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Altered pharmacology and GABA-A receptor subunit expression in dorsal midline thalamic neurons in limbic epilepsy

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ABSTRACT

The mediodorsal (MD) and paraventricular (PV) thalamic nuclei play a significant role in limbic epilepsy, and previous reports have shown changes in GABA-A receptor (GABAAR) mediated synaptic function. In this study, we examined changes in the pharmacology of GABAergic drugs and the expression of the GABAAR subunits in the MD and PV neurons in epilepsy. We observed nucleus specific changes in the sensitivity of sIPSCs to zolpidem and phenobarbital in MD and PV neurons from epileptic animals. In contrast, the magnitude of change in electrically evoked response (eIPSC) to zolpidem and phenobarbital were uniformly diminished in both MD and PV neurons in epilepsy. Immunohistochemical studies revealed that in epilepsy, there was a reduction in GAD65 expression and NeuN positive neurons in the MD neurons. Also, there was a decrease in immunoreactivity of the α1 and β2/3 subunit of GABAARs, but not the γ2 of the GABAAR in both MD and PV in epilepsy. These findings demonstrate significant alterations in the pharmacology of GABA and GABAARs in a key region for seizure generation, which may have implications for the physiology and pharmacology of limbic epilepsy.

Introduction

Temporal lobe epilepsy (limbic epilepsy) is a common form of human epilepsy that is frequently pharmacoresistant in that patients do not have their seizures completely controlled by current medications. The dorsal midline thalamus is an important component of the seizure circuit in limbic epilepsy (Bertram et al., 2001; Bertram et al., 2008). Previous experimental studies have found that focal inhibition of the mediodorsal (MD) thalamus by GABAA agonists inhibited seizures and reduced seizure durations(Cassidy and Gale, 1998; Patel et al., 1988). Supporting clinical evidence suggests potential alterations in GABA binding, as revealed by [^{11}C] flumazenil positron emission tomography, in the MD nucleus of patients with limbic epilepsy (Juhasz et al., 1999). In a recent study, we provided evidence for contrasting alterations in synaptic GABA-A receptor (GABAAR) mediated sIPSCs in the two adjacent regions of the dorsal midline thalamus in limbic epilepsy (Rajasekaran et al., 2007). There was a significant decrease in the frequency of sIPSCs in the MD neurons of epileptic animals (referred hereafter as 'epileptic' MD neurons). In contrast, there was an increase in the number and magnitude of sIPSCs in the PV neurons of epileptic animals (referred hereafter as 'epileptic' PV neurons). The changes in IPSC kinetics obtained in the study suggested altered postsynaptic responses to synaptically released GABA.

Regional heterogeneity of GABAAR in the dorsal midline thalamus has been previously reported (Gao et al., 1995; Pirker et al., 2000b; Peng et al., 2002) suggesting the possibility of potential alterations in pharmacosensitivity to GABAergic agents in this region in epilepsy. The allosteric modulators of the GABAAR exert their actions through increasing the conductance of the receptor, which are in turn influenced by the subunit composition of the receptor. Because GABAA agonists are common antiepileptics, we wished to determine whether the pharmacology of the GABAAR was altered in epilepsy in this region by evaluating the modulation of the postsynaptic responses by the α-subunit preferential positive allosteric modulator, zolpidem (Pritchett et al., 1989), and the β-subunit specific (Smith and Olsen, 1995) anticonvulsant barbiturate, phenobarbital (PB). Using immunohistochemistry, we also investigated for evidence of changes in the expression of some GABAAR subunits in epilepsy. The report is the first examination of the potential alterations in the pharmacology of a key subcortical component of the limbic seizure circuit.

Methods

All experiments were performed on age-matched, adult (6–9 months old) male Sprague–Dawley rats housed on standard light/dark schedule with free access to food and water, and handled according to a protocol approved by the University of Virginia Animal Care and Use Committee.

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Animal preparation

Adult male Sprague–Dawley rats were made epileptic using the continuous hippocampal stimulation method (Lothman et al., 1989). A bipolar electrode was implanted in the left mid-ventral hippocampus under ketamine–xylazine anesthesia using stereotaxic coordinates as described in Paxinos and Watson (2005) (in mm): AP 5.3 posterior to bregma; L 4.9; DV 5.0 below dura; incisor bar at −3.3. One week after surgery, status epilepticus was induced by stimulating the hippocampus at 50 Hz, 400 μA using 1 ms biphasic square waves in 10 second trains applied every 11 s for 90 min. Self sustaining limbic status epilepticus developed during the stimulation and lasted 10–14 h. Approximately 8–12 weeks later, animals developed spontaneous limbic seizures, which were documented by either continuous EEG monitoring or direct observation of behavioral seizures (Bertram and Cornett, 1994). Epileptic rats were used at least 4 months after stimulation to ensure that they had reached a comparable seizure maturity.

Electrophysiology

Thalamic slice preparation

Animals were anesthetized with halothane prior to decapitation, and brains were immersed into a low NaCl, high sucrose oxygenated dissection buffer (4 °C) containing (in mM) 65.5 NaCl, 2 KCl, 5 $MgSO_4$, 1.1 KH_2PO_4, 1 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$ and 113 sucrose (300 mOsm). The block containing the midline thalamus was mounted on a vibratome stage (Camden Instruments, UK) and cut at 300 μm coronally. The slices were kept in oxygenated ACSF at 29 °C for at least 1 h before being transferred to the recording chamber. The oxygenated ACSF contains (in mM) 127 NaCl, 2 KCl, 1.5 $MgSO_4$, 25.7 $NaHCO_3$, 10 dextrose, and 1.5 $CaCl_2$ (pH 7.4; 300 mOsm).

Recording and data acquisition

The slices were continuously perfused with oxygenated ACSF. The patch electrodes were prepared from thick walled borosilicate glass (World Precision Instruments, FL), pulled on a horizontal Flaming–Brown microelectrode puller (model P-97, Sutter Instruments, Novato, CA) using a two-stage pull protocol. Patch electrodes were filled with pipette internal solution containing (in mM) 153.3 CsCl, 1.0 $MgCl_2$, 10 HEPES, 5.0 EGTA, 2 ATP-Mg (buffered to pH 7.2 with CsOH, 285 mOsm) and had a resistance of 2–4 MΩ. Whole-cell voltage clamp recordings were made using an Axopatch 1D amplifier (Molecular Devices, CA). The temperature in the recording chamber was maintained at 24 °C using an inline heating system coupled with an automatic temperature controller (Warner Instrument Corporation, USA). Attempts to record at physiological temperature resulted in reduced viability and recording quality.

The recordings were performed under visual control through a video monitor to identify neurons by position. Cells were voltage clamped to −60 mV and for the combination of the internal and external solutions, the calculated E_{Cl}^{-} was 0 mV and the experimental E_{Cl}^{-} was +5 mV. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the presence of D-6-cyano-7-nitroquinoxaline-2, 3-dione (DNQX, 20 μM, AMPA/kainate antagonist) and DL-2-amino-5-phosphonovaleric acid (DL-AP5, 50 μM, NMDA antagonist) (both from Tocris, MO) to inhibit glutamate receptors. After access was obtained, the series resistance was compensated between 70 and 80% following capacitance compensation. The mean series resistance before compensation was 4–6 MΩ. Junction potential was not compensated for any experiment. Currents were low pass filtered at 5 kHz and digitized at a frequency of 10 kHz using 1322A Digidata (Molecular Devices, CA) A/D converter. The currents were recorded using pClamp 8.2 software (Molecular Devices, CA) 10 min after access was obtained to allow the pipette solution to equilibrate with the cell contents. Data for drug analysis were obtained from the recordings 10 min after the beginning of drug perfusion.

To obtain evoked IPSCs (eIPSCs), a bipolar concentric platinum electrode was placed adjacent to and within 500 μm of the recorded neuron. When recordings were performed from MD neurons, the stimulation electrode was positioned in the centrolateral nucleus, whereas the MD nucleus was stimulated when recording from PV neurons. The electrical stimuli consisted of single pulses (10–35 V, 100 μs duration) delivered at 0.1 Hz. In order to standardize the degree of receptor activation by the primary agonist (GABA) and facilitate comparison of drug effects, we adjusted the stimulus intensity for each cell such that it yielded a 475–500 pA postsynaptic response, which was at least 5 times greater that the average sIPSC amplitude of the MD and PV neurons. The responses were recorded using Clampex 8.02 (Molecular Devices, CA) such that the output current trace was a cumulative average of 10 consequent stimuli. Recordings in which access resistance changed >20% before drug application were rejected.

Data analysis

Tonic currents were determined using Clampfit 8.2 (Molecular Devices, CA) by measuring the mean holding current sampled every 50 ms at 500 ms intervals. Fifty data points were collected from immediately before drug application and 5 min after drug application. The epochs containing synaptic events or unstable baselines were eliminated from the analysis. The drug effects on individual neurons were assessed by comparing the distribution of 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 sIPSCs were analyzed using the MiniAnalysis (Synaptosoft, Decatur, GA). All events were identified visually to avoid errors in detection by automation. The threshold for detection of currents was set at 3 times the root mean square baseline noise, which was measured for each epoch of recording. An event was detected when there was at least a 100-ms interval between complete decay of the previous event and the rise of the next phasic current. All events were identified for determination of frequency, amplitude, half width, and 10–90% rise times; but decay constants were determined from events that did not have another overlapping event on the decay phase of the former. The 10–90% rise time was detected by the program by determining the time interval between the last data point with a value of 10% and the first data point with a value of 90% of the peak amplitude. The decay constants were derived by the software by evaluating 0.36 fraction of peak current. Charge transfer for each event was calculated by the software as the integral under the current–time-trace (pAms) during an event. eIPSCs were analyzed using Clampfit 8.2 (Molecular Devices, CA). The charge transfer by the eIPSC was calculated as the area under the curve from the peak current to its return within 10% of the initial baseline holding current.

Immunohistochemistry

Tissue preparation

Four animals with limbic epilepsy and 4 age-matched controls were used. The procedures of tissue preparation were performed as described in detail previously (Sun et al., 2004). Briefly, animals were anesthetized with a lethal dose of pentobarbitone sodium (220 mg/kg i.p.) and perfused through the ascending aorta with normal saline followed by 350–450 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and post-fixed in the same fixative for 2 h at 4 °C. After overnight incubation in 25% sucrose in 0.1 M phosphate buffer for cryoprotection, brains were frozen by immersion in −70 °C isopentane. Coronal sections containing PV and MD nucleus were collected at 40 μm thickness. The sections were put into 5 vials sequentially, and 4–5 sections from each animal were used for double labeling of a subunit of GABAR and GAD65 or NeuN.