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Recent Advances in Cannabinoid Research

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Key Words
Tetrahydrocannabinol (THC) · Arachidonylethanolamide (anandamide) · 2-Arachidonylglycerol (2-AG) · HU-211 · Emesis · Appetite enhancement · Multiple sclerosis · Spinal injury · Closed head injury

Summary
Although the active component of cannabis Δ^9-THC was isolated by our group 35 years ago, until recently its mode of action remained obscure. In the last decade it was established that Δ^9-THC acts through specific receptors – CB_1 and CB_2 – and mimics the physiological activity of endogenous cannabinoids of two types, the best known representatives being arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG).
THC is officially used against vomiting caused by cancer chemotherapy and for enhancing appetite, particularly in AIDS patients. Illegally, usually by smoking marijuana, it is used for ameliorating the symptoms of multiple sclerosis, against pain, and in a variety of other diseases.
A synthetic cannabinoid, HU-211, is in advanced clinical tests against brain damage caused by closed head injury. It may prove to be valuable against stroke and other neurological diseases.

Schlüsselwörter
Tetrahydrocannabinol (THC) · Arachidonylethanolamid (Anandamid) · 2-Arachidonylglycerol (2-AG) · HU-211 · Emesis · Appetitverstärkung · Multiple Sklerose · Rückenmarksverletzung · Schädel-Hirn-Trauma

Zusammenfassung
Jüngste Fortschritte in der Cannabinoid-Forschung
Obwohl die aktive Komponente von Cannabis, das Delta-9-THC, von unserer Gruppe vor 35 Jahren entdeckt worden war, blieb seine Wirkungsweise bis vor kurzem unklar. Im letzten Jahrzehnt wurde festgestellt, dass Delta-9-THC über spezifische Rezeptoren wirkt – CB_1 und CB_2 – und die physiologische Aktivität endogener Cannabinoide imitiert, von denen es zwei Typen gibt. Deren bekannteste Vertreter sind Anandamid (Arachidonoylethanolamid) und 2-AG (2-Arachidonoylglycerol).
THC wird offiziell gegen chemotherapieinduziertes Erbrechen und zur Anregung des Appetits, besonders bei Aids-Patienten, verwendet. Illegal, im Allgemeinen durch das Rauchen von Marijuana, wird es verwendet zur Linderung von Symptomen der Multiplen Sklerose, gegen Schmerzen und bei einer Reihe anderer Erkrankungen. Ein synthetisches Cannabinoid, HU-211, befindet sich in fortgeschrittenen klinischen Tests gegen Hirnverletzungen durch Schädel-Hirn-Trauma. Es könnte sich zudem bei der Behandlung des Schlaganfalls und anderen neurologischen Erkrankungen als nützlich erweisen.

The plant *Cannabis sativa* has been used for millennia in medicine, as a mood-modifying substance and in religious rites with considerable overlap between them, as expected for plant substances that affect emotions and cognition.
Research on cannabis as in most areas of science commenced early in the 19th century. On the chemical side the investigations did not lead very far. By contrast to morphine and cocaine, which were isolated from opium and coca leaves as crystalline salts, the active constituent of cannabis was quite elusive. It was present in an oily extract which could not be further purified.
The active cannabis constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated, apparently for the first time in pure form, and

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its structure was elucidated in 1964 by our group in Israel [1]. This, rather late, identification brought a renaissance to cannabis research: Most of the natural cannabinoids were isolated from the plant and their structures were elucidated. Total syntheses were achieved for most natural cannabinoids. The basic structure-activity relationships as regards cannabimimetic activity were established, and the metabolic routes were elucidated [2].

With the clarification of the chemical aspects of cannabis, interest focused on cannabinoid pharmacology, biochemistry, and medicinal properties. Although we have learned much on the overt behavioral effects, the neurochemistry, and the neurophysiology, until recently very little was known on the molecular basis of THC action.

Cannabinoid Receptors and Transmitters

Does THC act on a specific receptor or does it produce its actions by a non-specific change in the structure of lipid membranes? An answer, at least a partial one, was to be found in the stereospecificity of THC activity. THC is an asymmetric molecule, i. e. it can exist in two forms (enantiomers) which are mirror images of each other. As receptors, which are proteins, are also asymmetric, binding to a receptor will take place only with one of the two asymmetric components. Hence, if a compound shows stereospecific action, i. e. if only one of the two possible enantiomers produces the typical activity, it probably has a specific mode of action, possibly through a receptor [3].

By the mid-1980s work by several groups, including ours, indicated that cannabinoid action was indeed highly stereospecific. Thus, the very potent synthetic cannabinoid HU-210 is several thousand times more active than its synthetic mirror image (HU-211) in many tests in animals, as well as biochemical ones. These results prompted several groups to look for a specific receptor in brain and on neuronal cells, which would bind the psychoactive cannabinoids. In 1988 Howlett's group in St. Louis [4] established the presence of a specific cannabinoid receptor in rat brain by the use of a tritium labeled cannabinoid. The receptor is found in high concentrations (when compared to other receptors) in the cerebellum, the basal ganglia and other brain areas known to be associated with co-ordination of movement, memory, and emotions. Shortly after the cannabinoid receptor was identified, a group at the US National Institute of Health [5] achieved the cloning of the gene and established the amino acid sequence of the receptor now named CB_1 (or brain cannabinoid receptor). The cannabinoid receptor was shown to be highly specific for binding of compounds with cannabis-like activity, be they natural or synthetic. None of many known hormones, neurotransmitters or other biologically active compounds of various types were found to bind to the receptor. It was quite unacceptable to most neuroscientists that the brain will waste its resources to synthesize a receptor in order to bind a constituent of a plant. The only reasonable assumption which could be made was that the brain produces a neuronal mediator, a specific compound (or a family of compounds) which binds to and activates the cannabinoid receptor. The plant cannabinoid, THC, by coincidence happens to bind to the same receptor. In the late 1980s several groups initiated work aimed at the discovery of such a brain constituent.

Our group proceeded by preparing a novel, highly psychoactive compound for the cannabinoid receptor, which could be easily labeled with tritium. This probe binds to the receptor and the formed complex was used to assay brain fractions for cannabis-like activity. A lipid-soluble fraction isolated from pig brain exhibited cannabis-like activity: on incubation with the receptor, it displaced the radioactive probe. Purification of the constituents of this fraction over many different chromatographic columns ultimately led to the isolation of a single active constituent. With advanced physical techniques, such as nuclear magnetic resonance spectroscopy and mass spectrometry, it was possible initially to establish that the endogenous brain constituent was a fatty-acid derivative, and then to elucidate its complete structure as arachidonoylethanolamide [6]. The final proof of the suggested structure was its synthesis from readily available materials. We named this endogenous brain constituent 'anandamide' based on the Sanskrit term 'ananda', meaning 'bliss' and the amide moiety present in the novel compound.

When tested in animals anandamide paralleled THC as regards analgesia, sedation, motor coordination, and certain biochemical parameters. Later, using the same techniques, two additional compounds, structurally closely related to anandamide, were isolated from porcine brain. Both bind to the cannabinoid receptor. The brain apparently produces a whole family of anandamide-type compounds.

A second cannabinoid receptor (CB_2) has been identified in and cloned from rat spleen [7]. CB_2 has so far been found only in cells of the immune system. We have described the identification of a cannabinoid ligand from canine gut which binds to both CB_2 and CB_1. Its structure was elucidated as 2-arachidonyl glycerol (2-AG) [8]. Independently, a Japanese group later also identified 2-AG in brain [9].

What is the physiological role of the cannabinoid receptors and of anandamide and 2-AG? We do not know yet. However we can make certain assumptions on the basis of the anatomical distribution of the receptors and of the activity of THC and of the endogenous brain and peripheral cannabinoid ligands.

Presumably, in the brain the cannabis agonists act on coordination of *movement*, via the cannabinoid receptors in the cerebellum and in the basal ganglia, and on *memory*, via the receptors in the hippocampus. Both types of activity are well-known effects observed on heavy marijuana intoxication. These processes are very complicated sequences of biological events of which we know very little. We believe that the role of 2-AG is also associated with the immune system. It is also involved in *blood pressure* regulation [10, 11].

Cannabinoids as Medicinal Agents [12 a, b]

Many novel drugs are based on chemical modifications of transmitters. It seems reasonable to expect that, as with other receptor-transmitter systems, excess or lack of cannabinoid receptors or en-

dogenous ligands may be the cause for disorders in the CNS or those associated with the immune system.

Can the anandamides and cannabinoids be used as therapeutic drugs in CNS disorders? The answer so far is a tentative 'yes'. To date most investigations have been performed on THC, as anandamides were only recently discovered. As a result, it is not yet known whether these agents cause the same potentially therapeutic effects as THC.

Multiple Sclerosis and Spinal Injury

The Assyrians apparently used cannabis for neurological diseases. Azallu (an Assyrian term for cannabis used for medicinal purposes) was a drug against 'poison of all limbs' and against 'arimtu', which was probably also a neurological disease of the legs. Is one of these two disorders multiple sclerosis? We shall, of course, never know.

Multiple sclerosis is a disorder of the brain and the spinal cord that attacks the myelin sheath of nerve fibers. The cause is unknown – it is apparently an autoimmune disease triggered by an environmental factor. There is a genetic susceptibility. The disease is usually slowly progressive with exacerbations and remissions over many years. The symptoms vary. They include tiredness, spasticity, weakness in the legs and inability to walk, lack of balance, muscle pain, tremor, vision problems, urinary hesitancy and incontinence, slurred speech, and memory loss. Anxiety and depression are common.

A variety of partially effective drug treatments are in use. Corticosteroids (antiinflammatory drugs) are the cornerstone of therapy. However, their efficacy is limited and their chronic use entails considerable risk. Carbamazepine and phenytoin (antiepileptic drugs) are used for pain (including trigeminal neuralgia) and numerous other drugs are employed for specific symptoms. The lack of an efficient drug treatment has led many patients to search for additional therapies, one of which is cannabis. Today, cannabis is a medicinal agent used by an apparently large number of multiple sclerosis patients, although it is not legal and the medical basis of this use is not strong.

Consroe et al. [13] have reported and analyzed the answers to a questionnaire mailed to multiple sclerosis patients who use cannabis for their disease. The 112 patients who responded to the questionnaire were about equally divided between USA and UK and between male and female patients. Most of the patients reported strong improvement after cannabis consumption in spasticity and sleep onset and on awakening at night as well as reduction of pain and tremor. The patients also reported improvement in ability to walk, in recovery of balance and in sexual function. There was only minor improvement in memory loss, in fecal incontinence and in constipation. This report is in effect a compilation of anecdotal data. Yet, it cannot be disregarded, and should be viewed as a basis for future large scale clinical investigations.

There are also data from several small clinical trials on multiple sclerosis with Δ^9-THC or with nabilone – a synthetic cannabinoid agonist [12 a, b]. For example the effect of THC in patients with multiple sclerosis has been investigated in a placebo-controlled, double-blind, crossover trial involving 9 patients who were orally administered placebo or THC 5–10 mg/day. Clinical and electromyograph measurements indicated a significant reduction in spasticity, with negligible adverse effects. In another trial, patients with multiple sclerosis reported subjective improvement of tremor and sense of well-being. Smoking cannabis was reported to result in symptomatic improvement, as judged by clinical rating, electromyographic investigation of leg flexor reflexes and recording of hand action tremor, in a single patient with multiple sclerosis [12 a, b].

The ability of THC to suppress the effects of experimental autoimmune encephalomyelitis (EAE), the laboratory model of multiple sclerosis, has been investigated in Lewis rats and guinea pigs [14]. All animals treated with placebo developed severe clinical EAE 10–12 days post injection of myelin (the material that causes the multiple sclerosis-like effects). THC-treated animals had either no clinical signs or mild signs with delayed onset, with survival rates greater than 95%. Examination of CNS tissues revealed a marked reduction of inflammation in the THC-treated animals. As mentioned above multiple sclerosis is considered to be an autoimmune disease. As the cannabinoid CB_2 receptor has been found in the spleen (an organ involved in the immune system) it is possible that the effect of THC on multiple sclerosis is mediated through both CB_1 and CB_2 receptors.

Huntington's Chorea

A major effect of Huntington's chorea is the loss of neostriatal neurons, accompanied by the loss of dopamine D_1 receptors. A group of investigators in New Zealand has found that this loss of neurons also causes a 97.5% reduction of CB_1 receptors in the substantia nigra pars reticulata. On the basis of these data the authors suggested a role for cannabinoids in the therapeutics of hyperkinetic and dystonic disorders, such as Huntington's disease, as well as in the treatment of Parkinson's disease [14].

Neuroprotection

Cannabinoids may also cause effects via mechanisms distinct from the cannabinoid receptor pathways. The most extensively investigated compound is (+)-HU-211, a synthetic cannabinoid with a stereochemistry opposite to that present in the naturally occurring compounds. It does not produce THC-type effects in animals and shows insignificant binding to the CB_1 receptor. However, HU-211 blocks N-methyl-D-aspartate(NMDA) receptors. This receptor is one of the subreceptors of glutamic acid – the major stimulatory transmitter in the brain. Brain trauma and stroke cause severe overactivity of this system, which ultimately leads to cell death. Hence it is of importance to find compounds that block NMDA receptors. HU-211 is a potent blocker of NMDA-induced tremor, seizures and lethality in mice. It may therefore prove useful as a non-

psychoactive drug that protects against NMDA receptor mediated neurotoxicity.

Cerebroprotective effects of HU-211 have been demonstrated after experimental closed head trauma in rats. The drug is very effective in improving recovery of motor function. The drug was given 1 or 2 h after the trauma and the clinical status was evaluated 24 and 48 h later. The percentage of rats able to perform a beam walking task increased significantly. The drug was also effective in reducing by more than 4-fold the blood-brain barrier breakdown that is observed during head trauma. It also attenuates cerebral edema [12 a, 12 b, 14].

Phase II clinical studies in brain trauma, with HU-211 have been completed. HU-211 has shown significant improvement (Pharmos Corp., Rehovot, Israel, unpublished data).

Antiemetic Activity

Cannabis was used as an antiemetic drug in antiquity. It was well known to physicians in England during the 19th century. Yet, the discovery of the antiemetic effects of Δ^9-THC in 1975 was serendipitous and followed 'street reports' that marijuana helped to overcome the side-effects of anticancer chemotherapy. The original report was followed by numerous other clinical trials. In 1986, THC was approved for use by the U. S. Food and Drug Administration and is now being marketed under the generic name Dronabinol.

The number of clinical studies with Δ^9-THC published during the 1980s is considerable. In a typical report published in 1980, 46 patients (9–70 years old) in a double-blind, crossover study were administered either Δ^9-THC (10 mg/m^2 orally, 3 times daily) or prochlorperazine (10 mg, orally, 3 times daily). Δ^9-THC was more active than prochlorperazine, regardless of the emetogenicity of the chemotherapeutic agent, and young patients were more likely to show complete response than older patients [12 a, 12 b, 14].

From the above-mentioned and other clinical studies, several basic facts emerge: (a) Δ^9-THC is a potent antiemetic agent. It is very efficient in its action on nausea and vomiting caused by radiation therapy, as well as by many, but not all anticancer drugs. Thus, Δ^9-THC is only marginally active in patients administered potent emetogens such as cisplatin. Δ^9-THC is active in adults in total doses of about 15 mg orally. This dose causes side effects in many individuals. Indeed, most patients report somnolence and sedation. Psychological 'high', anxiety, increased heart rate and orthostatic hypotension are frequently encountered. In some cases, mental confusion, blurred vision, and even hallucinations are reported [12 a, 12 b, 14].

Surprisingly, in spite of the enormous public interest in 'medicinal marijuana' and numberless articles in the daily press and magazines focused predominantly on this aspect of marijuana use, little research progress has been reported on the antiemetic activity of cannabinoids in the last decade. In a review [15] the clinical experience gained over 7 years with dronabinol in antiemetic treatment was summarized. With doses of about 10–15 mg or below, complete response was noted in 36% of the patients, 32% showed partial response and 32% showed no response. However, 65% suffered from drowsiness and dizziness and 12% from dysphoric effects. Combination treatment of dronabinol with prochlorperazine (an antipsychotic drug with antiemetic effects) was more effective than either drug alone.

We and others have noted that in mice the cannabinoid receptors in the brain develop gradually and do not reach their maximal potency until adulthood. We assumed that if this observation is relevant for humans, children will not experience THC-type cannabimimetic effects until about the beginning of puberty. As the antiemetic effects are not mediated by the cannabinoid receptors it was reasonable to expect that the antiemetic effects of THC in young children will be observed while the cannabimimetic effects will be absent. We chose Δ^8-THC, rather than the marketed Δ^9-THC, as it is less psychotropic, far less expensive to produce, and much more stable than Δ^9-THC. We started the clinical trial with a low dose (5 mg) which in some adults already causes psychotropic effects. None were observed in children. Hence their antiemetic dose could be increased from about 5 mg up to 18 mg. At this very high dose, which cannot be administered to adults due to side effects, children showed no such effects. Vomiting and nausea were completely eliminated. Although this clinical trial was an open one, we believe that the results are highly significant, as almost without exception young children in the same hospital undergoing similar treatment vomit during cancer chemotherapy. The number of children was not large (n = 14), however, together they were administered Δ^8-THC about 400 times over a period of 2–3 years. No tolerance to the drug was noted [16].

THC can be considered an excellent, inexpensive, antiemetic drug in children, which does not cause cannabimimetic effects.

Other Medical Uses

Δ^9-THC is also legally used as an appetite stimulant, particularly in AIDS patients. Cannabis and/or THC have shown effects in various other medical conditions, such as glaucoma, asthma and epilepsy. There has not been much progress in these fields in the last decade.

'Medical Marijuana' – is it Better Than THC?

Over the last few years a heated public discussion has taken place concerning the legalization of marijuana for medical use – with emphasis on amelioration of nausea and vomiting in cancer patients, on appetite stimulation in AIDS patients, and on use against spasticity and pain in patients with multiple sclerosis and spinal cord injury. At least one, well-publicized, marijuana pub, selling the drug to patients, was closed in San Francisco. This resulted in a public outcry which ultimately led to a referendum on the issue. California and Arizona residents voted to legalize 'medical marijuana', but the Federal Government declared that it will take legal action against physicians who prescribe an illegal drug.

From the point of view of the medicinal chemist this fierce debate, as least as regards cannabis, is missing several central points. Most plant products of medical value are not usually administered as a crude plant extract but as a pure substance. In the Western world morphine, not opium, is used against pain. The same is true for a long list of important drugs found in plants or microorganisms. Why not should patients use THC rather than 'medical marijuana'. There are several advantages of 'medical marijuana' as compared to THC. Marijuana is usually smoked, which represents a very efficient way of administration and the effects are noted almost immediately; many users claim that the effects are 'milder' than those of THC; THC is considerably more expensive than marijuana. THC, however, has also significant advantages. It can be administered at exact dose levels and it contains no additional cannabinoids and other constituents, which are present in cannabis and which are difficult to quantify in each batch. THC and crude cannabis have not been directly compared in patients. Presumably THC in an aerosol form can be compared to smoked marijuana, with the amount of THC in the smoke determined.

Surprisingly in spite of the furor over 'medical marijuana' no comparative studies have been published, and we do not know whether (a) medical marijuana is indeed better that THC, as claimed; (b) whether the additional cannabinoids in crude cannabis (particularly cannabidiol) impart desirable qualities to the drug; and (c) whether better formulations of THC (with or without additional constituents) can be obtained.

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