Neurobiology of Disease 73 (2015) 334–347

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Analysis in conditional cannabinoid 1 receptor-knockout mice reveals neuronal subpopulation-specific effects on epileptogenesis in the kindling paradigm

E.L. von Rüden [a,b], M. Jafari [a,b], R.M. Bogdanovic [a,b], C.T. Wotjak [c], H. Potschka [a,*]

[a] Institute of Pharmacology, Toxicology and Pharmacy, Ludwig-Maximilians University, Munich, Germany
[b] Graduate School of Systemic Neurosciences, Munich, Germany
[c] Max Planck Institute of Psychiatry, Munich, Germany

ARTICLE INFO

Article history:
Received 4 July 2014
Accepted 1 August 2014
Available online 11 August 2014

Keywords:
Seizure
Hyperexcitable network
Ictogenesis
CB1
TRPV1
Endovanilloid
Neurogenesis
GABA
Glutamate
Amygdala

ABSTRACT

The endocannabinoid system serves as a retrograde negative feedback mechanism. It is thought to control neuronal activity in an epileptic neuronal network. The purpose of this study was to evaluate the impact of the endocannabinoid and endovanilloid systems on both epileptogenesis and ictogenesis.

Therefore, we modulated the endocannabinoid and endovanilloid systems genetically and pharmacologically, and analyzed the subsequent impact on seizure progression in the kindling model of temporal lobe epilepsy in mice. In addition, the impact of seizures on associated cellular alterations was evaluated.

Our principal results revealed that the endocannabinoid system affects seizure and afterdischarge duration dependent on the neuronal subpopulation being modulated. Genetic deletion of CB1-receptors (CB1Rs) from principal neurons of the forebrain and pharmacological antagonism with rimonabant (5 mg/kg) caused longer seizure duration. Deletion of CB1R from GABAergic forebrain neurons resulted in the opposite effect. Along with these findings, the CB1R density was elevated in animals with repetitively induced seizures. However, neither genetic nor pharmacological interventions had any impact on the development of generalized seizures. Other than CB1, genetic deletion or pharmacological blockade with SB366791 (1 mg/kg) of transient receptor potential vanilloid receptor 1 (TRPV1) had no effect on the duration of behavioral or electrographic seizure activity in the kindling model.

In conclusion, we demonstrate that endocannabinoid, but not endovanilloid, signaling affects termination of seizure activity, without influencing seizure severity over time. These effects are dependent on the neuronal subpopulation. Thus, the data argue that the endocannabinoid system plays an active role in seizure termination but does not regulate epileptogenesis.

Introduction

Temporal lobe epilepsy is the most common type of epilepsy with focal seizure onset (Tellez-Zenteno and Hernandez-Ronquillo, 2012). An initial brain-damaging insult can trigger a cascade of molecular and cellular alterations that form a hyperexcitable neuronal network and, eventually, the appearance of recurrent and spontaneous seizures (Pitkänen and Lukasiuk, 2009). The generation of this hyperexcitable neuronal epileptic network is called epileptogenesis. In order to develop new prophylactic treatment strategies, it is essential to understand the mechanisms underlying epileptogenesis.

The endocannabinoid system serves a central function as a key regulator of neuronal activity, synaptic transmission and neuronal plasticity in the central nervous system (Katona and Freund, 2008). The presynaptically located cannabinoid type 1 receptors (CB1Rs) are G protein-coupled and widely expressed within the central nervous system (Piomelli, 2003). When activated on demand by the binding of postsynaptically synthesized endocannabinoid ligands such as anandamide or 2-arachidonoylglycerol, CB1R signaling attenuates both inhibitory neurotransmission and excitatory neurotransmission (Monory et al., 2006).

The endocannabinoid anandamide can also activate transient receptor potential vanilloid (TRPV1, formerly vanilloid receptor VR1) channels (Smart et al., 2000). These channels are nonselective plasma

Abbreviations: CB1R, cannabinoid type 1 receptor; TRPV1, transient receptor potential vanilloid receptor 1; SS, seizure severity; SD, seizure duration; ADD, afterdischarge duration; ADT, afterdischarge threshold; Cum ADD, cumulative afterdischarge duration; DCX, doublecortin.

* Corresponding author at: Institute of Pharmacology, Toxicology, and Pharmacy, Ludwig-Maximilians-University, Munich, Koeninginstr. 16, D-80539 Munich, Germany. Fax: +49 89 2180 16556.
E-mail address: potschka@pharmtox.vetmed.uni-muenchen.de (H. Potschka).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2014.08.001
0969-9961/

membrane cation channels (Caterina et al., 1997). Stimulation of TRPV1 increases the release of glutamate in an activity-dependent manner (Peters et al., 2010). Thus, the duration of postsynaptic spiking is enhanced at glutamatergic synapses (Mori et al., 2012; Shoudai et al., 2010). It has been confirmed that TRPV1 modulate glutamatergic signaling not only in the rodent brain, but also in human cortical tissue (Mori et al., 2012). Therefore, targeting not only the endocannabinoid system but also the endovanilloid system may provide a promising strategy for the regulation of neuronal activity in epilepsies (Lutz, 2004).

Implying the cross-system functionality of endocannabinoids, target validation requires a comprehensive understanding of the complex neuromodulatory and protective properties of endocannabinoids against hyperexcitability and acute seizures (Hunt et al., 2012; Marsicano et al., 2003).

The detailed etiology of epileptogenesis remains unknown, and whether the endocannabinoid system is involved in epileptogenesis and promotes anti- or proconvulsant effects is still controversially discussed (Dudek et al., 2010; Echegoyen et al., 2009). This might be partly related to the dichotomous role of endocannabinoid signaling in different neuronal subpopulations. Conditional CB1R knockout mice with deletion in selected neuronal subpopulations offer an excellent opportunity to address the functional role of the endocannabinoid signaling cascade in glutamatergic vs. GABAergic forebrain neurons. Initial studies have already been performed in an acute chemical seizure model with systemic administration of kainate (Marsicano et al., 2003; Monory et al., 2006; Ruehle et al., 2013). The findings indicated that kainic acid-induced seizure severity is modulated by CB1-receptor expression on cortical glutamatergic neurons. Recently, Kow et al. (2014) reported that genetic loss of CB1Rs plays a role in pilocarpine-induced seizures and seizure severity. However, none of the studies provide information about the impact of CB1Rs on seizure thresholds or on epileptogenesis.

Considering CB1Rs as a putative target for anticonvulsant or antiepileptogenic interventions, respective information is urgently needed. In a previous study, we analyzed the impact of pharmacological CB1R agonism on kindling epileptogenesis. In this study we revealed that the direct CB1R agonist WIN55.212-2 delayed kindling acquisition whereas the indirect CB1R agonist URB597 did not affect seizure progression in the kindling paradigm (Wendt et al., 2011). These findings again raise the question about the impact of different neuronal subpopulations. Thus, we aimed to thoroughly assess the consequences of conditional CB1R-knockout in direct comparison with pharmacological blockade of CB1R on epileptogenesis in the kindling paradigm.

In view of the dual function of endocannabinoids as well as the suggested link between the endovanilloid system and epilepsy, there is only limited data from experiments with rather unspecific pharmacological tools available. Therefore, we aim to elucidate the effects of TRPV1 knockout as well as specific TRPV1 antagonism on epileptogenesis in the kindling paradigm.

In the above-mentioned epilepsy model, seizure discharges caused by electrical stimulation of the amygdala via a chronically implanted electrode lead to a progressive and permanent increase in the behavioral and electrographic seizure response (Loscher and Brandt, 2010). Thus, kindling assesses the impact of genetic and pharmacological modulation on seizure progression, which reflects the formation of a hyperexcitable network (McIntyre et al., 2002; Morimoto et al., 2004). In addition, one can precisely determine the impact on seizure thresholds before and following kindling acquisition based on application of systematically increasing stimulation intensities.

The aim of this study was to evaluate whether targeting the endocannabinoid and endovanilloid systems might (1) affect epileptogenesis (2) have an impact on ictogenesis and (3) render a basis for disease-modifying approaches.

Materials and methods

Animals and electrode implantation

Male NMRI mice were purchased at a body weight of 21–25 g (Harlan Netherlands, Horst, Netherlands). Male $CB1^{f/f;CaMKII\alpha Cre}$ mice (CamK-CB1 KO; (Marsicano et al., 2003)), $CB1^{f/f,Dlx5/6\text{-}Cre}$ mice (Dlx-CB1 KO; (Monory et al., 2006)), CB1R-null mutant mice $CB1^{-/-}$ (CB1-KO; (Marsicano et al., 2002)) as well as $VR1^{-/-}$ mice (Caterina et al., 2000) breeding pairs were obtained from The Jackson Laboratory, TRPV1-KO) and their respective wildtype littermate controls (CamK-CB1, Dlx-CB1, CB1- and TRPV1-WT) were bred at the Max Planck Institute of Biochemistry (Martinsried, Germany) and transferred with a weight of 21–25 g to our institute. CamK-CB1 KOs lack CB1Rs in principal forebrain neurons and Dlx-CB1 KOs in GABAergic forebrain neurons. CB1-KOs and TRPV1-KOs do not express the respective receptors at all. Mice were derived from heterozygous breeding pairs (TRPV1-KO and -WT) or from Cre-negative mothers and Cre-positive fathers (CamK-CB1 and Dlx-CB1). CamK-CB1 KO, Dlx-CB1 KO, CB1-KO and TRPV1-KO were genotyped by PCR using a slightly modified protocol as described previously by Casanova et al. (2001), Caterina et al. (1997, 2000), Marsicano et al. (2003) and Monory et al. (2006). The animals were kept under controlled environmental conditions (24–25 °C; humidity 50–60%; 12 h dark/light cycle, lights on from 7 a.m.–7 p.m.) with free access to water and standard laboratory food. Experimental procedures have been carried out in accordance with the EU directive 2010/63/EU for animal experiments, with the German Animal Welfare act and were approved by the responsible government (license numbers: 55.2-1-54-2532-186-09 and 55.2-1-54-2532-93-11).

To minimize the impact of circadian variations, all experiments were performed within the same time period (8 a.m. to 1 p.m.).

A teflon-isolated bipolar stainless steel electrode with a diameter of 280 μm was stereotactically implanted into the right amygdala as described previously (Jafari et al., 2012b). All animals received meloxicam injections (0.2 mg/kg, s.c., Metacam®, Boehringer Ingelheim, Ingelheim, Germany) 30 min prior and 24 h after the surgery to guarantee analgesia. Chloralhydrate (400 mg/kg in 10 ml saline i.p., Merck KGaA, Darmstadt, Germany) in combination with bupivacaine 2% (5 ml/kg s.c., Jenapharm®, Mibe, GmbH, Brehna) was used for anesthesia. The stereotaxic coordinates in millimeter relative to bregma were AP −1.0, L +3.2, DV −5.3 for NMRI mice, AP −1.2, L +3.5, DV −5.2 for CamK-CB1 mice and AP −1.4, L +3.5, DV −5.0 for Dlx-CB1 and TRPV1 mice. Following surgery, all the animals were allowed to recover for at least two weeks.

Kindling procedure

The initial afterdischarge threshold was determined following a stepwise protocol as described previously (Pekcec et al., 2007). The animals were kindled via daily electrical stimulation (1 ms, monophasic square-wave pulses, 50 Hz for 1 s), five times per week, either with a stimulation current 20% above the initial afterdischarge threshold (pharmacological modulation: rimonabant) or with 700 μA (all genetic modulations and pharmacological modulation: SB366791). In the pharmacological modulation with rimonabant the interindividual seizure thresholds varied. To exclude a bias, each mouse was stimulated with individual stimulation currents 20% above the afterdischarge threshold. For all the other experiments suprathreshold stimulation with 700 μA was used. The stimulation current of 700 μA was chosen based on the range of the initial afterdischarge seizure thresholds. In previous experiments in our lab, using individualized stimulation strength (20% above initial threshold) did not make a difference as compared to the suprathreshold stimulation with 700 μA, both, regarding the response to the first stimulation following threshold determination and regarding the kindling rate. The only difference was that kindling