

Role of the Cannabinoid System in Pain Control and Therapeutic Implications for the Management of Acute and Chronic Pain Episodes

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Abstract: Cannabis extracts and synthetic cannabinoids are still widely considered illegal substances. Preclinical and clinical studies have suggested that they may result useful to treat diverse diseases, including those related with acute or chronic pain. The discovery of cannabinoid receptors, their endogenous ligands, and the machinery for the synthesis, transport, and degradation of these retrograde messengers, has equipped us with neurochemical tools for novel drug design. Agonist-activated cannabinoid receptors, modulate nociceptive thresholds, inhibit release of pro-inflammatory molecules, and display synergistic effects with other systems that influence analgesia, especially the endogenous opioid system. Cannabinoid receptor agonists have shown therapeutic value against inflammatory and neuropathic pains, conditions that are often refractory to therapy. Although the psychoactive effects of these substances have limited clinical progress to study cannabinoid actions in pain mechanisms, preclinical research is progressing rapidly. For example, CB₁-mediated suppression of mast cell activation responses, CB₂-mediated indirect stimulation of opioid receptors located in primary afferent pathways, and the discovery of inhibitors for either the transporters or the enzymes degrading endocannabinoids, are recent findings that suggest new therapeutic approaches to avoid central nervous system side effects. In this review, we will examine promising indications of cannabinoid receptor agonists to alleviate acute and chronic pain episodes. Recently, *Cannabis sativa* extracts, containing known doses of tetrahydrocannabinol and cannabidiol, have granted approval in Canada for the relief of neuropathic pain in multiple sclerosis. Further double-blind placebo-controlled clinical trials are needed to evaluate the potential therapeutic effectiveness of various cannabinoid agonists-based medications for controlling different types of pain.

Key Words: Analgesia, cannabidiol, cannabinoid receptor, cannabis, endocannabinoid, inflammatory pain, neuropathic pain, tetrahydrocannabinol.

INTRODUCTION

Hemp, *Cannabis sativa*, is a coarse bushy annual plant with palmate leaves and clusters of small green flowers that grows wild in regions of mild or tropical weather and can attain a height of 3 metres. The genus name *Cannabis* is complemented by *sativa* (which means useful). Cannabis has indeed been used throughout history for a variety of purposes, including the production of fibre for paper and textile manufacture. However, its current popularity lies in its use as a recreational drug with psychoactive properties. The plant contains many chemical compounds that have different pharmacological properties, varying in quantity and quality depending on the strain and culture and storage conditions. Extracts of the dried flowers, buds, or leaves are known as either cannabis (British term) or marijuana (North American term, probably originating from Mexican slang). Hashish is made from a resin secreted by the flowers of female plants. Consumption of cannabis derivatives (by smoking, eating, or drinking) produces euphoria, relaxation, a general sense of well being, and time distortion. Heavy consumption may precipitate hallucinations, anxiety, depression, and psychoses.

Cannabis has been utilised for centuries throughout the world to alleviate disease. Its derivatives were named

“*panacea*”, or “cure-all”, and were sold as a legal medicine, mainly for pain [12, 135]. In the United States, cannabis has been illegal since 1937. In 1961, cannabis was added to the United Nations Single Convention on Narcotic Drugs (amended in 1972) and to Article 33 of the United Nations Convention on the Rights of the Child. One hundred fifty countries ratified these Conventions. Nonetheless, drug policies have varied. For example, during the late 1990s, 13 states of the United States made laws that permit cannabis for medical necessity, requiring to be prescribed by physicians; and being especially used to relieve AIDS patients treatment side effects. Regarding recreational use in Europe, some countries are being permissive, like Switzerland, Portugal, Spain, Italy, and Belgium, and others strict, such as Sweden, where it is illegal to consume cannabis. In the Netherlands, cannabis has long been decriminalised. The widely held view of cannabis-related products as drugs of abuse has slowed progress in the development of studies designed to take advantage of the properties of cannabinoid compounds for therapeutic purposes. Nonetheless, the discovery of the endogenous cannabinoid system in the early 1990s renewed interest in this field and strongly stimulated research. In 1964, Mechoulam and colleagues [42] found that delta-9-tetrahydrocannabinol (THC) was the major psychoactive ingredient of cannabis. THC causes a variety of effects in different animal species, such as antinociception, hypoactivity, catalepsy, hypothermia, and cardiovascular changes. In the late 1980s, Howlett and colleagues [29] identified and characterised a receptor in rat

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brain that met criteria for a high-affinity, stereoselective, pharmacologically distinct cannabinoid receptor, by means of radiolabelled agonist ligand binding and functional assays for G-protein coupled receptors. Moreover, they found a correlation between the potency of cannabinoid compounds in producing analgesia *in vivo* and the inhibition of adenylyl-cyclase *in vitro*, indicating the presence of a G-protein-coupled "cannabinoid analgesic receptor" in brain [64]. A few years later, in 1990, the cannabinoid type 1 (CB₁) receptor was cloned from brain tissue [100], followed by cloning of the type 2 (CB₂) cannabinoid receptor in 1993 [108]. Meanwhile, a torrent of new discoveries has yielded insight into the components and mechanisms of action of the endogenous cannabinoid system. Consequently, some endogenous ligands of cannabinoid receptors have been investigated, as well as their synthesis, transport, and degradation [for reviews see: 39, 120]. A number of synthetic cannabinoid receptor agonists and antagonists have been formulated and genetically modified mice have been produced [13, 27, 86]. Altogether, research has provided valuable tools for developing new pharmacologic cannabinoid products. One of the more promising applications appears to be the use of cannabis-based medicines to relieve pain, given the antinociceptive and anti-hyperalgesic effects of cannabinoid receptor agonists observed in animal models. Unfortunately, the underlying potential for abuse and dependence after heavy cannabis consumption is still a drawback in the search for effective pharmacologic preparations, which ideally would have minimal or no psychoactive side effects.

In this review, we will describe the different components of the endogenous cannabinoid system and their mechanisms of action, with especial emphasis on those implicated in analgesic effects. We will also discuss agents that may interfere pharmacologically with cannabinoid functions to enhance their antinociceptive effects and clinical conditions in which cannabinoids hold promise for effective therapeutic applications.

ENDOGENOUS CANNABINOID SYSTEM

Endogenous Cannabinoid Receptors

The biological effects of cannabinoid compounds are mediated by their binding to and further activation of cannabinoid receptors. Four subtypes of these receptors have been identified. Two have been cloned, type 1 (CB₁) and type 2 (CB₂) cannabinoid receptors [100, 108], while the other two, WIN and abnormal-cannabidiol (abn-CBD) receptors (the latter also known as anandamide receptor), have been characterised pharmacologically [8, 31, 51, 71, 107]. In addition, some truncated forms of the CB₁ receptor, like the CB_{1A}, have been found, resulting from alternative splicing [144]; and there may be more subtypes of cannabinoid receptors yet undiscovered [8, 51].

Cannabinoid receptors are G_{i/o}-protein coupled receptors anchored in the cell membrane. Structurally they consist of seven folded transmembrane helices with intra- and extracellular loops, functionally involved in signal transduction. The CB₂ receptor is located mainly in the immune system, but has been found in others sites, as in keratinocytes [67]. On the other hand, the CB₁ receptor, which is the canna-

binoid receptor that has been most studied, has high levels in brain but also lower levels in spinal and peripheral nervous tissue (including areas important for pain perception, as will be discussed below). CB₁ receptors are also disseminated in several other non-nervous tissues like endothelial cells, uterus, and others.

Neuroanatomic Distribution of CB₁ Receptors in the Nervous System

CB₁ receptors are abundant and widely dispersed throughout the brain. Their distribution has been mapped by autoradiographic studies, immunohistochemical techniques, *in situ* histochemistry, and electrophysiological studies [53, 78, 140]. CB₁ receptors have shown particularly high levels of expression in cortex, basal ganglia, hippocampus, and cerebellum and low levels of expression in brainstem nuclei. They are present in brain areas involved in nociceptive perception, such as the thalamus and amygdala (Fig. 1B) [93, 99]. CB₁ receptors are also expressed in cells of the midbrain periaqueductal grey matter (PAG), and in the substantia gelatinosa of the spinal cord (receiving nociceptive input from primary afferent neurons), which are key sites for modulating nociceptive information [87, 92, 106]. In the medulla oblongata and spinal cord, structures involved in processing pain signals, more dense concentrations of CB₁ receptors are detected in the superficial dorsal horn, and in the dorsolateral funiculus of the spinal cord (Fig. 1C) [35, 53, 59, 140, 159]. CB₁ receptors of the spinal cord dorsal horn are predominantly found in interneurons, particularly in a double band of CB₁ immunoreactivity in laminae I, II, and inner/III transition, and in lamina X [35]. In the superficial dorsal horn of rats, CB₁ receptors are located primarily on the axons of intrinsic interneurons [35, 159], indicating a presynaptic site of action that is consistent with modulation of neurotransmitter release by endocannabinoids. Furthermore, CB₁ receptors are synthesised in neurons of the rat dorsal root ganglia (that express neuropeptide markers found in nociceptive primary afferents) [59], and these receptors are transported both centrally, reaching superficial dorsal horn terminals [59] and peripherally towards peripheral nerve terminals of sensory nerves [58]. Interestingly, these sensory nerves are engaged in the ascent of nociceptive stimuli to the spinal cord (Fig. 1A, C, D). On the other hand, although CB₁ receptor mRNA expression has been described in the trigeminal ganglia in medium and large diameter neurons, the majority of these CB₁-expressing neurons do not seem to be involved in nociceptive neurotransmission in the non-injured animal [122]. Finally, CB₁ receptors are found on only a small percentage of C-fibres, while the majority are on axons of larger diameter neurons with myelinated A-fibres [11]. The described anatomical distribution of CB₁ receptors is consistent with their function of modulating pain perception at both peripheral and central (spinal and supraspinal) levels (Fig. 1).

Endocannabinoids

The endocannabinoids, or endogenous cannabinoids, are a family of bioactive lipids that activate cannabinoid receptors to exercise their effects, modulating neural transmission. They are present in only small amounts in brain and other tissues and participate in the regulation of various cerebral

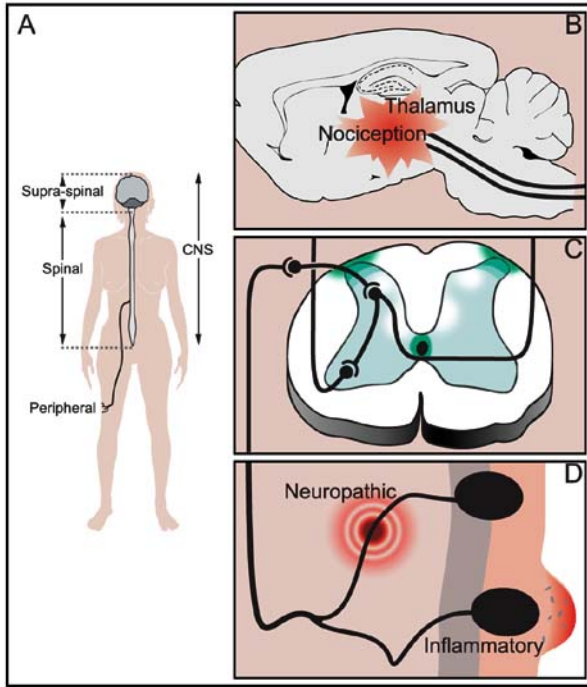


Fig. (1). Schematic representation of cannabinoid receptors distribution. **A:** Cannabinoid receptors are present in the pain pathway at the peripheral and central (spinal and supraspinal) levels. **B:** Supraspinal CB₁ receptors are distributed in areas of the brain and brainstem nuclei involved in nociceptive perception as thalamus, amygdala, and periaqueductal grey matter. **C:** The highest abundance of spinal CB₁ receptors are found in the dorsolateral funiculus, in the surroundings of the central canal and in the superficial dorsal horn. **D:** CB₁ receptors are also present in the peripheral sensory nerve endings, and both CB₁ and CB₂ receptors have been detected in non-neuronal cells participating in immune and inflammatory processes in the proximity of the primary afferent neurons nerve terminals.

functions, including pain perception, mood, appetite, and memory. Exogenously administered cannabinoid compounds of natural or synthetic origin mimic their effects. Even though we still have much to learn about the relative roles of different endocannabinoids, they appear to be promising potential targets for manipulation, for instance, to slow their degradation for analgesic purposes. Endocannabinoids possess submicromolar affinity for cannabinoid receptors and act as retrograde signal molecules in synapses. Despite the similarity of their chemical structures, endocannabinoids are produced by their own biochemical pathways. They are synthesised locally on demand in postsynaptic terminals, which requires Ca²⁺ influx, and released in selected regions to activate presynaptic cannabinoid receptors situated in specific small areas (Fig. 2). Thereafter, they are rapidly inactivated by a combination of a transporter mechanism and further enzyme degradation [32, 44, 118]. Noxious stimuli increase endocannabinoid release [168], which supports that they intervene in pain modulation.

The first endocannabinoid isolated (from porcine brain) and structurally characterised was arachidonyl ethanolamide

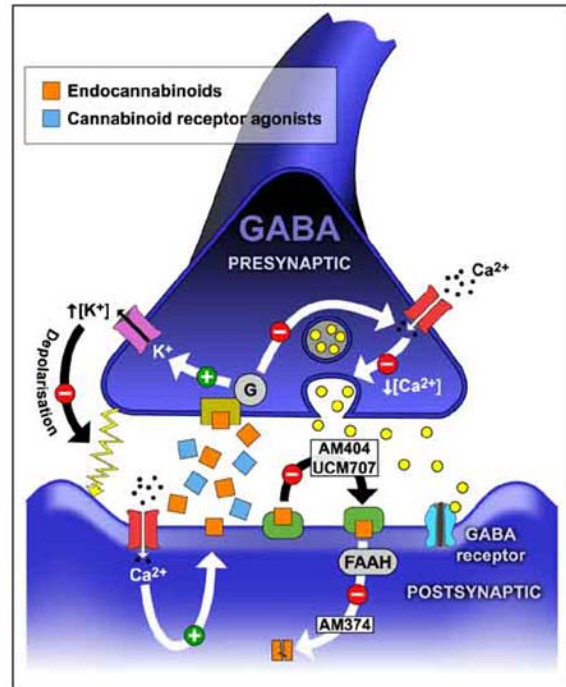


Fig. (2). Schematic representation of a GABAergic synapse, containing CB₁ receptors, to show potential targets for therapeutic intervention. Endocannabinoids are synthesised in membranes of neurons and other nervous cells and released to the synaptic space to activate presynaptic CB₁ receptors. Enhancement of cannabinoid receptors activity can be obtained by different pharmacological manipulations as, for example, administering exogenous cannabinoid receptor agonists or inhibiting either the reuptake or the degradation of the endocannabinoids.

(AEA), commonly designated anandamide [30]. The name comes from the Sanskrit word *ananda*, which means "bliss", and amide. Bliss means happiness that invokes physiologic and psychologic harmony and, in Buddhism, indicates an elevated consciousness since Ananda was one of the principal disciples of the Buddha. Anandamide acts in pain, depression, appetite, memory, and fertility (due to its uterine synthesis). Anandamide is synthesised enzymatically in brain areas that are important in memory and higher thought processes, and in areas that control movement. Anandamide, or arachidonyl ethanolamide, is an amide derivative of arachidonic acid and ethanolamine. It is synthesised by hydrolysis of the precursor N-arachidonoyl phosphatidylethanolamine, which is catalysed by the enzyme phosphodiesterase phospholipase D [16, 32]. After release from the postsynaptic terminal, anandamide interacts with presynaptic cannabinoid receptors. It is rapidly removed from the synaptic space by a high-affinity transport system present in neurons and astrocytes. Once internalised, anandamide is hydrolysed by the enzyme fatty acid amide hydrolase (FAAH), an intracellular membrane-bound enzyme. In cerebellum, hippocampus, and neocortex, FAAH is expressed at high levels in the somatodendritic regions of neurons postsynaptic to CB₁-positive axon terminals. Thus CB₁ receptors and FAAH have a close and complementary anatomical distribution [34].

Other endogenous ligands for cannabinoid receptors are 2-arachidonoylglycerol (2-AG), noladin ether, virodhamine, and N-arachidonoyldopamine (NADA) [103, 121, 168]; although anandamide is the endocannabinoid most extensively studied.

The endogenous cannabinoid 2-AG is produced by neurons and other nervous cells in a stimulus-dependent manner, binds to cannabinoid receptors and undergoes rapid biological inactivation through transport into cells and catalytic hydrolysis [103, 151]. It is synthesised by cleavage of an inositol-1,2-diacylglycerol, which is catalysed by phospholipase C [16, 32]. The levels of 2-AG found in some regions of the brain are much higher than anandamide levels, and the biosynthesis of these molecules is controlled separately [120].

Noladin ether, or 2-arachidonoylglyceryl-ether, has been isolated from porcine brain and is an endocannabinoid that binds to CB₁ cannabinoid receptor to signal food consumption and weight control [52].

Virodhamine is a partial agonist with *in vivo* antagonistic activity at the CB₁ receptor, but acts as a full agonist on the CB₂ receptor. Its concentrations are higher than those of anandamide, but its ability to alter pain sensitivity has not been tested. [168].

NADA was identified in rat and bovine brain [65], activates CB₁ (and VR₁ receptors) and elicits cannabimimetic effects, including analgesia, following systemic administration. It interacts with FAAH and the anandamide transporter. The highest levels are found in the striatum and hippocampus, and at low levels in the dorsal root ganglia [65, 168].

CANNABINOID PHARMACOLOGY

In this section we will describe the mechanisms of action and the effects that occur after endocannabinoids bind to their specific receptors, while highlighting their role in pain modulation.

Activated CB₁ receptors couple to G_{i/o}-protein to inhibit adenylate cyclase, decrease Ca²⁺ conductance, increase K⁺ conductance, and increase mitogen-activated protein kinase activity [for review see: 63]. The presynaptic localisation of CB₁ receptors indicates that cannabinoids modulate neurotransmitter release from axon terminals. The effect of cannabinoids on synaptic function consists of inhibition of the release of a variety of neurotransmitters and also the inhibition of electrical activity by a depolarisation phenomenon (Fig. 2) [79, 141]. The neurotransmitters whose release is inhibited by activation of cannabinoid receptors include L-glutamate, GABA, noradrenaline, dopamine, serotonin, and acetylcholine. Therefore, depending on the nature of the presynaptic terminal, endocannabinoids induce either suppression of inhibition or suppression of excitation, namely depolarisation-induced suppression of inhibition (DSI) or of excitation (DSE) [84, 85]. CB₁ receptor antagonists block DSI and DSE. However, if the CB₁ receptor agonist remains present, the depolarisation phenomenon is blocked by occlusion [115, 175] and inhibitory inputs are transient. This is why cannabinoid receptor agonists cannot mimic the same physiologic effects of locally released endocannabinoids. Since they cause long-lasting activation of CB₁ receptors in

all brain regions, their overall effect is persistent inhibition of neurotransmitter release from nerve endings that express CB₁ receptors and, as a consequence, they temporarily occlude and prevent DSI and DSE phenomena. On the contrary, endocannabinoids are involved in the rapid modulation of synaptic transmission in the central nervous system by a retrograde signalling pathway that can influence synapses in a local region, with inhibitory effects on both excitatory and inhibitory neurotransmitter release that persist for tens of seconds. This may be important in the control of neural circuits, such as nociceptive signalling.

Activation of cannabinoid receptors inhibits GABAergic synaptic transmission in a number of central nervous system regions, including areas participating in nociceptive signalling like the amygdala [93], periaqueductal grey matter [162], rostral ventromedial medulla (RVM) [163], and superficial dorsal horn [72]. Depolarisation of the postsynaptic neurons leads to DSI by inhibition of GABA release. However, even though cannabinoid receptor-mediated inhibition of glutamatergic transmission occurs in some brain regions including PAG neurons [162], it has not been detected in the medullary dorsal horn [143, 154, 162].

ANALGESIC EFFICACY OF CANNABINOID

Activation of Cannabinoid Receptors

Cannabinoid receptor agonists effects in the central nervous system (CNS) include disruption of psychomotor behaviour, short-term memory impairment, intoxication, stimulation of appetite, antiemetic effects, and antinociceptive actions [68]. Administration of natural or synthetic cannabinoid receptor agonists has shown therapeutic value for a number of important medical conditions, including pain (particularly against pain of neuropathic origin), anxiety, glaucoma, nausea, emesis, muscle spasms, and wasting diseases. Insofar as pain is concerned, it is well known that cannabinoid receptor agonists have antinociceptive and anti-hyperalgesic effects at the peripheral and central (spinal and supraspinal) levels, as has been demonstrated in acute and chronic pain models [69, 116]. Cannabinoid receptors and endocannabinoids are present in pain circuits from the peripheral sensory nerve endings up to the brain (Fig. 1). Cannabinoid receptor agonists modulate nociceptive thresholds by regulating neuronal activity [4], but they also relieve pain by acting on non-nervous tissues. CB₁ receptor is involved in the attenuation of synaptic transmission, and a proportion of the peripheral analgesic effect of endocannabinoids can be attributed to a neuronal mechanism acting through CB₁ receptors expressed by primary afferent neurons. However, recent findings suggest that CB₁ receptors are also present in mast cells and may participate in some anti-inflammatory effects. Thus, activated CB₁ receptors present in mast cells induce sustained cAMP elevation, which, in turn, suppresses degranulation [146]. On the other hand, although CB₂ receptors have been related traditionally to the peripheral effects of cannabinoids (mainly modulation of the immunologic responses), they also contribute to antinociception by inhibiting the release of pro-inflammatory factors by non-neuronal cells located near nociceptive neuron terminals. CB₂ receptors are expressed in several types of inflammatory cells and immunocompetent cells. For that reason, activation of peripheral CB₂ receptors

generates an antinociceptive response in situations of inflammatory hyperalgesia and neuropathic pain [66, 160], while selective CB₂ receptor agonists are not antihyperalgesic against chronic inflammatory pain in CB₂ knockout mice [160]. Possible mechanisms of this CB₂-mediated effect include the attenuation of NGF-induced mast cell degranulation and of neutrophil accumulation, both of which are processes known to contribute to the generation of inflammatory hyperalgesia [131]. Therefore, since activation of CB₁ receptors is associated with central side effects, including ataxia and catalepsy, selective CB₂ receptor agonists have the potential to treat pain without eliciting the centrally-mediated side effects. Furthermore, CB₂ receptors have novel pain control actions. A CB₂-mediated effect exists, consisting in the indirect stimulation of opioid receptors located in primary afferent pathways [67], as will be described in more detail in the next section. Thus, cannabinoid compounds can modulate hyperalgesia of various origins and they are effective even in inflammatory and neuropathic pain [10, 133], which are conditions often refractory to treatment. In the CNS, although CB₂ receptor mRNA has not been detected in the neuronal tissue of human or rat brain, a role in antinociception in inflammatory processes of the nervous system cannot be excluded due to its presence in activated microglia [166].

Animal Models of Pain

Different validated animal models are used to explore the analgesic effects of cannabinoid compounds. However, since most responses are quantified by behavioural tests, it is important to remember that cannabinoids may inhibit or enhance motor activity [139], depending on the dose and structure of the compound administered, which may influence behavioural reactions and mask the results of analgesic tests. In order to counteract these effects, complementary analyses are used to demonstrate the antinociceptive effects of cannabinoids. In this context, cannabinoids block spinal c-fos expression in response to noxious stimulation and suppress the electrophysiologic responses of spinal cord neurons [69, 116, 166]. In the spinal cord lamina receiving primary afferent fibres, noxious stimuli enhance c-fos expression, making it a good marker for spinal nociceptive activity. Following noxious heat stimulation, cannabinoid receptor agonists diminish stimulation in deep dorsal horn neurons, while the CB₁-specific antagonist SR141716A facilitates nociceptive responses [18, 60]. Temporary inactivation of neural activity in the RVM in rat brainstem circumvents the analgesic effects of systemically administered cannabinoids, while leaving motor activity effects untouched [104]. This reflects cannabinoid receptor agonists actions that specifically target sensory pathways passing through the RVM. Noxious stimulation evokes enhanced release of the anandamide, as observed in the PAG of brainstem [167], which is evidence that endocannabinoids modulate nociceptive information. Further confirmation of the role of the endocannabinoid system in the control of pain is that the blockade of cannabinoid receptors, whether by antagonists, antibodies, or genetic deletion, inhibits or attenuates pain perception [22, 33, 86]. Thus, the antinociceptive potency of a series of cannabinoid receptor agonists correlates strongly with their capacity to displace radioligands from the cannabinoid receptor

and to inhibit adenylate cyclase. Also, cannabinoid-induced antinociception can be attenuated by pertussis toxin and other substances that interfere with the signal transduction of CB₁ receptors connected to protein G [128]. Finally, cannabinoid receptors, both CB₁ and CB₂, are upregulated in models of chronic pain. Therefore, one response of the body to chronic pain is to increase the number of these receptors, suggesting that their function in such situations may be important. For example, animal models of neuropathic pain stimulate upregulation of CB₁ receptors expression in nervous structures involved in the processing of pain, like the ipsilateral superficial spinal cord dorsal horn [90] or contralateral thalamus [145], enhancing the analgesic effects of cannabinoid receptor agonists. This upregulation of central CB₁ receptors following peripheral nerve injury indicates a role for them in these pathologies and also explain the therapeutic effects of cannabinoid receptor agonists on chronic pain conditions as neuropathic pain. Chronic pain models associated with peripheral nerve injury, but not peripheral inflammation, induce CB₂ receptor expression in a highly restricted and specific manner within the lumbar spinal cord. Moreover, the appearance of CB₂ expression coincides with the appearance of activated microglia [178].

Sites of Pain Modulation

When investigating cannabinoid actions in the control of pain, special consideration should be given to the level at which such actions take place to determine whether the mechanisms are central or peripheral. Pain perception of a hot-plate is thought to be mediated by a central mechanism (eliciting supraspinally controlled behavioural responses to the noxious heat stimulus), whereas the second late phase of response to an intraplantar injection of formalin reflects peripheral inflammatory pain mechanisms [156]. The tail-flick assay consists of induction of a spinal tail flick response to noxious thermal stimuli (spinal reflex). Therefore, the analgesic response recorded in these different acute pain models allows identification of their location.

Another crucial factor in the approach to cannabinoid analgesia would be the route of administration, which not only provides information about the site of action but also helps to improve therapeutic results through selection of the most effective route for dispensation. Systemic and intracerebroventricular administration of cannabinoid receptor agonists both produce analgesia [56, 89], implying that they exercise antinociception centrally. Cannabinoid receptor agonists produce antinociception in spinal and supraspinal sites of action, as investigated in spinally transected and intact rats. In spinally intact animals, cannabinoid receptor agonists injected intravenously produced a potent, long-lasting elevation of tail-flick latencies, but spinal transection significantly attenuated antinociception [89].

The cellular actions of cannabinoids on supraspinal and spinal descending antinociception pathways have also been studied [56, 162, 163]. CB₁ receptors present in the PGA and dorsolateral funiculus intervene in the important descending controls in cannabinoid-mediated analgesia [35, 167]. Cannabinoid receptor-mediated processing of nociceptive stimuli at higher (supraspinal) levels, as the thalamus and sensory cerebral cortex, may indicate the existence of more specific,

finer-tuned, descending responses, which may be more effective in combating the origin of pain. Messages from the brain back to the periphery modulate the received nociceptive information by, for example, ordering release of chemicals with analgesic effects. Evidence that the cannabinoid system modulate the activity of neurons projecting from the brain to the spinal cord comes from an investigation in which CB₁ receptor agonist injected in the cerebral ventricle suppressed activity evoked, by application of noxious heat to the hind paw, in wide dynamic range neurones of the lumbar dorsal horn of urethane-anaesthetized rats [56]. Moreover, when the CB₁ cannabinoid agonist was given intravenously, the noxious heat-evoked activity of these neurons was not suppressed in animals with spinal transection or after administration of CB₁ receptor antagonist. The existence of supraspinal antinociceptive cannabinoid actions is also confirmed by cannabinoid administration directly injected on specific brain structures, as well as by the blockade of their analgesic effect when administered simultaneously with a cannabinoid receptor antagonist. Microinjection of cannabinoids into several brain regions, including the posterolateral ventral thalamus (an area with many nociceptive neurons receiving spinothalamic pathway inputs), amygdala, RVM, and PAG, produces antinociception [87, 98, 104].

Cannabinoid antinociceptive activity at the peripheral level has been investigated by intradermal administration of agonists selective for both CB₁ and CB₂ receptors, as well as by the lack of analgesic effects with simultaneous administration of specific CB₁ and CB₂ receptor antagonists. Peripheral cannabinoid receptors are less abundant than in central nervous system areas and non-neuronal cells located near nociceptive neuronal nerve endings are involved in the modulation of pro-inflammatory factors released [66, 146, 160].

Wind-up Phenomenon

Wind-up is an exaggerated neuronal response that occurs upon repeated noxious stimulation, residing at the synapse between the primary afferent and the spinothalamic neuron. Sensitisation causes the release of more neurotransmitters at this synapse enabling better transmittance of pain signals to the spinothalamic neuron and brain. There are no reliably satisfactory treatments for pain diseases showing no evidence of peripheral abnormalities (such as myofascial pain, irritable bowel syndrome, and fibromyalgia), and these diseases may be manifestations of an exaggerated central sensitisation mechanism or wind-up. Interestingly, CB₁ receptor agonists administered systemically exert analgesic effects on wind-up (elicited by transcutaneous stimulation) in the dorsal horn [56, 152]. The inhibition of wind-up is probably mediated by supraspinal actions. Intrathecal administration of cannabinoid receptor agonists also produces analgesia [89, 148], indicating spinal antinociceptive action.

Neurochemical Signalling

Superficial dorsal horn CB₁ receptors are mainly present in GABAergic neurons [157]. Since these presynaptic CB₁ receptors located in GABAergic neurons inhibit the GABAergic inhibitory effect, this disinhibition would result in activation of postsynaptic communication. For this reason, it has been suggested that cannabinoids are paradoxically hyperalgesic

at the level of the medullary dorsal horn because of their selective inhibition of GABAergic and glycinergic transmission [56, 89], an effect that appears to be mediated by CB₁ receptors located presynaptically in interneurons. However, a disinhibitory action on lamina II neurons, which emit local branches, may be necessary for modulating nociceptive information before it is transmitted to deeper laminae of the spinal cord or to higher centres. On the other hand, there is a controversy as to whether cannabinoid receptor-mediated antinociception maintains a baseline tone and, therefore, whether blockade of cannabinoid transmission will originate hyperalgesia. It has been suggested that although CB₁ receptor antagonists block the antinociceptive effects of cannabinoid receptor agonists, the antagonists by themselves do not alter baseline pain thresholds [22]. In opposition, other authors suggest that there is baseline endogenous cannabinoid activity at the spinal level, as intrathecal administration of the CB₁ receptor antagonist SR141716A induces hyperalgesic effects [132, 134]. Moreover, the CB₁ receptor agonist WIN 55, 212-2 has no effect on either primary afferent-evoked excitatory glutamatergic transmission or postsynaptic K⁺ conductance from primary afferents in lamina II neurons in the medullary dorsal horn, further supporting a lack of cannabinoid influence on excitatory synapses at this spinal level [72].

CB₁ receptor agonists presynaptically inhibit both GABAergic and glycinergic neurotransmission in lamina II neurons in the medullary dorsal horn, having no effect on either primary afferent-evoked excitatory glutamatergic transmission or postsynaptic K⁺ conductance [72]. Therefore, since the pathways used for cannabinoid receptors-mediated analgesia at the level of the superficial dorsal horn are different from those characterised for μ -opioid receptor-mediated analgesia, it may explain why cannabinoid receptor agonists retain their efficacy, in contrast with morphine, in animal models of neuropathic pain [95].

Vanilloid Receptor Type One (VR₁)

Not all antinociceptive effects of cannabinoid compounds are mediated by cannabinoid receptors. For instance, antagonists of the CB₁ receptor do not block antinociception induced by systemic administration of anandamide. This has been noted in CB₁ receptor knockout mice as well. In these mice, lacking functional CB₁ receptors, certain cannabinoid receptor agonists have antinociceptive effects in the hot-plate or formalin tests [179]. It has been proposed that some cannabinoid effects may be mediated by type one vanilloid receptors (VR₁). These receptors have been cloned [17]. They are calcium-permeable, non-selective cation channel present in primary afferent neurons and play an important role in nociceptive responses. While low concentration of anandamide results in inhibition of neurotransmitter release from nociceptive primary sensory neurons by a CB₁ receptor-mediated mechanism, high concentrations of anandamide increase the frequency of miniature excitatory postsynaptic currents recorded from substantia gelatinosa neurons by a VR₁ receptor-mediated mechanism [106]. Therefore, depending on the concentrations of anandamide it would activate different receptors and produce opposite effects. This may be an important presynaptic mechanism modulating pain perception at the spinal level. Indeed, nociceptive primary sensory neurons co-express CB₁ and VR₁ receptors to a

high degree, giving further support to a complementary role for these receptors [1]. However, the existence of undiscovered cannabinoid receptors has not been ruled out and some cannabinoid analgesic effects may be mediated in part by such receptors [8, 51].

SYNERGISM OF ANTINOCCEPTION BY CANNABINOIDS AND OTHER ANALGESIC SUBSTANCES

Cannabinoids and Opioids Analgesic Synergism

The combination of two antinocceptive drugs acting through specific receptor systems yields major benefits. When given in combination with synergistic substances, the required dose of each agent can be reduced to less than would be explained by a simple additive effect. The clinical benefit of this property is important in analgesic treatments because effective pain relief can be achieved with fewer or no side effects.

The opioid system is one of the systems interacting with the cannabinoids that has been most explored [for reviews see: 26, 41, 94]. Electrophysiologic analysis of the effects of cannabinoids on RVM has revealed that cannabinoids have effects similar to those elicited by morphine [104]. Cannabinoid and opioid receptors both exist at various levels in the pain circuits and these two systems may operate synergistically. THC and morphine have been shown to act synergistically, mutually potentiating their antinocceptive actions. Interestingly, this action is inhibited by either cannabinoid or opioid receptors antagonists separately, as demonstrated in acute [41] and chronic pain models [147, 174]. However, although cannabinoids and opioids both produce analgesia within the dorsal horn, their pharmacologic mechanisms of action differ. For instance, μ -opioid receptor agonists inhibit release of glutamate from primary afferent terminals at the level of the spinal and medullary dorsal horn, while CB_1 receptors do not have any inhibitory effect on those excitatory neurons [49, 81]. Moreover, μ -opioid receptors presynaptically inhibit both glycinergic and GABAergic synaptic transmission in the medullary dorsal horn [48], but not in the spinal cord [81], as occurs with CB_1 receptors. There are other differences at the supraspinal level, where both cannabinoid and opioid receptors presynaptically inhibit GABAergic synaptic transmission, disinhibiting nociceptive descending projection neurons, but only μ -opioid receptors directly inhibit putative GABAergic neurons in the PAG and RVM [19, 162, 163]. Depending on the cannabinoid receptor agonist, morphine effects are potentiated differently. Indeed, in the spinal cord, THC interacts synergistically with morphine and, in contrast, CP-55,940 and CP-56,667 do not interact if they are administered epidurally, but do interact if they are administered to the cerebral ventricles (the dose-response curve shows that the dose of morphine diminishes 10-fold) [173, 174]. This may be due to the physical and chemical characteristics of the cannabinoid receptor agonists and to variations in the release of endogenous opioids depending on the cannabinoid receptor agonist used. As for anandamide, it has not been possible to demonstrate a synergistic interaction with the opioids, probably because this substance degrades quickly. The biochemical mechanisms involved in the interaction between cannabinoid and opioid receptors relate to the transduction and release of

diverse mediators involved in the modulation of nociception and inflammation. On the other hand, cannabinoid receptor agonists induce the synthesis and/or release of endogenous opioid peptides [25, 94]. Subchronic treatment with THC produces an increase in opioids gene expression in the spinal cord, sustaining the hypothesis of an interaction between the cannabinoid and opioid systems in this region [25]. This relation is confirmed by observations, as a reduced cannabinoid and opioid receptor agonist synergistic analgesia in prodynorphin knockout animals or after administering opioid receptor antagonists like, nor-binaltor-phenimine (a kappa antagonist) or naltrindole (a delta antagonist), or antibodies against dynorphin, implies that cannabinoid receptor agonists act indirectly on opioid receptors [125, 126]. Interestingly, therefore, although the antinociception of morphine is mediated predominantly by μ -opioid receptors, it may be enhanced by THC through the activation of kappa and delta opioid receptors.

As mentioned earlier, new CB_2 locations and mechanisms of action, which are important to pain modulation, have been recently identified. CB_2 immunolabeling has been detected on β -endorphin-containing keratinocytes in stratum granulosum throughout the epidermis of the rat hindpaw. These CB_2 receptors, when activated, stimulate release of the endogenous opioid β -endorphin, which then acts at μ -opioid receptors on local primary afferent neurons to inhibit nociception. The antinociceptive effects of the CB_2 receptor-selective agonist AM1241 were prevented in rats when naloxone or antiserum to β -endorphin was injected in the hindpaw where the noxious thermal stimulus was applied, or was not observed in skin from CB_2 cannabinoid receptor-deficient mice and μ -opioid receptor-deficient mice, suggesting that β -endorphin is necessary for CB_2 receptor-mediated antinociception [67].

Cannabinoid and α_2 -Adrenergic Receptors Interactions

Interactions between CB_1 and α_2 -adrenergic receptors have also been postulated. Both receptors presynaptically modulate primary afferent neurons [62, 176] and occur in areas that process nociceptive information at spinal and supraspinal levels [5, 87, 97]. However, some studies suggest that the antinociceptive actions of α_2 -adrenergic receptors are mediated purely at the spinal level, mainly *via* A-subtype α_2 -adrenergic receptors present in the substantia gelatinosa [76, 155].

CB_1 , α_2 -adrenergic, and μ -opioid receptors are all G-protein coupled receptors sharing signal transduction pathways, such as those inhibiting adenylate cyclase and modulating K^+ and Ca^{2+} channels activity [24, 62, 80, 94, 124]. These receptors are distributed similarly in areas of the central nervous system that participate in antinociception, such as the PAG or substantia gelatinosa [5, 80, 87, 97, 116, 176]. Both α_2 -adrenergic or μ -opioid receptors agonists, when combined with a cannabinoid receptor agonist, show significant synergism in antinociception. Interesting findings have been obtained with combinations of these drugs. A cannabinoid receptor agonist combined with a μ -opioid receptor agonist displayed synergism in both the tail-flick and hot-plate assays, whereas a cannabinoid receptor agonist combined with an α_2 -adrenergic receptor agonist showed

simple additivity in the tail-flick assay and synergism in the hot-plate assay [88]. In both assays, combined α_2 -adrenergic and μ -opioid receptors activation was simply additive.

Interactions Between Cannabinoids and Prostaglandin Inhibitors and COX-2

An interaction between cannabinoids and inhibitors of prostaglandin biosynthesis (like NSAIDs) has been reported, and it appears to be due to the similarity in chemical structure of endogenous cannabinoid ligands and prostaglandins (arachidonic acid derivatives), and to the convergence of prostaglandin and endocannabinoids transduction signals [36]. In addition, there is evidence that the addition of cannabinoid compounds to brain tissue sections originates an accumulation of arachidonic acid [130]. The enhancement of CB₁ receptors activity by some NSAIDs (indomethacin, flurbiprofen) has been confirmed [37]. Moreover, the CB₁-receptor antagonist AM251 can block the antinociceptive effect of these NSAIDs administered intrathecally in a model of inflammatory pain (formalin test) [3]. In the same way, indomethacin loses efficacy in this model of pain in CB₁ knockout mice [50]. The explanation for this involves NSAIDs capacity to inhibit the FAAH [61]. However, this may not be the only mechanism because intraperitoneal administration of a nonselective FAAH inhibitor (phenylmethylsulfonyl fluoride) does not affect the response to the formalin test, while AM251 still antagonises its analgesic effect [3]. An alternative hypothesis suggests that the COX-2 enzyme can metabolise the endocannabinoids (like anandamide and 2-AG) and that epidural administration of NSAIDs prevents anandamide destruction by inhibiting the action of COX-2 [82]. Therefore, the administration of NSAIDs increases the amount of anandamide by impeding its metabolism through inhibition of the effect of COX-2 and/or FAAH.

Cyclooxygenase-2 (COX-2) is an enzyme associated with secondary damage after brain injury, as it facilitates the inflammatory response and delayed neuronal death. COX-2 exerts a negative influence on endocannabinoids because it catabolises them (as anandamide and 2-AG, that have shown neuroprotective properties in the injured brain) [83]. In a traumatic brain injury model, COX-2 inhibitor treatment protected 2-AG levels, enhanced functional recovery, and reduced cell death and inflammation [45], confirming an interaction between the endocannabinoid 2-AG and COX-2 enzyme. This also suggests that COX-2 inhibitors treatment may produce an indirect enhancement of cannabinoid receptors activity, by increasing endocannabinoid levels.

THERAPEUTIC USES OF CANNABINOIDS IN PAIN EPISODES

Together with morphine, cannabinoid receptor agonists have probably been one of the most common medicinal remedies since time immemorial. In recent years, especially since the decade of the 1990s when the endogenous cannabinoid system was discovered, basic knowledge of this system has advanced spectacularly. Now that there is no doubt that they are involved in the regulation of nociception, interest in using cannabinoid receptor agonists to manage pain has renewed. Therapeutic management using an antinociceptive mechanism different from the usual mechanisms opens a

new line of therapy for cases in which no other pharmacologic treatments are available. As mentioned previously, combinations of cannabinoid receptor agonists with other analgesic substances to achieve a synergistic effect and improve the efficacy and safety of treatment are also interesting.

Studies in experimental models of acute and chronic pain have demonstrated the efficacy of cannabinoid receptor agonists, even in neuropathic or inflammatory pain. Preclinical data obtained with cannabinoid receptor agonists in pain models have made it possible to assess their efficacy in humans. The results to date suggest that cannabinoid receptor agonists are analgesic substances *per se* whose potency is similar to that of opioids like codeine and their dose-dependent effect is limited by the appearance of adverse effects. Unfortunately, few clinical trials have been made and they have been carried out with major methodologic limitations. It is noteworthy that whereas cannabinoid receptor agonists have shown to be up to 10 times more potent than morphine in animal models of acute and neuropathic pain [38], analgesic efficacy has not been as good in some clinical trials. The main reason for this discrepancy is that doses are greatly reduced to avoid adverse effects, in particular psychotropic. In addition, there are other deficiencies in the scant clinical trials performed to date, such as important variations in doses, schedules, routes of administration, type of cannabinoid receptor agonists compounds, methods of testing analgesia, the selection of nonhomogeneous study groups (patients with diseases of diverse origin), and the extrapolation of results from models of experimental pain in healthy patients (pain circuits activity differ between healthy volunteers and patients). In Tables 1-5 are summarised some data collected from humans in situations of acute (either in patients suffering pain or in healthy volunteers submitted to noxious stimuli) and chronic pain treated with cannabinoid compounds, as well as from questionnaires from self-medicated patients.

THC is the substance with the greatest psychoactive potency of the natural cannabinoids, and exhibits the greatest analgesic activity [96]. In fact, THC administered epidurally (intrathecal, intraventricular) produces antinociception similar to that obtained with opioid compounds [173]. In addition, the epidural route of administration allows the use of much smaller doses to obtain a more lasting effect than with nonepidural routes. THC is lipophilic and relatively nonselective for CB₁ and CB₂ receptor subtypes, and has been widely used in experimental studies. Clinical trials have shown that nonselective cannabinoid receptor agonists are relatively safe and therapeutically efficacious, but they also induce psychotropic side effects [120]. However, if the THC is administered in small doses, it is well tolerated and efficient for neuropathic pain, without causing altered states of consciousness. Cardiovascular effects are generally moderate and well tolerated: hypotension can appear in comparison with placebo [158, 165], but no more than with codeine. However, since it may lead to significant increases in heart rate and a lowering of the blood pressure, patients with cardiovascular disease should probably not be treated with cannabinoid drugs. On the other hand, desired therapeutic effects and adverse effects both vary with the cannabinoid sub-

stance used. Thus, diverse THC analogues have been used for experimental or preclinical studies, some of which are being marketed in some countries for certain applications. The THC analogues most often used for preclinical or therapeutic purposes in humans are dronabinol, nabilone, levonantradol, CT-3 (or ajulemic acid), and HU211 [15, 117]. Another derivative, benzopyranoperidine, produces a degree of sedation similar to codeine but is ineffective as an analgesic [73].

Levonantradol is a synthetic analogue of THC that is administered intramuscularly. It has been used in clinical trials to palliate postoperative pain or pain due to trauma and has shown more analgesic efficacy than placebo [70]. Levonantradol causes adverse effects more often than THC in many patients, but none of them is considered serious.

Cannabidiol (CBD) is another major constituent of the *Cannabis sativa* plant, having the same therapeutic effects than THC (analgesic, anti-inflammatory, and others), but with a different pharmacologic profile. Studies have been made with cannabidiol derivatives developed to inhibit peripheral pain responses and inflammation after binding to cannabinoid receptors. Interestingly, some of these cannabidiol derivatives did not have central nervous system effects, but maintained their antinociceptive and anti-inflammatory properties. This means that centrally inactive synthetic cannabidiol analogues may be good candidates for the development of analgesic and anti-inflammatory drugs for peripheral conditions [40].

Medications have also been developed from extracts of the *Cannabis sativa* plant containing known amounts of THC and CBD [6, 111, 163, 164]. These medications are being used in clinical trials, and a form dispensed as sublingual spray has just been approved to be marketed in Canada.

Other possible therapeutic targets are CB₂ receptors by means of specific agonists. As there are no CB₂ receptors in neurons, the actions evoked by cannabinoid receptor agonists on the central nervous system seem to depend mainly on the activation of CB₁ receptors. Thus CB₂ receptor agonists merit special consideration for use as agents with absence of cognitive and psychotropic properties. Therefore one of them, HU-308 [102], does not produce hypothermia, catalepsy, or behavioural changes, while the role of CB₂ receptors is fundamental in other cannabimimetic actions, such as immunomodulatory and antiproliferative effects. On the other hand, as mentioned earlier, new CB₂ receptor properties are being discovered, as it has been confirmed that they indirectly stimulate opioid receptors located in primary afferent pathways [67].

Some interesting products are the anandamide reuptake inhibitors which inhibit its transport and potentiate its analgesic effects; as N-(4-hydroxyphenyl)-arachidonamide (AM 404), an anandamide analogue [119], and N-(3-furylmethyl) eicosa-5,8,11,14-tetraenamide (UCM707) (Fig. 2) [91]. Another promising target for therapeutic intervention is the fatty acid amide hydrolase (FAAH) enzyme, which is responsible for intracellular anandamide degradation [43]. AM374 (palmitylsulfonyl fluoride) is a potent FAAH inhibitor [28], preventing the hydrolysis of endocannabinoids and, therefore, increasing their synaptic levels and elevating cannabi-

noid receptors activity (Fig. 2). Indeed, reversible FAAH inhibitors produce analgesia in animal models [7]. In addition, other compounds like the N-acylethanolamines block anandamide degradation [118]. Knockout mice lacking FAAH display elevated concentrations of anandamide in brain and are more sensitive to the biological actions of anandamide [27].

An important detail to consider in cannabinoid treatment is the route of administration. Although oral dispensation is the route of choice for the clinical management of chronic pain, due to its prolonged action and ease of use, many patients prefer inhalation (smoking cannabis) and sublingual sprays have also been prepared for commercial preparations. Nonetheless, there are other possibilities, particularly when analgesia is deficient, oral intolerance exists, and/or the state of consciousness is altered, such as the epidural or intravenous routes. Other alternatives are delayed-release skin patches and, to a lesser extent, rectal suppositories [9].

Smoking cannabis remains the most efficient means of drug delivery since absorption is much more rapid and experienced users dose themselves by adjusting the frequency and depth of inhalation [68]. However, this route has the disadvantage of respiratory and other complications associated with the tobacco mixed with the product for smoking. On the other hand, the effects of natural cannabinoids (plant extracts) seems to be better than those of synthetic cannabinoids. The effects of cannabis, whether smoked or administered intravenously, appear 30 minutes to one hour after administration and last for 2 or 3 hours. Therefore, one disadvantage is a brief analgesic effect.

As with any other therapy, adverse effects must be taken into account. Some patients will suffer side effects, although most of them will appear only in the first days of treatment and disappear as the body adjusts to the drug. Short-term effects, such as unsteadiness, dizziness, difficulty concentrating, drowsiness, dryness of the mouth, and/or headache, are related to depression of the central nervous system. Chronic cannabis use does not produce serious cognitive disorders, as occurs with other substances like alcohol, but it can aggravate pre-existing mental disease. Therefore, treatment with cannabinoid receptor agonist with central actions may be contraindicated, or either rigorously controlled, in individuals predisposed to psychiatric disorders. No human deaths associated to cannabis use have been reported, and the lethal dose of THC in rodents is very high compared to other substances.

With respect to potentially addictive effects, therapeutic doses are smaller than those used for recreational purposes, and derivatives that have little or no central effects are the most valuable candidates for medicinal purposes. Controversy exists regarding the appearance of tolerance and dependence with the use of cannabinoid compounds. It has been claimed that tolerance develops after repeated administration of THC in humans, that 9-20% of regular cannabis users become dependent, and that a significant withdrawal syndrome occurs in human cannabis users [2, 136, 153]. However, it has also been suggested that many clinical studies are poorly controlled and the development of a cannabinoid withdrawal syndrome is debated [for reviews see: 74, 75].

As for clinical applications, one of the best documented therapeutic uses of cannabinoid derivatives, and the one most widely approved today, is as an antiemetic drug [12, 158, 172]. Indeed, cannabinoid-based prescription medicines are now marketed for this use in some countries. They have proven to be extremely effective for relieving nausea and emesis due to gastrointestinal distress caused by acquired immunodeficiency syndrome (AIDS) medications or cancer chemotherapy. These patients either smoke cannabis or take a THC synthetic analogue like dronabinol or nabilone, although they are prescribed only when other medications for nausea and vomiting do not work.

Acute Pain

Opioids are powerful analgesics widely utilised in clinical pain management, but they yield a poor analgesic response in conditions of certain pathologic pain, such as neuropathy. THC induces antinociception in rats with pathologic pain

after nerve injury. Moreover, THC antinociception is independent of opioid receptors in rats with some pathologic pain, as the antinociceptive effect after nerve injury is blocked by the CB₁ receptor antagonist SR141716A, but not by the opioid receptor antagonist naloxone, and there is no cross-tolerance between the antinociceptive effects of morphine and THC [95]. Therefore, THC and other cannabinoids may be superior to opioids in alleviating intractable pathologic pain syndromes.

What clinical trials are available on the efficacy of cannabinoid receptor agonists in the treatment of acute pain are scant and inconclusive with respect to their analgesic efficacy (Tables 1-2).

Postoperative Pain

One potential indication of cannabinoids would be as analgesic for postoperative pain. Morphine has been used

Table 1. Effects of Cannabinoid Receptor Agonists in Healthy Volunteers Submitted to Acute Noxious Stimuli

References	Pain Origin and Treatment	Results and Remarks
Zeidenberg <i>et al.</i> , 1973 [177]	Healthy volunteers n=4 THC 5 mg p.o. Thermal stimuli	Antinociceptive effects which remained after memory and psycholinguistic effects were returning to normal levels (i.e. longer time course for effect on pain).
Hill <i>et al.</i> , 1974 [55]	Healthy volunteers THC smoked 12 mg Electrical stimulation	THC increases pain sensitivity (hyperalgesia). The reason could be a biphasic effect, with initial stimulation followed by sedation.
Milstein <i>et al.</i> , 1975 [105]	Healthy volunteers - cannabis-experienced - naive subjects • cannabis smoked • placebo Pressure stimuli	Smoked cannabis increased antinociceptive effects. No statistical difference between drug effect and cannabis experience, but there was a definite trend towards a greater increase for the experienced (16%) compared to the naive group (8%).
Raft <i>et al.</i> , 1977 [129]	Healthy volunteers - THC 0.022 mg/kg i.v. - THC 0.044 mg/kg i.v. Electrical and pressure stimuli	No analgesic effect of THC, but methodological problems: pain assessment better performed by ratings intensity, as most pain is experienced in an intermediate range; while this study measured only the extremes of pain sensation, threshold (lowest intensity perceived as painful) and tolerance (maximum intensity of pain that a subject can withstand). They did not include a positive control; like an established analgesic (as opiate or narcotic).
Clark <i>et al.</i> , 1981 [20]	Healthy volunteers being regular cannabis users n=16 THC smoked 20mg/cigarette 3-12/day Thermal stimuli	One month (before starting experiment) drug free, second month smoking 3-12 cigarettes/day, third month drug free again. Antinociceptive effects during the first two weeks of smoking, then returning to presmoking pain level maintained during the postsmoking period. Heavy smoking caused increase in pain reports. (i.e. THC originates tolerance in cannabis regular users)
Greenwald and Stitzer 2000 [46]	Healthy volunteers being regular cannabis users n=5 THC smoked Sessions: placebo (9 puffs), THC 3, 6 and 9 puffs Combined with naltrexone 0, 50 or 200 mg p.o. Thermal stimuli	Cannabis dose-dependent antinociceptive effects. Naltrexone (randomised, double-blind) did not influence cannabis effects, suggesting no role of endogenous opiates in cannabis-induced antinociception under these conditions.
Naef <i>et al.</i> , 2003 [109]	Healthy volunteers n=12 - THC 20 mg - morphine 30 mg - THC + morphine - placebo Single dose, p.o.	Randomised, double-blinded, placebo-controlled, crossover. Pain tests (order randomised): heat, cold, pressure, single and repeated transcutaneous electrical stimulation. THC did not significantly reduce pain. In the cold and heat tests it even produced hyperalgesia, which was completely neutralized by THC-morphine. Psychotropic and somatic side effects (sleepiness, euphoria, anxiety, confusion, nausea, dizziness, etc.) were common, but usually mild.

Table 2. Effects of Cannabinoid Receptor Agonists on Acute Postoperative Pain

References	Pain Origin and Treatment	Results and Remarks
Raft <i>et al.</i> , 1977 [129]	Premedication for dental extraction n=10 - THC 0.022 mg/kg i.v. - THC 0.044 mg/kg i.v. - diazepam 0.157 mg/kg i.v. - placebo	Pain thresholds and psychiatric interviews were assessed, supplemented by tests of personality, depression and anxiety. THC analgesic effects less than that after diazepam and placebo, while pain detection thresholds were altered unpredictably with high THC doses. Three subjects at low-dose THC had a better analgesic effect than placebo but not diazepam. Six subjects preferred placebo to low-dose THC as an analgesic.
Jain <i>et al.</i> , 1981 [70]	Postoperative or trauma pain n=56 Levonantradol 1.5, 2.0, 2.5, or 3.0 mg or placebo Single dose, i.m.	Double-blind. Significant analgesic effects of each dose of levonantradol (a synthetic cannabinoid) as compared to placebo. No dose-response differences. Side effects in 57% levonantradol, especially drowsiness and, less frequently, dry mouth, dizziness, "weird dreams," mild hallucinations, nervousness, apprehension and confusion.
Buggy <i>et al.</i> , 2003 [14]	Postoperative pain n=40 Elective abdominal hysterectomy THC 5 mg p.o. single dose 48 h after surgery	Randomised double-blind, placebo-controlled. No analgesic effect of THC in this paradigm. Randomisation took place when postoperative patient-controlled analgesia was discontinued on the second postoperative day.

historically to relieve postoperative pain, but it renders unwanted side effects. Postoperative pain interfere with functioning and healing, and it can grow to intolerable levels. However, there is no drug for this condition which produces adequate analgesia with no side effects. Some clinical trials, using cannabinoid derivatives in postoperative pain, have been undertaken or are in progress (Table 2).

Chronic Pain

Multiple Sclerosis

Multiple sclerosis (MS) is a life-long chronic disease in which nerve cells are attacked by the immune system,

originating painful muscle spasms and many other problems, including neuropathic pain. There are about 1.1 million worldwide sufferers of MS. Clinical trials have tested the potential medical applications of cannabis for the treatment of MS symptoms, although some of them present a small number of patients ; and there are also data from responses to questionnaires (Tables 3-4) [68, 117]. Smoking cannabis not only has helped to stop spasms, but has halted the progression of multiple sclerosis. Although smoking cannabis is illegal in some countries, estimates suggest that 10% to 30% of MS patients in Europe smoke cannabis to ease the painful and disabling symptoms of the disease. Medications prepared from whole plant cannabis extract, containing known amounts

Table 3. Data from Questionnaires / Surveys of Humans, Taking Cannabis as a Medicine to Relief Chronic Non-Cancer Pain of Different Types, Especially as a Symptom of Multiple Sclerosis

References	Pain origin and Treatment	Results and Remarks
Consroe <i>et al.</i> , 1997 [23]	Questionnaire to 112 patients with MS taking cannabis for therapeutic reasons (in a self-medication basis), 2-3 times per day, 5-6 days per week, and usually smoked.	Approximately 95% reported cannabis improved spasticity and chronic pain of extremities; and other symptoms (as acute paroxysmal phenomenon, tremor, emotional dysfunction, anorexia/weight loss, fatigue states, double vision, sexual dysfunction, bowel and bladder dysfunctions, vision dimness, dysfunctions of walking and balance, and memory loss).
Ware <i>et al.</i> , 2002 [171]	Interviewed patients with chronic pain using smoked herbal cannabis therapeutically n=15 (median duration of use six years)	Twelve improved in pain and mood, while 11 improved in sleep. Eight reported a "high" (state of euphoric intoxication); six denied a "high". Tolerance to cannabis was not reported.
Ware <i>et al.</i> , 2003 [170]	Anonymous cross-sectional survey to determine the prevalence of medicinal cannabis use among patients with chronic non-cancerous pain n=209	35% ever had used cannabis; 15% had used cannabis for pain relief (pain users), and 10% were currently using cannabis for pain relief; 18% denied using cannabis for pain relief (recreational users). Largest group using cannabis had pain by trauma and/or surgery (51%), and predominantly in neck/upper body (68%) and myofascial (65%). Pain, sleep and mood were most frequently reported as improving with cannabis use, and "high" and dry mouth were the most commonly reported side effects.
Clark <i>et al.</i> , 2004 [21]	Survey to estimate the patterns and prevalence of cannabis use among patients with multiple sclerosis (MS) n=220	36% ever had used cannabis for any purpose; 14% use cannabis for symptom treatment. Medical cannabis use associated with male gender, tobacco use, and recreational cannabis use. Symptoms most effectively relieved: stress, sleep, mood, stiffness/spasm, and pain.

Table 4. Data from Clinical Trials of Humans Treated with Cannabinoid Receptor Agonists to Relief Chronic Non-Cancer Pain of Different Types, Especially as a Symptom of Multiple Sclerosis or Other Neuropathic Pain

References	Pain origin and Treatment	Results and Remarks
Maurer <i>et al.</i> , 1990 [101]	Spinal cord injury n=1 - THC 5 mg p.o. - Codeine 50 mg p.o. - placebo	Single case double-blind trial. THC and codeine both had an analgesic effect as compared with placebo. Only THC showed a significant beneficial effect on spasticity. THC did not alter consciousness.
Notcutt <i>et al.</i> , 1997 [110]	Neuropathic pain n=50 Nabilone 0.25 to 3 mg/day	Observational study. Analgesic effect in 30% of patients. A significant number of patients abandon the drug because dysphoria and drowsiness. Benefits: analgesia, pain distancing, compressing the pain, sleep relief muscle spasm, anxiolysis, relief constipation, etc. Patients who had used cannabis for chronic pain prior to trying nabilone (a synthetic analogue of THC) preferred the former
Karst <i>et al.</i> , 2003 [77]	Neuropathic pain n=21 (all with hyperalgesia and 7 with allodynia). - Placebo - CT-3 40 mg first 4 days and 80 mg following three days Two 7-day treatment (plus 1 week washout)	Randomised, placebo-controlled, double-blind crossover trial (preliminary). Ajulemic acid (AJA or CT-3 or IP-751), a THC analogue, more analgesic effect than placebo, mainly 3 h after intake of CT-3; while 8 h after intake, the pain scale differences between groups were less marked. No dose response. Adverse effects, mainly transient: dry mouth and tiredness, significantly more often during CT-3 treatment. No major adverse effects were observed.
Wade <i>et al.</i> , 2003 [165]	Neurogenic symptoms unresponsive to standard treatment n=24 Sublingual sprays. - Placebo - THC (cann. extract) - CBD (cann. extract) - THC+CBD (1:1) 2.5-120 mg each/day Two-week treatment periods	Double-blind, randomised, placebo-controlled single-patient cross-over trials. Pathologies were: MS (18), spinal cord injury (4), brachial plexus damage (1), and limb amputation (1). Pain relief by both THC and CBD significantly superior to placebo. Three patients had transient hypotension and intoxication with rapid initial dosing of THC. Cannabis medicinal extracts improved neurogenic symptoms unresponsive to standard treatments. Unwanted effects are predictable and generally well tolerated.
Wade <i>et al.</i> , 2004 [164]	Multiple sclerosis outpatients n=160; Sublingual sprays. - placebo - THC+CBD (1:1), cann. extract: 2.5-120 mg of each daily, in divided doses.	Parallel group, double-blind, randomised, placebo-controlled study, undertaken in three centres. MS patients experiencing significant problems: spasticity, spasms, bladder problems, tremor or pain. The primary symptom score reduced more with CBME, but not significantly different than placebo. Spasticity was significantly reduced by CBME compared with placebo. No significant adverse effects on cognition or mood, and intoxication was generally mild.
Notcutt <i>et al.</i> , 2004 [111]	Chronic pain (mainly neuropathic) n=34 Sublingual sprays 12-week period - Placebo - THC 2.5 mg (cann. extract) - CBD 2.5 mg (cann. extract) - THC+CBD (1:1) Randomly change of one of these treatments each week	Randomised, double-blind, placebo controlled, crossover trial. Extracts containing THC proved most effective in symptom control. Wide range of dosing requirements was observed. Side effects were generally acceptable and little different to those seen when other psychoactive agents used for chronic pain. The authors suggests that these initial experiences with Cannabis Based Medicinal Extracts (CBME) open the way to more detailed and extensive studies.
Berman <i>et al.</i> , 2004 [6]	Central neuropathic pain from brachial plexus avulsion, with intractable symptoms n=48 Sublingual sprays -Placebo - THC (cannabis extract) - THC+CBD (cann. extract) Each patient each treatment for 2 weeks.	Randomised, double-blind, placebo-controlled, three period crossover study. Significant improvement on pain severity and on sleep disturbances. Generally well tolerated, majority of adverse events being mild to moderate in severity and resolving spontaneously. Studies of longer duration in neuropathic pain are required to confirm a clinically relevant, improvement in the treatment of this condition.

of THC and CBD as the principal components have been prepared to be administered by oral spray to relieve MS symptoms, as well as for the treatment of other disorders with severe neuropathic pain. This product has undergone phase III placebo-controlled trials, which show that it reduces neuropathic pain, spasticity, and sleep disturbances. Its use

has been approved only in Canada so far. Furthermore, animal model of multiple sclerosis, have found other advantage of cannabinoid receptor agonists, since they appear to exert CB₁ receptor-mediated neuroprotective effects that would be beneficial for the neurodegeneration occurring in MS [123].

Neuropathic Pain

Neuropathic pain, which is frequently chronic, arises when neurons in the brain or peripheral nervous system become hypersensitised and generate abnormal or prolonged impulses. In Europe, about 4 million patients suffer neuropathic pain. There are many causes of neuropathic pain, including diabetic neuropathy, post-herpetic neuralgia, brachial plexus lesion, fibromyalgia, multiple sclerosis, and cancer.

Severe neuropathic pain has proved difficult to treat and evidence suggests that none of the available drugs, mainly opioids, is effective in more than 50% of patients. This is thus an area of significant unmet clinical need. It is important to emphasise that cannabinoid receptor agonists are more effective than opioids in the management of neuropathic pain. Different hypotheses have been proposed to explain this phenomenon [95]. One of them is based on the presence of cannabinoid receptors in primary afferent myelinated A-fibres, since this pain is partly due to spontaneous discharge of these fibres [59]. The A-fibres contain fewer μ -opioid receptors than cannabinoid receptors. There is evidence confirming this hypothesis. Thus, WIN 55,212-2, a cannabinoid receptor agonist, which is less potent than morphine in the inhibition of the acute pain response conducted by C-fibres, is a more effective inhibitor of the wind-up phenomenon (a phenomenon that contributes to the development of hyperalgesia and allodynia) [152]. Another hypothesis is based on the fact that, unlike opioids, cannabinoids do not lose effectiveness in the management of neuropathic pain because fewer receptors disappear in this situation (the destruction of primary afferent fibres by rhizotomy [10] or the administration of neonatal capsaicin [57] reduce CB₁-receptor expression less than opioid expression). In contrast, there is less expression of the opioid receptors in the posterior horn [10].

This means that cannabinoid receptor agonists have more capacity for suppressing pathophysiologic mechanisms like the wind-up phenomenon linked to this type of pain [152].

Behavioural studies have shown that cannabinoids reduce thermal and mechanical allodynia in rat models of neuropathic pain [38, 54, 69]. Intrathecal administration of CP55,940 in models of neuropathic pain (chronic L5/6 spinal nerve ligation) or acute pain (tail flick) attenuated tactile allodynia and induced thermal antinociception. These antinociceptive effects appears to be mediated chiefly by the CB₁ receptor, but some data also suggest a role for CB₂ receptor-mediated antinociception in both acute and neuropathic pain [142].

Cancer Pain

Pain is one of the most frequent symptoms in patients with cancer and the World Health Organisation recommends that they receive adequate pain relief. Cannabinoids are among the compounds under development for the treatment of these patients, and they seem to have analgesic activity (Table 5) [127]. Clinical trials are also under way to assess the effectiveness of cannabis extract preparations (containing THC and CBD) for the relief of cancer pain (neuropathic-related cancer pain). Around 40% of cancer patients suffer some degree of neuropathic pain.

Fibromyalgia

This disease is characterised by the presence of generalised pain throughout the body, confirmed by the presence of tender and painful points on digital palpation in at least 11 of the 18 points established for diagnosis. Pharmacologic treatment usually consists of tricyclic antidepressants combined with NSAIDs. Although there are no specific clinical studies of the use of cannabinoid receptor agonists for symp-

Table 5. Data from Clinical Trials of Humans Treated with Cannabinoid Receptor Agonists to Relief Chronic Cancer Pain

References	Treatment	Results and Remarks
Noyes <i>et al.</i> , 1975 [114]	n=10 - placebo and THC 5, 10, 15, and 20 mg p.o. Each patients all treatments	Double blind placebo-controlled trial (preliminary). Analgesic effect of THC at high doses (15 and 20 mg), significantly superior than placebo. At these doses, substantial sedation and mental clouding. No nausea or emesis. Increased appetite in some patients.
Noyes <i>et al.</i> , 1975 [113]	n=36 - placebo - THC 10 or 20 mg p.o. - codeine 60 or 120 mg p.o.	Mild analgesic effect of THC. At 20 mg THC (similar to 120 mg codeine) induced side effects that would prohibit its therapeutic use, including somnolence, dizziness, ataxia, and blurred vision; and even some alarming adverse reactions. At 10 mg THC (similar to 60 mg codeine): analgesic potential; well tolerated, sedative effect.
Jochimsen <i>et al.</i> , 1978 [73]	n=35 - placebo - benzopyranoperidine (2 or 4 mg) p.o. - codeine sulfate (60 or 120 mg) p.o.	Double-blind, 5-way crossover designed study. Significant analgesic relief with 120 mg of codeine, but no differences between placebo and benzopyranopyridine (analogue of THC). Pain perception even appeared to be augmented by both doses of benzopyranopyridine.
Staquet <i>et al.</i> , 1978 [150]	NIB (nitrogen analogue of THC) 1 mg p.o. - Trial I: NIB vs. codeine - Trial II: NIB vs. secobarbital - Placebo (trials I and II)	Two consecutive, randomised, double-blind trials. Evaluation of mild, moderate, and severe pain. Trial I: NIB superior to placebo and equivalent to 50 mg of codeine. Trial II: NIB superior to placebo and to 50 mg secobarbital (a short-acting barbiturate). However, NIB is not useful clinically because of the frequency of side effects.

tomatic relief of this disease, data that support their therapeutic potential thanks to their anti-inflammatory and sedative properties [138].

Migraine

Migraine is defined as vasomotor headache characterised by its pulsatile nature, the presentation of crises, and its periodic occurrence. It is usually hemicranial and accompanied by generalised sensorial hyperesthesia, sensitivity to light and noise, and nausea and/or vomiting. Ergot alkaloids and 5-HT_{1D} serotonin receptor agonists are used for its treatment. NSAIDs, codeine, and caffeine are also generally used. Historically, cannabis was prescribed for its management in the early 20th century. At present, the antimigraine properties of cannabis have been recognised [137] and there are studies that affirm that the pain relief it produces is comparable, or better than, that achieved with ergotamine and aspirin [112]. The most effective route of administration is by inhalation because of its rapid action. The antiemetic and vasodilator properties of cannabinoid compounds are additional benefits that support its use as an alternative medication in migraine refractory to conventional therapy.

Spasticity

Spasticity entails increased resistance to passive movement. Among other disadvantages, it causes pain *per se* and also secondary to joint stiffness. Among the therapeutic measures proposed are the elimination or reduction of nociceptive stimuli, rehabilitation (occupational physical therapy), and the use of antispasmodic medication. Clinical trials have evaluated the efficacy of cannabinoids in diverse musculoskeletal entities accompanied by severe spasticity [149, 161, 164].

Phantom Limb Syndrome

Patients who suffer amputation of an extremity can experience a type of referred neuropathic pain in the amputated zone. Pharmacologic management of this condition is complex and it is based mainly on anticonvulsants. The use of cannabinoids is supported by their efficacy in alleviating neuropathic pain. Promising results have been obtained in clinical practice [47].

CONCLUDING REMARKS

Pain severely impairs quality of life. Currently available treatments, generally opioids and anti-inflammatory drugs, are not always effective for certain painful conditions. The discovery of the cannabinoid receptors in the 1990s led to the characterisation of the endogenous cannabinoid system in terms of its components and numerous basic physiologic functions. CB₁ receptors are present in nervous system areas involved in modulating nociception and evidence supports a role of the endocannabinoids in pain modulation. Basic research on how cannabinoid receptors and endocannabinoids intervene in pain mechanisms is progressing rapidly. Clinical progress, however, is advancing slowly. Cannabinoids have antinociceptive mechanisms different from that of other drugs currently in use, which thus opens a new line of promising treatment to mitigate pain that fails to respond to the pharmacologic treatments available, especially for neuropathic and inflammatory pains. The combination of can-

nabinoids with synergistic analgesic substances is interesting because it may improve the efficacy and safety of treatment. One of the drawbacks of investigating cannabinoids is their typification as substances of abuse. However, compounds blunting severe pain allow patients to perform daily activities more easily, so the potential benefits should be weighed against possible adverse effects. Our current understanding of the physiology and pharmacology of the endogenous cannabinoid system has motivated cannabis-based therapeutic drug design, in which attempts are being made to synthesise compounds with the desired therapeutic actions but without psychoactive adverse effects. Medications prepared with cannabinoid receptor agonists or with drugs that enhance endocannabinoid function (by either increasing release or diminishing reuptake of endocannabinoids) may afford the novel therapeutic approaches demanded by disorders in which pain is a prominent symptom. Clinical trials seem to indicate that either extracts of the *Cannabis sativa* plant containing known amounts of the active compounds (mainly THC and CBD) or diverse synthetic derivatives of THC are promising treatments for painful conditions that do not respond to available treatments, such as neuropathic, inflammatory and oncologic pain. Specifically, cannabis extracts have shown effectiveness to relief some symptoms of the patients with multiple sclerosis, mainly for pain and spasticity. Pharmacologic manipulation directed to elevate endocannabinoids levels like, for example, with anandamide reuptake inhibitors, or by inhibiting the enzyme fatty acid amide hydrolase (FAAH), which is responsible for intracellular anandamide degradation, may well become a valuable therapeutic tool. CB₂ receptor selective agonists with no central effects are other promising pain treatment under investigation. Adequately sized and designed, double-blind placebo-controlled clinical trials are needed to evaluate the potential applications of cannabis-based medications as novel and effective therapeutic drugs for controlling different types of pain.

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ABBREVIATIONS

2-AG	=	2-arachidonoylglycerol
AEA	=	Arachidonylethanolamide or anandamide
cann. Extract	=	Extract of <i>Cannabis sativa</i>
CB ₁ receptor	=	Cannabinoid receptor type 1
CB ₂ receptor	=	Cannabinoid receptor type 2
CBD	=	Cannabidiol
CBME	=	Cannabis Based Medicinal Extracts
CNS	=	Central nervous system
COX-2	=	Cyclooxygenase-2
CT-3 or AJA or IP-751	=	Ajulemic acid

DSE	=	Depolarisation-induced suppression of excitation
DSI	=	Depolarisation-induced suppression of inhibition
FAAH	=	Fatty-acid amide hydrolase
GABA	=	Gamma-aminobutyric acid
MS	=	Multiple sclerosis
NADA	=	N-arachidonoyldopamine
NIB	=	Synthetic nitrogen analogue of tetrahydrocannabinol
NSAIDs	=	Non steroidal anti-inflammatory drugs
PAG	=	Periaqueductal grey matter
RVM	=	Rostral ventromedial medulla
THC	=	Delta-9-tetrahydrocannabinol
VR ₁	=	Vanilloid receptor type 1

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