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Review Article

Cannabinoids CB2 Receptors, One New Promising Drug Target for Chronic and Degenerative Pain Conditions in Equine Veterinary Patients

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ABSTRACT

Osteoarticular equine disease is a common cause of malady; in general, its therapy is supported on steroids and nonsteroidal anti-inflammatories. Nevertheless, many side effects may develop when these drugs are administered. Nowadays, the use of new alternatives for this pathology attention is demanded; in that sense, cannabinoid CB2 agonists may represent a novel alternative. Cannabinoid belongs to a group of molecules known by their psychoactive properties; they are synthetized by the *Cannabis sativa* plant, better known as marijuana. The aim of this study was to contribute to understand the pharmacology of cannabinoid CB2 receptors and its potential utilization on equine veterinary patients with a chronic degenerative painful condition. In animals, two main receptors for cannabinoids are recognized, the cannabinoid receptor type 1 and the cannabinoid receptor type 2. Once they are activated, both receptors exert a wide range of physiological responses, as nociception modulation. Recently, it has been proposed the use of synthetic cannabinoid type 2 receptor agonists; those receptors looks to confer antinociceptive properties but without the undesired psychoactive side effects; for that reason, veterinary patients, whit chronical degenerative diseases as osteoarthritis may alleviate one of the most common symptom, the pain, which in some cases for several reasons, as patient individualities, or side effects produced for more conventional treatments cannot be attended in the best way.

1. Introduction

Part of the current knowledge about cannabinoids originates from the studies on the psychotropic effect of *Cannabis sativa* plant; however, its ancient therapeutic purpose is known about 2600 B.C. In the XIX century, the systematization of their therapeutic use described by Sir William Brooke O'Shaughnessy showed the potential of these compounds in the management of epilepsy, inflammation, and pain [1].

Pain is a common symptom of equine veterinary patients with chronic degenerative diseases. In these patients, the treatment of first choice are nonsteroidal anti-inflammatory drugs (NSAIDs); however, the prolonged use of NSAIDs produces diverse side effects, including gastric irritation and peptic ulcer. Hence, the identification of alternative treatments is crucial. In this sense, recently, it has been proposed cannabinoids CB2 receptors as therapeutic targets for treatment of pain because these receptors look to confer antinociceptive properties but without the psychoactive side effects.

Therefore, the objective of this article was to contribute to understand the pharmacology of cannabinoid CB2 receptors and its potential utilization on equine veterinary patients with a chronic degenerative painful condition.

Animal welfare/Ethical statement: The research was performed in accordance with the ethical standard laid down in the 1996 Declaration of Helsinki and its later amendments.

Conflict of interest statement: There was no conflict of interest.

Databases used: Pubmed and science direct, using the terms: Cannabinoids, Cannabinoids agonist, Cannabinoids and osteoarthritis, CB1 receptors, CB2 receptors, equine osteoarthritis, osteodegenerative disease, 40 scientific references were included, from several editorial publishers including Elsevier, Wiley, and Springer.

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2. Cannabinoids, Receptors, and Function

In the 60th decade, the abuse in the recreational use of the plant triggered the research about the *C. sativa* derivatives including the Δ-9-tetrahydrocannabinol (Δ9-THC), compound responsible of the psychotropic effects, and the discovery of the endocannabinoid system in vertebrates [2]. The endocannabinoid system is formed by cannabinoid receptors, endogenous agonists, and enzymes involved in their metabolism. Endocannabinoids are synthesized from phospholipids in cytoplasmic and intracellular membranes. High concentrations of Ca^{2+} ions or activation of metabotropic receptors coupled to phospholipase C can stimulate enzymes necessaries for endocannabinoids synthesis (Fig. 1). The two major known endocannabinoids include anandamide (from Sanskrit Ananda: that gives inner happiness) and 2-arachidonoylglycerol (2-AG); however, other endogenous compounds such as virodhamine, N-arachidonoyl-dopamine, and Nolandin ether meet endocannabinoid criteria. Anandamide synthesis in inner membranes requires N-arachidonoyl-phosphatidylethanolamine-PLD and N-acyl transferase. 2-Arachidonoylglycerol synthesis in cytoplasmic membranes requires phospholipase C and diacylglycerol lipase [3].

Current knowledge suggests that endocannabinoids are synthesized and released "on demand"; it means that cellular activity triggers their synthesis in response to specific stimulus. Once synthesized, endocannabinoids reach the extracellular space in cells, particularly in the nervous system at the synapse site by means of their lipophilic properties or specific transport systems. After a transient activation of cannabinoid receptors, presynaptic and/or postsynaptic cells reuptake endocannabinoids by means of specific transporter systems. Inside the cell, anandamide and 2-AG are degraded by the fatty acid amidohydrolase, given ethanolamide and arachidonic acid as metabolic compounds [4]. In the central nervous system (CNS), 2-AG can also be degraded by monoacylglycerol lipase [5].

Cannabinoid receptors can be viewed in two fashions, in one, "the classical", there are only two recognized receptors: the CB1 and CB2 receptors, members of the seven transmembrane G-protein–coupled receptors [6]. In a more extensive point of view, cannabinoid and endocannabinoid compounds can activate a more widespread range of receptors, including the nuclear peroxisome proliferator–activated receptors that act as transcription factors for regulating lipid metabolism and that can be activated by prostaglandins, leukotrienes, N-acylethanolamine, and Δ9-THC [7]; the ionotropic receptors of the family of transient receptor potential channels (TRP), which generate a cationic current in mechanic, thermic, nociceptive, and chemical sensory neurons and that can be activated by anandamide and synthetic cannabinoids [8]; and finally, two G-protein–coupled orphan receptors and putative endocannabinoid receptors: the G-protein–coupled receptor 55 and the G-protein–coupled receptor 119 [9]. CB1 and CB2 receptors are classical Gi protein–coupled receptors, which inhibit the activity of adenylyl cyclase by Giα; in addition, through the βγ complex can close ionic channels (K+ and Ca2+) modulate release of Ca^{2+} from intracellular compartments and activate phosphatidylinositol 3-kinase and the mitogen-activated protein kinase (MAPK) pathways and in consequence signaling to the nucleus [6].

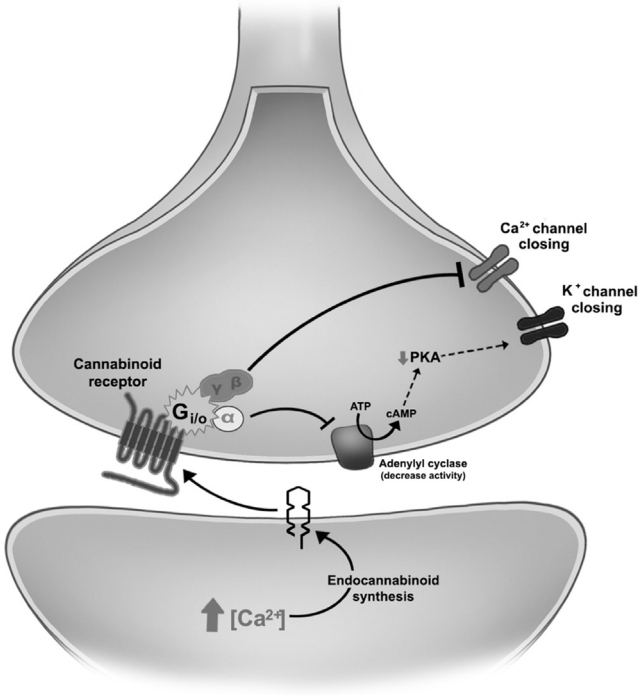

Fig. 1. The endocannabinoid system.

Cannabinoid type 1 receptors are mainly located in the CNS, and in, to a lesser extent, in the periphery; in fact, the more widespread G-protein–coupled receptor in CNS is the CB1 [10]. Their subcellular location is presynaptic in neurons; by their location, a close relationship between the receptor and the neurotransmitter release has been found. At glial cells, CB1 membrane receptors modulate immune responses participating in the neuroinflammatory process [11]. By their coupling to Gi protein, CB1 receptors decrease neurotransmitter release from the terminals; almost all kind of neurotransmitters are regulated by endocannabinoids and their receptors. Regulation of neurotransmitter release can be acute or in a long-term fashion; in fact, short- and long-term depressions and usual neuronal forms of plasticity related with learning and memory are modulated by CB1 receptors [12]. In addition, CB1 receptors also modulate neurotransmitter uptake [13].

The location of CB1 receptors in CNS is closely related with the brain areas related with symptoms of cannabis intoxication and in consequence with their functions; for example, increase of appetite produced by cannabinoids is related with the expression of receptors in nuclei related with eating behavior including hypothalamus, solitary nucleus tract, and accumbens nucleus [2]. In addition, increased locomotor activity and/or incoordination of movement induced by cannabis in patients and experimental animals is related with location of CB1 receptors in basal ganglia [14] and cerebellum [15]. In fact, this knowledge leads the proposal of using cannabinoid compounds for the management of eating disorders such as obesity and anorexia, disorders of the control of movement [16], and also as antiemetic by their effects related with the location of the receptors in the centers controlling these reflexes [17]. In the same way, the addictive properties of cannabis compounds are related with the receptors located in the reward system nuclei which produce the common effect of addictive drugs: the increase in dopamine, neurotransmitter responsible for the pleasure [18]. Cannabinoid receptors also modulate mood and neuronal excitability, hence their proposed use for treatment of depression and epilepsy [19].

On the other side, CB2 receptors are on the contrary located mainly in periphery and in a lesser extent but in more specific areas in the CNS [20]. In the periphery, they are located in immune cells such as macrophages, monocytes, myeloid cells, and spleen cells; in fact, all organs and structures related with immune response have CB2 receptors [21]. In the CNS, their location is in astrocytic cells and their expression is induced by a tissue insult. Therefore, it can

be said that the CB2 receptors in a general fashion modulate immune and inflammatory responses from the organism including the CNS.

3. Cannabinoid Receptors Inflammation and Pain

Plant-derived cannabinoids are a group of compounds isolated from the *C. sativa* plant better known as marijuana. There is no doubt about the historically plant-derived cannabinoids use for inflammation and pain relief in many cultures from ancient times. Until the discovery of the endocannabinoid system, the explanation for their anti-inflammatory and analgesic effects remained unknown, but now, the rationale for their use seems to be clearer. Now it is known that location and signaling of cannabinoid receptors are the basis of these effects.

It is well-known that tissue lesions cause inflammation and perception of pain. Initially, it was found that these lesions induce the release of proinflammatory mediators such substance P, histamine, bradykinins, prostaglandins, and in general cytokines that can induce changes in the permeability of nerve endings from peripheral tissue, in part by changes in the K^+ fluxes, increasing the excitability of nociceptive afferents to the spinal medulla and giving the perception of pain. From this point of view, the activation of CB1 receptors can counteract the excitability of the nerve by their action on K^+ channels by Gi proteins and in consequence decreasing the pain perception [22]. However, the action of cannabinoids on CB1 receptors can also be explained on basis of the location of their receptors not only in the periphery, but also at the spinal and supraspinal level; for example, CB1 receptors are located at serotonergic descending afferents that modulate pain peripheral afferents [23] besides intramedullary receptors, at the thalamic sensory afferent relay station and in the somatosensory cortex [24]. In consequence, the modulation can occur at several levels, including functions modulated by cannabinoids not directly related with the perception of pain such as mood. Intoxicated patients by cannabis have a decreased threshold of pain, a decreased perception of pain, and a dysphoric response to nociceptive perception of pain. In fact, activation of CB1 receptors in the periphery, spinal, and supraspinal sites induce antinociceptive properties in a variety of experimental pain models [25], but the main disadvantages of CB1 receptors agonists are the psychoactive side effects, which restrict their therapeutic use. Even so, management of allodynia and neuropathic pain with Δ9-THC in patients with multiple sclerosis and cancer is a reality; however, the dissociation between the psychoactive and antinociceptive effect is difficult to separate and a disadvantage that should solve the clinician.

Cannabinoids compounds can also modulate pain through other receptors, particularly activation of TRP channels can induce antihyperalgesia in the peripheral nerves, and however, the altered perception after modification of other sensorial modalities can preclude their use because no functional selectivity of their effects can be obtained [26].

CB2 receptor activation can reduce inflammation and pain by means of their action on MAPK enzymes and also by the observed modification in K^+ and Ca^{2+} channel activity as do CB1 receptors. This can be the basis of a selective use in pain conditions among their location in selective CNS structures as thalamus, and the lack of apparent or at least poor psychoactive effect [27]. With this basis and the discovery of selective ligands which provide a selective activation, recent studies suggested the use of CB2 receptor agonist in animal models of pain [28] and it has been widely demonstrated that CB2 analgesia is interestingly related to peripheral mechanism without CNS effects [29]. Another possible advantage of CB2 receptors activation is the lack of rapid tolerance [27] compared with CB1 receptor.

3.1. Actual State of Pain Management in Equine Veterinary Patients

Equine veterinary patients who suffer from painful diseases including osteoarthritis-related or any other degenerative diseases represent an important class of patients in veterinary attention, as many authors have pointed out; in those patients, the cornerstone for the therapy is NSAIDs or corticosteroids [30], with main disadvantage for this therapy being the side effects associated with the long-term treatments. The most frequently observed are at the gastrointestinal tract as anorexia, gastric irritation, and peptic ulcer. Less-frequent side effects, but with sensible implications, are nephrotoxicity, hepatotoxicity, and hematologic diseases [31,32].

4. Cannabinoids for Equine Veterinary Patient?

There is a public health concern about using and prescribing cannabinoids for therapeutically purposes because of the recreational use and abuse of plant cannabinoids that leads to undesired behaviors. Those effects are mediated by the psychotropic properties of plant compounds in humans; nevertheless, the therapeutic importance for this group of drugs cannot be denied. In some countries, therapeutic marijuana has a legal use for medical purposes [33] that may include osteodegenerative conditions such as degenerative arthritis, which represents an important public health issue; besides some conditions with a very painful component are also treated with cannabinoid receptor–based therapies. Another potential field for cannabinoid therapy is for the patient attention, which in painful situations, requires a potent analgesic support.

For such pathologies associated with painful stimulus, there are some more conventional treatments, including nonsteroidal anti-inflammatories or opiates; however, there are specific inconveniences associated with the therapy; NSAIDs are related to side effects such as gastritis, gastric ulcer, or coagulopathies. Moreover, sometimes they cannot relieve the painful stimulus related to the disease because of its intrinsic and pharmacological characteristics, leaving a group of patients without appropriate attention and impact in their quality of life.

In the case of the equine patient, osteoarthritis is related with the 60% of the lameness being the tarsal disease one of the most common [34], and is considered one of the most harmful articular diseases because of the slow healing activity of the articular surfaces, coupled with it; there is an inflammatory component which leads to cartilages degradation, pain, and loss of the function [35,36].

As can be seen, some conditions with an acute or chronic profile accompanied by a powerful painful component cannot be adequately managed by the patients' singularities or by the intrinsic pharmacologic characteristics of the drugs used in pain management that are not compatible. In those cases, alternative therapies or drugs should be excellent resources and in that order of ideas, cannabinoids agonists represent an excellent opportunity.

Recently, CB1 cannabinoid receptor–based drugs have emerged as an attractive therapeutic target for human pain management, and interest in the use of cannabinoids is gradually increasing particularly in patients where conventional treatments fail [37].

On the other hand, as we mentioned before, CB2 receptors are expressed in a more restricted fashion; they are less expressed in the CNS but in some peripheral tissues such as spleen, tonsils, and thymus [38]. Because of its restricted distribution in the CNS, the pharmacological profile of CB2 receptors agonist is different. The main advantage is conferring an important antinociceptive activity with less psychoactive side effects, and that anatomical and structural differences between both receptors may result in a more suitable therapeutic target for equine veterinary patients. Moreover, that peculiar distribution through the body makes the CB2

receptors an excellent option for pain-related conditions especially when conventional therapies fail.

Moreover, based on the CB2 receptors pharmacologic profile, there is another particular property related to the CB2 receptor activation that may give positive results particularly for the osteoarticular pathologies. Based on several studies, CB2 receptors have displayed immunomodulatory properties, modifying the inflammatory and the infiltrative response in chronic or progressive articular degenerative conditions moreover, and the CB2 receptors appear to be overexpressed in animal models of this disease [39]. CB2 receptor agonist may give positive results for the treatment of equine veterinary patients with osteoarticular pathologies, conferring additional level of protection against the degenerative process modifying not only the main symptom of the disease but the progression and finally the outcome of the degenerative condition.

With all the evidences generated on several lines of investigation which demonstrate the analgesic efficacy of systemically administered CB2 receptors agonists in acute and chronic pain models [40]. It is possible to hypothesize the beneficial use of specific CB2 receptor agonists in the osteoarticular degenerative disease in horses; surely, many controlled and well-conducted investigations are needed to clarify the equine endocannabinoid system and its function, but at the moment, it is possible to expect a similar behavior and physiological function, between the documented and studied endocannabinoid system in other mammals and horses.

5. The Future in the Attention on Equine Veterinary Patients With Painful Conditions

We believe that the use of selective CB2 receptors agonists represents a potential field for the veterinary patient attention, especially those that transit for a painful disease. In our working group, we are trying to understand the basic mechanism involved in the pain modulation associated with CB2 receptor activation and many questions remain unsolved, for example, is the analgesic effect directly related with the anti-inflammatory effect or is inverse as the case of NSAIDs? What are the specific specie side effects? It's very important to understand all the underlying mechanisms by which CB2 receptors may alleviate chronic degenerative-related symptoms such as pain. With that point of view, veterinary practitioners and veterinary researchers may contribute by means of well-designed and controlled protocols to improve the quality of life of a selected group of patients which for several reasons may not reach a satisfactory therapy.

To date, it is remarkable that there is not enough scientific literature about the importance or the treatment of using cannabinoids in the veterinary patient, showing a forgotten group of molecules waiting to be used. That situation is difficult to explain; perhaps, the main reason relies on the facts related to the use and abuse of cannabinoids in humans by itself and the stigmatization around the misuse of that kind of drugs. Nevertheless, we believe that there is much information and evidence which supports the idea of taking advantage of the pharmacologic properties of CB2 receptors and selective agonists which may exert a protective action against pain but without all the psychoactive and undesired side effects of CB1 agonists. The only aim of this effort to incorporate cannabinoid receptors agonist is to provide a more reliable alternative for painful conditions in veterinary patients, and in many cases to limit some side effects related to the nature of other more conventional treatments.

6. Conclusions

Cannabinoids belong to a group of molecules known by their psychoactive properties; they are synthesized in the *C. sativa* plant, better known as marijuana. In animals, two main receptors for cannabinoids are recognized: the cannabinoid CB1 and the CB2 receptors. Once they are activated, both receptors exert a wide range of physiological responses such as nociception modulation. Recently, it has been proposed the use of cannabinoids CB2 receptors for treatment of pain; these receptors look to confer antinociceptive properties but without the psychoactive side effects. For this reason, equine veterinary patients with chronic degenerative diseases such as osteoarthritis may experience alleviation of one of the most common symptoms, the pain, which in some cases for several reasons, such as patient individualities or side effects produced by more conventional treatments, cannot be attended in the best way.

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