International Journal of Developmental Neuroscience 63 (2017) 1–7

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Consequences of perinatal hypoxia in developing brain: Changes in GABA transporter functioning in cortical, hippocampal and thalamic rat nerve terminals

Natalia Pozdnyakova

Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovicha Str. 9, Kiev, 01030, Ukraine

ARTICLE INFO

Keywords:
Perinatal hypoxia
Plasma membrane GABA transporters
Nerve terminals
Rat brain cortex
Hippocampus
Thalamus

ABSTRACT

Perinatal hypoxia leads to behavioral abnormalities, cognitive disabilities, and epilepsy resulting from alterations in neurodevelopment, maturation and construction of the network. Considering a particular role of γ-aminobutyric acid (GABA) for an immature brain, we analysed transporter-mediated [^{3}H]GABA uptake in the cortical, hippocampal and thalamic nerve terminals isolated from rats of different age in the control and after perinatal hypoxia. The state of hypoxia was induced by exposure of rats at the age of 10 postnatal days (pd) (that corresponds approximately to the time of birth in humans) to a respiratory medium with low O_2 content (4% O_2 and 96%N_2) for 12 min (up to the initiation of clonico-tonic seizures). Here, we found that the initial rate of [^{3}H]GABA uptake was higher in the young rats (pd 17–19) as compared to the older ones (pd 24–26, 38–40 and 66–73) in both control and hypoxia groups. It decreased abruptly by 50% in the thalamus and by 25% in the cortex for the period from pd 17–19 to pd 66–73. In the hippocampus, a decrease in the rate during the same time interval was 25%. Exposure to hypoxia had no effect on the intensity of [^{3}H]GABA uptake by the cortical and thalamic nerve terminals, but caused a significant age-dependent attenuation (by 35%) of the uptake intensity in the hippocampal ones. Significant age-dependent hypoxia-independent decrease in [^{3}H]GABA uptake with step-like dynamics of changes was shown in the thalamus and cortex. Gradual age-dependent hypoxia-dependent decrease in [^{3}H]GABA uptake was revealed in the hippocampus, and so a particular vulnerability of the latest structure to hypoxia as compared to the cortex and thalamus was revealed.

1. Introduction

Normal neuronal function based on a tight balance between excitation and inhibition. The latest one in the mammalian central nervous system is typically mediated by neurotransmitter γ-aminobutyric acid (GABA). Definite ambient level of this neurotransmitter is keeping up by specific high-affinity, Na^+/Cl^--dependent GABA transporters in the plasma membrane of the presynaptic nerve terminals. Four types of GABA transporters (GATs) belong to the SLC6 superfamily of Na^+-dependent transporters, and are expressed in the nerve terminals and glial cells, that is, GAT1, GAT2, GAT3, and GAT4. The transporters rapidly terminate inhibitory synaptic transmission after exocytotic release of GABA removing the latest from the synaptic cleft, and thereby maintaining optimal ambient level of the neurotransmitter (Borden, 1996; Dalby, 2003; Kersanté et al., 2013; Richerson and Wu, 2004).

Frahm and Draguhn (2001) have showed that the GABAergic system undergoes deep functional and structural alterations during the first 2 weeks of postnatal development including changes in GABA transporter expression and subcellular distribution of interneurons. Impairment of transporter-mediated uptake of neurotransmitters can be registered during hypoxia (Borisova et al., 2004; Borisova and Himmelreich, 2005; Borisova and Krisanova, 2008; Krisanova et al., 2009).

Perinatal hypoxia leads to numerous chronic neurological deficits which include learning and memory disabilities, mental retardation, behavioral abnormalities and epilepsy. A population-based analysis in children of Ronen et al. (2007) on a long-term prognosis from neonatal seizures examined outcome in a cohort following neonatal seizures. The development of epilepsy, mental retardation, cerebral palsy, learning disorders and even death was demonstrated. Premature infants with seizures show high rates of subsequent long-term disability and mortality. The severity of pathologic processes is the main determinants for outcome prognosis (Ronen et al., 2007). Early life hypoxia pathological consequences can result from deregulation of maturation process. Misbalance between the processes of excitation and inhibition after

Abbreviations: GABA, (γ-aminobutyric acid); GATs, (GABA transporters); NO-711, (Tetrahydro-1-(2-(((diphenylmethylene)amino)oxy)ethyl)-3-pyridinecarboxylic acid hydrochloride); ANOVA, analysis of variance; pd, post-natal day; S.E.M., standard error of the mean

E-mail address: nataly.pozdniakova@gmail.com.

http://dx.doi.org/10.1016/j.ijdevneu.2017.09.002
Received 27 January 2017; Received in revised form 26 June 2017; Accepted 5 September 2017
Available online 06 September 2017
0736-5748/

hypoxia and seizures at early age is crucial aspect in the etiology of neurological disorders. Changes in the functioning of GATs are considered to be a reason of the development of chronic neurological abnormalities after early age hypoxia (Dalby, 2003; Richerson and Wu, 2004).

In the present study, the hypoxia-induced neonatal seizure model developed by Jensen et al. (1998) was used. A single brief episode of moderate graded global hypoxia in rats at the age of 10–12 postnatal days (pd) causes a long-lasting increase (70–80 days after hypoxia) in seizure excitability in hippocampal slices (Jensen et al., 1998). The model exhibits a seizure-free latent period following by an increase in the frequency of electroclinical seizures. The prevalence of epilepsy in cortical and hippocampal recordings using epidural cortical electroencephalography and hippocampal depth electrodes was 94.4% in adult rats (pd 60–180) (Rakhade et al., 2011). This model mimics the age-related specificity of the proepileptogenic effects of global hypoxia, and the chronic increase in seizure excitability and epilepsy risk were clinically observed (Sanchez et al., 2012). The age in rat pd 10 corresponds approximately to the time of birth in humans by a range of anatomic and biochemical parameters (Romijn et al., 1991). In humans, this latent period can extend for months to years. The presence of the latent period in epileptogenesis offers opportunities for targeted modulation of GABA transporter activity and in general GABA-ergic neurotransmission by specific medicines to prevent the development of clinical epilepsy. The administration of specific anticonvulsant agents in latent period attenuates the hippocampal hyperexcitability in the rodent model (Rakhade et al., 2008; Sanchez et al., 2005).

In our previous study, rats underwent to perinatal hypoxia/seizures showed a long-lasting increase in the ambient level of [^{3}H]GABA in cortical and hippocampal nerve terminals. Thalamic GATs had lower affinity to GABA in contrast to the cortical and hippocampal ones (Pozdnyakova et al., 2011). Also, we recently demonstrated that the efficacy of β-alanine (the inhibitor of GAT3) to affect uptake of [^{3}H]GABA was augmented in the hippocampal and thalamic nerve terminals after perinatal hypoxia, whereas the related capacity of NO-711 (GAT1 blocker) was lowered in the thalamic nerve terminals. These facts may be associated with the changes in the ratio of active GAT1 and GAT3 expressed in the nerve terminal plasma membranes after perinatal hypoxia. A principal possibility of non-GAT1-targeted modulation of activity of GABA transporters in different brain regions by both exogenous and endogenous β-alanine was suggested (Pozdnyakova et al., 2014).

Two main questions are rising from abovementioned facts, the first one is whether or not perinatal hypoxia differently affects transporter-mediated [^{3}H]GABA uptake in the cortical, hippocampal and thalamic nerve terminals, and the second one – whether perinatal hypoxia-induced changes are age-dependent? Therefore, the aim of the research was to analyze comparatively the initial rate of transporter-mediated [^{3}H]GABA uptake by the cortical, hippocampal and thalamic nerve terminals isolated from rats of different age in the control group and animal group preliminary underwent to hypoxia/seizures at the early age (10–12 postnatal days).

2. Materials and methods

2.1. Materials

Aminooxyacetic acid, *N*-2-hydroxyethylpiperazine-*n*-2-ethanesulfonic acid (HEPES), ethylenediaminetetraacetic acid (EDTA), D-glucose, sucrose, Whatman GF/C filters, Ficoll-400, analytical grade salts were purchased from Sigma (St. Louis, MO, USA.). [^{3}H]GABA (γ-[2,3-^{3}H(*N*)]-aminobutyric acid) was from Perkin Elmer (Waltham, MA, USA), and Organic Counting Scintillant (OCS) were received from Amersham (Little Chalfont, UK).

2.2. Ethics statement

The experiments were carried out in accordance with the European Guidelines and International Laws and Policies. Wistar rats from the vivarium of M.D. Strazhesko Institute of Cardiology, Medical Academy of Sciences of Ukraine were used in the study. Animals were kept in special facilities of the Palladin Institute of Biochemistry of NAS of Ukraine in Kyiv in accordance with the European Guidelines and International Laws and Policies. Animals were kept in a quiet temperature-controlled room (22–23 °C) and provided with water and dry food pellets *ad libitum*. All procedures conformed to the guidelines of the Palladin Institute of Biochemistry. The experimental protocols were approved before starting the experiments by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol №1 from 19/09-2012).

2.3. Exposure to hypoxia

Wistar rat litters of 8–10 male pups were divided in two equal control and experimental sub-groups containing 4–5 pups. In each subgroup, animals were used in the experiments at the age of pd 17–19, pd 24–26, pd 38–40 and pd 66–73 with proportion one animal per one pd period. In this study, we used 6 litters referred in the result section as 6 independent experiments (n = 6). Animals exposed to hypoxia and their control littermates were taken in the experiments at pd 17–19, pd 24–26, pd 38–40 and pd 66–73, and so four pd periods were analysed in the control experiments and after perinatal hypoxia. Summarising, the experiments with rat brain cortical, hippocampal and thalamic nerve terminals (synaptosomes) were performed at pd 17–19, pd 24–26, pd 38–40 and pd 66–73 in the control (24 animals) and after hypoxia (24 animals).

At postnatal days 10–12 (pd 10–12), males from “hypoxia” subgroup were removed from the litter and placed in an airtight chamber infused by atmosphere composed of 4% O_2 and 96% N_2. The exposure in the chamber lasted for 12 min up to development of strongly pronounced tonico-clonic seizures (Jensen et al., 1998). Only those animals that showed pronounced tonicoclonic seizures were used in the experiments. Among pups exposed to hypoxia-induced neonatal seizures, the mortality was not observed either during hypoxic exposure or in the subsequent post-hypoxic period. The synaptosomal preparations from hippocampus, thalamus and the motor zone of the cortex were isolated from one animal. The synaptosomes from control and experimental animals from each litter were analysed simultaneously.

The synaptosomes retain all features of the intact nerve terminals. They are able to maintain the membrane potential, to accomplish exocytotic release and uptake of the neurotransmitters (Pastukhov et al., 2016; Horák et al., 2017). The synaptosomes are one of the best systems to investigate the relationship between the structure of a protein, its biochemical properties and physiological role (Südhof, 2004).

2.4. Isolation of nerve terminals

Rats were rapidly decapitated before removing the brain. The brain was quickly removed after decapitation and immediately placed in ice-cold solution (0.32 M sucrose, 5 mM HEPES-NaOH, pH 7.4, 0.2 mM EDTA). Then, the motor zone of the cortex, hippocampus and thalamus were rapidly removed and homogenized in ice-cold solution (0.32 M sucrose, 5 mM HEPES-NaOH, pH 7.4, 0.2 mM EDTA) taken in the ratio of 1:10 (weight/volume). The homogenates were centrifuged (2500*g*, 5 min), the supernatants were removed and again centrifuged at 15,000*g* for 12 min in order to isolate crude synaptosomal fraction. Purified synaptosomes from the motor zone of the cortex were prepared by Ficoll-400 density gradient centrifugation of crude preparations according to the method of Cotman (1974) with slight modifications (Borisova and Krisanova, 2009; Borisova, 2014; Borisova et al., 2014, 2006). The synaptosomes were suspended in the standard salt solution