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Short communication

Endocannabinoid system protects against cryptogenic seizures

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Abstract:
Effects of the cannabinoid antagonist rimonabant on the EEG were investigated in healthy, non-epileptic rats. The drug was administered orally at 30 mg/kg/day for 3 weeks. The EEG was recorded continuously. In 3 out of 13 rats, limbic convulsive seizures, which were not related to the time of drug administration, were observed after 5–8 days. We hypothesize that an accumulation of micro-injuries in the brain is responsible for these "spontaneous" seizures.

Key words:
cannabis, rimonabant, endocannabinoid system, epilepsy, cryptogenic seizures

Introduction

The effects of the long-term use of cannabinoids are of current scientific and clinical interest. Rimonabant (SR141716A, Acomplia®, Zimulti®), which has been licensed for the treatment of overweight adult patients [11], was withdrawn from the market in January 2009 because a number of cases of depressive disorders had been reported that were ascribed to its use [12]. However, a plea for the continuation of clinical research on cannabinoid antagonists was immediately heard [7] because the drugs have high therapeutic potential for the treatment of metabolic disorders. With respect to epilepsy, it was stated that rimonabant should be used with caution [11]. In line with this recommendation, Katona and Freund [16] expressed their concern that cannabinoid antagonists might counteract the beneficial effects of endocannabinoids in individuals with a history of convulsions. Indeed, the cannabinoid system has been shown to have a vital role in dampening the effects of pro-epileptic events and *vice versa*: agonists of cannabinoid receptor type 1 (CB1) retard the development of kindling [3]. In the pilocarpine rat model for epileptogenesis, in which chronic seizures develop days after an initially acute status epilepticus, cannabinoid agonists completely abolished the late "spontaneous" epileptic seizures, whereas rimonabant increased both the seizure frequency and duration [31]. Mutant mice, lacking CB1 receptors in the hippocampus, are more vulnerable to kainic acid-induced seizures than their

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wild-type counterparts [1, 18], while endocannabinoid enhancement protected against kainic acid-induced seizures [15]. In addition, rimonabant induced status epilepticus-like activity in a neuronal culture model of acquired epilepsy [5].

Herein, we report that 23% of healthy rats, which neither were prone to epilepsy nor had spontaneous seizures of any type, experienced severe convulsive seizures after a few daily doses of the cannabinoid antagonist rimonabant. Therefore, our observation compels us to issue a warning about *de novo* seizures in non-epileptic but healthy subjects using cannabinoid antagonists.

Experimental procedures

The study was performed in accordance with the guidelines of the European Community for the use of experimental animals and was approved by the ethical committee for animal studies (RUDEC-2008-020). Adult female Crl:WI Wistar rats (Charles River Laboratories, Sulzfeld, Germany) that were 8–9 weeks old at the start of treatment were administered daily, by gavage, pure vehicle (a semi-solid oral solution with Cremophor) or vehicle containing rimonabant at a dose of 30 mg/kg. Recordings of controls were taken from our database to reduce the number of animals needed. The half life of rimonabant in rats is 7.3 h. With once-daily dosing, steady state pharmacokinetics is reached within one week [11]. Female animals were used because they have a higher bioavailability of the drug than males [11]. Rimonabant was kindly donated by Solvay Pharmaceuticals Weesp, The Netherlands.

Two experiments were conducted. In the first explorative experiment, rats (n = 6 with rimonabant) were observed daily at various times during the day for 20 days. The observed behavioral seizures in this experiment caused us to conduct the next experiment. In this second experiment, rats (n = 13) were equipped with a permanent tripolar EEG electrode allowing free movement during recording. The active electrode was in the hippocampus, mm relative to bregma: AP: –4.2; ML: –3.6; and –4.1 under skull surface; the reference and ground electrodes were located above the cerebellum. EEGs (band pass 1–100 Hz, 512 samples/s) were registered continuously from day 0 (24 h baseline) until days 11 to 21 of treatment. Video recordings were made simultaneously.

Results and Discussion

In the first experiment, two episodes of convulsions were observed in one of the six rats (on days 15 and 20). In the second experiment, 3 out of the 13 animals showed convulsive seizures, simultaneously behaviorally and on the EEG (Fig. 1a–e). We observed severe bilateral clonic muscle twitches of the forelimbs and the facial area and occasionally of the hind limbs, with full rearing in a kangaroo-like posture with the neck curled backwards and a rigid curled tail, Racine stage 5 [26]. In one animal, three short seizures were observed on day 5, each with a duration of about half a minute. In another animal, 6 seizures were observed on day 8. The duration of these seizures varied between 20 and 125 s (Fig. 1). A third animal had three seizures: one on day 5 with a duration of about 1 min, one on day 8 and one on day 9, each with a duration of about 3 min. The occurrence of the seizures was scattered over day and night between 3 and 23 h after drug administration. It is notable that multiple interic-

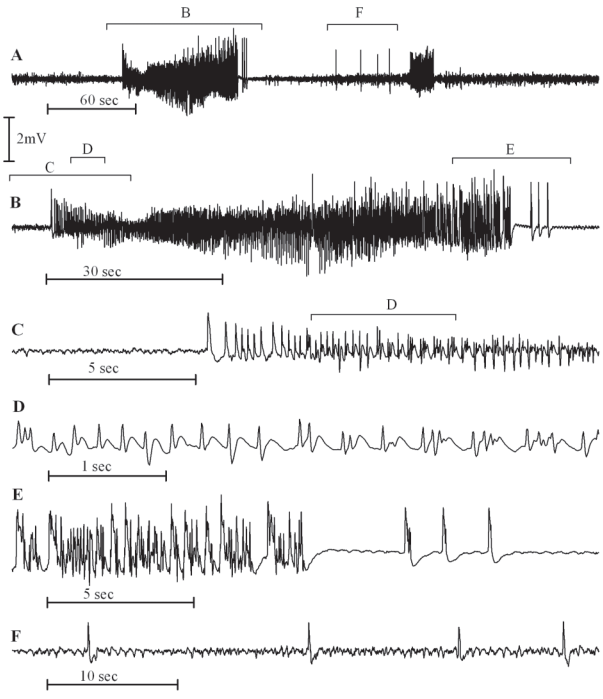

Fig. 1. Electrophysiologically recorded hippocampal seizure activity. The seizure was registered on day 8 of treatment with rimonabant, 10 h after the last gavage (at approximately 9:00 pm). (**A**) A fragment of about 5 min. (**B**) The seizure had a total duration of approximately 85 s. (**C**) Seizure onset was acute. (**D**) A clear spike and wave activity were observed. (**E**) After the seizure ended, the EEG showed post-ictal depression. (**F**) Interictal spikes were observed in this animal only

tal spikes were observed in the animal with 6 seizures (Fig. 1a and 1f). In our database with matched controls, none of the 14 animals gavaged with pure vehicle for up to 6 months showed any behavioral seizure during this time period, nor was any seizure seen in repeated 24 h EEG recordings.

The number of animals with seizures in the rimonabant group (3 out of 13 animals) was higher than that in the control group (0 out of 14 animals) (Barnard's test comparing two independent binomial proportions, one sided $p = 0.04$, StatXact, 9) [21, 27].

European Medicines Agency (EMEA) documents have reported behavioral convulsions in rodents and in macaques, but these documents neither confirm nor deny pre-existing seizures or seizure susceptibility [11]. Moreover, the epileptic nature of these convulsions was not confirmed by EEG recordings. It has even been stated that no adverse effects of rimonabant treatment were observed on the EEG patterns. We, however, not only observed spontaneous behavioral seizures but also confirmed their epileptic nature by EEG. To ascribe a cause to the seizures, in the EMEA documents, it is stated that in some, but not all, cases, the initiation of convulsions appeared to be associated with procedural stress such as handling of the animals [11]. In our study, however, the seizures had no temporal relationship to the administration of the drug or with any other observable external stress.

We propose another cause for the observed convulsions, which stems from the mechanism of the endocannabinoid system. It has been postulated that active CB1 receptors on excitatory terminals provide neurons with on-demand protection against the consequences of a variety of injuries [8, 20, 22–24, 28]. In view of this, we hypothesize that a normally functioning brain is subject to continuous micro-threats of, for example, oxidative or hypoxic nature and that these threats are warded off by the endocannabinoid system. Elimination of this protection system might lead to an accumulation of micro-injuries, and these micro-injuries might lead to spontaneous seizures by triggering an endogenous kindling process. This hypothesis would explain the individual differences in the expression of the seizures during treatment with the CB1 antagonist rimonabant (seizures were observed in only 3 out of 13 animals) as well as the difference in the time delay after starting the treatment (5 to 9 days in the measured group). This hypothesis also fits in with the observation that the incidence of epilepsy in humans increases with age, especially the incidence of "idiopathic" seizures [4, 10]. It has been suggested that the etiology of age-related seizures is often cryptogenic rather than idiopathic; thus, an underlying non-genetic cause is suspected [17]. Since the limbic system is especially vulnerable to injuries [19], it is not surprising that it was limbic seizure that was observed in our animals. In humans, age-dependent seizures are indeed often of a limbic nature [14, 25].

Results from animal models for brain injuries are in line with this hypothesis as well. In a model of chronic brain injury, viral encephalopathy, rimonabant induced spontaneous seizures [29]. Moreover, impairment of endocannabinoid synthesis increased the seizure susceptibility [30]. G protein $G\alpha q/G\alpha 11$ knockout mice, which have impaired endocannabinoid synthesis, showed spontaneous epileptic seizures. It is notable that both the frequency of seizures in each animal and the number of affected animals increased with age [30].

Numerous reports show that the role of the endocannabinoid system is far from clear, and contradictory results have been reported concerning its protective role; e.g., although controlled modulation of fatty acid amide hydrolase (FAAH), the enzyme metabolizing the breakdown of endogenous cannabinoids, promotes protective cannabinergic signals, FAAH knockout mice exhibit proconvulsant activity [15]. Moreover, in neonatal animals both proconvulsant [2] and protective effects [9] of CB1 receptor blockade have been reported.

In clinical trials reported in the EMEA documents, no difference was seen in the incidence of seizures in patients receiving rimonabant or placebo [11]. However, the US Food and Drug Administration (FDA) document NDA 21-888 [13] mentions 11 possible cases of seizures among participants in clinical trials. Rimonabant has been withdrawn from the market, but new cannabinoid antagonists will enter development [6, 7]. Clinical vigilance for any future clinically applied CB1 antagonist should also focus on this severe adverse effect, even in non-epileptic human users. We hypothesize that the endocannabinoid system is crucial in the protection of the normal brain against the development of seizures.

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