesized and released from the post-synaptic neuron to activate pre-synaptic cannabinoid CB_1 receptors, in most cases via inhibition of voltage-activated calcium channels, reduce

the release of both excitatory (e.g., glutamate) and inhibitory (e.g., GABA) neurotransmitters. This "*retrograde*" signaling has been suggested to participate in several physiological and pathological functions at brain level, including the control of food-intake, excitotoxicity, hormones and neuropeptides release and also stress and immune responses.

The CB_2 receptors were previously thought to be predominantly expressed in immune cells in the periphery and act to modulate immune function [16]. CB_2 receptors are also expressed in tonsils, bone marrow, thymus, pancreas, adult rat retina, and peripheral nerve terminals in the mouse vas deferens [17]. However, it has currently been demonstrated the expression of CB_2 in neuronal, glial and endothelial cells in the brain. The role of CB_2 receptors in the immune system and its therapeutic potential in pain, inflammation, autoimmune and neurodegenerative disorders is receiving a great deal of attention in current researches. Since high expression of CB_2 are found on B and natural killer (NK) cells, its signaling is thought to mediate cannabinoid inhibition of T cell proliferation, modulation of proinflammatory cytokine secretion and B cell responses. Also, several studies provide evidence about the neuronal involvement of CB_2 receptors and its possible role in drug addiction, eating disorders, psychosis, depression and autism [18]. Furthermore, the role of CB_2 receptors in central nervous system disturbances such as neuroinflammation and neuropathic pain has been reported. Both CB_1 and CB_2 receptors seem likely to work independently or in opposite directions and/or cooperatively in different neuronal and/or glial cell populations to regulate relevant physiological activities in the central nervous system.

Besides the classical cannabinoid receptors CB_1 and CB_2, there is evidence that TRPV1 channels also participates in endocannabinoids signaling. Interestingly, anandamide behaves as a partial agonist of CB_1 receptors but a full agonist of TRPV1 [19]. These receptors could bind endocannabinoids that appear to act in a non-retrograde manner to modulate post synaptic transmission as well as trigger gliotransmission. These channels are largely expressed in afferent peripheral sensory neurons and also been found in central nervous system where they regulate synaptic function such as the release of substance P (SP) responsible for local vasodilator and pro-inflammatory effects. Therefore, their physiopathological relevance still needs to be fully understood [20].

Following their release and CB receptor binding, endocannabinoids are prone to putative re-uptake into cells where they are rapidly degraded. Several hypotheses of transport of anandamide-like endocannabinoids have evolved overtime. The most insightful hypothesis involves the passive diffusion of lipophilic endocannabinoids across the plasma membrane. This model of transport requires FAAH alone to bind anandamide directly from the extracellular space and then FAAH-mediated hydrolysis into ethanolamine and arachidonic acid. Alternatively, an endocytotic uptake mechanism has been proposed, based on the cytoplasmic and predominantly perinuclear localization of FAAH. In this model, the sole function of FAAH is to establish a concentration gradient for anandamide transport through its hydrolysis. Since the delivery of anandamide to perinuclear FAAH may require a special protein (or a currently undefined process), other model proposed that anandamide enters the cell through the plasma membrane via a rapid endocytotic process, which may involve ancillary binding proteins, and then delivered to cytoplasmic FAAH for hydrolysis. Finally, using a small-molecule inhibitor of anandamide uptake, a high-affinity binding site that is independent of FAAH expression has been identified, indicating a role for a specific protein-mediated uptake process. This last model involves a specific binding protein facilitating anandamide transport across the plasma membrane. Although the highest concentration of FAAH has been localized into perinuclear compartments, this work suggests that sufficient FAAH resides at the plasma membrane closely associated with the transport protein or otherwise moves to the plasma membrane based on use-dependency to facilitate transport and hydrolysis [17]. Very recently, it has been described a cytosolic variant of the intracellular anandamide-degrading enzyme fatty acid amide hydrolase-1 (FAAH-1), termed FAAH-like anandamide transporter (FLAT), that lacked amidase activity but bound anandamide with low micromolar affinity and it could also facilitate its translocation into cells. In the central nervous system, after binding to CB_1 receptors, the endocannabinoids are internalized into synaptic terminals and possibly surrounding glial cells by this late selective transport system described above [21].

The activity of lipid mediators on the cannabinoid receptors is essentially terminated following their hydrolysis by several lipases. As we have previously mentioned, the anandamide degrading-enzyme is fatty acid amide hydrolase (FAAH) primarily found in post synaptic terminals while other endocannabinoids degrading enzymes seems to be predominantly located in presynaptic terminals. These findings support the idea that the lifetime of each endocannabinoid may be differentially regulated in order to serve to different functions. FAAH is a membrane-bound serine hydrolase, which belongs to a distinct class of enzymes characterized by the amidase signature which confers to FAAH the ability to hydrolyze amide bonds from various endogenous bioactive lipids [22]. FAAH has a vast spectrum of inhibitors and comprises numerous potent and selective compounds. The most widely used is the carbamate derivative URB597, which inactivates FAAH by carbamylation of the Ser nucleophile. Using an inhibitor of this degrading enzyme could allow increasing local levels of anandamide due to their on-demand production. Advantages of inhibiting the endocannabinoid hydrolyzing enzymes exist since, a subset of the effects obtained following agonists administration are observed by selectively inhibiting FAAH alone . Looking at the cannabinoid tetrad of effects - i.e. antinociception, catalepsy, hypo-locomotion and hypothermia - all the effects are present following CB_1 agonist administration, but only antinociception is induced upon FAAH inhibition [23].

The endocannabinoid network is suitable for multiple points of interaction with another signaling and neuroimmunoendocrine systems and it has been recognized as a major neuromodulatory complex, mastering homeostasis maintenance; including metabolic balance; crucial for an organism's survival and normal functioning. Unfortunately, due to various environmental and internal interfering factors, disruption of homeostasis is quite common and often leads to development of diseases. The stress and immune responses are the best examples for recovery of homeostasis; however the intensity and duration of these responses must be strictly controlled to re-establish the balance point to avoid extensive tissue damage. An exacerbated or improper activation of these responses increase the risks for developing devastating diseases such as neuroinflammation, neurodegeneration, chronic inflammatory and autoimmune diseases [24]. Interestingly, research in the cross-disciplinary field of neuroendocrinology and immunology has unexpectedly made quite significant contributions in this area since both, the neuroendocrine and the immune systems are involved in the pathophysiology of these diseases [25]. Relationships between the endocrine, nervous and immune systems are mediated by complex networks of primary and accessory cells and specific agents, which are in constant communication to develop a variety of coordinated responses to danger [26]. This biological dialogue implies that the immune system informs the neuroendocrine command when a systemic immune/inflammatory response to infection or tissue injury is occurring. The nervous system responds to this information with the orchestration of the febrile response and its subsequent effects on behavior, such as sleep, mating, locomotion and feeding [27]. The hypothalamus and particularly the paraventricular nucleus (PVN) is a critical node for regulation and coordination of these physiological responses. Neurons dwelling in the PVN control three major physiological effector pathways, as follow: the hypothalamic-pituitary-adrenal (HPA) axis, the neurohypophyseal system via

vasopressin (VP) and oxytocin (OT) neurons in the anterior, medial and posterior magnocellular cells groups; and autonomic regulation using brainstem and spinal cord projecting neurons in the parvocellular divisions. The intermingling between three physiologically distinct systems places the hypothalamic PVN at the crossroads for signal integration in a coordinated and inter-regulated fashion [28]. Meaningfully, the endocannabinoid system emerges as a robust modulator for the signaling on these three fundamental systems mentioned above and a striking interdependence between the components of the endocannabinoid system with the HPA axis and the neurohypophyseal system; they were strongly demonstrated by several studies. In the following content we focus on the current knowledge about these interactions.

HYPOTHALAMIC-PITUITARY ADRENAL AXIS

As we previously mentioned, the hypothalamus is brain structure functioning as the crucial "headquarters" in the regulation of many fundamental physiological activities and some of them are directly related to body homeostasis; such as stress and immune responses. Through a broad network of hormonal and neural communications, the hypothalamus receives a variety of peripheral information. Stress and immune stimuli both activate major neuronal clusters in the hypothalamus to trigger a constellation of molecular pathways resulting in immunoregulatory neuro-endocrine responses. Cytokines are a particular set of molecules that provide afferent information to the brain about the surveillance activity of the immune system. Distinct groups of hypothalamic neurons are responsible for sensing and regulation of these inflammatory signals and they are particularly located in the mediobasal hypothalamus (MBH) and PVN. The regulation of the neuroendocrine response is mediated by controlled synthesis, release, and actions of hypothalamic neuropeptides and neurotransmitters that end with the release of hormones to the periphery. The cytokine activation of the HPA axis and the secretion of glucocorticoids is one of the best examples [29]. A large number of immunological effects of the neuropeptides and endocrine mediators released after activation of the HPA axis are well known. These neuroendocrine mediators act systemically as well as locally to shape an immune response that is optimally suited to overcome the challenges. Following an insult, the resulting immunological events are complex and include proinflammatory as well as anti-inflammatory effects. This interaction between immune and neuroendocrine systems is bidirectional, and is maintained through a mutual biochemical language, a sort of code where cytokines/interleukins produced by immune cells are recognized by receptors expressed on neuroendocrine cells and; vice versa, cells of the immune system recognize neurotransmitters, neuropeptides and hormones produced by the nervous and endocrine systems.

The HPA axis comprises neurons in the hypothalamic PVN and cells in the anterior pituitary and adrenal gland cortex. In the PVN, there are parvocellular neurons that mainly synthesize corticotrophin-releasing hormone (CRH) and other peptides as well as vasopressin (VP) and oxytocin (OT) and they send axonal projections to the pituitary portal blood in the external zone of the median eminence. Various infectious agents that include endo- or exotoxins, microorganisms and antigens cause a rapid immune activation that precedes the HPA axis activation. The same series of events could be triggered by noninfectious challenges such as psychological stress. Upon activation, these neurons release CRH, VP and other secretagogues, which reach the corticotrope cells on the anterior pituitary to activate the synthesis and release of the adrenocorticotropic hormone (ACTH). Circulating levels of ACTH act on the zona fasciculata of the adrenal cortex to stimulate the synthesis and secretion of glucocorticoids and mineralocorticoids. The main glucocorticoid is cortisol in most mammals, including humans; but there are also some levels of corticosterone too. In rodents, the predominant glucocorticoid is corticosterone, and curiously in the case of rats and mice they only have corticosterone [30]. Once glucocorticoids are released into circulation its availability at the cellular level has multiple effects in the brain, as well as in the periphery, producing a range of diverse developments on cardiovascular, metabolic, neural and immune systems safeguarding and enhancing optimal responses [31]. Glucocorticoids exert these immunomodulatory effects through a binding to a cytosolic receptor ligand, consequently it dissociates from a protein complex, dimerizes and translocates to the nucleus, where it binds to specific DNA sequences to regulate gene transcription. Glucocorticoids receptors also interact with other components such as nuclear factor kappa B and activator protein 1 to suppress gene transcription acting as anti-inflammatory feature [32]. Elevations of glucocorticoids and catecholamines direct the movement of various cell types of the immune system such as lymphocytes, monocytes and NK cells and also, other soluble local mediators for further activation of immune functions such as interferon gamma and cytokines [33]. These cytokines produced in the periphery are known to be dispatched and distributed into the brain stimulating central pathways that may contribute to HPA activation. In addition, there is an *in situ* brain production of cytokines by a variety of cell types, mainly by microglia, astrocytes and also by neurons and endothelial cells [34]. The proinflammatory cytokine interleukin-1, especially its β form (IL-1β), is probably the most important molecule capable of modulating cerebral functions during systemic and localized inflammation process. Other cytokines implicated in neuroendocrine and febrile responses include tumor necrosis factor-α (TNF-α) and IL-6 [35]. These proinflammatory cytokines could stimulate glucocorticoids release by acting at all three levels of the HPA axis; for example IL-1 can release CRH from the hypothalamus and ACTH from the pituitary [36]. Furthermore, IL-2, IL-6, TNF-α and interferon gamma have been shown to stimulate the adrenocortical axis. Finally, glucocorticoids establish a negative feedback on the immune system to suppress the further synthesis, release and/or efficacy of cytokines and other mediators promoting immune and inflammatory reactions. Along with their role during stress and immune responsiveness, excessive and unchecked secretion of glucocorticoids can produce detrimental effects on health [37]. To prevent such effects, glucocorticoids exert potent suppression of HPA axis activity since they regulate their own production through a negative feedback on the upper levels of the HPA axis, including CRH at the PVN of the hypothalamus and ACTH in the anterior pituitary [31, 37].

HYPOTHALAMIC-NEUROHYPOPHYSEAL SYSTEM

It is interesting that, in concert with the activation of the HPA axis, the stimulation of the hypothalamic-neurohypophyseal system also occurs and that is an important integrative brain structure that coordinates responses to perturbations in homeostasis too. It consists of the hypothalamic supraoptic nuclei (SON) situated laterally to the optic chiasm, and the PVN on each side of the third ventricle. Magnocellular neurons in those nuclei synthesize oxytocin (OT) and vasopressin (VP) and send axonal projections to the neurohypophysis from where those peptidic hormones are released into the systemic circulation. Also, magnocellular neurons release oxytocin and vasopressin from their perikarya, dendrites, and/or collateral axons to several areas in the central nervous system where oxytocin and vasopressin have neurotransmitter functions, thus controlling complex neuroadaptive processes. In addition, parvocellular OT- and VP-containing cells are found in the hypothalamus and project into the external zone of the median eminence where these peptides are released into the portal vessels system to regulate anterior pituitary function [38]. These hormones regulate relevant functional processes that range within the maintenance of body fluid homeostasis, modulation of neuroendocrine, immune and stress responses, memory and also social behaviors. In addition to its vital role in reproduction, parturition and lactation, oxytocin also participates in fluid and electrolyte balance, fever, inflammation and stress response [39, 40]. Moreover, very recently it was demonstrated that

oxytocin has alleviating and preventing effects on the subsequent health problems caused by excessive HPA axis activation during chronic stress or inflammatory responses [41]. Vasopressin is critical for maintenance of water balance and cardiovascular function and also participates in febrile response. It is known that vasopressin secreted to portal circulation from parvocellular neurons is the responsible of the stimulation to ACTH secretion through its interaction with V3 subtypes of vasopressinergic receptors in the pituitary corticotropes; however is it possible that vasopressin secreted to the peripheral circulation by magnocellular neurons, acting on V1 vasopressinergic receptors located in other tissues contribute to the effects during stress and immune responses [42].

In addition to vascular elements, the other major cell types present in both mentioned hypothalamic nuclei are glial astrocytes, oligodendrocytes and a very few microglial cells. The soma and dendrites of both oxytocin and vasopressin producing-neurons are close intermingled by glial cells and remain separated by neurophil elements such as fine lamella-like processes of astrocytes. This neuronal-glial arrangement is not limited to the hypothalamic nuclei since it also occurs in the neurohypophysis. Under basal conditions, neurosecretory axons are enclosed by the operation of pituicytes, an astrocytic-like glia [43, 44]. Oxytocin and vasopressin secretion depends on the particular electrical activity of magnocellular neurons, under the influence of excitatory glutamatergic and inhibitory GABAergic synaptic afferent inputs [45]. Astrocytes play an important role in regulating the function of magnocellular neurons by permitting intercellular communication. Glial wrapping of synapses and neuronal elements limit the amount of neurotransmitters and its diffusion into the extracellular surrounding space. Under certain physiological conditions, a diminished astrocytic coverage of neurons and synapses may impact magnocellular system. In the hypothalamic nuclei, reduction in glial coverage modifies extracellular ionic homeostasis and glutamate clearance and, therefore, the overall excitability of magnocellular neurons. In the stimulated neurohypophysis, pituicytes retraction results in an increased levels of extracellular potassium ion which can enhance neurohormones release and also an enlarge neurovascular contact zone facilitating the diffusion of oxytocin and vasopressin hormones into the general circulation [44].

As we previously mentioned the hypothalamic neurohypophyseal system plays an important role in maintaining homeostasis under inflammation and infection. The cell bodies of the cytokine producing-neuron are located particularly in the PVN, the integrating center for brain reaction evoked by immune challenge [34]. The response of the organism to disturbance of its homeostasis caused by a toxin or microorganism activates the PVN and SON nuclei, and neurohypophyseal hormones secretion is increased [46-48]. Indeed, we have already demonstrated an enhancement of oxytocin release from hypothalamus following immune challenge with the concomitant increase in TNF-α production in this tissue, reaching 150-fold the initial values after 2 hours. Plasmatic levels of oxytocin are also elevated indicating its release to general circulation by its secretion from neurohypophysis [47]. Oxytocin production occurring during inflammation is considered to promote insulin and glucagon secretion and thus regulate peripheral metabolism during an infection [49].

It is known that the oxytocin system promotes and regulates social relationships. When a psychological stressor (i.e. immobilization stress) induced a rise in corticosterone levels, this HPA axis activation persists longer if the individual is socially isolated. In fact, oxytocin can directly attenuate the activation of the HPA axis, reducing the stress-induced psychological health risks. Oxytocin appears to function as an anxiolytic during long periods of stress since its action in brain regions that release and receive it during stress (PVN and amygdala) reduced the HPA axis chronic activation [41]. Also, the reduced incidence and mortality of stroke among humans with social support have been well documented. In fact, it was determined the oxytocin-mediated neuroprotective effect of social interaction on stroke outcome [50]. Finally, there are synaptic contacts between oxytocin and CRH-expressing neurons, furthermore oxytocin receptors are colocalized on CRH-expressing neurons in the PVN. Moreover, oxytocin dendritic release during immune challenge or stress could act on adjacent neurons. Finally, plasma oxytocin can act directly on the adrenal cortex to regulate the release of glucocorticoids. This provides an anatomical and neural basis for a PVN-originating effect of oxytocin on the HPA axis activity [51].

In addition to increased understanding of its classical physiologic effects, vasopressin has recently been found to be involved in modulating the inflammatory response. Vasopressin is released into both the systemic and the portal circulation to the anterior pituitary gland from the neurohypophysis. Sepsis initially rises plasma vasopressin levels by 20-fold to 200-fold to supraphysiologic levels. This occurring-increment of vasopressin could be induced by stimulus such as increased levels of mediators of inflammation and decreased cardiac output. It was shown that cytokines such as IL-1 β increase vasopressin expression and could be an important additional modulator of its levels in septic shock [52]. Furthermore, vasopressin is also an important neurotransmitter in the brain being a key regulator of the behavioral responses to stress and inflammation; in fact it has been shown that endotoxin stimulates the release of vasopressin into hypophyseal portal blood. The effects of vasopressin on the corticosteroid axis are clearer. When vasopressin binds to V3 subtype of vasopressinergic receptors expressed in the anterior pituitary gland, this molecular complex increases corticotroph responsiveness to CRH, thus elevating ACTH release, even in conditions of stress when corticosteroid levels are high. The vasopressin-induced increase of ACTH (unlike effects of CRH on ACTH) is resistant to corticosteroid negative feedback [53]. Vasopressin is released both in the brain and in the circulation to counteract some of the inflammatory processes. However, vasopressin-producing neurons appear to be less sensitive to inflammatory signals than CRH neurons. This peptidic hormone release to the periphery down-regulates the peripheral inflammatory response, however, it mechanism of action still needs further investigation. There are few studies on the effects of vasopressin on cytokine expression. Astrocytes are the key immune response cells of the brain whose function is regulated by vasopressin, since this peptide decreased both mRNA and protein expression of TNF-α and IL-1 β via V1 subtype of vasopressinergic receptor; so vasopressin may be anti-inflammatory in the brain. Also, it has been well described its action within the brain as an endogenous antipyretic to reduce the febrile response [49, 52].

ENDOCANNABINOID SYSTEM IN THE REGULATION OF HYPOTHALAMIC-PITUITARY AXES

A body of evidence indicates that the endocannabinoid system modulates many neuroendocrine axes. In addition to regulate release of classical neurotransmitters such as glutamate and GABA, it also controls the release of neuromodulators, including serotonin, dopamine and opioids and finally regulates plasma levels of diverse hormones, too. In particular, several lines of investigation suggest a role of this system in the modulation of the hypothalamic-pituitary-gonadal and adrenal axes and hypothalamic-neurohypophyseal axis both in basal activity and during its activation by stress and immune response. Since the discovering of the endocannabinoid system components in the hypothalamus as well as in brain structures involved in the regulation of reproduction and stress and immune responses [13], and the presence of this system in pituitary and adrenal glands, this newly intercellular system was consider a neuromodulatory mediator involved in physiological processes and in diseases associated with the activation of the axes aforementioned.

The endocannabinoid system appears to regulate levels of diverse hormones. Earlier studies indicate that administration of can-

nabinoid compounds is generally associated with inhibition of neuroendocrine functions [54]. Exogenously administered cannabinoids were shown to alter the normal production and release of hormones, in fact, early studies of our group reported the inhibitory effects of THC on luteinizing hormone (LH) and prolactin (PRL) secretion [55, 56]. We demonstrated that a single dose of THC (2 µl of 10^{-6} M), injected into the third cerebral ventricle (intracerebroventricularly, icv), decreased serum LH temporarily but did not alter serum follicle stimulating hormone (FSH) levels. The hypothalamic luteinizing hormone releasing hormone (LHRH) content was elevated by 30 min after the injection and persisted for 1 h, indicating that THC alters pituitary LH release by inhibiting the release of hypothalamic LHRH [56]. Furthermore, our *in vitro* studies performed in 1990 showed that THC (10^{-8} M) inhibited the norepinephrine ($5x10^{-5}$ M) as well the dopamine ($5x10^{-5}$ M)-stimulated LHRH release from medial basal hypothalamic (MBH) explants of male rats [57]. With the discovery of the presence of cannabinoid receptors and the production of endogenous cannabinoids in the hypothalamus, pituitary and adrenal gland, it became evident that endocannabinoids control the release of neuropeptides, neurotransmitters and hormones at hypothalamic, pituitary and adrenal levels [58]. Actually, we showed the presence of CB_1 receptors in the preoptic hypothalamic area and in the periventricular MBH of male rats, regions that contain the neuronal somas and terminals involved in the synthesis and release of LHRH, respectively. Using double immunohistochemical techniques, no co-localization of CB_1 receptors with LHRH immunoreactive neurons was observed. However, CB_1 receptor immunoreactive neurons were shown adjacent to the third ventricle, an area that contains axons from LHRH neurons [59]. Based on our older results obtained using THC, we considered to study the effects of the endocannabinoid anandamide on LHRH release. Firstly, we showed that anandamide (10^{-9} M) decreases by 70% the NMDA-stimulated LHRH release from MBH incubated *in vitro*. Secondly, we demonstrated that the same concentration of anandamide significantly decreases the forskolin-induced cAMP content and LHRH release. These inhibitory effects of anandamide were prevented by the selective CB_1 receptor antagonist (AM251, 10^{-5} M), confirming the participation of the endocannabinoid system as inhibitor of LHRH release in male rats [58]. Also, we demonstrate that the endocannabinoid anandamide (10^{-9} M) significantly increased the release of GABA from MBH but had no effect on β-endorphin release. Moreover, bicuculline (10^{-4} M), a GABAergic antagonist, completely blocked the inhibitory effect of anandamide on NMDA-stimulated LHRH release. However, the opioid receptor antagonist, naltrexone (10^{-6} M), did not modify the inhibitory effect of anandamide. These data confirm that GABA mediates the endocannabinoid induced inhibition of LHRH release [58, 59]. Since the activity of the anterior pituitary is under the influence of circulating sex-steroids and several differences in the endocannabinoid system were registered between sexes, we focused on the role played by the endocannabinoid system in hormone secretion in male and ovariectomized (OVX) female rats [60-62]. Anandamide, like THC, suppressed the release of LH in male and ovariectomized female rats [61]. However, in OVX females, administration of the CB_1 receptor antagonist AM251 produced even a greater inhibition of LH release. The effect of anandamide on LH could be reversed by priming with estradiol; but this reversal was blocked if administraion was simultaneous with AM251. Ovariectomy reduced CB_1 receptor density in the hippocampus, amygdala and limbic forebrain, whereas the opposite was seen in the hypothalamus. These results indicate that changes to estrogen functions can influence central endocannabinoid signaling [54]. In the beginning of the history of cannabinoids studies, we have demonstrated by *in vitro* and *in vivo* experiments that THC inhibits prolactin release from the adenohypophysis of male rats by acting at hypothalamic level [55]. In subsequent investigations we have shown that anandamide centrally microinjected also inhibits this hormone release from the adenohypophysis of male rats. Anandamide acts on CB_1 receptors located on dopaminergic neurons in the MBH and activates dopamine release into the portal vessels; therefore inhibits prolactin release. This effect of anandamide on dopamine release from the MBH is similar to acute effects of THC on dopaminergic neurons in several other brain areas of the rat. The presumed effect of anandamide to increase synthesis and release of dopamine from the MBH was confirmed by incubating MBH explants in the presence of anandamide (10^{-9} M); showing an increased release of dopamine [60]. This interaction between endocannabinoid system with gonadal hormones and neurotransmitters open our landscape to elucidate how this system orchestrates its role to interplay with other endocrine axes.

The commonly accepted view attributes to the cannabinoids as having a general inhibitory role on neuroendocrine functions, however it has been suggested that cannabinoids, on the contrary, are able to stimulate the HPA axis. Smoking marihuana or THC administration both have long been known to stimulate ACTH and glucocorticoid secretion in humans and experimental animal models activating the stress/immune response executed by the HPA axis. Cannabinoids are not only able to increase corticosterone secretion induced by mild stress, but they also increase the corticosterone response to more severe stressors being of physical, psychological, social and endotoxic nature. The cellular and molecular mechanisms underlying the effects of endocannabinoids on HPA axis have just started to be deciphered and needs future research. There is no doubt that the endocannabinoid system participates in the endocrine regulation of the adrenal axis, especially under stressful and immunological threats conditions. However, by several reports showing a different action of endocannabinoids on the HPA axis, the function of this system in the regulation of adrenal hormones secretion has just recently begun to be understood. The current whole picture is very complex because, is important to remark, the presence of CB_1 receptors on both GABAergic inhibitory and glutamatergic excitatory hypothalamic neurons are responsible for both, CRH neuronal release and also glucocorticoid-mediated negative feedback regulation of the HPA axis [63-65]. It has been proposed that endocannabinoids negatively modulate HPA axis activity in a context-dependent manner, with a basal endocannabinoid tone which exerts an inhibitory action over HPA axis activation, but upon exposure to stress or infection, endocannabinoid levels rapidly decline through an undetermined mechanism, resulting in a disinhibition of glutamatergic projections to the PVN and allowing activation of the HPA axis [66]. Mice lacking CB_1 receptor displayed a deregulation of the HPA axis activity with a central impairment of glucocorticoid feedback plus an enhancement of circadian HPA axis activity peak resulting in elevated plasma levels of corticosterone as onset at night [64, 65, 67]. The expression of CB_1 receptors and the synthesis of both anandamide and 2-AG in the hypothalamus, including locally within the PVN as well as SON nuclei, suggest that this system could be regulated by mediators of the stress and immune responses. In fact, glucocorticoids evoked a rapid induction of the endocannabinoid synthesis and release through a non-genomic mechanism involving a G_s coupled membrane bound receptor [68, 69]. The glucocorticoid-mediated release of endocannabinoids within the PVN, in turn, resulted in the suppression of incoming excitatory neurotransmission to CRH secretory-neural cells and provided a mechanism by which glucocorticoids could exert a rapid shut down of the HPA axis. On the other hand, further investigations are needed on the effects of anandamide targeting CB_2 and TRPV1 receptors, regarding its role in the regulation of HPA axis.

The CB_1 receptors and the synthesis of endocannabinoids can also be found in the somatotrophs, lactotrophs, corticotropes and folliculostellate cells in human pituitary gland indicating that the endocannabinoid system modulates the axis at pituitary level [70]. In contrast to the pituitary, much less is known about the endocannabinoid system in the adrenal glands. Until 2009 it was unknown

whether endocannabinoids influence adrenal glucocorticoid synthesis directly at the level of this gland. A possible direct effect on aldosterone secretion at the adrenal level has been suggested by the fact that a CB_1 receptor antagonist reduces blood pressure [71]; however it has not been directly studied. For that reason, our group investigated the expression of cannabinoid receptors and the effects of endocannabinoids on the release of cortisol and aldosterone in human adrenals and the human adrenocortical cell line NCI-H295R. Our data also indicated, in addition to the central and pituitary regulation of the HPA axis, endocannabinoids, via CB_1 receptors, directly inhibit adrenocortical steroid genesis at adrenal level. Similar to the normal human adrenal, the human adrenocortical cell line NCI-H295R expressed CB_1 but not CB_2 receptors. The endocannabinoid anandamide reduced basal as well as angiotensin II- and forskolin-stimulated cortisol and aldosterone secretion. Ang II stimulates adrenocortical steroidogenesis via inositol-1,3,4-phosphate (IP3) formation and subsequent Ca^{2+} mobilization while forskolin is an activator of the PKA pathway. The inhibition of Ang II- and forskolin-induced steroidogenesis by anandamide indicates a further effect downstream in steroid synthesis. The inhibitory effect of anandamide was reversed by concomitant incubation with a CB_1 receptor antagonist confirming the participation of this subtype of cannabinoid receptor [72]. A very recent work demonstrated, in concordance with the findings in our studies, that disruption of endocannabinoids signaling through CB_1 receptor antagonism results in potentiated neural and endocrine responses to stress; and substantial increases in the activity in various brain regions and in adrenal gland [64]. Interestingly, and of particular importance is the endocannabinoids involvement in rapid feedback mechanisms of corticosterone that seems to represent a regulatory principle that could be observed not only on CRH release but also on magnocellular, vasopressinergic and oxytocinergic neurons. In conclusion, the endocannabinoid system acts constraining the HPA axis activity under basal conditions; however facilitates its activation during stress-immune response and finally counteracts overshooting for this response.

As we previously mentioned, the endocannabinoid system plays a pivotal role in the regulation of the hypothalamic neurohypophyseal axis. In 1991, Herkenham *et al.* [13] first reported that CB receptors are localized in the PVN of the hypothalamus as well as in anterior and posterior pituitary lobes. Endocannabinoids have also been found in these tissues [73]. It has been reported that endocannabinoids are released as retrograde messengers in the SON by magnocellular neurons and CB_1 receptors are localized within the SON, suggesting that endocannabinoids could modulate the physiology of magnocellular neurons; in fact it was reported that there is an interaction of endocannabinoid system with central hormone release in the modulation of magnocellular SON neurons synaptic physiology [32, 74]. In regard with this axis, we also studied the effect of endocannabinoids on oxytocin release. We performed *in vitro* studies testing the effect of anandamide on oxytocin release from neurohypophysis (NH) [75] and MBH *in vitro* in normal conditions [47]. Several doses of anandamide ranging from 10^{-11} to 10^{-8} M significantly decreased oxytocin release from NH, but we found 10^{-9} M as the most effective inhibitory dose. On the contrary, anandamide (10^{-9} M) stimulated oxytocin release from MBH and this effect was mediated by CB_1 receptor since it was blocked by AM251, its selective antagonist. Moreover, the inhibition of FAAH, the enzyme that degrades anandamide, by URB597 (10^{-10} and 5.10^{-10} M) increased oxytocin release from MBH [47]. Otherwise, since nitric oxide (NO) acts like a local modulator of magnocellular neuronal activity [76], NO donors reduce oxytocin secretion from both NH and MBH *in vitro* as we have demonstrated several years ago [77]. Therefore, we studied the mutual relationship between the endocannabinoid system, NO and oxytocin release. Our study showed that anandamide increases NO synthase activity in the NH as well as in the hypothalamus. We found that the inhibitory effect of anandamide on oxytocin secretion from the NH is mediated by NO since the scavenging of NO by hemoglobin or the inhibition of NO synthase by L-NAME completely blocked this inhibitory effect. On the other hand, the production of NO induced by anandamide could act as negative feedback on oxytocin release from MBH. In fact, in the presence of hemoglobin the stimulatory effect of anandamide on oxytocin release from MBH is much higher [47]. Still little is known about the expression and function of cannabinoid receptors in the posterior pituitary lobe. We have performed pharmacological experiments *in vitro* in which the NH was incubated in the presence of anandamide and cannabinoid or selective antagonist for vanilloid receptors. The CB_2 and the TRPV1 receptors antagonists, AM630 and capsazepine respectively, completely blocked the inhibitory effects of anandamide on oxytocin release from NH. However, in the presence of the CB_1 receptor antagonist AM251, the inhibitory effect of anandamide persisted, suggesting that this subtype of cannabinoid receptor is not involved in oxytocin release from the NH. In summary, anandamide acting through CB_2 and TRPV1 receptors, effectively increases the activity of NO synthase, increasing NO production that inhibits oxytocin release from the NH. At hypothalamic level, anandamide acting through CB_1 receptor increases oxytocin release and NO synthase activity, and the consequent NO increase produces a negative feedback, ending the stimulatory effect of anandamide on oxytocin release from the MBH (See Diagram) [75].

A variety of stressors and immune challenges with ability to stimulate the hypothalamic-pituitary axis induce oxytocin and vasopressin secretion, not only into the blood, but also within the brain. Infection has been shown to increase plasma levels of oxytocin and vasopressin and also stress induces intracerebral oxytocin release [78]. However, the mechanism involved in the activation of oxytocin and vasopressin producing-neurons by an immune or stress challenge has not been well elucidated yet. This prompted us to consider that oxytocin might be involved in the control of neuroendocrine responses to inflammation and furthermore, that the endocannabinoid system is actually involved as modulator of oxytocin response to infection. The neuroendocrine response to infection can be mimicked by exogenous administration of bacterial lipopolysaccharide (LPS), which is a commonly used as standard model of immune challenges [79]. LPS activates the synthesis and release of IL-1, IL-6 and TNF-α in the periphery which then are transported into the brain and stimulate central pathways contributing to the hypothalamic neurohypophyseal axis activation. In addition, there is a brain production of these cytokines, mainly by microglia, astrocytes and also by endothelial cells and neurons located particularly in the PVN; as we mentioned before, those have been recognized as part of an integrating center for brain reaction evoked by immune challenges [34]. Previously, we have demonstrated the increase of anandamide synthase activity in hypothalami obtained from adult male rats peripherally injected with LPS [80]. We have also showed that TNF-α is the first cytokine to be produced in large quantities, reaching high levels in circulation after 30 min following LPS injection. TNF-α activates PVN hypothalamic neurons and triggers the release of CRH, oxytocin and vasopressin [81]. For that reason, we tested *in vivo* the effects of the intracerebroventricular administration of TNF-α and *in vitro* with the incubation of hypothalamic fragments with the same cytokine also demonstrating increased hypothalamic anandamide synthase activity [80, 82].

We have previously mentioned that exogenous cannabinoids exert effects on hormone secretion from pituitary gland having, predominantly an inhibitory impact. Several years ago it has been shown that THC produced an increase in diuresis and suppression of milk ejection reflex by inhibiting the release of vasopressin and oxytocin, respectively [83, 84]. These effects are possible due to the fact that CB_1 receptors are located in oxytocin-secreting parvocellular and magnocellular neurons of the PVN and SON nuclei [85]. On the other hand, it was reported a stimulatory effect of cannabinoids on stress-induced hypothalamic pituitary axis, with enhanced secre-

tion of hypothalamic pituitary hormones. These results are in concordance with the findings in our studies, where we observed that endocannabinoids enhance the stimulatory effect of LPS on hypha-lamo-neurohypohyseal axis, since the blockade of hypothalamic cannabinoid receptors CB_1 and CB_2 prevents the LPS-augment in oxytocin plasma levels. This complete prevention of LPS-induced increase of oxytocin plasma levels due to CB_1 and CB_2 receptors blockade induced with both antagonists centrally injected indicates that, the hypothalamic endocannabinoids control oxytocin release under endotoxic conditions with the participation of both subtypes of receptors. Moreover, the approach to attenuate the degradation of anandamide by the FAAH inhibitor URB597 administered into the lateral ventricle, (which only promotes endocannabinoid signaling specifically in the brain area of our interest), a further increase of oxytocin plasma levels in LPS-challenged rats was observed. The fact that the blockade with both antagonists was equal and partial suggest that endocannabinoids signaling through CB_1 subtype of receptors is sufficient to mediate LPS-induced increases in TNF-α plasma levels, however probably other receptors could be involved at-large, such as vanilloid TRPV1 or GPR55.

With respect to vasopressin hormone, studies from our group demonstrated that anandamide decreased vasopressin secretion from incubated NH. Anandamide action was mediated by NO since the inhibition of NO synthesis completely blocked this inhibitory effect. CB_2 and TRPV1 are involved in this inhibitory effect since AM630 and capsazepine, but not AM251, blocked anadamide inhibitory effect, as was also observed studying oxytocin release [75].

Taken together, all the studies presented here reveal that the endocannabinoid signaling is vital to the regulation of brain function, metabolism and the immune system. Also, that the endocannabinoid system is altered in response to environment, exhibits genetic diversity and modulates many physiological functions. An increasingly complex role of the endocannabinoid system, mostly in terms to the effects on hypothalamic-pituitary axes activity modulation it was shown. A dual role of the endocannabinoid system regulation of these axes is noticed. A tonic level of endocannabinoids is needed and appears to be a gatekeeper, which must be lowered before the stress and immune responses can occur. A rapid decrease in endocannabinoids brain levels following stress or immune challenge exposure promotes activation of hypothalamic-pituitary axes. Finally, glucocorticoid mediated elevation in endocannabinoids levels, primarily at hypothalamus, contributes to a fast negative feedback inhibition of the axes [86]. Here, endocannabinoids serve as preventers of maladaptive excess activation of the axes. Similarly, endocannabinoids maintain gonadal hormones at the correct physiological levels preventing an overload in the activation process of this axis. It is clear that a deficiency in neural endocannabinoid system signaling may be an important step in understanding the interrelationship of physiopathological factors involved in the onset of several entities derived from chronic activation of hypothalamic-pituitary axes. Pharmacological agents targeting the components of the endocannabinoid system have high potential expectation to act as treatments for dysfunctions and disorders related to altered hormones, neuropeptides and neurotransmitters released by the mentioned axes. The administration of selective cannabinoids receptor antagonists or agonists, and inhibitors of endocannabinoid metabolism and transport are useful tools for therapeutic treatment or intervention of a wide range of diseases. The list of pathological conditions that could be ameliorated by the modulation of the activity of the endocannabinoid system is extensive and diverse, including movement disorders such as Parkinson´s and Huntington´s diseases; myocardial infarction; eating disorders such as obesity/metabolic syndrome and anorexia; pain and inflammation, reproductive disorders and hypertension; as well as others. The classical behavioral effects of marihuana and the particularly high density of cannabinoid receptors and endocannabinoids synthesis within the brain provide a certain clue about the key components of the endocannabinoid system as potential therapeutic targets in central nervous system disorders such as neurotoxicity, stroke, multiple sclerosis, Alzheimer´s disease, epilepsy, mental disorders such as schizophrenia, anxiety, insomnia, alcoholism and autism [87]. In very ancient civilizations, extracts of the plant *Cannabis sativa* were used for a variety of medicinal effects including improving mood, to reduce pain, to increase apetite and to diminish inflammation. However; there is actually an impediment to the development of cannabinoids medications due to its psychotropic properties. This issue was avoided when CB_1 receptor antagonists or selective CB_2 receptor agonists and FAAH inhibitors, which lack psychoactive properties were developed, representing a promising treatment for those conditions previously discussed.

The CB receptors agonists and antagonists and the increase of endocannabinoid tone by agents that interfere with the degrading enzyme FAAH were among the most promising drug targets in the last decade. There are cannabinoid receptors signaling pathways that are linked to neuronal survival and repair. The activation of presynaptic CB_1 receptors leads to the inhibition of neuropeptides and neurotransmitters release and by this mechanism there is a reduced post synaptic neurons excitability being responsible for the effects on cognition, reward and anxiety behaviors. In contrast, activation of CB_2 receptors produces immunosuppression associated to limit inflammation and tissue damage. Chronic activation of microglia plays a major role in disorders characterized by nervous tissue inflammation. CB_1 is constitutively expressed in microglial cells while CB_2 expression is absent in resting cells and it is related to the cell activation stage. The main corollary of CB_2 receptors activation seems to be a protective effect in neuroinflammation being a promising therapy approach to some neuroinflammatory diseases such as multiple sclerosis [88]. The endocannabinoid system serves to facilitate habituation to stress, reduction of anxiety, extinction of fear, analgesia, etc. however; the bulk of our knowledge comes from experimental animal models and there is relatively scarce in terms of clinical human studies. Moreover, at present, studies point to contradicting effects of cannabinoids depending on the dose, animal strain and test, and cannabinoid receptors pharmacological manipulation also yields to conflicting results [14]. To date, cannabis-based medicines are in the clinic, including a plant-derived cannabinoid preparation that has already gained regulatory approval in Canada and Europe, and is used as antiemetic following chemotherapy. Also, a CB_1 antagonist that has been explored and found to be effective as therapeutic agent for obesity problems has been approved too. However, clinical studies have reported that in obese patients that receive a treatment with this CB_1 receptor antagonist developed mood disorders such as depression and anxiety, and was removed from the market; thus it is recommended a special care in those studies since long-term effects of this kind of drugs require further clinical trials [89]. It is our hope that we could exploit the role of the endocannabinoid system as a modulator of all fundamental processes previously discussed here. Future studies dissecting mechanisms and overcoming adverse effects of pharmacologic intervention will allow an improvement in the survival of affected individuals and the manipulation of components of this system to treat a wide range of disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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