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Glycine transporter 1 is a target for the treatment of epilepsy

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ABSTRACT

Glycine is the major inhibitory neurotransmitter in brainstem and spinal cord, whereas in hippocampus glycine exerts dual modulatory roles on strychnine-sensitive glycine receptors and on the strychnine-insensitive glycine$_B$ site of the *N*-methyl-D-aspartate receptor (NMDAR). In hippocampus, the synaptic availability of glycine is largely under control of glycine transporter 1 (GlyT1). Since epilepsy is a disorder of disrupted network homeostasis affecting the equilibrium of various neurotransmitters and neuromodulators, we hypothesized that changes in hippocampal GlyT1 expression and resulting disruption of glycine homeostasis might be implicated in the pathophysiology of epilepsy. Using two different rodent models of temporal lobe epilepsy (TLE) – the intrahippocampal kainic acid model of TLE in mice, and the rat model of tetanic stimulation-induced TLE – we first demonstrated robust overexpression of GlyT1 in the hippocampal formation, suggesting dysfunctional glycine signaling in epilepsy. Overexpression of GlyT1 in the hippocampal formation was corroborated in human TLE samples by quantitative real time PCR. In support of a role of dysfunctional glycine signaling in the pathophysiology of epilepsy, both the genetic deletion of GlyT1 in hippocampus and the GlyT1 inhibitor LY2365109 increased seizure thresholds in mice. Importantly, chronic seizures in the mouse model of TLE were robustly suppressed by systemic administration of the GlyT1 inhibitor LY2365109. We conclude that GlyT1 overexpression in the epileptic brain constitutes a new target for therapeutic intervention, and that GlyT1 inhibitors constitute a new class of antiictogenic drugs. These findings are of translational value since GlyT1 inhibitors are already in clinical development to treat cognitive symptoms in schizophrenia

1. Introduction

Epilepsy treatment is limited by poor response to available antiepileptic drugs and limited tolerability due to major cognitive side effects (Arif et al., 2009; Hirsch et al., 2003; Loscher and Schmidt, 2011; Ortinski and Meador, 2004). The development of therapies that combine anticonvulsant with pro-cognitive properties would therefore be a significant advancement. Glycine is the major inhibitory neurotransmitter in brainstem and spinal cord (Betz et al., 2001). In the hippocampus glycine has evolved into a homeostatic modulator of neuronal function by assuming potentially opposing activities that depend on the extracellular concentrations of glycine: *(i)* Glycine, but also D-serine, act as obligatory co-agonists of the N-methyl-D-aspartate receptor (NMDAR) by binding to its strychnine-insensitive glycine$_B$ site (Labrie and Roder, 2010). Since this binding site is normally not saturated (Bergeron et al., 1998), increases in extracellular glycine can potentiate impulse-dependent NMDAR activation, with resulting pro-cognitive effects (Black et al., 2009). The indirect modulation of NMDAR function via glycine is a promising strategy to improve cognition (Labrie and Roder, 2010; Mohler et al., 2008). Importantly, and in contrast to direct NMDAR agonism, the allosteric modulation of NMDARs through activation of the glycine$_B$ site does not bear risks for seizure generation or neurotoxicity (Yang and Svensson, 2008). *(ii)* Low concentrations of extracellular glycine (10 μM) activate presynaptic glycine receptors (GlyRs) and thereby promote pro-convulsant mechanisms (Chen et al., 2014; Kubota et al., 2010; Winkelmann et al., 2014). *(iii)* Under higher extracellular concentrations (100 μM) glycine binds to

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extrasynaptic GlyRs in the postsynaptic compartment providing tonic suppression of network excitability (Eichler et al., 2008; Kirchner et al., 2003). Together, those mechanisms implicate that low concentrations of extracellular glycine favor epileptiform activity and impairment of cognitive function. Consequently, the maintenance of glycine homeostasis plays a crucial role for the regulation of excitability in the hippocampal formation. Accordingly, *in vitro* studies demonstrated that exogenous glycine suppressed neuronal excitation in the dentate gyrus (Chattipakorn and McMahon, 2003) and reduced the firing of action potentials in hippocampal neurons (Song et al., 2006), whereas blockade of glycine-reuptake depressed excitatory postsynaptic potentials (Zhang et al., 2008).

Hippocampal glycine is largely regulated by its reuptake transporter GlyT1 found in both excitatory neurons and astrocytes (Aragon and Lopez-Corcuera, 2005; Betz et al., 2006; Cubelos et al., 2005; Eulenburg et al., 2005; Martina et al., 2005; Tsai et al., 2004). Consequently, the genetic deletion of GlyT1 increased synaptic glycine availability (Gomeza et al., 2003). Engineered mice with a genetic deletion of GlyT1 in forebrain were characterized by a decrease in hippocampal glycine uptake, an increase in hippocampal NMDAR function, and a wide spectrum of pro-cognitive effects (Mohler et al., 2011, 2008; Yee et al., 2006). Therefore, GlyT1 has emerged as a promising target for the treatment of cognitive symptoms in schizophrenia and several compounds are currently in phase II and III clinical trials (Black et al., 2009; Mohler et al., 2011; Singer et al., 2009).

Whereas the role of glycine regulation within the context of schizophrenia has received much attention, glycine may also play an underappreciated role in epilepsy. In patients with TLE changes in hippocampal glycine receptor expression have been reported, suggesting dysregulation of glycinergic signaling in epilepsy (Eichler et al., 2008). In line with those findings activation of glycine receptors modulated spontaneous epileptiform activity in the immature rat hippocampus (Chen et al., 2014), whereas GlyT1 inhibitors demonstrated anticonvulsant properties in a rat maximal electroshock test (Kalinichev et al., 2010). However, the role of GlyT1 in human epilepsy and in clinically relevant rodent models of chronic epilepsy has not been studied to date.

The present study was designed to investigate the role of GlyT1 in TLE and to evaluate whether GlyT1 inhibition might be a feasible strategy for seizure control in chronic epilepsy. Using two different rodent models of TLE and samples from human TLE patients, we demonstrate robust increases of GlyT1 in the epileptogenic hippocampus. Consequently, the genetic deletion or pharmacological inhibition of GlyT1 suppressed both induced and chronic seizures.

2. Materials & methods

2.1. Studies in mice

All animal procedures were conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Legacy Research Institute and the principles outlined by the National Institutes of Health (NIH). Eight to 10 week old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) as well as CamKIIαCre:GlyT1$^{fl/fl}$ ("GlyT1-KO") mice (Yee et al., 2006) and their wild-type littermates GlyT1$^{fl/fl}$ ("GlyT1-WT") were used. All mutant animals were in an identical C57Bl/6 background. GlyT1-KO mice are characterized by a pro-cognitive phenotype as described previously (Yee et al., 2006). All animals were housed in temperature- and humidity-controlled rooms with a 12 h light/dark cycle (lights on at 6:30 AM).

2.2. Mouse model of TLE

Chronic epilepsy in adult male C57Bl/6 mice was induced by intrahippocampal kainic acid (KA) injection according to our standard procedures (Gouder et al., 2003) with modifications. Briefly, under anesthesia with 68.5% N_2O, 30% O_2, and 1.5% isoflurane the animals received stereotactic injections into the right dorsal hippocampus (coordinates: AP = −2.10 mm; ML = ± 1.80 mm; DV = −1.70 mm to Bregma) with 400 ng of KA (K0250, Sigma, USA) in a volume of 200 nl 0.9% NaCl using a 1-μl microsyringe (Hamilton, Reno, NV, USA). Injections were performed over a period of 3 min. At the end of the injection, the cannula was left in place for an additional 3 min to limit reflux along the cannula track. Control mice received intrahippocampal injection of 200 nl saline. The KA injection triggered non-convulsive status epilepticus, which is the precipitating event for subsequent epileptogenesis. Four weeks after the injection of KA or vehicle, the animals were implanted with bipolar coated stainless steel electrodes (80 μm in diameter; Plastics One) into the right dorsal hippocampus using the same coordinates as the previous KA injection. A cortical screw electrode was placed over the frontal cortex and a ground electrode over the cerebellum. All electrodes were secured to the skull with dental cement.

2.3. Electroencephalography and drug treatment

Electroencephalography (EEG) monitoring was performed according to our previous publications (Shen et al., 2014; Theofilas et al., 2011). Six weeks after KA-injection and two weeks after electrode implantation the animals were subjected to blocks of 24 h of EEG monitoring (Grass Technologies). Electrical brain activity was amplified and digitized (PowerLab; AD Instruments). Quantification of EEG records was performed blinded to the experimental treatment. EEG seizure activity was defined as high-amplitude rhythmic discharges that clearly represented a new pattern of tracing lasting for >5 s (repetitive spikes, spike-and-wave discharges, or slow waves). Epileptic events occurring with an interval <5 s without the EEG returning to baseline were defined as belonging to the same seizure. Seizures were primarily electrographic in nature, and only occasionally accompanied by arrest or staring episodes; thus seizure quantification was performed exclusively by intrahippocampal EEG recordings. Before each recording session the animals were habituated in the recording cage for 4 h. After habituation the EEGs were recorded for 24 h. To assess the antiepileptic effect of GlyT1 inhibition the animals received daily injections with the GlyT1 inhibitor [2-(4-benzo[1,3] dioxol-5-yl-2-tert-butylphenoxy)ethyl]-methylamino-acetic acid LY2365109 (Tocris Bioscience, Bristol, UK) (3–30 mg/kg i.p, dissolved in 5% DMSO), or vehicle.

2.4. Chemoconvulsant seizure tests

To quantify the anticonvulsive effect of pharmacological inhibition or genetic disruption of GlyT1 in an acute seizure model we used standard pentylenetetrazole (PTZ) seizure tests (White, 2003; White et al., 2007) in WT and GlyT1-KO mice. PTZ (P6500, Sigma, USA) was freshly dissolved daily in sterile saline. Mice were subjected repeated PTZ-injections (10 mg/kg, i.p.) every 10 min until they reached a behavioral seizure score of 5 according to Racine (Racine, 1978). The cumulative PTZ dose (in mg PTZ per kg body weight) needed to trigger stage 5 seizures was considered the convulsant threshold dose. To quantify the anticonvulsant efficacy of GlyT1 inhibition, the GlyT1 inhibitor LY2365109 (10 mg/kg, single i.p.) was administered 30 s prior to the first PTZ injection, whereas 5% DMSO in saline was used as vehicle control.