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On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures

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Abstract

Neurons intensively exchange information among each other using both inhibitory and excitatory neurotransmitters. However, if the balance of excitation and inhibition is perturbed, the intensity of excitatory transmission may exceed a certain threshold and epileptic seizures can occur. As the occurrence of epilepsy in the human population is about 1%, the search for therapeutic targets to alleviate seizures is warranted. Extracts of *Cannabis sativa* have a long history in the treatment of various neurological diseases, including epilepsy. However, cannabinoids have been reported to exert both pro- and anti-convulsive activities. The recent progress in understanding the endogenous cannabinoid system has allowed new insights into these opposing effects of cannabinoids. When excessive neuronal activity occurs, endocannabinoids are generated on demand and activate cannabinoid type 1 (CB_1) receptors. Using mice lacking CB_1 receptors in principal forebrain neurons in a model of epileptiform seizures, it was shown that CB_1 receptors expressed on excitatory glutamatergic neurons mediate the anti-convulsive activity of endocannabinoids. Systemic activation of CB_1 receptors by exogenous cannabinoids, however, are anti- or pro-convulsive, depending on the seizure model used. The pro-convulsive activity of exogenous cannabinoids might be explained by the notion that CB_1 receptors expressed on inhibitory GABAergic neurons are also activated, leading to a decreased release of GABA, and to a concomitant increase in seizure susceptibility. The concept that the endogenous cannabinoid system is activated on demand suggests that a promising strategy to alleviate seizure frequency is the enhancement of endocannabinoid levels by inhibiting the cellular uptake and the degradation of these endogenous compounds.

Keywords: Endocannabinoids; Neurotransmitters; Epileptic seizures

1. Introduction

Intensive neuronal activity is a prerequisite for proper brain function. However, excessive neuronal activity may endanger individual neurons, entire neuronal networks and even the organism's life. Therefore, the brain needs to protect itself against this danger. Appropriate neuronal activity is based on a tight balance between excitatory and inhibitory communication between neurons. Excessive excitatory activity is harmful for neurons, because it triggers molecular pathways that eventually lead to neuronal death, through a process known as excitotoxicity [1,2]. It is thought that excitotoxicity participates in the progress of many neurological and degenerative central nervous system diseases and disorders including Alzheimer's disease, Parkinson's disease, and various forms of epilepsy.

The large interconnected networks of the forebrain are able to generate synchronized activities. A transformation of otherwise normal brain rhythms may lead to epileptic seizures. Cortical, hippocampal and thalamocortical networks are particularly prone to the generation of such synchronized bursts of activity. In fact, epilepsy affects about 1% of the human population, with a cumulative lifetime incidence approaching 3%. The incidence is highest during the first year of life and in elderly persons (for reviews see [3,4] and refs. therein). Febrile (i.e. fever-induced) seizures are the most common seizures during childhood, affecting 3–5% of infants and young children [5]. More than 40 recognized types of epileptic syndromes can be grouped into two basic categories [4]: partial and generalized. Partial seizures occur within localized brain regions, whereas generalized seizures appear in the entire forebrain. In severe forms of epilepsy, the patient with a

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grand mal seizure becomes unconscious and experiences muscular convulsions all over the body. An absence seizure (also called *petit mal*) is much milder and leads only to a temporary lapse in consciousness, often lasting for a few seconds only. The occurrence of seizures can be acutely life threatening in particular circumstances. The long-term effects, however, also threaten brain function, as excessive neuronal activity during seizure induces excitotoxic pathways, eventually leading to the degeneration of neurons.

There are several drugs available that help to control epilepsy and that lessen the risk of seizures. However, as epilepsy constitutes a complex disease of multiple origin, the current medical control of seizures remains ineffective or not fully effective for a considerable number of patients (in the range of 30–40%) [3]. Therefore, further investigations in seeking novel therapeutic targets for the treatment of epilepsy are warranted, and, indeed, several new antiepileptic drugs have been approved during the last decade [3].

There is evidence from animal models and partly from clinical studies that treatments with cannabinoids may help to alleviate nervous system diseases and disorders such as multiple sclerosis [6], stroke [7], traumatic brain injury [8] and neuroinflammation [9]. However, the potential of cannabinoids in treating epilepsy is currently controversial (for reviews see also [10,11]). The present review will focus on this topic and aim to shed light on the understanding of why cannabinoids can exert both anti- and pro-convulsive activities. The elucidation of the anti- or pro-convulsive mechanisms is a prerequisite for the development of better strategies in targeting the endogenous cannabinoid system to treat epilepsy.

2. Marijuana and the body's own cannabinoids

Δ^9-Tetrahydrocannabinol (Δ^9-THC) was identified as the major psychoactive component in *Cannabis sativa* [12]. In 1990, it was found that a seven transmembrane G protein-coupled receptor (called cannabinoid receptor type 1 or CB_1 receptor) is the endogenous receptor for Δ^9-THC [13]. The CB_1 receptor is predominantly expressed in the nervous system. A second cannabinoid receptor, named CB_2 receptor, was later cloned and was found to be mainly expressed in immune cells [14]. In 1992, the first endogenous ligand for cannabinoid receptors was identified as an amide of arachidonic acid with ethanolamine, named anandamide (from the Sanskrit word ''ananda'', meaning ''bliss'') [15]. Subsequently, the biosynthesis and degradation pathways of endocannabinoids have been identified and characterized (reviewed in [16]). Anandamide is thought to be synthesized in two steps. First, *N*-arachidonoyl phosphatidyl ethanolamine is formed from the precursor phosphatidyl ethanolamine by the enzyme *N*-acyltransferase. In a second step, anandamide is synthesized from *N*-arachidonoyl phosphatidyl ethanolamine by a recently cloned phospholipase D [17]. The second major endocannabinoid, 2-arachidonoyl glycerol (2-AG), is synthesized in two steps from the precursor phosphatidyl inositol. However, two different pathways have been described for the synthesis of 2-AG. 1,2-diacylglycerol and lysophosphatidyl inositol were found to be intermediate products. Two diacylglycerol lipase enzymes (DGLα and DGLβ) catalyzing the synthesis of 2-AG from 1,2-diacylglycerol have recently been cloned and characterized [18]. For the degradation of endocannabinoids, fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL) were found to hydrolyze anandamide and 2-AG, respectively (for review see [16]).

In neurons, the typical intracellular effects after binding of agonists to CB_1 receptors are (i) inhibition of adenylyl cyclase leading to decreased levels of intracellular cAMP, (ii) stimulation of potassium channels (A-type and inwardly rectifying potassium channels) leading to an increased efflux of potassium, and (iii) inhibition of voltage-dependent calcium channels (N- and P/Q-type) leading to a decreased calcium influx. Collectively, CB_1 receptor agonists render neurons less excitable (reviewed in [16,19]).

The synthesis of endocannabinoids appear to occur on demand when neurons are stimulated (reviewed in [16,19]). Excitation leads to increased levels of intracellular calcium ions and induces the production of endocannabinoids. Activation of metabotropic glutamate receptors and muscarinic acetylcholine receptors may also lead to a calcium-independent enhancement of endocannabinoid synthesis. Thereupon, endocannabinoids are released from the neurons, then bind to CB_1 receptors and finally modulate neurotransmission (reviewed in [20–22]).

As the predominant expression of CB_1 receptors is on GABAergic neurons at presynaptic sites, activation of CB_1 receptors inhibits the release of GABA from presynaptic terminals (reviewed in [20,21]). CB_1 receptor function was also described on glutamatergic terminals of corticostriatal projections [23,24], and transcripts encoding CB_1 receptors were shown to be present in glutamatergic neurons of the hippocampus and amygdala [25]. Thus, the overall effect after CB_1 receptor activation can be a reduction of both the inhibitory and the excitatory transmission [26].

3. Cannabinoids: aggravation or improvement of epilepsy?

The use of *Cannabis* for the treatment of various diseases has a long history reaching back about 5000 years [27]. The treatment of epileptic seizures was mentioned in the 15th century, where the medication of hashish was reported to cure the sick son of the Caliphate Councillor in Baghdad [28]. In the early nineteenth century, W. B. O'Shaughnessy, an Irish scientist and physician working at the Medical College of Calcutta, investigated the drug's

impact on many maladies, and described the use of hashish as analgesic, anti-convulsing, anti-spasmodic and anti-emetic [28]. After these observations were published in 1842, the medicinal use of *Cannabis* expanded rapidly. In 1890, J.R. Reynolds, a prominent British neurologist, reported the role of *Cannabis* in the therapy of epilepsy [29]. At that time, however, the peak of *Cannabis* as a prescribed medicine and home remedy had already passed. Finally, its use was prohibited in the early twentieth century in the USA. and later in other countries. After the discovery of the active component of marijuana in 1964 as Δ^9-THC [12], several clinical studies and case reports investigated its effects on epileptic seizures, using naturally occurring cannabinoids. Disappointingly, no convincing randomized controlled trials have been performed so far. Nevertheless, it is worth mentioning a few results from clinical trials and patient's reports (for reviews see also [10,11,30]).

3.1. Cannabidiol

This is a major constituent of marijuana, but it does not bind to cannabinoid receptors and is not psychotropic [31]. The molecular targets of cannabidiol have not been identified yet [32]. Small clinical studies examined the effect of cannabidiol and found that cannabidiol treatments were able to reduce seizure frequencies as compared to placebo treatments, while other studies did not observe any differences between cannabidiol and placebo treatments [11,33,34]. In a randomized controlled study with 16 patients, the effect of cannabidiol on epilepsy frequency was tested [33]. Using 200–300 mg cannabidiol per day for 4.5 months, in addition to the already prescribed anti-epileptic medication, four out of eight cannabidiol-treated patients were without seizures during the time of medication, while only one out of eight patients in the placebo group showed reduced seizure frequency during this time.

3.2. Marijuana and Δ^9-THC

No large double-blinded studies have evaluated marijuana or Δ^9-THC in the treatment of epilepsy patients. Only clinical anecdotes and single case reports are currently available. An epidemiological study on patients hospitalized after the first *grand mal* convulsion in their lives evidenced that marijuana use is a protective factor against seizures [35]. In informal interviews of 215 patients with active epilepsy (i.e. within the past 5 years or current use of anti-epileptic drugs) who have used marijuana regularly or intermittently, 90.2% of the patients did not report any relationship between marijuana use and seizure frequency, while 7.4% of the patients believed in a reduction of seizure frequency, and 2.3% reported an increased seizure frequency [11]. Two case reports described a reduced seizure frequency after marijuana use [36,37], while another case report suggested a positive correlation between smoked marijuana and the occurrence of seizures [38].

Taken together, the limited evidence suggests that marijuana, Δ^9-THC and cannabidiol may have anti-epileptic effects in some individuals. Cannabinoids may be used as an adjunctive medicine to prevent epileptic seizures. Some patients may find that they can reduce dosage of other seizure-control medication while using *Cannabis*. However, these reports do not allow stringent conclusions.

4. The role of the endogenous cannabinoid system: insights from animal models

During the last three decades, many investigations focussed on the application of exogenous cannabinoids in animal models of seizure. Similarly to the results obtained from human studies, cannabinoids exerted both pro- and anti-convulsive activities, depending on the model used (for review see [10,11]).

During the recent few years, several investigations have addressed the role of the endogenous cannabinoid system in the control of neuronal excitability and seizure threshold in vivo. These studies have helped to further understand the ambiguous activities of cannabinoids. Several selected studies are discussed below in detail.

4.1. Febrile (fever-induced) seizure

In this model, rats are exposed on postnatal day 10 to heat for about 30 min (core temperature is increased to 41–42 °C), and are then analyzed several weeks later. It was found that treated rats displayed alterations in inhibitory neurotransmission in the hippocampus that lasted into adulthood [39]. This change in GABA transmission was attributed to a potentiation of endocannabinoid signalling that was caused by an increase in the number of presynaptic CB_1 receptors on hippocampal GABAergic interneurons [40]. CB_1 receptor-mediated short-term suppression of GABA transmission (also called depolarization-induced suppression of inhibition, DSI) [20] was selectively enhanced, but no changes of glutamatergic transmission were observed. These results implicate a protective role of the endogenous cannabinoid system in this particular form of seizure. It remains to be investigated whether a pharmacological enhancement of the endogenous cannabinoid system helps to alleviate febrile seizures, and whether the blockade of CB_1 receptor function worsens febrile seizures.

4.2. Maximal electroshock model of seizure initiation and spread

In this rat model, an electric current is applied via the cornea to produce tonic hind limb extension. The suppression of the hind limb extension is considered as a measure

of the anti-convulsive activity of a tested compound. In a series of experiments, it was established that anandamide (EC_{50} = 50 mg/kg, intraperitoneal injection) and the metabolically stable analogue of anandamide, O-1812 (EC_{50} = 1.5 mg/kg, intraperitoneal injection), rendered dose-dependently anti-convulsive effects in a CB_1 receptor-mediated mechanism, as the CB_1 receptor antagonist SR141716A abolished this protection [41].

4.3. Pilocarpine model of temporal lobe epilepsy

In this model, a *status epilepticus* (i.e. a continuous or repetitive seizure activity for at least 30 min without regaining consciousness) is induced in rats by a single intraperitoneal injection of pilocarpine, a muscarinic acetylcholine receptor agonist. After 30 min, the *status epilepticus* is terminated by several injections of diazepam to activate $GABA_A$ receptors [42]. The seizure activity induces permanent neuronal plasticity changes, a process called epileptogenesis, resulting in a neuronal hyperexcitability and a recurrent seizure activity. This model is reported to resemble to the pathology in humans with epilepsy. Both pathologies display similar patterns of neuronal injury in the hippocampus and persistent spontaneous recurrent seizures [43]. It is also reported that this model represents a refractory epileptic condition that is not readily treated by conventional anti-convulsions [44]. In the study by Wallace et al. [42], the seizure activity was measured for several days to a few weeks in the freely moving rats by electroencephalographic and video monitoring. During this time, various pharmacological treatments were performed, including injections of CB_1 receptor antagonists, CB_1 receptor agonists and/or clinically used anti-convulsive drugs. The major findings were as follows: (i) treatments of epileptic rats with CB_1 receptor agonists strongly reduced seizure frequency; (ii) CB_1 agonists were more efficient in the reduction of seizure frequency than phenobarbital and phenytoin, two clinically used anti-consultants; (iii) pharmacological blockade of CB_1 receptors increases seizure frequency; and (iv) seizure activity increased the levels of the endocannabinoid 2-arachidonoyl glycerol and of CB_1 receptor protein in the hippocampus. These findings suggest that the increase of intracellular calcium levels, a hallmark of epilepsy [45], is able to induce the synthesis of 2-arachidonoyl glycerol [46], which in turn lowers intracellular calcium levels again by the activation of CB_1 receptors. Consequently, neurons are less excitable. It appears that CB_1 receptors act in a negative feedback mechanism to guard excitability. Remarkably, exogenous CB_1 agonists were able to support the effects of the endocannabinoids in this model of epilepsy. However, the dose used in these experiments was rather high (10 mg/kg Δ^9-THC, intraperitoneal injection) and induced psychotropic effects. Nevertheless, these findings are very interesting, and it remains to be investigated whether long-term treatments with CB_1 receptor agonists are feasible and whether hyperexcitability after withdrawal or tolerance would develop.

4.4. Kainic acid model of excitotoxic epileptiform seizures

In this model, kainic acid (KA) is systemically applied to mice or rats to induce strong activation of excitatory pathways, leading to acute seizures. The hippocampus appears to be centrally involved in the generation of seizures [47]. Using this model, CB_1 receptors were shown to be essential for the protection against seizures [48]. CB_1 receptor-deficient mice were more susceptible to KA-induced seizures than wild-type littermates. In wild-type mice, KA induced a transient increase in the levels of the endocannabinoid anandamide, but not of 2-arachidonoyl glycerol and palmitoyl ethanolamide (an endocannabinoid-related compound), while pharmacological prolongation of anandamide action using the anandamide uptake inhibitor UCM707 was protective against KA-induced seizures. This suggests that the endogenous cannabinoid system is activated on demand when KA induces strong neuronal activation. Importantly, using another mutant mouse line lacking CB_1 receptors specifically in all principal forebrain neurons, it was found that CB_1 receptors expressed on glutamatergic hippocampal neurons predominantly mediate the protective effects of the endogenous cannabinoid system, as these conditional mouse mutants showed the same susceptibility to KA-induced seizure as animals lacking CB_1 receptor in all the cells of the body. Consistently, UCM707 was unable to provide any protective effect in the conditional mutants. Furthermore, the excitation of hippocampal glutamatergic neurons was also increased in conditional CB_1 receptor mutant mice after KA treatment as compared to control mice, indicating a hyperexcitability of hippocampal glutamatergic neurons lacking CB_1 receptor. It was also shown that known neuroprotective signaling pathways were not properly induced in these mutants. In particular, phosphorylation of p42 and p44 extracellular signal regulated kinases (ERKs) and the transcription of the c-fos, zif268 and BDNF genes were not induced to the same degree in mutants as in wild-type littermates. In a follow-up study, using organotypic hippocampal explants, BDNF was shown to mediate, at least in part, the CB_1 receptor-dependent neuroprotection against KA-induced excitotoxicity [49]. In summary, these recent investigations established (i) that CB_1 receptors expressed on glutamatergic neurons are important mediators of the protective function in the KA model of epileptiform seizures, (ii) that the endogenous cannabinoid system is activated on demand when strong neuronal activation occurs, and (iii) that BDNF is a central mediator of the long-term neuroprotective function (Fig. 1).

These findings may also explain the results recently obtained from experiments using FAAH-deficient mice

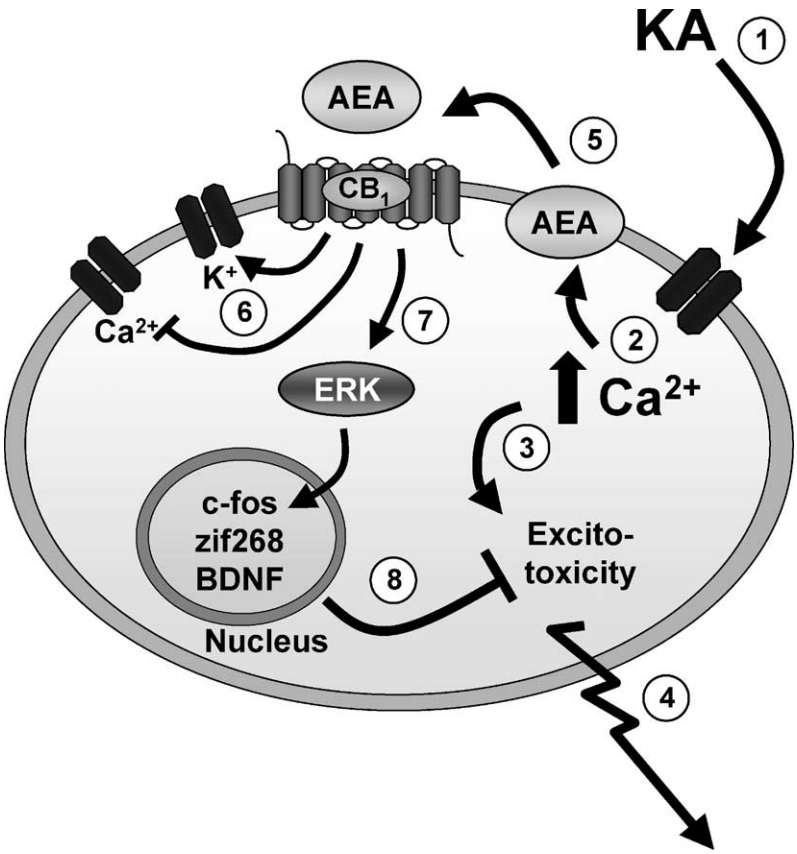

Fig. 1. Schematic representation of the mechanisms involved in CB_1 receptor-dependent protection from excitotoxicity and seizures. In the model of kainic acid (KA)-induced epileptiform seizures, KA activates KA receptors (1), leading to an increase in intracellular calcium ion concentration (2), which then induces excitotoxic events (3). The strong neuronal depolarization will activate interconnected neurons, and seizures may spread (4). Intracellular pathways will also be induced, leading to neuronal cell damage or even death. As a protective mechanism, the increase of calcium (2) induces the on-demand synthesis of anandamide (AEA) (5), which will be released to activate in an autocrine or paracrine manner CB_1 receptors on forebrain principal neurons. Specifically, in hippocampus and cerebral cortex, CB_1 receptors are expressed on glutamatergic neurons. CB_1 receptor activation leads to an inhibition of calcium channels and stimulation of potassium channels to decrease neuronal excitability (6). CB_1 receptors also activate the extra cellular signal regulated kinase pathway (ERK) (7), which leads to the transcriptional stimulation of immediate early genes encoding the transcription factors c-fos and zif268, and the neurotrophin brain-derived neurotrophic factor (BDNF). These gene products will be able to counteract damaging excitotoxic event (8). Taken together, the endogenous cannabinoid system constitutes an on-demand protection system against excessive neuronal activities.

and using mice treated systemically with CB_1 receptor agonists in the KA model of epilepsy [50]. Here, both genetic enhancement of the endocannabinoid levels and systemic activation of CB_1 receptors by exogenously applied CB_1 agonists resulted in an increased susceptibility to KA-induced seizures. It appears that the lack of protection in these two models is due to the fact that the activation of the endogenous cannabinoid system was not cell-type specific (i.e. CB_1 receptors on both glutamatergic and GABAergic neurons were activated) and not in the appropriate time course (i.e. CB_1 receptors were activated for a long period of time). Thus, from the therapeutic point of view, the most promising strategy to convey protection against seizures is the enhancement of the on-demand action of endocannabinoids by a treatment with anandamide uptake inhibitors. To the present, the anandamide transporter has not yet been cloned, but such a putative protein seems to be distinct from the anandamide-degrading enzyme fatty acid amide hydrolase (FAAH) [51–53] and appears to facilitate the transport of anandamide across the membrane (for further discussion see also [16]). Several uptake inhibitors have been characterized, including AM404 [54], UCM707 [55], and OMDM-1 and OMDM-2 [56] (Fig. 2). It is interesting to note that uptake inhibitors evoke much less or even no psychotropic effects as compared to CB_1 receptor agonists, as shown for UCM707 [55], and OMDM-1 and OMDM-2 [56]. This is very promising for the possible therapeutic use of such compounds.

5. Perspectives

It has not yet been established by large clinical studies in humans whether CB_1 receptor agonists or the non-psychotropic cannabidiol are beneficial in the treatment of epilepsy. CB_1 receptor agonists have been shown to be both pro- or anti-convulsive, and the reasons for this have not been fully understood yet. Cannabidiol has been reported either to have no effect or to be beneficial.

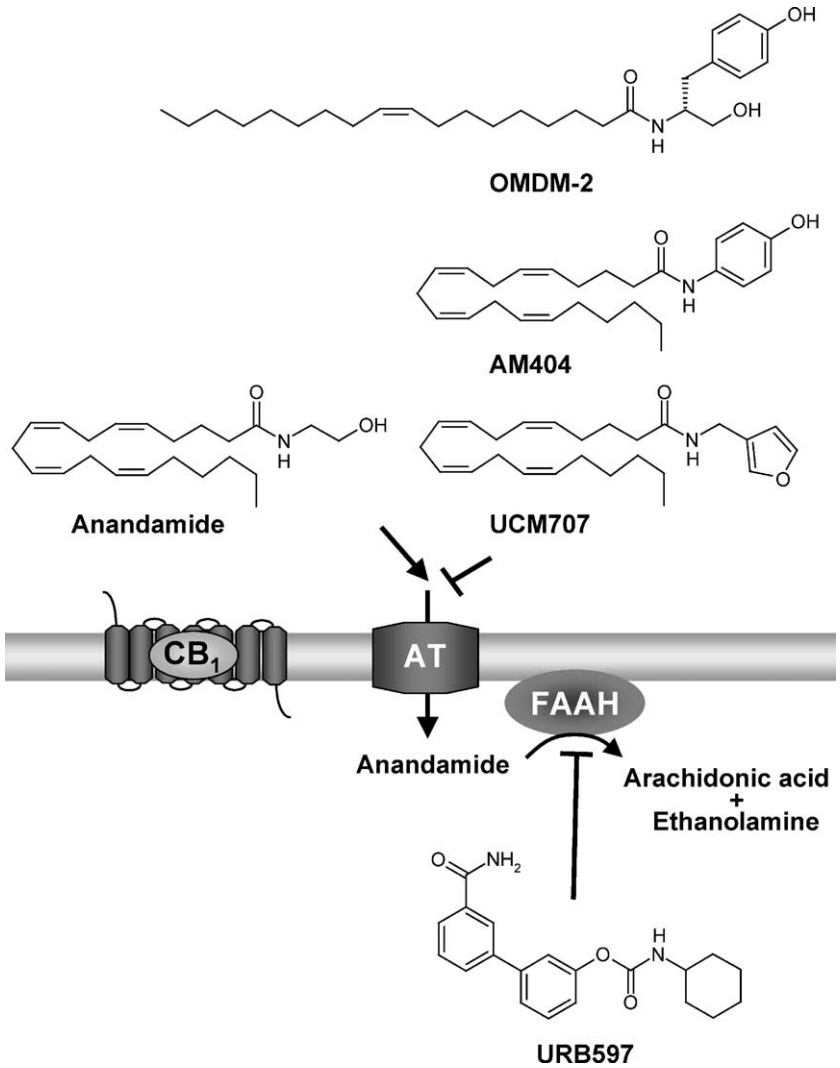

Fig. 2. Chemical structures of inhibitors of endocannabinoid inactivation. The endocannabinoid anandamide, which is released on demand into the extracellular space, binds to CB_1 receptors. To terminate its effect, anandamide is degraded intracellulary by the degrading enzyme fatty acid amide hydrolase (FAAH). An anandamide transporter (AT) facilitates the uptake of anandamide into the cell. OMDM-2, AM404 and UCM707 are inhibitors of AT, while URB597 inhibits FAAH.

The various recent studies conducted in animal models of seizures, however, have helped to better understand the controversial results obtained from human studies. As CB_1 receptors are present in two very different neuronal subpopulations (i.e. inhibitory GABAergic neurons and excitatory glutamatergic neurons), and as the activation of CB_1 receptors generally reduces the release of neurotransmitters, the systemic application of CB_1 agonists may not target the appropriate neuronal subpopulation containing CB_1 receptors to induce a protective effect. In fact, the opposite may happen, and the treatment will even worsen the seizure frequency, if GABAergic transmission is diminished. It appears furthermore that the endogenous cannabinoid system is activated during seizures in a distinct time course. Thus, prolonged application of CB_1 receptor agonists may not be beneficial and may lead to a down-regulation of CB_1 receptor levels (reviewed in [19,57]). As the endogenous cannabinoid system constitutes an on-demand protective system, the most promising strategy will be to increase the activity of endocannabinoids at the sites where this system has been activated. This is most likely to be accomplished by using anandamide uptake inhibitors, such as UCM707, as exemplified in the protection from KA-induced seizures by this compound [48]. It has not yet been tested whether inhibition of FAAH, e.g. using the compound URB597 [58] (Fig. 2), provides protection against seizures. The increased susceptibility to KA-induced seizures in FAAH-deficient mice [50] would suggest that the treatment with URB597 may not be protective, but further investigations are needed.

Currently, it is not known why cannabidiol is able to alleviate seizure frequency in certain conditions. The mechanism of action of cannabidiol remains to be investigated. Furthermore, the different functional properties of the two major endocannabinoids anandamide and 2-ara-

chidonoyl glycerol in neuroprotection have not been understood yet. Apparently, most if not all activities of anandamide regarding neuroprotection are mediated by CB_1 receptors, as the other ''anandamide receptor'', the vanilloid receptor type 1 (called VR1, or TRPV1), has so far not been implicated in neuroprotection [59,60]. It is worth mentioning that the involvement of the endogenous cannabinoid system was also investigated in various other animal models of excitotoxicity. In these experiments, remarkable species differences and age dependencies have been reported (reviewed in [61]; discussed also in [48,62]). It will be challenging to understand these intriguing and partly conflicting findings in more detail.

To date, over 70 epilepsy susceptibility genes have been found in humans [63]. However, CB_1 receptors and other components of the endogenous cannabinoid system have not yet been found to be mutated in epilepsy subjects. Thus, an extensive analysis regarding small nucleotide polymorphisms (SNPs) [64] in the genes encoding components of the endogenous cannabinoid system is warranted. Also, as epilepsy has different origins, such an analysis may also provide some information on a classification of those forms of epilepsy that might be treatable with cannabinoids. Thus, the recent progress in cannabinoid research has helped to understand better how the endogenous cannabinoid system controls seizure threshold and neuronal excitability. Whether or not it will be eventually possible to apply this knowledge to clinical applications, such as for the treatment of epilepsy, will need, however, much more research.

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