Allodynia Lowering Induced by Cannabinoids and Endocannabinoids (ALICE)

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\textbf{A B S T R A C T}

Neuropathic pain is a neurological disorder that strongly affects the quality of life of patients. The molecular and cellular mechanisms at the basis of the neuropathic pain establishment still need to be clarified. Among the neuromodulators that play a role in the pathological pain pathways, endocannabinoids could be deeply involved in both neuronal and non-neuronal mechanisms responsible for the appearance of tactile allodynia. Indeed, the function and dysfunction of this complex system in the molecular and cellular mechanisms of chronic pain induction and maintenance have been widely studied over the last two decades. In this review article, we highlighted the possible modulation of the endocannabinoid system in the neuronal, glial and microglial modulation in neuropathic pain treatment.

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1. Chronic pain management

Neuropathic pain is a devastating condition which has a serious impact on the quality of life of patients. It represents a very tricky-to-treat consequence of damages to the peripheral or central nervous systems (PNS or CNS) that are associated with a rearrangement of spinal and supraspinal areas resulting in the exacerbation of pain message [1]. After CNS or PNS injuries, thermal and mechanical painful stimuli are integrated as amplified (hyperalgesia), while innocuous one are elaborated as aching (allodynia). Thus, neuropathic pain represents a real neurological dysfunction that is characterized by as yet scarcely investigated neurophysiological and neurobiochemical pathways. Nowadays, weak pharmacological approaches exist to manage abnormal pain, which is very often not responsive even to opioids and other pain killers commonly used in several pain conditions. Therapeutic compounds available include:

1) gabapentinoids such as gabapentin and pregabalin, representing the therapy of choice for the post-herpetic neuralgia.

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2) opioid derivatives, in particular, those with the hybrid mechanism of action and lower affinity for the opioid receptor, such as tramadol and tapentadol
3) several types of antidepressants such as amitriptyline – which still represents one of the most useful drugs in the clinic but is associated with several adverse reactions – duloxetine and others
4) non-steroidal anti-inflammatory drugs (NSAIDs), some of which used for chronic pain conditions
5) carbamazepine as the first choice for trigeminal neuralgia
6) local anaesthetics such as lidocaine patches.
7) TRPV1 modulators such as capsaicin patches
8) adjuvant drugs such as vitamin B12 complex, acetyl-carnitine, bisphosphonates and corticosteroids.

Despite this huge therapeutic arsenal, several forms of neuropathic pain are still scarcely treated and need an improvement of the target-specific molecules.

2. Endocannabinoid and neuropathic pain establishment

Among the pharmacological approaches that have been proposed for neuropathic pain treatment, the pharmacological manipulation of cannabinoid receptors, by natural or synthetic agonists, or indirectly by selective blockers of enzymes involved in the endocannabinoid degradation fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), is largely sustained by recent preclinical studies in experimental animal models [2-4]. Evidence highlights that cannabinoid receptor-targeted ligands might be effective in several animal models of neuropathic pain [5,26-9]. Reports of cannabinoid-based therapies are also available [9,10]. However, even though there is evidence of endocannabinoid level changes in tissues from neuropathic pain-affected animals [12], the role of these lipids in tactile allodynia induction and maintenance is still highly debated. Indeed, recent evidence showed that the CB1 deletion is associated with enhancement of cold allodynia, whereas a recovery of tactile allodynia was also observed after two weeks in the spared nerve injury (SNI) model of neuropathic pain [13], whereas in other models of neuropathic pain this discrepant effect is not evident. The phenotype of the global deletion of CB1 receptor (CB1R) might be surely associated with compensatory mechanisms that could explain, at least in part, the paradoxical effect revealed in SNI-induced allodynia (e.g. increased levels in microglia of CB2 receptors [CB2R]), whose stimulation has been reported to be antiallodynic [14,15].

Until now, the main symptoms associated with neuropathic pain have been thought to derive mainly from the neuronal maladaptive processes. Recent reports, however, have clearly demonstrated that central and peripheral immune system cells represent the key regulators of the establishment of symptoms like hyperalgesia and allodynia following an injury to the nervous system. Indeed, it has been shown that astrocytes and microglia drive the neuroinflammatory processes within the spinal cord and actively contribute to the development and progression of the tactile allodynia [16]. Interestingly, microglia have been shown to express cannabinoid receptors [17] and to produce and inactivate endocannabinoids [18,19].

Originally, the endocannabinoid system was believed to be composed of the two G-protein-coupled cannabinoid (CB) receptors, CB1R and CB2R; their endogenously produced ligands, anandamide (arachidonylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG) and the metabolic machinery for these lipids including enzymes such as FAAH and MAGL [20]. The latest version of the system is more complex and includes several other endocannabinoid-like mediators involved in pain processes, putative receptors and enzymes as well as endocannabinoid metabolites with their own receptors. Furthermore, AEA and 2-AG have been shown to exert several effects via other targets as well, such as the transient receptor potential vanilloid-type 1 (TRPV1) channel, other TRP channels (i.e.: TRPM8 or TRPA1), GPR18, GPR55, T-type calcium channels, glycine receptors and GABA_A receptor [21-24]. Other mediators, such as two other endolamidines, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), the lipoaminoacid N-arachidonoyl-glycine [25,26], as well as cycloxygenase-derived metabolites "prostamides" [27], have been shown to be involved in the abnormal pain perception associated with the peripheral injuries by modulating many receptors, including TRPV1 channels, peroxisome proliferator-activated nuclear receptor-α (PPAR-α), and yet-to-be-finally characterized proteins [28]. Therefore, the term "endocannabinoidome" has been recently proposed for this complex lipid system [26]. The two recognised receptors CB1 and CB2 share very similar, although not identical, signal transduction pathways but have different localization in mammals. In particular, CB1R is abundantly expressed in the CNS areas involved in pain modulation such as the periaqueductal grey (PAG), rostral ventromedial medulla (RVM), hippocampus and cortex and in the spinal cord [29,30]. However, evidence shows that CB1R is also located in the peripheral sites and could play an important role in some pathological states [31,32].

The CB2R is mainly expressed in immune cells of either the periphery, such as macrophages, dendritic cells and T-cells, or the CNS, such as microglia. Indeed, CB2R over-expression in the dorsal horn of spinal cord of neuropathic animals has been shown in microglial cells and astrocytes and its modulation results in a neuroprotective effect [33,34]. Moreover, it has been reported that CB2R play a key role in driving the neuro-immune response in the spinal dorsal horn during neuropathic pain [14,15]. Other receptors have been suggested to mediate endocannabinoid actions. In particular, TRPV1 and other TRP channels might be activated by high concentrations of AEA and other endocannabinoid-like compounds such as OEA, as well as several plant-derived phytocannabinoids such as cannabidiol (CBD) [35], and seems to be involved in the enhancement of phagocytosis in microglia cells [36]. Importantly, the modulation of the biochemical machinery for the production and degradation of the endocannabinoids (e.g. FAAH or MAGL inhibitors) has been shown to alter pain transmission. Besides this indirect pharmacological strategy, also the direct activation of CB2Rs showed efficacy at reducing pain in preclinical animal models. Unfortunately, CB1R-induced analgesia is often linked with unpleasant effects, such as sedation, psychotopic like behaviour, addiction, tolerance and cognitive impairment. These effects limit the clinical use of compounds that activate the CB1R. Also, CB2R activation has been reported to alleviate inflammatory and neuropathic pain and to reduce motor impairments in models of neurodegenerative diseases [37-40,14,15,41,42,58]. Importantly, CB2R-mediated effects seem to be associated with less psychotropic actions since are mainly due to non-neuronal pathways. Peripheral sites of action of CB2R agonists in both inflammatory and neuropathic pain models have been identified [43,44], even though the specific mechanisms are still debated. This evidence is supported by studies suggesting an over-expression of CB2R mRNA and/or protein in the spinal dorsal horn in animal models of neuropathic pain. In particular, the over-expression of both CB2R mRNA and protein has been shown to be localized in the spinal dorsal horn activated microglia [33,14,15] (Fig. 1). Overexpression of CB2R or application of its agonists reduces microglial activation and neuropathic pain symptoms while treatment with CB2R antagonists blocks these effects in various neuropathic pain models. [45-47]. Also systemic administration of CB2R agonists
prevents microglial activation and alleviates neuropathic pain symptoms in STZ rats [48].

The function of CB2R has been also investigated by a recent research by Landry and collaborators, who demonstrated that CB2R-mediated anti-allodynic effects were due to the reduction of mitogen-activated protein kinase phosphorylation in the spinal dorsal horn [42]. The role of CB2R in the induction of abnormal pain through an immune mechanism has been linked to IFN-γ activity [14]. The activity of CB2R in microglial cells would reduce the activation of these cells during neuropathic pain by regulating the expression of iNOS and CCR2 [14]. Results of in vitro studies have shown that CB2R activation in microglia stimulates phosphatases that inhibit the ERK pathway, thus decreasing TNFα expression and chemotaxis mediated by ADP in microglia [49]. Recently, several new CB2R modulators have been proposed also based on new medicinal chemistry approaches [50]. Despite the promising therapeutic potential showed by CB2R agonists, their translational success depends also by some limitations, such as immune suppression upon chronic use- or paradoxical pro-inflammatory actions [50].

Moreover, a CB2R-mediated effect was also shown on the modulation of the endocannabinoid biosynthetic machinery [51].

Indeed, another opportunity to limit the CB1R-associated side effects could be to pharmacologically modulate the enzymes responsible of the endocannabinoids degradation [12,52]. In fact, these molecules are synthesized and metabolized on demand [53] in several pathophysiological conditions, including inflammatory and abnormal pain [12,54]. Thus, inhibition of enzymes catalysing endocannabinoid degradation would activate CB1R specifically at sites of elevated endocannabinoid turnover, avoiding the interference with other populations of these receptors and, in turn, limiting their side effects [53]. Other strategies have been suggested with hybrid drugs blocking FAAH and antagonizing TRPV1 channels that also exert antinociception in several models of neuropathic pain [55,56]. Moreover, also the dual blockade of FAAH and COX2 have been proposed [57,58]. This strategy would be beneficial since the increase in the endocannabinoids such as anandamide or 2-AG can generate, via COX2-mediated routes, several pro-hyperalgesic products such as prostamide F2α [27].

3. Cannabinoid and neuroinflammatory processes

It is recently recognized that several forms of chronic/neuropathic pain are also associated with neuroinflamm-
malian brain and exerts important pharmacological effects when supplied exogenously as a drug [73,74], or when its endogenous levels are increased by the inhibition of its metabolism [75]. In particular, PEA has been shown to be involved in the neuroprotective mechanisms activated under several pathological states, including chronic and neuropathic pain [76,77,78] and other CNS-related disorders associated with neuroinflammation [79].

4. Cannabinoid use in human pain-associated diseases

The high amount of evidence reporting the efficacy of the cannabinoid system pharmacological manipulation in preclinical models of chronic and neuropathic pain, allowed the clinicians to start several clinical trials using cannabinoids in several chronic pain-associated diseases. In particular, it has been reported their efficacy in complex multi-pain syndrome such as fibromyalgia [11]. It has been reported the possibility to use nabilone for the management of pain [80]. Interestingly, nabiximols has been approved as a botanical drug in the United Kingdom in 2010 as a mouth spray to alleviate neuropathic pain, spasticity, overactive bladder and other symptoms associated with multiple sclerosis [81,82].

5. Conclusions

The pharmacological manipulation of the endocannabinoid system could represent a new target in the management of those types of neuropathic pain that remain untreatable with the commercially available pain killers. The advantage of endocannabinoid-based therapies would consist of the targeting not only neurons but also astroglia and microglia that are the early players in the establishment of tactile allodynia. Intriguingly, the cannabinoid-based therapy is also available for human diseases associated with abnormal pain such as fibromyalgia and multiple sclerosis.

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