CB2 RECEPTORS IN NEURONS OF THE CENTRAL NERVOUS SYSTEM.

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ABSTRACT

The endocannabinoid system is formed by endogenous ligands, biosynthetic enzymes and two classical receptors: CB1 and CB2. From a classical point of view, CB1 receptors are the "central cannabinoid receptors" expressed in neurons, whose function is the regulation of neurotransmitter release. On the other hand, CB2 receptors are considered the "peripheral cannabinoid receptors", expressed in peripheral tissues. However, this classical view has changed over the years; new endogenous ligands, receptors isoform variants, new cellular locations such as mitochondria and new putative receptors GPR119 and GPR55 have been described. Thus, evidence indicates that the endocannabinoid system is more complex than once thought.

In this regard, over the years, CB2R were found in glial cells where they modulate immune response; moreover, with the development of more selective ligands, antibodies and the characterization of their genes, the expression of CB2R in central neurons became evident. Thus, several questions arise concerning these findings. What is the function of these receptors in neurons? Do they also control the release of neurotransmitters and others? Here we review the state of the art for these findings and the possible future direction of this new line of research that contributes to understanding the complexity of the endocannabinoid system.

Keywords: CB2 receptors, endocannabinoid system, central nervous system.

RESUMEN

El sistema endocanabinoide está formado por sus ligandos endógenos, enzimas biosintéticas y dos receptores clásicos denominados CB1 y CB2. Clásicamente el RCB1 es el "receptor canabinoide central" expresado en neuronas, que regula la liberación de neurotransmisores en la plasticidad sináptica. Por otro lado, el RCB2 es el "receptor canabinoide periférico" expresado en el sistema inmune. Sin embargo, esta visión clásica ha cambiado con los años; nuevos ligandos endógenos, isoformas de los receptores, nuevas localizaciones celulares como la mitocondria, y nuevos receptores putativos: GPR119 y GPR55 han sido propuestos. Así, el sistema endocanabinoide es más complejo de lo que se pensaba.

Al paso del tiempo, los RCB2 se encontraron en las células gliales donde modulan la respuesta en casos de insulto, más todavía con el desarrollo de ligandos más selectivos, anticuerpos y caracterización de sus genes, la expresión de RCB2 en neuronas centrales se hizo evidente. Así, diversas preguntas surgieron de estos hallazgos. ¿Cuál es la función

de estos receptores en las neuronas? ¿Controlan la liberación de neurotransmisores? Y otras más. En esta revisión mostramos el estado del arte de estos hallazgos y la dirección futura de esta línea de investigación que contribuirá a entender la complejidad del sistema endocanabinoide.
Palabras clave: receptores CB2, sistema endocanabinoide, sistema nervioso central

Original received: September 13, 2019; Accepted in its final form: October 22, 2019

Introduction

There is now a great deal of evidence that the endocannabinoid system is more complex than CB1 and CB2 receptors (CB1R and CB2R), their ligands, and the enzymes responsible for their synthesis. Findings regarding their location and function in intracellular organelles, new endogenous ligands, putative new receptors, and the mechanisms of uptake and release have changed our classical perspective of endocannabinoids and their functions. This is important, not only with respect to the knowledge of the system itself, but for their potential application in solving health problems. In this regard, a role for endocannabinoids in neural function has been attributed only to CB1R, and is basically related with the control of neurotransmitter release and their implications in synaptic plasticity. In addition, the CB1R mitochondrial location contributes to controlling neural metabolism. However, in recent years, the appearance of CB2R in central neurons opened the doors to important questions about their function. Here we summarize findings and open the discussion to the assessment of the implications of these new data.

The endocannabinoid system

Classically, the endocannabinoid system refers to cannabinoid receptors, endogenous ligands and enzymes that produce and metabolize endogenous agonists. Two principal cannabinoid receptors have been described and fully accepted: the CB1R and CB2R. They are members of the seven transmembrane domain receptors (GPCR) that are classically coupled to $G_{i/o}$ proteins, and their activation results in an inhibition of adenylyl cyclase [1]. Other orphan GPCR that have been proposed as cannabinoid receptors include GPR55, GPR119, and GPR18; in addition, TRPV channels and PPAR receptors can also be modulated by endocannabinoids [2-4]. CB1R is the most abundant cannabinoid receptor in the central and peripheral nervous systems, it is considered among the most abundant GPCR in the brain. CB1R was cloned in 1990 from rat cerebral cortex and, one year later, from human brain. In rodent brain, high expression has been found in basal ganglia, striatum, substantia nigra, globus pallidus, cerebellum, hippocampus, and cerebral cortex [5]. This expression is consistent with areas that are involved in motor control, cognition, and motivation [6], thus explaining the psychoactive effects of Δ^9 tetrahydrocannabinol, the main agent of marihuana. On the other hand, CB2R was thought to be only expressed in cells of the peripheral immune system and was initially considered solely as a tissue peripheral receptor [7]. However, to date, CB2R have been identified in the central nervous system (CNS), microglia, and neurons at lower levels of expression compared with CB1 [8]. CB2R was cloned three years later after CB1R from HL-60 cells, and share 68% amino-acid sequence homology with CB1R and only 44% in total amino-acid sequence [7]. Both receptors, CB1R and CB2R, are activated by endogenous cannabinoids: 2-arachidonilglicerol (2AG) and anandamide (AEA), which are the most abundant endocannabinoids. In the central nervous system,

endocannabinoids act as retrograde messengers; namely they are synthetized at postsynaptic neurons when intracellular Ca^{2+} rises and/or by activation of metabotropic receptors, and then released into synaptic space where they activate the presynaptic receptors [9]. In response, the activation of presynaptic CB1R, decreases neurotransmitter release mediating short- and long-term plasticity [10].
AEA was discovered by Mechoulam and co-workers in 1992. It is synthetized from N-acyl-phosphatidyl-ethanolamine (NAPE) and catalyzed by the NAPE-PLD enzyme at post- and presynaptic sites [11]. AEA is degraded into arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH). On the other hand, 2-AG is 200 times more abundant than AEA [15]: it is synthetized from PIP2 by PLC-β to give rise to diacylglycerol (DAG), and finally, through the action of both DAG lipases (DAGLα and DAGLβ), is converted into 2-AG. The 2-AG is degraded into arachidonic acid and glycerol by monoacylglycerol lipase (MAGL) [15]. In the peripheral tissues, endocannabinoids are released paracrinally.

CB2R in neurons and glia

The first evidence of CB2R in elements of CNS reported their presence in perivascular glia [8]. Interestingly, it was postulated that their glial expression was low and that it increased under pathological conditions, such as Alzheimer disease and brain injury [12], since in healthy brains, the expression of these receptors was not observed [7]. Lately, the expression of these receptors has been found particularly in microglia cells where they participate in the regulation of tripartite synapse and the immune response [13]. It is noteworthy that CB2R expression is lower than that of CB1R in CNS, but under certain pathological conditions, CB2R expression is found to be greatly increased.
The expression of CB2R in CNS elements remained controversial until it was found in glial cells. After that, with the advances in selective antibodies for Western blotting, electronic microscopy, and immunohistochemistry, as well as better primers for RT-PCR and *in-situ* hybridization techniques, the presence of these receptors in neurons could be demonstrated. Van Sickle et al. [14] were the first to demonstrate the presence of CB2R in neurons from brainstem, cerebellum, and cortex. Interestingly, these authors suggested that those CB2R participate in an antiemetic effect when co-activated with CB1R. One year later, two works reaffirmed the presence of CB2R in neurons. One reported a much broader expression in different subtypes of neurons [15]. It is noteworthy that, although CB1R and CB2R co-localize in the same brain structure, they may have differences in their distribution patterns. On the other hand, Brusco et al. [16] showed by electron microscopy, the subcellular distribution of CB2R in CA1 hippocampal area and in substantia nigra. Even though CB2R are mainly expressed in cytoplasm and neuronal cell bodies, they have been observed in dendrites, where they have a postsynaptic location and also in some unmyelinated axons, suggesting a presynaptic localization that other functional pharmacological studies have also confirmed.
Today, CB2R protein and/or mRNA expression in neural elements of CNS has been detected in Purkinje and granular neurons in the cerebellum, hippocampal pyramidal neurons, pyramidal neurons of the cerebral cortex, nervous vagus nuclei, and also in elements of the basal ganglia such as striatal neurons, pallidal complex, substantia nigra pars reticulata neurons, subthalamic nucleus neurons, and dopaminergic neurons of the VTA and of the substantia nigra pars compacta (Figure 1).

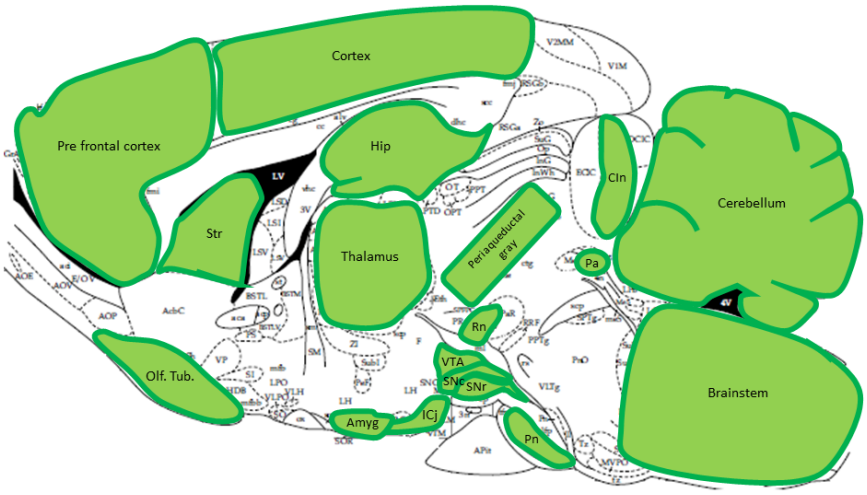

Figure 1. Main location of central nervous system nucleus that expresses CB2R in neuronal elements. Hip, hippocampus; Str, striatum; Olf. Tub., olfactory tuberculum; Amyg, amygdala; ICj, Islands of Calleja; VTA, ventral tegmental area; Rn, Red nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; Pa, paratrochlear nucleus; Cln, inferior colliculus nucleus; Pn, pontine nucleus.

CB2R and neuronal function

Many questions have arisen concerning the function of CB2R in neurons, mainly with respect to their role in neuronal excitability and presynaptic effects. To date, the majority of studies have been conducted in neural soma, where their activation decreased neuronal activity in prefrontal cortex, nucleus accumbens, hippocampus, VTA, prefrontal cortex, and striatum, and more recently in cortex [17].

Several mechanisms have been attributed to CB2R in terms of the control of excitability (see figure 2). Stempel et al. [18] showed, in hippocampus and specifically in pyramidal neurons, a long-lasting hyperpolarization that reduces neural excitability by a mechanism involving the sodium-bicarbonate co-transporter. First, the action potential depolarizes the neuron; this depolarization evokes the synthesis and paracrine release of 2-AG, which activates CB2R, leading to the activation of the sodium-bicarbonate co-transporter in order to hyperpolarize the neuron [19]. On the other hand, Marinelli et al. [20] initially described that CB1R mediate the self-regulation of activity in layers II/III of the cortex, and now this function appears to be shared with CB2R, whose activation leads to hyperpolarization that is dependent on the activation of GIRK channels [21]. den Boon et al. [22] found that CB2R are expressed in pyramidal mPFC neurons, and that their activation leads to the IP3R-dependent opening of calcium-activated chloride channels to control the firing rate. Interestingly, these receptors are intracellularly located and, unlike CB1R that are highly expressed, CB2R are poorly expressed in plasma membrane. Additionally, the mechanism of reduction of neuronal activity involves the activation of PLC, IP3R activation, calcium rise, and finally, activation of calcium-activated chloride channels. Last, by means of the activation of CB2R in VTA dopaminergic neurons, Zhang et al., both in mouse [23] and in rat [24], demonstrated an inhibition of firing of these neurons mediated by potassium channels, which is related to the decrease of dopamine release in target areas. This effect is clearly related to motor behavior, addiction, depression, anxiety, alcohol intake, cocaine, and psychomotor behaviors [17, 23, 24]. Thus, it appears that a variety of mechanisms is involved in CB2R control of excitability.

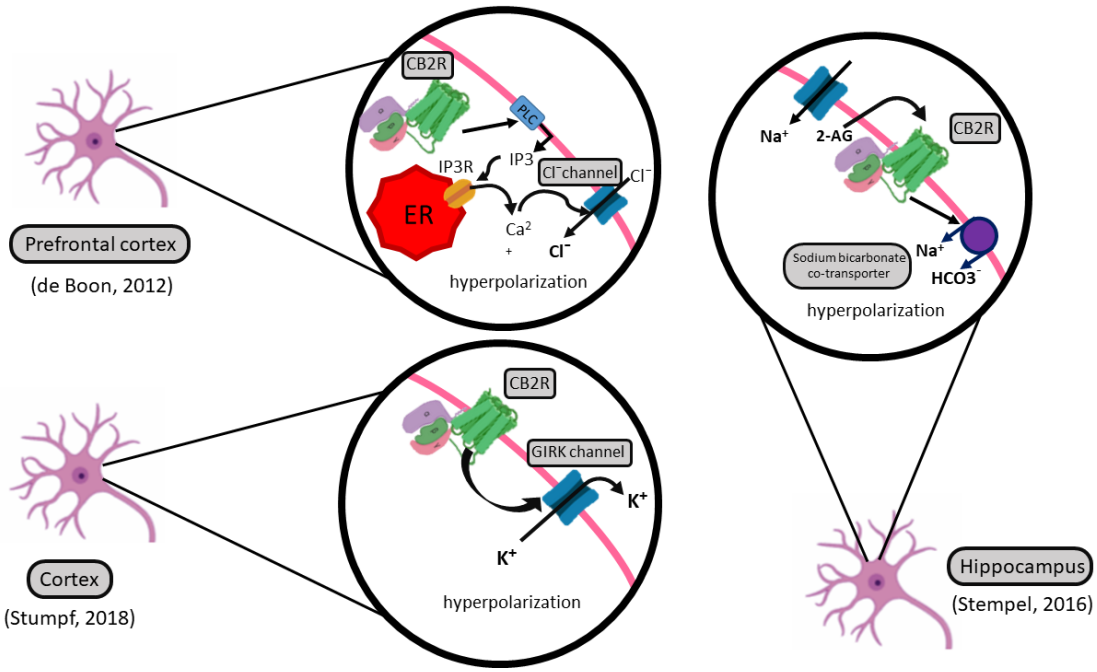

Figure 2. Proposed post-synaptic mechanism by which CB2 post-synaptic receptors control neuronal excitability.

Another very important component of neuronal function is the neurotransmitter released at terminals. The presence of CB2R in presynaptic terminals has been controversial, although widely suggested, but there is now increasing evidence about their presence and functionality, as well as their role in certain pathologies. Focusing on the presence of CB2R in presynaptic terminals, we can find some recently reported examples where these receptors can modulate either plasticity of the synapse and/or the release of neurotransmitters. One of the first reports corresponds to Morgan et al. [25]. These authors showed that activation of CB2R in the entorhinal cortex gives rise to a decrease of GABA release. To date, it has been described that CB2R regulate GABA, glutamate, and dopamine release at the presynaptic level [24-27].

Interestingly, not all CB2R expressed presynaptically are inhibitory, Kim and Li [27] found that CB2R in the hippocampus, specifically in CA1, increase excitatory synaptic transmission (mEPSC) and the number of dendritic spines when activated chronically (7-10 days) via ERK-dependent signaling pathways. This intriguing result highlights the importance of CB2R in the maintenance of dendritic spines and glutamate release. It is noteworthy that CB2R also inhibit GABA release in the hippocampus [26]. The signaling of CB2R at the presynaptic level is less studied. Sánchez-Zavaleta et al [28] were the first to show that the subthalamo-nigral terminals express CB2R, and that their activation inhibits glutamate release. Through the use of pharmacological tools, these authors also demonstrated the inhibition of P/Q calcium channels through subunit βγ of the Gi/o protein. This finding is in agreement with the idea previously suggested by Atwood et al. [29], who noted that if CB2R are expressed at terminals, the regulation of neurotransmitter release is related with voltage-gated calcium channels (see figure 3). It is important to mention that the control of neurotransmitter release by CB2R has consequences on behavior and pathology. For example, the inhibitory regulation of glutamate release in substantia nigra by CB2R modifies motor behavior. In addition, as previously mentioned, the regulation of dopamine release in VTA relates CB2R with a wide broad of dopamine-related behaviors.

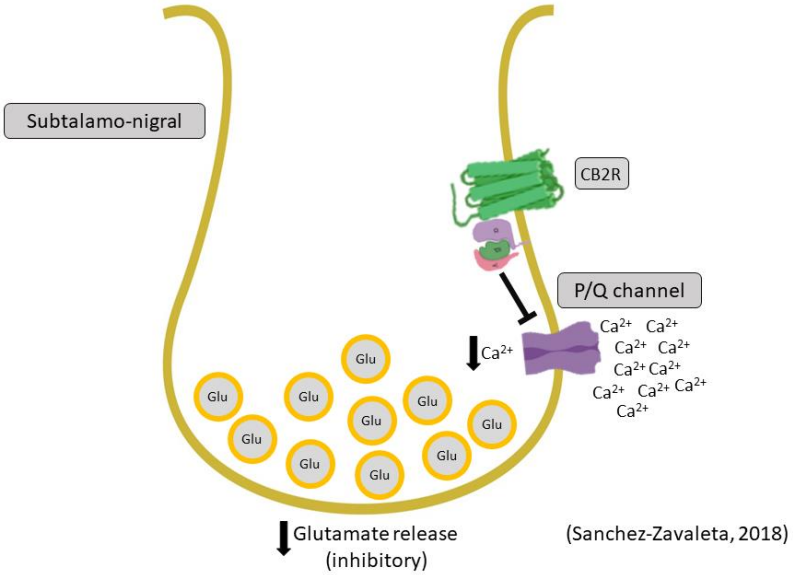

Figure 3. Presynaptic mechanism for couple to Ca^{2+} channels in the control of neurotransmitter release at CNS terminals by CB2R.

In sum, CB2R modulate neural excitability and neurotransmitter release, suggesting an important participation of these receptors in the regulation of various neural physiological processes and functions, that ranging from neurodegenerative diseases, anxiety, depression, locomotor behaviors, pain, and aversive effects stimulated by dopamine-related behaviors, among others [23, 24, 28, 30-33].

Future directions

It is now clear that CB2R are expressed in neurons of the CNS, regulating the excitability and neurotransmitter release that impact behavior. However, some aspects should be studied in greater detail and there are questions to be solved. Some reports are only based in one pharmacological technique, and/or protein or messenger expression, or the functional effects of CB2R, but a more rigorous demonstration is required to establish adequately the role of these receptors in normal neural functions or in pathology [34, 35]. In terms of questions, the co-expression of CB1R and CB2R in the same neurons claims to clarify their meaning; in this regard, a dimeric interaction appears to be a good response, and the characteristics of and meanings regarding the neuronal element should be studied in more detail. Also, the mechanism of activation of these receptors by the endogenous agonist, the condition, the type of endocannabinoid, the requirements to activate one type or another of receptors, and their interactions remain to be elicited. It is additionally important to clarify the meaning of the splicing subtypes of the receptors described, both functionally and pharmacologically. Finally, the role of neuronal CB2R in other pathologies remains to be elucidated. Therefore, there is an extensive panorama of research for clarifying the function and role of CB2R in neuronal function.

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