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Cannabinoids to treat spinal cord injury

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ABSTRACT

Spinal cord injury (SCI) is a devastating condition for which there is no standard treatment beyond rehabilitation strategies. In this review, we discuss the current knowledge on the use of cannabinoids to treat this condition. The endocannabinoid system is expressed in the intact spinal cord, and it is dramatically upregulated after lesion. Endogenous activation of this system counteracts secondary damage following SCI, and treatments with endocannabinoids or synthetic cannabinoid receptor agonists promote a better functional outcome in experimental models. The use of cannabinoids in SCI is a new research field and many questions remain open. Here, we discuss caveats and suggest some future directions that may help to understand the role of cannabinoids in SCI and how to take advantage of this system to regain functions after spinal cord damage.

1. The endocannabinoid system

Although the effects of *Cannabis* derivatives and their use for medicine have been known from ancient times, it was the discovery and synthesis of their major constituent, tetrahydrocannabinol (THC), that established the foundations of an exponentially growing research field (Mechoulam and Parker, 2013). During this time, two types of G protein-coupled receptors (CB_1 and CB_2 cannabinoid receptors) have been identified as mediators of THC actions in the organism (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993). The receptors together with their main endogenous ligands (endocannabinoids), and the specific enzymatic machinery for their synthesis and degradation (Fig. 1) form the core of what is currently known as the endocannabinoid system. This system is widely distributed in the organism (Pacher et al., 2006) and phylogenetically widespread (Elphick, 2012). The main endocannabinoids are 2-arachidonoylglycerol (2-AG) and anandamide (arachidonoylethanolamine or AEA), lipid mediators produced on-demand from membrane precursors in response to cell activation. Anandamide is currently accepted as a high affinity, CB_1-selective partial agonist, whereas 2-AG is a moderate affinity, CB_1/CB_2 full agonist (Sugiura et al., 2006; Di Marzo and De Petrocellis, 2012). However, in vitro experiments using cells transfected with human CB_2 show that anandamide may bind this receptor with moderate affinity (Griffin et al., 2000). These compounds display a very short half-life in the CNS and are degraded by specific enzymes (Fig. 1; Di Marzo and De Petrocellis, 2012). The endocannabinoids behave as retrograde messengers in synaptic plasticity and act as autocrine and paracrine signals between neurons and glial cells (Gomez et al., 2010; Di Marzo and De Petrocellis, 2012; Navarrete et al., 2014).

It is well established that the endocannabinoid system is modulated in response to a variety of neurological insults and this enhancement or the activation of cannabinoid receptors may have therapeutic effects (Pacher et al., 2006; Pacher and Kunos, 2013). In addition, in spinal cord injury (SCI), cannabis has been used by patients for years as a non-conventional treatment against pain and spasticity (Petro and Ellenberger, 1981; Maurer et al., 1990; Cardenas and Jensen, 2006; Pooyania et al., 2010). In this review we present a summary of the current status on cannabinoid research in SCI, a growing body of data, still incomplete, that could harbor a high therapeutical interest.

Before beginning the review, we would like to stress two previous considerations:

First, we will discuss here cannabinoids as molecules that show affinity for one or two of the described cannabinoid receptors (CB_1 and CB_2). In its broadest sense, the term "cannabinoid" may also include a

Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; ABHD4, α/β-hydrolase domain-containing protein 4; ABHD6, α/β-hydrolase domain-containing protein 6; ABHD12, α/β-hydrolase domain-containing protein 12; AEA, arachidonoylethanolamine; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CGRP, calcitonin gene-related peptide; DAG, diacylglycerol; DAGL-α, diacylglycerol lipase alpha; DAGL-β, diacylglycerol lipase beta; DPL, days post-lesion; EA, ethanolamine; EAE, experimental autoimmune encephalomyelitis; FAAH, fatty acid amidohydrolase; GABA, gamma-aminobutyric acid; GDE1, glycerophosphodiester phosphodiesterase 1; IL-1, interleukin 1; IL-4, interleukin 4; iNAT, calcium-independent N-acyltransferase; MAGL, monoacylglycerol lipase; MHC-II, major histocompatibility complex class II; NAAA, N-acylethanolamine-hydrolyzing acid amidase; NAPE, N-arachidonoylphosphatidylethanolamine; NAPE-PLD, N-acylphosphatidylethanolamine-phospholipase D; NAT, calcium-dependent N-acyltransferase; NO, nitric oxide; Olig2, oligodendrocyte lineage transcription factor 2; PKCγ, protein kinase C gamma; PPAR, peroxisome proliferator-activated receptor; PTPN22, protein tyrosine phosphatase, non-receptor type 22; ROS, reactive oxygen species; SCI, spinal cord injury; THC, tetrahydrocannabinol; TRPV1, transient receptor potential cation channel, subfamily V, member 1.

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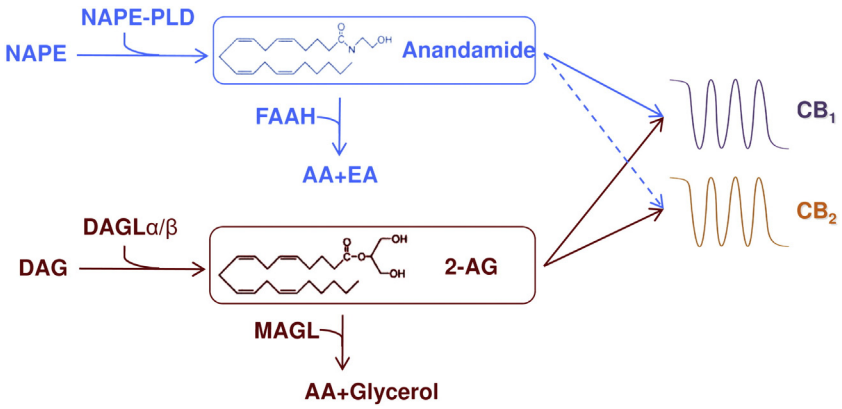

Fig. 1. The endocannabinoid system is mainly formed by two ligands, anandamide and 2-arachidonoylglycerol (2-AG) that bind cannabinoid receptors 1 (CB1) and 2 (CB2). Anandamide and 2-AG are formed from the membrane precursors N-arachidonoylphosphatidylethanolamine (NAPE) and diacylglycerol (DAG), respectively, by the synthesizing enzymes NAPE-PLD and DAGL-α and -β. Anandamide is degraded by FAAH into arachidonic acid (AA) and ethanolamine (EA), while 2-AG is degraded by MAGL into AA and glycerol. Dashed line represents low affinity for CB_2 receptors in human, very low in mice and lack of affinity in rats. NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D; FAAH: fatty acid amide hydrolase; DAGLα/β: diacylglycerol lipase-α and -β; MAGL: monoacylglycerol lipase.

variety of molecules derived from *Cannabis sativa*, synthetic ligands and endogenous lipids that are structurally related to 2-AG or anandamide but show no affinity for CB_1 nor CB_2 cannabinoid receptors (Di Marzo et al., 2014). We will not consider compounds that lack affinity for CB_1 or CB_2 in the current review, despite they can show interesting biological properties. In addition, proper endocannabinoids like anandamide may signal through non-CB_1, non-CB_2 receptors, activating the vanilloid receptor 1 (TRPV1), with variable potency and efficacy depending on the context. This anandamide capacity may play a relevant role in pain regulation and establish a crosstalk with cannabinoid signaling (Di Marzo and De Petrocellis, 2012), but will not be discussed here.

A second consideration is that SCI can be produced by traumatic or non traumatic causes with potential heterogeneity in the mechanisms of damage and in neurological outcomes (McKinley et al., 2001). However, the experimental models used to study cannabinoids in SCI have been limited so far to traumatic models (contusions, compressions and hemisections), the most frequent types of spinal damage in patients. The data originating from traumatic SCI comprise the bulk of the results included in this review. Nevertheless, some conclusions and caveats that we underline in the following pages could be (at least partially) extended to non traumatic SCI and even to other type of degenerations affecting spinal cord.

2. The endocannabinoid system in the intact spinal cord

The first evidence for the presence of cannabinoid receptors in the spinal cord was obtained with ^{3}H-CP55,940, a radioactive ligand that binds CB_1 and CB_2 receptors. ^{3}H-CP55,940 strongly binds to dorsal horn and central gray matter (lamina X), moderately to ventral horn and intermedio-lateral nucleus, and diffusely to other locations of gray matter in rats and humans (Herkenham et al., 1991; Glass et al., 1997). Furthermore, ^{3}H-CP55,940, binding can be prevented with antisense oligonucleotides against CB_1 mRNA or pharmacological inhibitors of this receptor (Richardson et al., 1998). The arrival of immunohistochemistry supported these observations and provided new data on the cell types that expressed CB_1 or CB_2 receptors. Antibodies against CB_1 revealed an intense immunoreactivity in the dorsal horn, dorsolateral funiculus, lamina X, intermediolateral nucleus, and a moderate signal in other laminae and white matter regions (Tsou et al., 1998; Ong and Mackie, 1999; Farquhar-Smith et al., 2000; Salio et al., 2002a,b; Furuse et al., 2009; Garcia-Ovejero et al., 2009; Nyilas et al., 2009; Pernia-Andrade et al., 2009). Due to the important role of cannabinoids in nociceptive circuitry, most studies have been focused in the dorsal horn. There is a general agreement that CB_1 in this region is expressed by inhibitory interneurons co-expressing GABA, PKCγ and NO (Farquhar-Smith et al., 2000; Furuse et al., 2009; Nyilas et al., 2009; Pernia-Andrade et al., 2009). However, CB_1 expression in sensory afferents is still under discussion: while some studies report low levels or absence of CB_1 in substance P, CGRP, isolectin B4, β-subunit of cholera toxin and TRPV1 terminals (Ong and Mackie, 1999; Farquhar-Smith et al., 2000; Salio et al., 2002a,b; Furuse et al., 2009; Pernia-Andrade et al., 2009), others show CB_1 expression in primary afferents coming from dorsal root ganglion neurons projecting to the upper layers of the dorsal horn and in DRG-derived large myelinated fibers, that project to deeper layers of the spinal cord (Salio et al., 2002a,b; Bridges et al., 2003; Nyilas et al., 2009; Veress et al., 2013). Additionally, CB_1 in the intact spinal cord is expressed by glial cells: astrocytes (Salio et al., 2002a, b; Hegyi et al., 2009), microglia (Hegyi et al., 2009), oligodendrocytes (Garcia-Ovejero et al., 2009) and a subpopulation of ependymal cells (Garcia-Ovejero et al., 2013).

The presence of CB_2 receptor inside the nervous system has been a matter of controversy (Van Sickle et al., 2005). In the spinal cord, this gene is expressed in very low amounts (Zhang et al., 2003; Yiangou et al., 2006; Shoemaker et al., 2007; Baty et al., 2008; Romero-Sandoval et al., 2008; Garcia-Ovejero et al., 2009; Brownjohn and Ashton, 2012) but may be strongly induced after neurodegeneration or peripheral nerve damage in microglia (Zhang et al., 2003; Yiangou et al., 2006; Shoemaker et al., 2007; Palazuelos et al., 2008; Romero-Sandoval et al., 2008; Luongo et al., 2010; Hsieh et al., 2011; Paszcuk et al., 2011), immune infiltrates and astrocytes (Garcia-Ovejero et al., 2009; Luongo et al., 2010). CB2 has also been proposed to be upregulated in axon terminals at the dorsal horn after nerve ligation (Wotherspoon et al., 2005).

Together with the expression of cannabinoid receptors, a basal tone of their endogenous ligands anandamide and 2-AG has been described in non-injured spinal cords (Huang et al., 1999; Baker et al., 2001; Cravatt et al., 2004; Witting et al., 2004; Mestre et al., 2005; Bilsland et al., 2006; Suplita et al., 2006; Petrosino et al., 2007; Garcia-Ovejero et al., 2009). Accordingly, the enzymatic machinery for 2-AG formation and degradation is also present in the spinal cord: diacylglycerol lipase alpha (DAGL-α), a major synthesizing enzyme for 2-AG (Fig. 1) is strongly expressed in the dorsal horn, the intermediolateral nucleus and laminae VIII and IX, moderately in the rest of gray matter and with lower intensity in white matter oligodendrocytes and subpial astrocytes (Garcia-Ovejero et al., 2009; Nyilas et al., 2009; Hegyi et al., 2012). Some authors report also that a notable proportion of dorsal horn DAGL-α belongs to microglia and astrocytes (Hegyi et al., 2012). Non-injured spinal cords also express monoacylglycerol lipase (MAGL), the main degradative enzyme for 2-AG (Fig. 1) (Garcia-Ovejero et al.,

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