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Inhibition of different GABA transporter systems is required to attenuate epileptiform activity in the CA3 region of the immature rat hippocampus

Salim Sharopov, Rongqing Chen, Haiyan Sun, Sergei N. Kolbaev, Sergei Kirischuk, Heiko J. Luhmann, Werner Kilb*

Institute of Physiology, University Medical Center of the Johannes Gutenberg University Mainz, Duesbergweg 6, D-55120 Mainz, Germany

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Summary GABA transporters (GATs) are an essential element of the GABAergic system, which regulate excitability in the central nervous system and are thus used as targets for anticonvulsive therapy. However, in the immature nervous system the functions of the GABAergic system and the expression profile of GATs are distinct from the adult situation, obscuring to predict how different GAT isoforms influence epileptiform activity. Therefore we analyzed the effects of subtype specific GAT inhibitors on repetitive epileptiform discharges using field potential and whole-cell patch-clamp recordings in the CA3 region of hippocampal slices of immature (postnatal days 4—7) rats. These experiments revealed that inhibition of GAT-1 with either tiagabine (30 μM) or NO-711 (10 μM) exhibited only a minor anticonvulsive effect on repetitive epileptiform discharges. Blockade of GAT-2/3 with SNAP-5114 (40 μM) had no anticonvulsive effect, but significantly prolonged the decay of spontaneous GABAergic postsynaptic currents. In contrast, the combined application of 10 μM NO-711 and 40 μM SNAP-5114 blocked epileptiform activity in 33% of all slices and reduced the occurrence of epileptiform discharges by 54% in the remaining slices. In addition, the input resistance decreased by 10.5 ± 1.0% under this condition. These results indicate that both GAT-1 and GAT-2/3 are functional in the immature hippocampus and that only the combined inhibition of GAT 1—3 is sufficient to promote a considerable anticonvulsive effect. We conclude from these results that both GAT-1 and GAT-2/3 act synergistically to regulate the excitability in the immature hippocampus.

* Corresponding author. Tel.: +49 211 3926101; fax: +49 211 3926102.
E-mail address: wkilb@uni-mainz.de (W. Kilb).

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Introduction

The amino acid γ-amino butyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain and mediates its action via two distinct classes of receptors: ionotropic $GABA_A$ (and $GABA_C$) and metabotropic $GABA_B$ receptors (Farrant and Kaila, 2007). In the immature rodent hippocampus, however, activation of $GABA_A$ receptors elicits a depolarizing and in many cases excitatory response (Mueller et al., 1984; Ben-Ari et al., 1989; Lamsa et al., 2000; Achilles et al., 2007). These depolarizing GABAergic responses are caused by Cl^- efflux via $GABA_A$ receptors due to the high intracellular Cl^- concentration in developing neurons (Blaesse et al., 2009; Ben Ari et al., 2012). A variety of studies suggest that such a depolarizing GABAergic action is essential to generate spontaneous activity transients, which are required for the adequate development of the brain (see Ben-Ari et al., 2007; Kilb et al., 2011 for review). The higher incidence for epileptic seizures during the first postnatal weeks in human development has been attributed to these depolarizing GABAergic responses (Sanchez and Jensen, 2001; Ben Ari et al., 2007), although there is no clear evidence for depolarizing GABAergic responses in human fullterm newborns (see Loscher et al., 2013 for critical review) and a variety of studies demonstrated that the GABAergic system can mediate an inhibitory action already in the immature rodent brain with clear depolarizing actions (Baram and Snead, 1990; Khalilov et al., 1997; Wells et al., 2000; Kilb et al., 2007; Richter et al., 2010). In addition, the poor pharmacological responsiveness of childhood seizures (Booth and Evans, 2004; Silverstein and Jensen, 2007) has been attributed to the distinct molecular and functional properties of the GABAergic system during early development. Beside depolarizing GABAergic responses, the late development of $GABA_B$ receptors (Fukuda et al., 1993; Gaiarsa et al., 1995), different properties of synaptic and extrasynaptic GABAergic currents (Marchionni et al., 2007; Kolbaev et al., 2012) as well as an altered molecular composition of $GABA_A$ receptors (Fritschy et al., 1994; Taketo and Yoshioka, 2000) can also contribute to the higher seizure susceptibility and the poor pharmacological control in the immature brain.

Another important element of the GABAergic system is GABA transporters (GATs), which mediate the sequestration of released GABA from the synaptic cleft (Borden, 1996), but also directly regulate interstitial GABA levels (Richerson and Wu, 2003). GATs belong to the superfamily of solute carriers and mediate the symport of 1 molecule GABA with 1 Cl^- ion and 2 Na^+ ions (Nelson, 1998). Four different GAT isoforms are identified in rodents and humans, with GAT-1, GAT-2 and GAT-3 described in the CNS (Borden, 1996). In the adult brain GAT-1 is preferentially synaptically located, suggesting a role for fast removal of GABA from the synaptic cleft and GABA uptake to the presynaptic terminal, while GAT-2 immunoreactivity was mainly detected in leptomeninges, suggesting that GAT-2 may regulate GABA levels in the cerebrospinal fluid, and GAT-3 is predominantly expressed in distal astrocytic processes (Conti et al., 2004). According to their essential function within the GABAergic system, GATs have been linked to the etiology of epilepsy (Allen et al., 2004; Kim et al., 2011) and blockers of these transporters are used for antiepileptic medication (Gram, 1994; Dalby, 2003). However, during postnatal development substantial changes in the expression of GATs occur. In the rat neocortex GAT-1 expression is rather low at birth and reaches adult levels approximately by the end of third postnatal week, while GAT-2 and GAT-3 expression is already relatively high at birth and reaches the adult levels during second postnatal week (Minelli et al., 2003; Conti et al., 2004). In addition, during this developmental period an expression of GAT-1 in astrocytes and of GAT-3 in neurons has been reported (Minelli et al., 2003; Conti et al., 2004).

Given this distinct GAT expression and the heterogeneous GABA actions in the immature brain, the consequences of GAT blockade on the excitability of the immature brain are difficult to predict. To address the question, how different GATs influence the excitability of the immature hippocampus, we investigated the effects of specific blockers of GAT subtypes on repetitive epileptiform discharges using field potential recordings in the CA3 region of hippocampal slices of immature rats. In addition, we performed whole-cell patch-clamp recordings from CA3 pyramidal cells to analyze the effect of GABA transport blockers on isolated GABAergic postsynaptic currents.

Experimental procedures

Slice preparation

All experiments were conducted in accordance with EU directive 86/609/EEC for the use of animals in research and were approved by the local ethical committee. All efforts were made to minimize the number of animals and their suffering. Wistar rat pups of postnatal days 4—7 (P4-7) were obtained from the local breeding facility and were deeply anesthetized by enflurane (Ethrane, Abbot Laboratories, Wiesbaden, Germany). The brains were quickly removed and immersed for 2—3 min in ice-cold standard artificial cerebrospinal fluid (ACSF, composition see below). Coronal slices (400—600 μm thickness) including the hippocampus were cut on a vibratome (Vibroslicer 752 M, Campden Instruments Ltd., Leicester, UK, or HR2, Sigmann Elektronik, Hüffenhardt, Germany). The slices were transferred to an interface-type recording chamber where they were continuously superfused with ACSF at a rate of 1—2 ml/min at $31 \pm 1\,^{\circ}C$. The slices were allowed to recover for at least 1 h under these conditions. For the patch-clamp experiments 400 μm thick slices were stored in an incubation chamber filled with oxygenated ACSF at room temperature for at least 1 h before they were transferred to a submerged recording chamber.

Data acquisition and analysis

Extracellular field potentials were recorded with tungsten microelectrodes (impedance 4—5 MΩ; FHC, Bowdoinham, ME) in the stratum radiatum of the hippocampal CA3 region as described before (Kilb et al., 2006). Signals were amplified by a purpose built amplifier in AC mode (cutoff frequency 3.8 Hz), low-pass filtered at 3 kHz and stored on a PC using an AD/DA board (ITC-16, HEKA, Lamprecht, Germany) and TIDA software (HEKA). Extracellular field potentials were recorded simultaneously from maximally 4 separate