Cannabinoid-Receptor Agonists

Inhibitors of Endocannabinoid Degradation: Potential Therapeutics for Neurological Disorders

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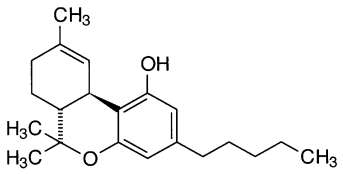
Introduction

For a long time, *Cannabis sativa* preparations have been used as drugs for medicinal and recreational purposes. Its dried blossom tips are known as marijuana, and the dried resin as hashish. For 60 years, chemical and physiological effects of cannabinoids have been investigated. Recently, this research has focused on cannabinoid receptors in the human body and their endogenous lipid ligands. Evidence is accumulating that interference with endocannabinoid metabolism offers novel prospects for the treatment of a multitude of disorders such as pain, cancer, epilepsy, and multiple sclerosis. Recently, a series of highly potent inhibitors of endocannabinoid degradation has been described, which promises the development of new strategies for the treatment of anxiety and neurological disorders.[1]

Cannabinoids and Their Receptors

The consumption of cannabinoids leads to a highly complex spectrum of pharmacological effects, which could be of therapeutic benefit for a variety of disorders. They exhibit sedative and mood-altering properties, reduce pain sensation, alleviate nausea and vomiting, stimulate the appetite, alleviate cramps, and relax vascular muscles. At the same time, however, they impair cognitive and motor functions and interfere with sensory perception and short-term memory.[2] The underlying causes of the effects of cannabinoids have only been explored partially. In 1964, after about 20 years of research, the structure of the main psychoactive ingredient of marijuana, (−)-Δ^9-tetrahydrocannabinol (**1**) was finally determined.[3] This laid the foundation for the chemical synthesis of high-affinity cannabinoid derivatives, which in turn led to the identification of the first cannabinoid receptor, CB1, in the central nervous system.[4]



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Another receptor, CB2, was discovered in 1993.[5] Both receptors are glycoproteins with seven transmembrane helices. Their stimulation leads to the activation of G proteins belonging to the $G_{i/o}$ family, thereby inhibiting the production of adenylyl cyclase and cAMP (cyclic adenosine-3′,5′-monophosphate) and thus regulating numerous signal-transduction pathways.[6] In addition, CB1 receptors are known to inhibit voltage-gated calcium channels and to activate potassium channels. Whereas CB1 is expressed predominantly in the central nervous system and represents one of the most abundant neuromodulatory receptors in the brain, CB2 occurs mainly on immune cells where it contributes to the mediation of the immunosuppressive effect of cannabinoids.[5]

Ligands for Cannabinoid Receptors

Cannabinoid-receptor agonists can be assigned to different classes of compounds. Tetrahydrocannabinol (**1**) belongs to a family of over 60 bi- and tricyclic secondary metabolites that are biosynthetically derived from geranyl pyrophosphate and the polyketide olivetol. Known under the name of marinol, **1** is employed for the alleviation of nausea in cancer patients undergoing chemotherapy and for the treatment of anorexia in patients with AIDS. Structural variations of the cannabinoid backbone led to the synthesis and pharmacological characterization of over 300 compounds.[6] Many of them bind to both receptors, CB1 and CB2, but some ligands with high selectivity for one or the other receptor type have been described.[6]

Synthetic analogues are, for example, CP-55,940 (**2**), a full agonist for both receptors that is 4-50 times as potent as **1**. The radio-labeled form of **2** allowed the identification of the first cannabinoid receptor.[7] Another derivative, HU-210 (**3**), is currently one of the most potent cannabinoids.[8] Agonists with aminoalkylindole structure, such as WIN-55,212–2 (**4**),[9] as well as selective antagonists of the CB1 receptor, for example, SR141716A (**5**),[10] have proven to be valuable pharmacological tools.

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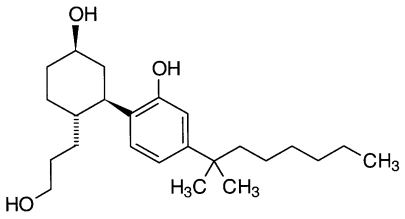

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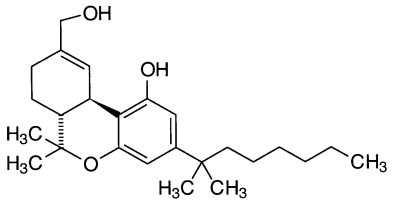

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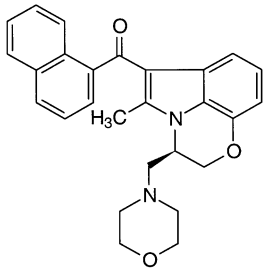

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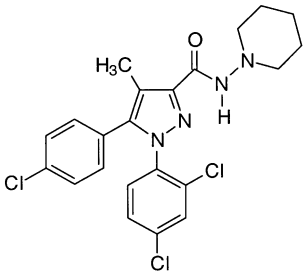

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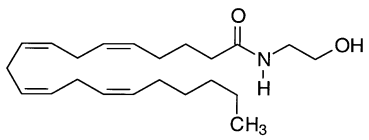
Endogenous Cannabinoids

Several endogenous lipids present in mammalian brain tissue have been identified as cannabinoid-receptor agonists. They mimic the pharmacological effects of synthetic or plant-derived cannabinoids, but are metabolically less stable. The first endocannabinoid to be identified was *N*-arachidonoylethanolamine (anandamide, **6**).[11] Later, 2-arachidonoylglycerol (**7**),[12,13] and 2-arachidonylglycerylether (**8**) were found.[14] In contrast to classical neurotransmitters, they



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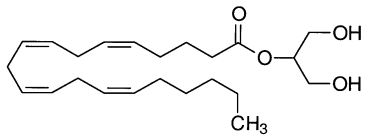

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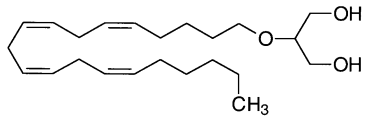

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are not stored in synaptic vesicles, but are rapidly synthesized in neurons in response to membrane depolarization and calcium influx.[15] Compounds **6**[16,17] and **7**[18] are biosynthetically derived from different membrane lipids. In the case of anandamide **6,** a transacylase transfers arachidonic acid from the *sn*1-position of phosphatidylcholine to the amino group of phosphatidylethanolamine. Subsequently, **6** is released by the action of a phospholipase D.

Another peculiarity of endocannabinoids is that they act as retrograde synaptic messengers: They are released by postsynaptic neurons, diffuse across the synaptic cleft, and activate the CB1 receptors on presynaptic nerve cells.[19] Endocannabinoid action is terminated within several minutes[20] through intracellular degradation: Endocannabinoids are transported across the cell membrane by a protein-mediated,[21] bidirectional process,[22] then anandamide **6** and related substances are degraded by an enzyme, fatty acid amide hydrolase (FAAH).[23]

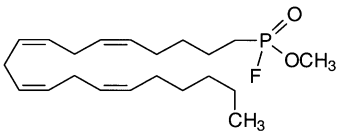
Fatty Acid Amide Hydrolase

The concentration and signaling duration of several endogenous cannabinoids is regulated primarily by FAAH, a membrane protein that is predominantly expressed in brain and liver. Genetically engineered mice that lack FAAH show significantly elevated levels of anandamide and related fatty acid amides in the brain.[24] If these animals are intraperitoneally treated with anandamide, they show an array of CB1-dependent responses such as hypomotility, catalepsy, and hypothermia. Remarkably, these animals display a lower pain perception, an effect that can be eliminated by the CB1 antagonist **5**. These results conclusively indicate that FAAH plays a key role in the regulation of anandamide action and that the latter modulates pain sensation. Therefore, together with FAAH and with the CB1 receptor, two protein members of the endocannabinoid system are targets for the development of novel analgesics.[25]

FAAH is an integral membrane protein, a homodimer composed of two 63-kD subunits and was previously crystallized in the presence of the irreversible active-site-directed inhibitor methoxy arachidonyl fluorophosphonate **9**.[26] The crystal structure reveals that the active site is accessible from both the cytosolic leaflet of the membrane, and from the aqueous phase of the cytosol. The nucleophilic side chain of Ser241 is part of an unusual Ser-Ser-Lys catalytic triad. The structure of the enzyme suggests that fatty acid amides destined for degradation can directly reach the active site from the membrane and need not be transported through the aqueous phase.

Inhibitors of Fatty Acid Amide Hydrolase

During the last few years, research concentrated on FAAH inhibitors with the aim of developing drugs that amplify the pharmacologically useful effects of endogenous cannabinoids by inhibiting their degradation. These substances would have the advantage of avoiding the unwanted psychotropic effects displayed by Δ^9-THC and other direct-acting exogenous cannabinoid agonists.[27] Previously described FAAH inhibitors, however, did not show the required target selectivity or bioavailability,[28–30] or were not sufficiently investigated with respect to their biological effects.[31]



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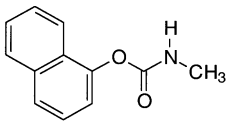

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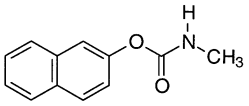

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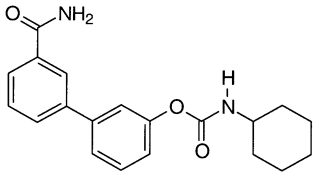

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Piomelli and co-workers recently reported the discovery of a novel series of selective FAAH inhibitors with carbamate structure.[1] Carbamates are known as covalent inhibitors of serine esterases. In this way, for example, the alkaloid of the calabar bean physostigmin inhibits acetylcholinesterase activity.[32] The cholinesterase inhibitor carbaryl **10** was employed as a lead structure for inhibitor design. Even though this compound proved to be inactive, its positional isomer **11** displayed a weak inhibitory effect ($IC_{50} = 18.6$ μM), which could be enhanced by replacing the *N*-methyl group with an *N*-cyclohexyl substituent. Further optimization of the lead structure resulted in URB597 (**12**), which proved to be the most potent FAAH inhibitor in this series of compounds with an IC_{50}-value of 4 nM in membrane preparations and 0.5 nM in intact neurons.

Kinetic analyses suggest that a nucleophilic attack of the serine residue of the active site at the carbamate irreversibly inactivates the enzyme. Most notably, other serine hydrolases are not inhibited by this compound. In experiments with mice, intraperitoneal injections of **12** resulted in a strong, dose-dependent inhibition of FAAH activity in the brain and a significant increase in the levels of anandamide and other fatty acid ethanolamides in the brain. This was accompanied by anxiolytic and mild analgesic effects, which could be reversed by the CB1 receptor antagonist **5**. These studies demonstrate that the endocannabinoid system plays a major role in the modulation of emotional states.

Future Prospects

In spite of their promising properties,[2] the unwanted psychotropic side effects of cannabinoids prevent their broad clinical application. The increase in endocannabinoid concentration by selective inhibition of their degradation promises to circumvent these disadvantages, but the complexity of the endocannabinoid system must be considered prior to clinical application of inhibitors: Not only anandamide **6**, but also a series of related fatty acid amides such as palmitoylethanolamine, oleamide, and the sleep-inducing *N*-oleoylethanolamine, are degraded by FAAH and might compete together with oxygenated metabolites for the endocannabinoid transporter. Receptors for these bioactive lipids have not yet been identified, but it is likely that inhibition of FAAH might also affect other cellular signal-transduction pathways. In addition to CB1 and CB2 receptors, anandamide also binds to the vanilloid receptor with partly opposing cellular effects. Moreover, some data suggest the existence of nonclassical cannabinoid receptors,[6] whose influence on signal transduction and cell fate is largely unknown.

Apart from the potential treatment of neurological disorders, endocannabinoids promise new concepts in cancer therapy. In the same way as their exogenous analogues, endocannabinoids show significant antitumour effects on some cell lines in vitro through selective inhibition of growth-factor-dependent cell proliferation or the induction of apoptosis.[33] In this respect, metabolically stable analogues that can be administered in very low, nonpsychotropic concentrations, or derivatives that cannot pass the blood–brain barrier, are particularly important.

A better knowledge of the endocannabinoid system will reveal the full therapeutic potential of its modulation and will facilitate the rational design of potent inhibitors with improved biological properties.

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