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Inhibition of monoacylglycerol lipase mediates a cannabinoid 1-receptor dependent delay of kindling progression in mice

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ABSTRACT

Endocannabinoids, including 2-arachidonoylglycerol (2-AG), activate presynaptic cannabinoid type 1 receptors (CB1R) on inhibitory and excitatory neurons, resulting in a decreased release of neurotransmitters. The event-specific activation of the endocannabinoid system by inhibition of the endocannabinoid degrading enzymes may offer a promising strategy to selectively activate CB1Rs at the site of excessive neuronal activation with the overall goal to prevent the development epilepsy.

The aim of this study was to investigate the impact of monoacylglycerol lipase (MAGL) inhibition on the development and progression of epileptic seizures in the kindling model of temporal lobe epilepsy.

Therefore, we selectively blocked MAGL by JZL184 (8 mg/kg, i.p.) in mice to analyze the effects of increased 2-AG levels on kindling acquisition and to exclude an anticonvulsive potential.

Our results showed that JZL184 treatment significantly delayed the development of generalized seizures ($p = 0.0066$) and decreased seizure ($p < 0.0001$) and afterdischarge duration ($p < 0.001$) in the kindling model of temporal lobe epilepsy, but caused only modest effects in fully kindled mice. Moreover, we proved that JZL184 treatment had no effects in conditional CB1R knockout mice lacking expression of the receptor in principle neurons of the forebrain.

In conclusion, the data demonstrate that indirect CB1R agonism delays the development of generalized epileptic seizures but has no relevant acute anticonvulsive effects. Furthermore, we confirmed that the effects of JZL184 on kindling progression are CB1R mediated. Thus, the data indicate that the endocannabinoid 2-AG might be a promising target for an anti-epileptogenic approach.

Introduction

Anandamide and 2-arachidonylglycerol (2-AG), the two most studied endocannabinoids in the brain, are small lipid molecules, which retrogradely traverse the synapse and act presynaptically on metabotropic cannabinoid type 1 receptors (CB1Rs) (Kreitzer and Regehr, 2001; Maejima et al., 2001; Wilson and Nicoll, 2001). The activation of CB1Rs inhibit adenylyl cyclase and voltage-gated Ca^{2+} channels, resulting in a decreased release of neurotransmitters, thus modulating neuronal excitation and inhibition (Bidaut-Russell et al., 1990; Sugiura et al., 1997; Lauckner et al., 2005). As a consequence, the activation of the endocannabinoid system may be a safeguard against hyperexcitability, acute seizures and excitotoxity (Ameri and Simmet, 2000; Marsicano et al., 2003; Monory et al., 2006), raising the idea to slow down or even to prevent the development or progression of epilepsy. This is of particular interest, when considering that during excessive excitation endocannabinoid signaling has stronger effects on cortical principal neurons than on GABAergic interneurons (von Rüden et al., 2015). Therefore, the endocannabinoid system is discussed as a potential target for the prevention of epilepsy (Hofmann and Frazier, 2013). The process of epilepsy development (= epileptogenesis) refers to molecular and cellular alterations, which transform a physiological neuronal network into an epileptic state, with an increased risk of recurrent spontaneous seizures (Goldberg and Coulter, 2013). The detailed etiology of epileptogenesis and if the endocannabinoid system may be involved in epileptogenesis remains incompletely understood.

There are only few experimental studies in rodents which indicate anti-epileptogenic properties of direct CB1R activation (Bhaskaran and Smith, 2010; Wendt et al., 2011). Recently, we evaluated the impact of the CB1R agonist WIN55.212-2 as well as of the indirect agonist URB597 on kindling progression (Wendt et al., 2011). WIN55.212-2

Abbreviations: 2-AG, 2-arachidonylglycerol; CB1R, cannabinoid type 1 receptor; MAGL, monoacylglycerol lipase; SS, seizure severity; SD, seizure duration; ADD, afterdischarge duration; ADT, afterdischarge threshold; Cum ADD, cumulative afterdischarge duration.

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delayed kindling acquisition whereas URB597, which inhibits the catabolic enzyme fatty acid amide hydrolase and thereby reduces the degradation of anandamide, had no effect on the generation of a hyperexcitable neuronal network. In contrast to anandamide, 2-AG acts as a full agonist on CB1Rs and exhibits higher CB1R efficacy (Sugiura et al., 1999, 2000, 2006). A molecular and morphological study of mossy cell-granule cell synapses revealed that 2-AG mediates retrograde signaling at these synapses and may reduce the excitability of granule cells and prevent seizures (Uchigashima et al., 2011).

Although these experimental data are only limited, they strongly suggest a potential therapeutic role for the endocannabinoid 2-AG in epilepsy. However, there is, to our knowledge, no information about the impact of 2-AG on seizure thresholds or on the progression of seizure development in epilepsy. Considering this information, it is of particular interest to evaluate whether increased 2-AG levels affect these parameters in a chronic epilepsy model with excellent predictive validity for temporal lobe epilepsy.

The aim of this study is to evaluate if pharmacological inhibition of monoacylglycerol lipase (MAGL) by JZL184 has an impact on the progression of epileptic seizures in the kindling mouse model of temporal lobe epilepsy and to demonstrate that JZL184-mediated effects are CB1R dependent. For this purpose, the indirect CB1R agonist JZL184 was used. JZL184 potently and selectively inhibits the 2-AG degrading enzyme MAGL and this results in increased 2-AG levels up-to an 8-fold (Long et al., 2009). This strategy offers a clear benefit compared with direct CB1R agonism namely event-specific effects, which are based on inhibition of the endocannabinoid degrading enzyme at CB1Rs localized on excessively activated glutamatergic synapses.

Methods

Animals

Animal experiments have been performed in accordance with the EU directive 2010/63/EU and with the German Animal Welfare act. They were approved by the responsible government (license numbers 55.2-1-54-2532-93-11 and 55.2-1-54-2532-173-11). Male mice with a body weight of 21–15 g were used in all experiments and maintained in standard conditions with food and water ad libitum (24–25 °C; humidity 50–60%; lights on from 7 am to 7 pm). NMRI mice were acquired from Harlan Netherlands (Horst, Netherlands). $CB1^{f/f;CaMKII\alpha Cre}$ mice (CamK-CB1 KO), and their respective wild-type littermate controls (CamK-CB1 WT) were bred at the Max Planck Institute of Biochemistry (Martinsried, Germany). These mice were derived from cre-negative mothers and cre-positive fathers (CamK-CB1) and were genotyped by PCR as described previously (Marsicano et al., 2003). They lack CB1Rs in principal forebrain neurons. All mice were allowed to habituate to the new environmental conditions for at least 1 week.

Electrode implantation

Stereotactical implantation of the kindling electrode into the right amygdala was performed as described previously (Jafari et al., 2012). The electrode consists of teflon-isolated stainless steel with a diameter of 280 μm. Mice were anesthetized with chloral hydrate (400 mg/kg in 10 ml saline i.p., Merck KGaA, Darmstadt, Germany) and bupivacaine (5 ml/kg s.c., Jenapharm®, Mibe, GmbH, Brehna). For analgesia meloxicam (1 mg/kgm, s.c. Metacam®, Boehringer-Ingelheim, Ingelheim, Germany) was administered 30 min prior to and 24 h post-surgery. The stereotaxic coordinates in millimeter relative to bregma according to the atlas of Paxinos and Franklin (2005) were AP -1.0, L +3.2, DV -5.3 (NMRI) and AP -1.2, L +3.5, DV -5.2 (CamK-CB1). Seven mice were euthanized following surgery due to alterations in their general condition.

Kindling

Kindling of mice was initiated following a postsurgical recovery period of 2 weeks. During the experiments, body weight varied between 30 and 50 g (NMRI) and between 20 and 35 g (CamK-CB1 WT and KO). Mice were housed separately.

The initial afterdischarge threshold (initial ADT) was determined for each animal using an ascending stair-step procedure with an initial current of 8 μA and an increase by 20% of the previous current every minute until afterdischarges were elicited (Pekcec et al., 2007).

The amygdala was electrically stimulated once daily, five times per week with 700 μA (1 ms, monophasic square-wave pulses, 50 Hz for 1 s). The seizure severity was scored according to the Racine scale (Racine, 1972): (1) mouth and facial movements, (2) head nodding, (3) forelimb clonus, (4) rearing and (5) rearing and falling. In addition to seizure severity, seizure duration and afterdischarge duration were recorded for each seizure event. The cumulative afterdischarge duration was calculated as the sum of all afterdischarge durations throughout the experiment. Following kindling acquisition, we determined the post-kindling afterdischarge threshold by the stepwise procedure (Pekcec et al., 2007).

Experimental details: kindling acquisition

To minimize the impact of circadian variations, all experiments were performed within the same time of the day (1 pm to 6 pm). The initial afterdischarge threshold was determined twice on two consecutive days. Following determination of the first initial afterdischarge threshold without JZL treatment, the animals were distributed into a vehicle-treated and a JZL184-treated group with a comparable mean of the initial afterdischarge threshold and afterdischarge duration (Figs. 2A, B and Figs. 4A, B). At the following day, the second afterdischarge threshold was evaluated 60 min after drug or vehicle application. Due to the different genetic background, NMRI mice received twelve and CamK-CB1 mice seven electrical stimulations until the majority of mice in the control group exhibited generalized stage (4) or (5) seizures (Fig. 1A). At stimulation day twelve, eleven out of twelve NMRI mice and at day seven nine out of ten CamK-CB1 WT mice in the control group exhibited generalized stage (4) or (5) seizures. We expected a misplacement of the electrode in the animals not exhibiting generalized seizure activity. Subsequent analysis revealed a correct localization in the right amygdala. Thus, data from these animals were included in further analyses.

JZL184 or vehicle was administered intraperitoneally with a volume of 10 ml/kg bodyweight in a dosage of 8 mg/kg once per day 60 min prior to the electrical stimulation. Following a washout phase of 6 days, we determined the post-kindling afterdischarge threshold.

The experimental groups consisted of 12 animals per group for NMRI mice (vehicle-treated and JZL184-treated mice) and of 6–10 animals per group for CamK-CB1 mice (WT vehicle $n = 10$, WT JZL $n = 7$, KO vehicle $n = 6$ and KO JZL184 $n = 8$).

Experimental details: fully kindled mice

To minimize the impact of circadian variations, all experiments were performed within the same time period (8 am to 1 pm). Following the determination of the initial afterdischarge threshold, NMRI mice ($n = 10$) were electrically stimulated with 700 μA once daily, five times per week, until they exhibited at least ten generalized stage (4) or (5) seizures (the number of stimulations to reach this kindling criterion ranged between 10 and 31) and were considered fully kindled. Please note that known from several years of experience with different mouse strains, mice are kindled to reproducibly exhibit generalized seizures of stage (4) or (5); however, the highest seizure score (5) cannot always be elicited in fully kindled animals. In contrast, rats reliably exhibit the highest seizure score once the fully kindled state is reached.