Neurobiology of Disease 40 (2010) 490–501

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Receptors with low affinity for neurosteroids and GABA contribute to tonic inhibition of granule cells in epileptic animals

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ARTICLE INFO

Article history:
Received 28 April 2010
Revised 22 July 2010
Accepted 26 July 2010
Available online 1 August 2010

Keywords:
Temporal lobe epilepsy
Dentate granule cells
GABA-A receptor
Tonic inhibition
Neurosteroids

ABSTRACT

Neurosteroid sensitivity of $GABA_A$ receptor mediated inhibition of the hippocampal dentate granule cells (DGCs) is reduced in animal models of temporal lobe epilepsy. However, the properties and subunit composition of $GABA_A$ receptors mediating tonic inhibition in DGCs of epileptic animals have not been described. In the DGCs of epileptic animals, allopregnanolone and L-655708 sensitivity of holding current was diminished and δ subunit was retained in the endoplasmic reticulum and its surface expression was decreased the in the hippocampus. Ro15-4513 and lanthanum had distinct effects on holding current recorded from DGCs of control and epileptic animals suggesting that the pharmacological properties of $GABA_A$ receptors maintaining tonic inhibition in DGCs of epileptic animals were similar to those containing the α4βxγ2 subunits. Furthermore, surface expression of the α4 subunit increased and a larger fraction of the subunit co-immunoprecipitated with the γ2 subunit in hippocampi of epileptic animals. Together, these studies revealed that functional α4βxδ and α5βxγ2 receptors were reduced in the hippocampi of epileptic animals and that novel α4bxγ2 receptors contributed to the maintenance of tonic inhibition. The presence of α4βxγ2 receptors resulted in low GABA affinity and neurosteroid sensitivity of tonic currents in the DGCs of epileptic animals that could potentially increase seizure vulnerability. These receptors may represent a novel therapeutic target for anticonvulsant drugs without sedative actions.

Introduction

Temporal lobe epilepsy (TLE) is a common form of epilepsy and nearly a third of patients may be refractory to anticonvulsants. An array of pathological changes in neuronal circuits occurs in the hippocampal dentate gyrus of experimental animals with TLE including, sprouting of mossy fibers and loss and dysfunction of GABAergic interneurons (Dudek and Sutula, 2007; Sutula and Dudek, 2007; Obenaus et al., 1993; Kobayashi and Buckmaster, 2003; Sun et al., 2007a; Zhang and Buckmaster, 2009). These changes render the hippocampus susceptible to generating and propagating seizures. However, there are endogenous molecules such as neurosteroids that can suppress seizures. Neurosteroids are molecules derived from circulating steroids and synthesized de novo from cholesterol. Neurosteroids modulate $GABA_A$ receptors and have an anticonvulsant action (Kokate et al., 1994; Lawrence et al., 2010; Majewska et al., 1986; Reddy et al., 2004).

There is growing evidence that neurosteroid modulation of $GABA_A$ receptors on hippocampal dentate granule cells (DGCs) is diminished in TLE (Mtchedlishvili et al., 2001; Peng et al., 2004; Sun et al., 2007b; Zhang et al., 2007). $GABA_A$ receptors are pentamers composed of subunits derived from α, β, γ, δ, ε and π gene families, and the majority of receptors are composed of 2 α, 2 β and a γ or δ subunit (Sieghart and Sperk, 2002). Receptors containing the δ subunit are particularly sensitive to neurosteroids (Belelli et al., 2006; Mihalek et al., 1999; Wohlfarth et al., 2002). In DGCs, $GABA_A$ receptors containing the α4 and δ subunit are targeted to the extrasynaptic membrane, where they contribute to neurosteroid-sensitive leak conductance often referred to as tonic inhibition (Herd et al., 2007; Mtchedlishvili and Kapur, 2006; Stell and Mody, 2002; Sun et al., 2004; Wei et al., 2003). In TLE, there is diminished expression of δ subunit mRNA and polypeptide in DGCs that is associated with diminished neurosteroid sensitivity of tonic currents (Nishimura et al., 2005; Peng et al., 2004; Schwarzer et al., 1997a; Zhang et al., 2007).

Previous studies investigating the neurosteroid modulation of tonic inhibition have not addressed several issues. Despite loss of neurosteroid sensitivity and reduced expression of the δ subunit, tonic inhibition of DGCs appears to be preserved in animals with TLE however; the identity and properties of these $GABA_A$ receptors have not been described. Studies in δ subunit knockout animals demonstrate a residual tonic current suggesting that $GABA_A$ receptors containing subunits other than the δ subunit can maintain tonic inhibition (Stell et al., 2003; Wei et al., 2004). Recent studies indicate that $GABA_A$ receptors containing the α5 subunit maintain residual tonic currents in DGCs (Glykys et al., 2008), suggesting the possibility that upregulation of α5 subunit-containing receptors can maintain

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2010.07.016

tonic inhibition in DGCs of epileptic animals (Zhan and Nadler, 2009). Alternately, a previous study proposed that tonic inhibition of epileptic DGCs could be maintained by $GABA_A$ receptors containing the α4 and γ2 subunits (Zhang et al., 2007). These possibilities have not been tested directly, and assembly of the γ2 subunit with the α4 subunit remains untested.

The current study demonstrates that receptors containing the α4 and γ2 subunit contribute to the maintenance of tonic inhibition of DGCs in epileptic animals. These receptors have a lower affinity for GABA and neurosteroids, which is likely to increase vulnerability to seizures.

Materials and methods

Induction of TLE

All experimental procedures were performed on adult male Sprague–Dawley rats (150–200 g) in accordance with the protocol approved by the University of Virginia Animal Use and Care Committee. TLE was induced in rats using the continuous hippocampal stimulation (CHS) protocol as described previously (Lothman et al., 1989). The rats were anesthetized with ketamine (50 mg/kg) and xylazine (40 mg/kg) and implanted with a pair of bipolar stimulating electrodes in the left posterior ventral hippocampus (AP 3.6, ML 4.0, DV 5.0 from dura; incisor bar at +5.0). After 1 week of recovery, the left hippocampus was stimulated with 10 s trains of 50 Hz, 1 ms, 400 mA biphasic square wave current pulses delivered every 13 s for 90 min to induce status epilepticus (SE) (Lothman et al., 1990). Approximately 4–6 weeks after stimulation, the rats developed spontaneous limbic seizures with a motor component. For this study, seizures were documented by either continuous EEG recording or by direct observation of the spontaneous seizure (Bertram et al., 1997). The epileptic animals were sacrificed at least 24 hours after the last seizure. All animals were housed individually under conditions of a 12 h/12 h light/dark cycle and had free access to food and water. Age-matched rats were used as controls.

Electrophysiology

Animals were anesthetized with isoflurane and decapitated. The brain was dissected free and immersed in cold (2–4 °C) ACSF composed of (in mM) 65.5 NaCl, 2 KCl, 5 $MgSO_4$, 1.1 KH_2PO_4, 25 $NaHCO_3$, 10 dextrose, 113 sucrose, and 1 $CaCl_2$ (osmolarity 300 mOsm) saturated with 95%O_2–5%CO_2. Brains were mounted on a vibratome stage (Camden Instruments, UK) and 300 μM thick horizontal sections containing the right ventral hippocampus were cut. Slices were maintained in continuously oxygenated ACSF containing (in mM) contains (in mM) 127 NaCl, 2 KCl, 1.5 $MgSO_4$, 25.7 $NaHCO_3$, 10 Dextrose, 1.5 $CaCl_2$ (pH 7.4; 300 mOsm) at 32 °C in a holding chamber for 30–45 min and then at room temperature in a recording chamber mounted on the stage of an Olympus BX51 microscope equipped with a 40× water-immersion objective, IR-DIC optics, and video. Patch electrodes (final resistances 4–6 MΩ were pulled from borosilicate glass (Sutter Instruments, Novato, CA) on a horizontal Flaming-Brown microelectrode puller (model P-97, Sutter Instruments), using a 2-stage pull protocol. Electrode tips were filled with a filtered internal recording solution consisting of (in mM): CsCl 153.3, $MgCl_2$ 1.0, N-[2-Hydroxyethyl] piperazine-N-[2-ethansulfonic acid] (HEPES) 10.0, and glycol-bis (α-aminoethyl ether) *N,N,N,N*-tetraacetic acid (EGTA) 5.0, pH 7.2 (with CsOH), osmolarity was 285–295 mOsm. The electrode shank contained 3 mM ATP Mg^{2+} salt. $GABA_A$ receptor mediated currents were recorded from DGCs voltage-clamped to −65 mV with an Axopatch 200B amplifier (Molecular Devices, CA) in the presence of 50 μM DL-2-Amino-5-phosphonopentanoic acid (DL-AP5) and 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris-Cookson Ellisville, MO). Whole cell capacitance and series resistance were compensated by 80% at a 7 μs lag. Recordings were performed when series resistance after compensation was less than 20 MΩ. Access resistance was frequently monitored with a 10-ms −5 mV test pulse. If the series resistance increased by 25% at any time during the experiment, the recording was terminated. Currents were filtered at 5 kHz, digitized at 10 kHz using a Digidata 1440A digitizer, and acquired using Axoscope 10.2 software (Molecular Devices, CA) on an IBM PC-compatible computer hard drive.

Determination of tonic inhibition

To assess the potential alterations in tonic inhibition, we evaluated either the shift in baseline holding current (I_{hold}) (Mtchedlishvili and Kapur, 2006; Rajasekaran et al., 2009), or changes in its root mean square amplitude of noise (RMS) (Mtchedlishvili et al., 2006) following application of antagonists or agonists. The $GABA_A$ receptor mediated component of the holding was confirmed by applying bicuculline and demonstrating a reduction in holding current. To determine the shifts in I_{hold} (I_Δ), the digitized current traces were analyzed with Clampfit 10.2 (Molecular Devices, CA). The mean I_{hold} for each recording was determined by measuring the mean current (I_{avg}) sampled every 50 ms at 500 ms intervals. Fifty such data points were collected immediately before drug application and at least 10 min after drug application to allow steady state response after drug equilibration. To minimize the contribution of synaptically present receptors to our measures of I_{hold}, epochs containing synaptic events that could be visually identified or were larger than the RMS noise were eliminated from the analysis. Epochs having an unstable baseline were also eliminated from the analysis. The drug effects on individual neurons were assessed by comparing the distribution of the mean holding current before and after drug application by means of a Kolmogorov–Smirnov (KS) test (available online at http://www.physics.csbsju.edu/stats/KS-test.n.plot_form.html). The changes in I_{hold} between the experimental groups were not normalized to cell capacitance since we did not find any difference in whole cell capacitance between control and epileptic DGCs (data not shown). The difference in the mean I_{hold} after drug application relative to baseline was calculated and I_Δ in the two experimental groups were then compared using an unpaired *t*-test.

RMS noise (I_{rms}) was defined by the equation

$$I_{rms} = \left[\frac{\left(I_{avg}-I_1\right)_2 + \left(I_{avg}-I_2\right)_2 + \ldots + \left(I_{avg}-I_n\right)_2}{n}\right]^{1/2}$$

where I_{avg} is the average current amplitude, I_n is the amplitude of an individual point and n is the number of measurements in an epoch. Each epoch was 50 ms in duration and contained 2500 amplitude measurements. The time interval between 2 epochs was 500 ms. Sixty epochs were analyzed for each experimental condition (60 control and 60 after the drug application in each cell). In order to asses the effect of a drug on I_{rms} in individual neurons, the distribution of I_{rms} in epochs before application of the drug (during the baseline period) was compared to that following drug application by means of a KS test. In order to compare data obtained from a group of neurons, I_{rms} values in individual epochs before and after drug application were averaged. Mean I_{rms} values were compared using unpaired *t*-test.

Since differences in the washout of slices or pathophysiological changes leading to altered GABA synthesis, release, or clearance could potentially alter ambient GABA concentrations and confound experimental determination of changes in tonic currents (Glykys and Mody, 2007), most experiments were performed in the presence of 10 μM of the GABA uptake inhibitor, NO-711 (Sigma, St. Louis, MO), and 3 μM GABA (Sigma, St. Louis, MO). However, in experiments designed to measure RMS noise, the uptake blocker or GABA was not included in the ACSF.