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The endogenous cannabinoid system

Cannabis preparations: drug of abuse and medicine?

Preparations of the hemp plant *Cannabis sativa* such as hashish and marijuana belong to the most widely used illicit drugs of abuse worldwide. Marijuana consists of the dried flowers of cannabis plants; hashish is the concentrated resin that is typically pressed into plates. The risks induced by cannabis consumption are conversely discussed, often in complete ignorance of scientific data. Depending on the political view, backers of a liberal drug policy either trivialize or negate the harmful properties, whereas supporters of strict drug controls take the opposite stand. Both views, as usual, might be wrong and the truth lies somewhere in between. Hashish and marijuana are certainly less threatening than alcohol or tobacco, which both possess a high abuse potential and cause thousands of deaths every year. Nevertheless, several epidemiological and long-term studies as well as animal experiments also taught us that cannabis is not that harmless as some might think. Especially teenagers are at a risk of being affected by this drug. Cannabis consumption reduces intelligence, decreases performance level, increases the risk to develop psychiatric disorders and can lead to addiction! These threats cannot be ignored.

It is annoying for committed scientist working in cannabis research that there is a lot of misuse under the guise of medical benefit. The medical use, however, is a serious concern and of great importance for many patients. For thousands of years, cannabis has been cultivated and used for medical purposes, which might also be ascribed to its euphoric effects. During the last decade the medical application of cannabis experienced a renaissance and thus, despite a general prohibition, there are special cultivation programs for medical purposes in many countries. These products are still mainly consumed via smoking. The EU and Canada further approved an oral spray with cannabis extract (Sativex) that is used for the treatment of multiples sclerosis in Europe. In Canada it is also used to treat neuropathic pain and pain caused by tumor. Further indications for cannabis are the treatment of nausea due to chemotherapy and appetite stimulation in HIV patients.

Molecular components of the endogenous cannabinoid system

About 50 years ago Raphael Mechoulam, an Israeli scientist, isolated and characterized the psychoactive compound of hashish and marijuana. It was identified as the lipophilic and thus liposoluble molecule Δ^9-tetrahydrocannabinol (THC), whose mechanism of action is well studied today. THC activates receptors at the surface of neurons or several other cell types including immune cells. Virtually all psychoactive effects of THC are mediated via activation of the CB1 cannabinoid receptor that is found on almost all neurons. This receptor belongs to the family of G-protein coupled receptors, which are characterized by seven transmembrane domains coupling the receptor to the cell membrane. Another very similar cannabinoid receptor (CB2) is found on immune cells.

Cannabinoid receptors are not only activated by THC, but also by endogenous molecules. The two major molecules, arachidonoyl ethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), are metabolic products of the cell membrane. Like THC, AEA and 2-AG are lipophilic and closely associated with proteins or lipid structures of the cell. It has long been discussed that endocannabinoids are not stored within the cells but are synthesized on demand. The activity of the cannabinoid system thus largely depends on the regulation of the endocannabinoid synthesis and the amount of degrading enzymes. The synthesis and degradation pathways of AEA and 2-AG are quite different and involve different enzymes. On the other hand, there is evidence for the storage of endocannabinoids within the cells, e.g. in specific lipid stores called lipid droplets. Although AEA and 2-AG are able to activate both cannabinoid receptors, they have different functions. Before we discuss these differences in detail, we will take a closer look on how the endocannabinoid system modulates communication between neurons.

Endocannabinoids as feedback signals in the cell-to-cell communication

Endocannabinoids modulate the signal transduction between neurons that takes place at specialized contacts called synapses. Neurons pass signals in terms of current changes at its cell membrane. At the synapse, signal transduction occurs through chemical neurotransmitters from the sending (presynaptic) to the receiving (postsynaptic) neuron. The electric signal is thus converted into a chemical signal. Due to the electric pulse the presynaptic neuron releases neurotrans-

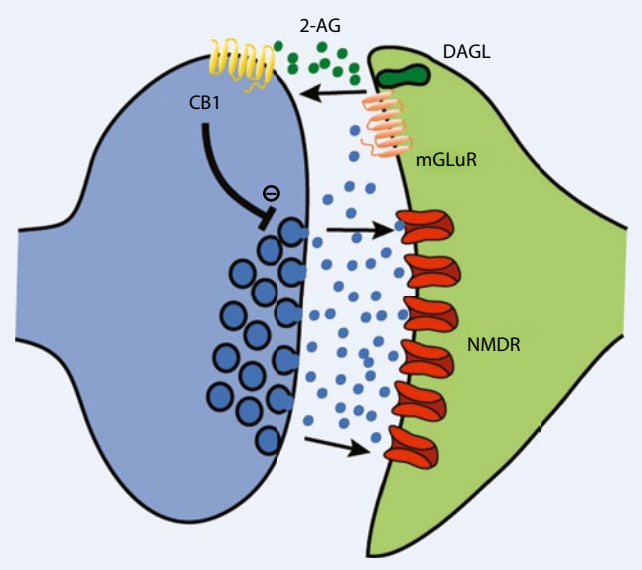

Fig. 1 ▲ Retrograde modulation of cell-cell communication via endocannabinoids. Strong activation of the presynaptic neuron (*blue*) leads to the release of high amounts of glutamate (*blue circles*) into the synaptic cleft. Besides activation of ionotropic NMDA glutamate receptors (*NMDAR*) also metabotropic glutamate receptors (*mGluR*) located in the periphery of the synapse are activated. Thereby activated intracellular signal transduction pathways stimulate endocannabinoid-synthesising enzymes (diacylglycerol lipases, *DAGL*) at the postsynaptic cell membrane (*green*). The endocannabinoids synthesised by DAGL (*2-AG*) are transported out of the postsynaptic cell and in turn bind to CB1 receptors at the presynaptic membrane (retrograde signalling). The activation of CB1 receptors now inhibits the further release of glutamate, resulting in the inhibition of synaptic activity

mitters, which travel through the synaptic cleft and activate ion channels at the postsynaptic membrane. This in turn results in a change in current in the postsynaptic neuron. The neurotransmitter is usually degraded very rapidly and the synapse is ready for the next signal to arrive. There are different neurotransmitters and correspondingly various synapses. The role of the endocannabinoid system can be best explained by means of glutamatergic synapses that use glutamate as the neurotransmitter (◘ **Fig. 1**), and which we will focus on. Endocannabinoids modulate the activity of glutamatergic synapses if their signal burst reaches a high frequency. As a result, the presynaptic neuron releases a great amount of glutamate leading to high glutamate concentrations in the synaptic cleft. Under these circumstances glutamate can reach the periphery of the synapse and activate so-called metabotropic receptors, which also belong to the family of G-protein coupled receptors. This receptor activation does not directly cause a change in the membrane potential, but rather initiates intracellular signal transduction pathways that results in long-lasting changes in cell functioning: the metabotropic receptors amongst others stimulate endocannabinoid synthesizing enzymes that are located nearby at the postsynaptic membrane. Via a yet unknown mechanism the synthesized endocannabinoids are transported out of the postsynaptic neuron and activate CB1 receptors at the opposing presynaptic membrane. Endocannabinoids thus travel against (retrograde) the usual neurotransmitter-based communication. The activation of CB1 receptors in turn inhibits a further release of glutamate. Accordingly, endocannabinoids are signalling molecules in a retrograde negative feedback mechanism that inhibits synaptic activity.

There is evidence that endocannabinoids constitute a similar feedback mechanism at other cell–cell contacts. One good example is the neuronal regulation of bone remodelling, which has been studied by Itai Bab together with our laboratory for many years [5]. Although it has been long known from clinics that bone remodelling is modulated by the central nervous system, this knowledge has not caught on in the scientific communities yet. Patients suffering from traumatic brain injuries for example usually exhibit improved healing of bone fractures and often even abnormal bone growth at soft tissues or joints. In contrast, depressive patients often show decreased bone density and osteoporosis. The vegetative nervous system, composed of the parasympathetic and the sympathetic nervous system, is responsible for the communication between brain and skeleton. The bones are full of sympathetic nerve fibres that are in close contact with the bone-forming osteoclasts. The sympathetic neurons release noradrenaline, which activates β2-adrenergic receptors at the cell membrane of osteoblasts and hence inhibits their activity. The activation of the sympathetic nervous system thus inhibits bone growth. At the same time, however, it stimulates the synthesis of endocannabinoids by osteoblasts by activating the respective enzymes in these cells. The endocannabinoids in turn bind to CB1 receptors at sympathetic nerve endings and inhibit the further release of noradrenaline. This condition thus resembles that in the central nervous system.

Due to the lipophilic properties of endocannabinoids one can assume that these signalling molecules only have a short range. For the CB1 receptor-mediated feedback mechanism it is therefore crucial to precisely arrange the single components on both cells involved. Whether this is equally true for CB2 receptors on immune cells is completely unknown so far. The function of CB2 receptors is generally less studied and understood, but most animal studies have shown that after activation by cannabinoids, CB2 receptors reduce immune responses and inflammation.

Not only because of their potential anti-inflammatory properties are CB2 receptors an interesting therapeutic target. Experiences with cannabis preparations acting on both CB1 and CB2 receptors have shown that substances specific for CB2 receptors should have no or only minor side-effects, since virtually all psychoactive effects are mediated via CB1 recep-

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Abstract
The hemp plant *Cannabis sativa* has been cultivated for thousands of years and is used as a medical plant and intoxicant. Scientific research on the psychoactive substances of *Cannabis sativa* and their effects on the brain started around 50 years ago and led to the discovery of the endogenous cannabinoid system. Today we know that this system represents an important feedback mechanism that modulates the communication between neurons. However, this system is not only active in the brain, but is known to be activated in different tissues and organs during specific disease states. Consequently, there is increasing interest in this system as a possible target for the development of new drugs. The currently commercially available drugs are based on cannabis extracts or synthetic compounds of the plant's active components and are mainly used to treat chronic pain. In this review, the mechanisms of the endogenous cannabinoid system in pain perception are elucidated and a new herbal (phyto)cannabinoid which is a constituent of our daily food is presented.

Keywords
Endocannabinoids · Chronic pain · Inflammation · β-caryophyllene

tors. However, there are only few clinical studies using CB2 receptor-specific agonists that unfortunately do not show the desired results.

Changes in pain perception after inflammation and nerve injury

Chronic pain, and especially neuropathic pain, is an indication for CB2 receptor-specific agonists which are of special interest. To better understand this topic, we first have to discuss how pain and particularly chronic pain develops.

Specialized neuronal cells, the nociceptors, are responsible for the perception of pain stimuli and are present in almost all tissues [6]. The cell bodies of the nociceptors in the skin and skeletal muscles are located in a chain of ganglia, which travel across the spinal cord. These cells possess an axon that projects in two directions (**Fig. 2**). One branch projects to the periphery and creates small ramifications in the skin and muscles. The topography of these projections resembles those of our segmented physique, which is also reflected in the vertebral bodies. Axons from one ganglion hence innervate a relatively small and well-defined area of the body. The other branch of a nociceptor ends at the back part of the spinal cord, the so-called dorsal horn. After tissue damage or strong tissue strain that may lead to injury the nociceptors are activated and send a signal to the spinal cord. From there the signal is transmitted to pain areas in the brain. A thermal pain stimulus thereby activates different nociceptors than a mechanical stimulus, meaning that nociceptors are specialized in different pain modalities.

The whole system is not static but rather very dynamic. Injuries or tissue inflammation can have an impact on the sensitivity of nociceptors as well as on the signal transduction efficacy in the spinal cord. First we will consider the processes in the periphery. Here, different pro-inflammatory molecules such as prostaglandins, ATP or cytokines are released upon injury or inflammation. These molecules are recognized by immune cells, which then migrate into the affected tissue and enhance the inflammation by releasing additional signals. At the same time the pro-inflammatory molecules activate receptors at the cell membrane of nociceptors and thus coverts them into an activated state, allowing them to react to usually subliminal stimuli.

In the spinal cord, ongoing activation of nociceptors or a nerve injury likewise leads to an inflammatory response. Therefore, the interaction of neurons and microglia cells is of special importance. Microglia cells are immune cells of the central nervous system though with different functions [7]. In healthy tissue, microglia cells are in a state commonly described as the "resting state". This description unfortunately is rather misleading, since the cells are still active. They control the integrity of synapses and resorb those that are not functional anymore. Upon peripheral nerve injury, the injured neurons in the spinal cord release different chemical signals that now shift the microglia cells into an "activated" state. In this activated state the microglia cells then secrete additional pro-inflammatory signals that again recruit other immune cells, activate astrocytes and change the synaptic characteristics of neighbouring cells. The nerve injury therefore causes an activation of microglia cells and astrocytes in their projection area, leading to a sustained enhancement of the synaptic transduction of pain stimuli.

Everyone probably knows the consequences of such a peripheral and central sensitisation. The perception of pain stimulus in the damaged site appears more painful than in the healthy tissue (hyperalgesia). Stimuli that are rarely perceived by healthy tissues might be unpleasing or even cause pain in injured areas (allodynia). As a result, a warm shower after sunburn can suddenly become painful or a shirt causes the skin to itch.

This kind of change in pain perception is always annoying, but rather reasonable. Due to the enhanced sensitivity the affected tissue is preserved and healing is accelerated. However, if the sensitisation becomes chronic, it will become problematic and of clinical interest. This often occurs due to accidents, diabetes or chemotherapy, as well as chronic inflammation, e.g. caused by arthritis. The resulting long-lasting and hardly treatable pain burden tremendously affects the patient's quality of life.

The role of the endocannabinoid system in the modulation of pain perception

Mouse lines with a genetic deletion of the cannabinoid receptors were of great importance for the elucidation of cannabinoid receptor functioning. These mice are viable, but show a variety of deficits and have an increased mortality rate [8]. Since CB1 and CB2 receptors are active on different cell types the endocannabinoid system can impair pain management at different levels. Via CB1 receptors cannabinoids can directly modulate the activity of nociceptors and the pain areas of the brain. Thus, activation of CB1 receptors in acute pain should also have analgesic

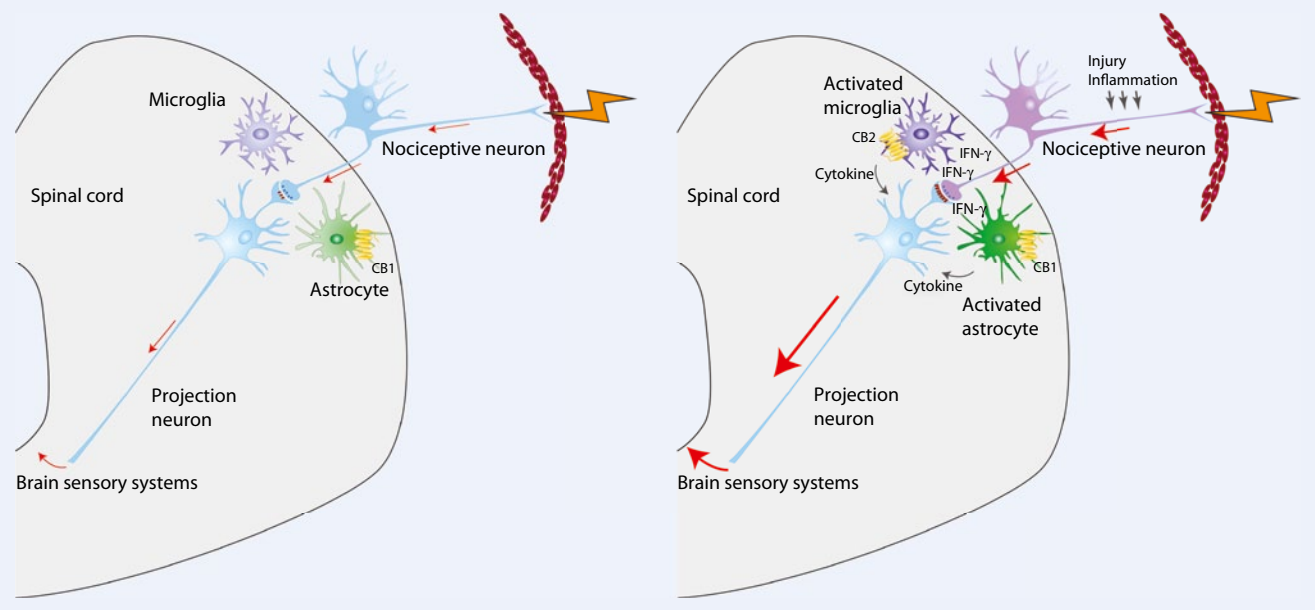

Fig. 2 ▲ Modulation of pain perception. In the healthy tissue, microglia cells are in a state commonly described as "resting state" (*left*). Upon peripheral nerve injury the microglia cells are shifted into an "activated" state, due to the release of different chemical transmitters such as interferon-γ (*IFN-γ*; *right*). At this state the microglia cells secrete additional pro-inflammatory cytokines, leading to a sustained enhancement of the synaptic transduction of pain stimuli (sensitisation). The perception of a pain stimulus will then appear more painful than in the healthy tissue (hyperalgesia). Activation of cannabinoid receptors modulates the activity of immune and microglia cells and thus antagonizes the inflammatory response and the sensitisation process (see text for details)

effects without tissue inflammation. Indeed, neither the basal pain threshold was altered in CB1 receptor-deficient mice, nor did they show differences in animal models of neuropathic pain. These results demonstrate that for normal pain perception, basal CB1 receptor activity does not play an important role. Conversely, the analgesic effects of THC or synthetic cannabinoids were much lower in these animals [8], indicating that CB1 receptors mediate, at least in part, the analgesic effects of THC.

An activation of CB2 receptors mainly influences the activity of microglia and immune cells and thus the sensitisation of pain perception upon tissue damage and inflammation. Selective CB2 agonists are therefore only slightly effective in acute pain, but alleviate inflammation-induced and neuropathic pain [9]. These pharmacological results were also validated in studies using mouse lines lacking CB2 receptors. These mice indeed did not show any changes in acute pain experiments, but exhibited interesting alterations in neuropathic pain models.

One of the most commonly used methods to model neuropathic pain in mice is the partial ligation of the sciatic nerve. For this method the nerve is first exposed in minor surgery. Next, a thin filament is guided through the nerve and on half is ligated without separating it. As a result of this surgery, an inflammation of the nerve and its projection area in the spinal cord develops within a few days. The resulting enhanced reaction of the hind paw (being the projection area of the nerve) to thermal (thermal hyperalgesia) and mechanical stimuli (mechanical allodynia) is assessed after 5–8 days by respective behavioural experiments. Either a bundled infrared beam is directed to the paw of the animal, or the paw is stimulated using a mechanical actuator. Thermal hyperalgesia or mechanical allodynia has developed if the paw at the nerve-ligated site reacts faster to the beam or actuator than the paw at the opposing control site. Nerve-ligated wild type and CB2 receptor deficient mice showed a similar stimulus response with the paw of the nerve-ligated site [10], indicating that these receptors are not critical for the development of neuropathic pain. Interestingly, in contrast to the control mice, animals without CB2 receptors also developed hyperalgesia and mechanical allodynia at the opposing control site. This phenomenon was rather exceptional and indicated that the inflammation in the spinal cord has spread unreasonably far to the opposite site. Immunohistochemical studies indeed revealed a spreading of microglia and astrocyte activation throughout the whole spinal cord. Such an extensive expansion is physiologically obviously not reasonable, because a higher sensitivity of tissue that is not affected by the nerve injury cannot contribute to the process of healing. The physiological role of CB2 receptor-mediated processes thus seems to be the limitation of the inflammation in the spinal cord. It can be assumed that also during inflammation the endocannabinoid system provides a negative feedback mechanism.

Molecular and genetic experiments further demonstrated that this mechanism mainly antagonizes the effects of interferon-γ [11]. This pro-inflammatory signal molecule is produced by neurons and astrocytes and contributes to the activation of microglia cells. The effects of interferon-γ were much stronger in mice lacking the CB2 receptors compared to respective control animals. In contrast, microglia ac-

tivation in mice deficient in both CB2 receptors and interferon-γ was markedly reduced. The efficacy of CB2 receptor-selective agonists in chronic pain states is thus probably based on an enhancement of the feedback mechanism.

β-caryophyllene, a CB2 receptor-specific phytocannabinoid

There are many reports in which patients react differently to synthetic THC and cannabis preparations. These observations prompt the suggestion that in addition to THC some of the many other substances of *Cannabis sativa* may also have bioactive action. One substance apparently matching this assumption is cannabidiol, which is thought to have sedating effects without knowing its exact mechanisms of action. So far, the receptor of this molecule has not been identified.

The Swiss scientist Jürg Gertsch wondered whether cannabis sativa might even produce a CB2 receptor-specific molecule. To answer this question he established a test that is based on the displacement of a CB2-specific, radiolabelled agonist [12]. This test was relatively easy to perform with a high amount of extracts and was at the same time very sensitive. The results of the experiments were as clear as astonishing. Another compound of *Cannabis sativa*, β-caryophyllene, indeed showed strong binding affinities for CB2, but not for CB1 receptors. Especially interesting about this discovery was the fact that β-caryophyllene is not only present in cannabis extracts, but also in many vegetable oils at high concentrations. These include various spice and food plants such as avocado, basil, rosemary, cinnamon, oregano, caraway and pepper. In these plants β-caryophyllene probably helps in defending against herbivores. Corn for example secrets β-caryophyllene upon attack of the corn rootworm, thereby attracting nematodes, which are the natural enemies of the corn rootworms.

Comprehensive cell biological experiments demonstrated that β-caryophyllene does not only bind to CB2 receptors with high affinity, but also activates them. Furthermore, animal studies revealed excellent anti-inflammatory characteristics of β-caryophyllene, which are mediated by the CB2 receptor. Consequently, these effects are absent in mice lacking CB2 receptors. The amount of β-caryophyllene used in the animal experiments reflected those of humans ingested by daily food. Taken together, these results imply that β-caryophyllene present in our food substantially contributes to the tonic activity of CB2 receptors and their effects on the immune system.

Conclusion

By means of some selected examples we have tried to explain how the endogenous cannabinoid system is activated under pathological conditions and how it contributes to the reconstitution of the healthy state. Such examples can be extended almost at will, since this system is active in various tissues and organs. Much about this system still needs to be investigated. We do not know for example, how exactly the synthesis of endocannabinoids takes place and how it is regulated, how the molecules are transported in and outside the cells and whether and where endocannabinoids are stored. We also do not know how the exact subcellular localisation of the single components is regulated. However, the most important question might be if our knowledge today is only the tip of the iceberg. We have left unmentioned that both AEA and 2-AG not only modulate cannabinoid receptor activity but also those of other receptors. Endocannabinoids therefore also have an impact on completely different cellular networks. Finally there are various other hydrophobic molecules whose structure is similar to endocannabinoids. Whether and how these molecules contribute to cell–cell communication is still entirely unknown.

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Conflict of interest. On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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