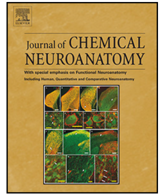
Journal of Chemical Neuroanatomy 64–65 (2015) 1–11

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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



Interstrain differences of ionotropic glutamate receptor subunits in the hippocampus and induction of hippocampal sclerosis with pilocarpine in mice

Endre Dobó *, Ibolya Török, András Mihály, Norbert Károly, Beáta Krisztin-Péva

Department of Anatomy, Faculty of Medicine, University of Szeged, Szeged, Hungary

ARTICLE INFO

Article history:
Received 18 October 2014
Received in revised form 16 January 2015
Accepted 6 February 2015
Available online 16 February 2015

Keywords:
Hippocampus
Pilocarpine
Glutamate receptor
Interstrain difference
Hippocampal sclerosis

ABSTRACT

Rodent strains used in epilepsy research have various neurological characteristics. These differences were suggested to be attributed to the diverse densities of the ionotropic glutamate receptor (iGluR) subunits. However, previous studies failed to find interstrain differences in the hippocampal receptor levels.

We supposed that a detailed layer-to-layer analysis of the iGluR subunits in the hippocampus might reveal strain-dependent differences in their base lines and reactions induced by pilocarpine (PILO) between two mouse strains without documented ancestors.

Levels of iGluR subunits in Balb/c and NMRI mice were compared using semiquantitative immunohistochemistry. The alterations in the neuronal circuitry were validated by neuropeptide Y (NPY) and neuronal nuclear antigen (NeuN) immunostainings.

Immunohistochemistry showed interstrain laminar differences in some subunits of both the control and PILO-treated animals. The seizure-induced irreversible neuronal changes were accompanied by reduced GluA1 and GluA2 levels. Their changes were inversely correlated in the individual NMRI mice by Pearson's method. Increase in NPY immunoreactivity showed positive correlation with GluA1, and negative correlation with GluA2. The NMRI strain was susceptible to PILO-induced hippocampal sclerosis, while the Balb/c animals showed resistance.

Basal levels of iGluRs differ in mouse strains, which may account for the interstrain differences in their reactions to the convulsant.

© 2015 Elsevier B.V. All rights reserved.

Introduction

The rodent PILO model of temporal lobe epilepsy reproduces the main neuropathological features of the human epileptic disorder therefore it has been widely used (Schauwecker, 2012; Curia et al., 2008; Winawer et al., 2007; Scharfman et al., 2001; Cavalheiro et al., 1991, 1996). The muscarinic cholinergic agonist PILO induces status epilepticus, which is followed by characteristic neuropathological changes that may lead to the appearance of spontaneous recurrent seizures. After PILO-induced status epilepticus, neuronal cell loss, gliosis and MF sprouting were observed predominantly in the hippocampus (Borges et al., 2003; Turski et al., 1984). The neuronal loss of hilus, one of the common neuropathological features of the rodent models, was reported to correlate with the development of spontaneous seizures (Mello et al., 1993; Buckmaster and Dudek, 1997; Borges et al., 2003).

Significant increase of glutamate release is involved in status epilepticus, which may play a crucial role in the development and maintenance of chronic epileptic seizures (Carvalho et al., 2011; Costa et al., 2004; Kovacs et al., 2003). Several studies reported that PILO-induced status epilepticus also resulted in increase of glutamate release (Cavalheiro, 1995; Smolders et al., 1997). Moreover, alterations in expression and synaptic functions of glutamate receptors (GluRs) were associated with glutamate excitotoxicity and neuronal death (Zhang et al., 2004; Ding et al., 2007).

Abbreviations: AMPAR, AMPA receptor; DG, dentate gyrus; GC, granule cell; GluR, glutamate receptor; iGluR, ionotropic glutamate receptor; IML, internal molecular layer; KAR, kainate receptor; MC, mossy cell; MF, mossy fibre; ML, molecular layer; NMDAR, NMDA receptor; NMDAR1, NMDA receptor 1, GluN1; NeuN, neuronal nuclear antigen; NPY, neuropeptide Y; PC, pyramidal cell; PILO, pilocarpine; SGZ, supragranular zone; SL, stratum lucidum; SLM, stratum lacunosum-moleculare; SO, stratum oriens; SR, stratum radiatum.

* Corresponding author at: Department of Anatomy, Faculty of Medicine, University of Szeged, H-6724 Szeged, Kossuth L. sgt. 40, Hungary.
Tel.: +36 62 545665; fax: +36 62 545707.
E-mail address: dobo.endre@med.u-szeged.hu (E. Dobó).

http://dx.doi.org/10.1016/j.jchemneu.2015.02.002
0891-0618/© 2015 Elsevier B.V. All rights reserved.

GluRs may operate as canonical ion channels and/or metabotropic receptors, which elicit diverse signalling cascades. Based on the pharmacological properties and structural homology, iGluRs can be grouped into four distinct classes: AMPA receptors (AMPARs), kainate receptors (KARs), NMDA receptors (NMDARs) and δ-receptors (Traynelis et al., 2010). AMPARs are characterized by very low Ca^{2+} permeability and fast kinetics, while NMDARs are characterized by large Ca^{2+} permeability and slow kinetics. While these two iGluRs are postsynaptic, the KARs are localized both pre- and postsynaptically and may operate as metabotropic receptors, too. The functions of the δ-receptors are still unresolved. The subunit composition of the iGluRs is crucial to their function (Kortenbruck et al., 2001; Su et al., 2002).

Several studies found important species differences in the responses to convulsants (Curia et al., 2008). The species differences include various behavioural properties, e.g. susceptibility to convulsants and neuronal damages. In addition to the phylogenetic characteristics in the reactions, significant genealogical and even source-dependent intrastrain variations in seizure and cellular susceptibility were reported (Winawer et al., 2007; Portelli et al., 2009; Schauwecker, 2012). In previous experiments, we found marked individual differences between PILO-injected mice of the CFLP strain; in spite of the symptoms of acute status epilepticus, only a small fraction of the treated animals developed spontaneous recurrent seizures (Karoly et al., 2011, submitted).

Since the hippocampal principal neurones are glutamatergic, the discrepancies between the responses of the animals to convulsants may be based on the amount and/or the composition of the functional iGluRs. However, previous studies failed to find explanation for strain differences on the hippocampal receptor level (Schauwecker, 2003; Kurschner et al., 1998). In the present experiments, we supposed that a detailed layer-to layer analysis may reveal the possible strain-dependent differences in the densities of the iGluRs.

We investigated the hippocampal distribution of iGluR subunits in two mouse strains lacking registered common ancestors by means of semiquantitative immunohistochemistry. The effects of the chemoconvulsant PILO on the densities of iGluRs were evaluated in both strains after a 2-month post-treatment period, which is thought to be sufficient for the development of spontaneous recurrent seizures (Curia et al., 2008). NPY immunohistochemistry was used to indicate the incidence of spontaneous recurrent seizures and to validate the neuropathological alterations of the hippocampal neuronal circuitry, and NeuN immunohistochemistry was applied to detect the intense neuronal loss.

Materials and methods

Animal treatment with PILO

Adult, male Balb/c and NMRI mice (25–30 g) were kept in a temperature controlled room under standard light/dark cycle, with food and water ad libitum. All experimental procedures were conducted according to the EU Directive (2010/63/EU) and to the Hungarian Animal Act. Specific approval of care and use of animals was obtained in advance from the Faculty Ethical Committee on Animal Experiments (University of Szeged). Animals were injected with a single intraperitoneal (i.p.) dose of PILO (Sigma–Aldrich Co., St. Louis, MO, USA) that was adjusted so as to cause at least a single occurrence of status epilepticus in only two-thirds of the animals in order to decrease the death rate. In preliminary experiments, 180 mg/kg and 195 mg/kg PILO were found to be appropriate for Balb/c and NMRI strains, respectively. Ninety minutes after the first onset of status epilepticus, the animals were injected i.p. with diazepam (Seduxen, Gedeon Richter, Budapest, Hungary; dose: 10 mg/kg). The NMRI strain exhibited a higher incidence rate of status epilepticus, though reacted less severely than the Balb/c strain during the PILO-induced initiation period. This protocol resulted in comparable seizure mortality: 38% in the Balb/c mice and 32% in the NMRI strain. Post-treatment of the animals included i.p. injections with Ringer lactate solution. The control animals received the same volume of physiological saline, the solvent of PILO. The animals, which developed status epilepticus during the treatment were termed "PILO-responsive" animals.

Tissue preparation

The PILO-treated and the control animals were sacrificed 2 months after the injections. The animals were deeply anaesthetized with diethyl ether, and perfused through the ascending aorta with physiological saline, and then with 4% formaldehyde in 0.1 M phosphate buffer (PB). The brains were dissected and cryoprotected overnight in 30% sucrose in PB at 4 °C. Coronal brain sections were cut on a freezing microtome at a thickness of 24 μm. Section planes were selected according to the Mouse Brain Atlas of Franklin and Paxinos (1997).

Immunohistochemistry

The free-floating sections were treated with 0.5% Triton X-100 and 3% hydrogen peroxide, then with normal swine serum (diluted: 1/10). The following primary antisera were used: rabbit anti-NPY (Abcam, Cambridge, UK, dilution: 1/10,000); mouse anti-NeuN (Chemicon, Temecula, CA, USA, dilution: 1/8000); rabbit anti-GluA1 (Millipore, Temecula, CA, USA, 1/500); mouse anti-GluA2 (Chemicon, dilution: 1/200); rabbit anti-GluA2/3 (Chemicon, dilution: 1/400); monoclonal rabbit anti-GluK2 (clone: EPR6307; Abcam, dilution: 1/3000); mouse anti-NMDAR1 (Abcam, dilution: 1/5000). The sections were incubated under continuous agitation at room temperature overnight. After washing, the sections were incubated with the appropriate biotinylated secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA, dilution: 1/400) for 60 min, and finally with peroxidase-labelled streptavidin (Jackson ImmunoResearch, dilution: 1/1000) for 60 min. The sites of immunoreaction were visualized with diaminobenzidine in the absence or presence of nickel (Adams, 1981). At one particular series of a given immunostaining for assessment, all the sections were incubated in aliquots from the same solutions of either the immunoreagents or the chromogens synchronously for exactly the same time. The same number of sections of the control and PILO-responsive animals of both strains was incubated in the same volume of the solutions.

Image analysis

Pictures were taken with an image-capture system (Olympus DP50) attached to an Olympus BX-50 microscope (Soft Imaging System GmbH, Münster, Germany). Image analysis was performed with Adobe Photoshop 7 (Adobe Systems Incorporated, San Jose, CA, USA). A researcher blind to the experimental conditions of the animals measured the pixel density of immunostained images. Briefly: through use of the "marquee" tool, 8–12 circular, 0.1 mm diameter areas were selected in adjacent positions inside the hippocampal layers. The average of 10 background determinations (carried out near the layers in unstained neuropil-containing sites) was subtracted from the average pixel densities measured within the hippocampal layers. Differences between the corresponding hippocampal regions of the control and PILO-responsive, but non-sclerotic animals were assessed by using the unpaired one-tailed Student's *t*-test. Pearson's correlation analysis was used to evaluate the relationship between the optical densities of different hippocampal layers. Data were analyzed and plotted with the aid of GraphPad 4.0 (GraphPad Software, Inc., CA, USA).

Results

NPY immunohistochemistry

According to previous data (Scharfman and Gray, 2006), only a few, small NPY-immunoreactive neurones were scattered throughout the hippocampus of the control animals (Fig. 1A). These interneurones displayed short non-varicose branches. Dramatic increase in hippocampal NPY synthesis was reported to be a diagnostic tool to confirm the incidence of spontaneous recurrent seizures (Sperk et al., 1992; Borges et al., 2003; Scharfman and Gray, 2006). After the PILO treatment the NPY immunoreactivity greatly increased in the whole area of the dentate gyrus (DG) and in the stratum lucidum (SL) of CA3 in every PILO-responsive mouse (Fig. 1B), irrespective of the strains.

The vast majority of the increased immunostaining was localized in the synaptic fields of the MFs. We did not observe the NPY staining in the perikarya of dentate granule cells (GCs). Apart from the heavy staining of the MF areas, NPY-immunoreactive puncta were seen in the thin supragranular zone (SGZ) within the internal molecular layer (IML) of the DG. The immunoreactivity increased in the molecular layer (ML) too, but in much less extent than in the areas supplied by MFs. Furthermore, the NPY-immunoreactive cells in the CA1 region displayed stronger staining

Download English Version:

https://daneshyari.com/en/article/1988827

Download Persian Version:

https://daneshyari.com/article/1988827

Daneshyari.com