Functional and Morphological Changes of the Brain in Rats Exposed to Intermittent Hypobaric Hypoxia after the Repetitive Magnesium Administration

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Abstract: Intermittent hypobaric hypoxia induces functional and morphological changes of the brain in 25-day-old rats. Administration of magnesium has partial pro-convulsion effect in hypoxia not exposed rats and it practically does not influence the excitability of cortical neurones in rats exposed to intermittent hypoxia. Magnesium administration decreases the number of NADPH-diaphorase neurones in rats exposed to hypoxia in all studied areas of the hippocampus and dentate gyrus. In control rats this effect was only in CA1, CA3 and in the ventral blade of the dentate gyrus. Increased concentration of magnesium in cells of the hypoxia exposed rats after the repeated magnesium administration was found.

Key words: Rats – Hypoxia – Magnesium – Epileptic seizures – Brain – Neurons – Nitrogen oxide - Red blood cells

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Introduction

Early and late consequences of the hypoxic injury in the foetal and newborn brain can be observed in babies as mental retardation, seizure disorders, cerebral palsy etc. [1, 2]. The brain is more susceptible to hypoxia than other organs, as it has high oxygen demand and only little energy is produced by anaerobic mechanisms [3, 4]. Brain damage results from a cascade of metabolic events induced by calcium influx into the cells as an effect of altered ion homeostasis and membrane depolarization and due to the activation of glutamate receptors of calcium channels [5, 6].

Hypoxia in experimental animals evokes series of behavioural, morphological [7, 8] and electrophysiological changes [9, 10]. A histological analysis of the central nervous system in animals exposed to hypoxia demonstrated signs of fine damage in the cytoarchitectonics of the neurones and macroglia in the cerebral cortex and in the hippocampus [11], changes in the arborization of neuronal dendritic processes and changes in the spine density [12].

One of the possible mechanisms of the brain functional changes during hypoxia and during post hypoxic period (reperfusion) is the activation of some enzymes as are xantine oxidase or nitric oxid synthase (NOS). Activity of this enzyme is a criterion of the production of the nitric oxid (NO), one of modulators of synaptic transmission [13].

Clinical and experimental studies described the role of different cations on the function and structure of the brain in hypoxic/ischemic conditions and during the reperfusion period. Important regulatory function in many intracellular mechanisms has magnesium [14]. Magnesium deficiency causes central nervous hyperexcitability, which results from a sum of direct cellular effects that induce depolarization and hyperexcitability [15].

Magnesium may also act my means of vascular effect – increasing cerebral blood flow by vasodilatation of cerebral arteries and by lowering blood pressure [16]. Neuronal effects include the block of NMDA receptor ion channel, calcium antagonism at voltage-gated channels, enhanced buffering of intracellular calcium ions and enhanced regeneration of adenosine triphosphate [17].

In the present study we analysed possible protective effect of magnesium on the developing brain exposed to hypoxic conditions on the basis of functional and morphological changes of the brain.

Materials and Methods

1. Functional study

Experiments were performed on freely moving 25-day-old male rats (Wistar strain). Rats, together with their mother, were exposed to the simulated altitude of 7 000 m for 8 hours per day since birth to the age of 17 days, excepting the 6th, 7th, 13th and 14th day.

Excitability of cortical neurones was tested by the repeated electrical stimulation of the sensorimotor cortex [9]. Following groups of rats were studied:

1. Control rats not exposed to hypoxia (C)
2. Control rats repeatedly injected with magnesium sulphuricum – 300 mg/kg i.p. (C + Mg)
3. Rats exposed to repeated hypobaric hypoxia (H). Excitability was tested 8 days after the last exposition to hypoxia
4. Rats exposed to repeated hypobaric hypoxia as above and repeatedly injected with magnesium sulphuricum – 300 mg/kg i.p. before the hypoxia exposition (H + Mg)
5. Rats exposed to repeated hypobaric hypoxia and repeatedly injected with saline solution before the hypoxia exposition (H + S)

The duration and shape of evoked epileptic seizures – cortical afterdischarges (ADs) was estimated. ANOVA and t-test were used for evaluation of results. Significance was set on 5 % level.

2. Morphological study
Animals were studied the 25th day, 8 days after the last exposure to hypoxia. Brains were processed for NADPH-diaphorase staining [18]. CA1 and CA3 area of the hippocampus, dorsal, ventral blade and hilus of the dentate gyrus were subjected to quantification of the NADPH-d positive neurones.

Results obtained at experimental animals were compared with that of intact controls.

3. As the reference value of the intracellular magnesium concentration we determined its concentration in red blood cells of animals not exposed to hypoxia (C), in rats exposed to repeated hypoxia (H) and in rats exposed to repeated hypoxia with magnesium pre-treatment (H + Mg).

Results

1. Electrophysiological study
Repeated exposition of young animals together with their mothers to hypobaric hypoxia seriously retards the somatic development. Hypoxic animals had lower body weight and magnesium administration was not effective in the prevention of the weight loss (Fig. 1).

Stimulation of the sensorimotor cortex elicits cortical afterdischarges – evoked epileptic seizures. In control animals the significant decrease ($p < 0.001$) of the 2nd, 3rd a 4th AD (in correlation to the first AD) was observed – a phase of the postictal depression was registered (Fig. 2). Repeated magnesium administration increased the duration of evoked epileptic seizures in animals not exposed to hypoxia after the 2nd and 3rd stimulation ($p < 0.001$) and the phase of the postictal depression was not registered (Fig. 3).

Exposition to hypobaric hypoxia led to the prolongation of cortical afterdischarges ($p < 0.001$) (Fig. 2). Pre-treatment with saline did not change the duration of

evoked epileptic seizures. Magnesium pre-treatment brought about shortening of AD only after the 3rd stimulation ($p < 0.05$) (Fig. 4).

2. Histological Study

Repeated magnesium administration to intact rats did not change the number of NADPH-diaphorase positive neurones in the hilus of dentate gyrus (DG) or in the dorsal blade of DG (Fig. 5, 6). However, it brought about the decrease of NADPH-diaphorase positive neurones in the ventral blade of DG, in CA1 and in CA3 areas of the hippocampus ($p < 0.001$) (Fig. 7, 8, 9).

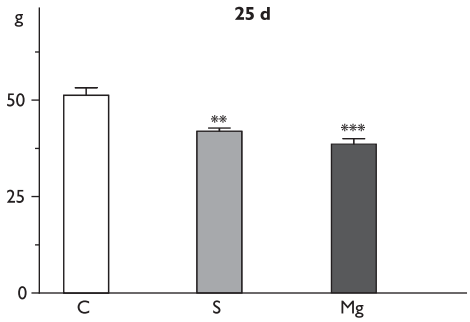

*Figure 1 – Body weight in rats not exposed to hypoxia (white column) and in rats exposed to intermittent hypobaric hypoxia after the administration of saline solution (shaded column) and magnesium (black column). *** significance level of differences at p < 0.05 – 0.001*

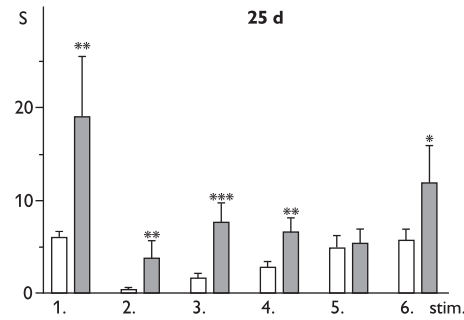

*Figure 2 – The duration of evoked cortical afterdischarges in 25-day-old rats. White columns – rats not exposed to hypoxia, shaded columns – rats exposed to repeated hypobaric hypoxia. *** significance level of differences at p < 0.05 – 0.001*

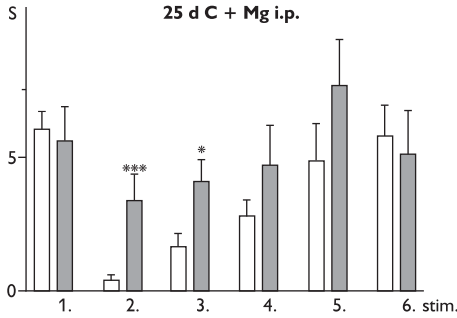

*Figure 3 – The duration of evoked cortical afterdischarges in 25-day-old rats. White columns – rats not exposed to hypoxia, shaded columns – rats not exposed to hypoxia after magnesium administration. *** significance level of differences at p < 0.05 – 0.001*

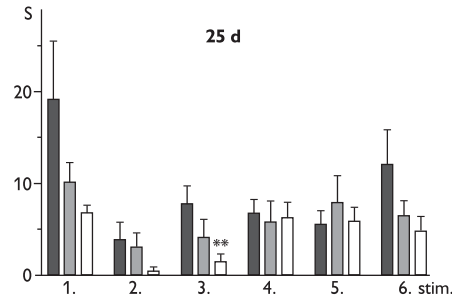

Figure 4 – The duration of evoked cortical afterdischarges in 25-day-old rats. Black columns – rats exposed to repeated hypoxia, shaded columns – rats exposed to hypoxia after saline solution, white columns – rats exposed to hypoxia after magnesium administration.

Perinatal exposition to hypoxia resulted in the increased density of NADPH-diaphorase positive neurones in the hilus of dentate gyrus by 37% ($p < 0.001$) (Fig. 5). At the same time the density of NADPH-diaphorase positive neurones was lower in the dorsal blade of DG by 18% ($p < 0.001$) (Fig. 6) and in the ventral blade of DG by 19% ($p < 0.01$) (Fig. 7) when compared to hypoxia not exposed rats. Hypoxia did not interfere with the density of NADPH-diaphorase positive neurones in the CA1 (Fig. 8) and CA3 (Fig. 9) areas of the hippocampus.

Magnesium pre-treatment in hypoxia exposed rats reduced density of NADPH-diaphorase positive neurones in the hilus by 22% ($p < 0.001$) (Fig. 5), in the dorsal

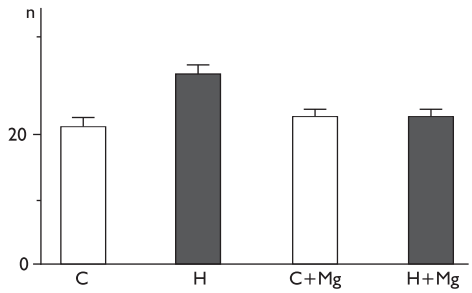

Figure 5 – Number of NADPH-diaphorase positive neurones in hilus in 25-day-old rats. White columns – rats not exposed to hypoxia (C) and after magnesium administration (C + Mg), black columns – rats exposed to hypoxia (H) and after magnesium administration (H + Mg).

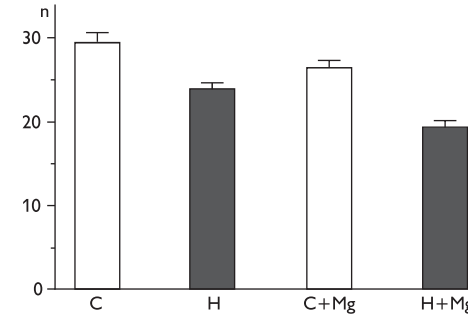

Figure 6 – Number of NADPH-diaphorase positive neurones in the dorsal blade of the dentate gyrus in 25-day-old rats. White columns – rats not exposed to hypoxia (C) and after magnesium administration (C + Mg), black columns – rats exposed to hypoxia (H) and after magnesium administration (H + Mg).

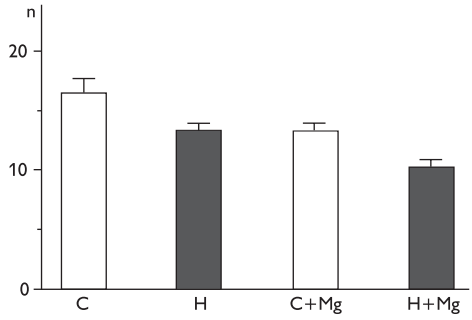

Figure 7 – Number of NADPH-diaphorase positive neurones in the ventral blade of the dentate gyrus in 25-day-old rats. White columns – rats not exposed to hypoxia (C) and after magnesium administration (C + Mg), black columns – rats exposed to hypoxia (H) and after magnesium administration (H + Mg).

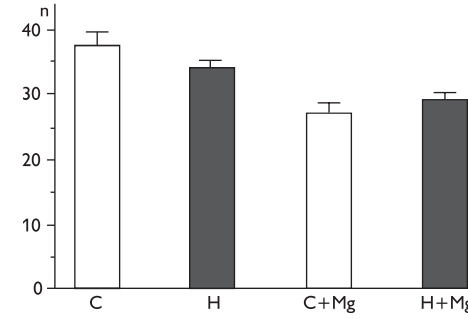

Figure 8 – Number of NADPH-diaphorase positive neurones in CA1 area of the hippocampus in 25-day-old rats. White columns – rats not exposed to hypoxia (C) and after magnesium administration (C + Mg), black columns – rats exposed to hypoxia (H) and after magnesium administration (H + Mg).

blade by 20% ($p < 0.001$) (Fig. 6) and in the ventral blade of the DG by 23% ($p < 0.001$) (Fig. 7). Similarly in the CA1 hippocampal area the density of NADPH-diaphorase positive neurones was lower by 14% ($p < 0.005$) (Fig. 8) and in CA3 area by 26% ($p < 0.001$) (Fig. 9) when compared with animals exposed to hypoxia only.

3. Concentration of magnesium in red blood cells: The concentration did not differ between the controls (C) and hypoxia exposed rats (H). The pre-treatment with magnesium in animals repeatedly exposed to hypoxia increased ($p < 0.001$) its intracellular concentration (Fig. 10).

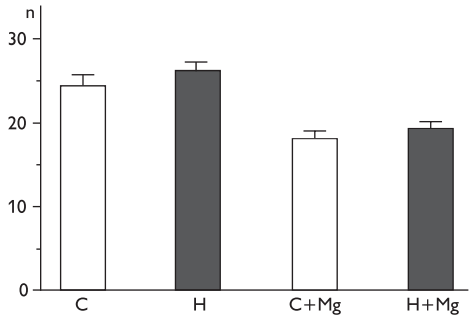

Figure 9 – Number of NADPH-diaphorase positive neurones in CA3 area of the hippocampus in 25-day-old rats. White columns – rats not exposed to hypoxia (C) and after magnesium administration (C + Mg), black columns – rats exposed to hypoxia (H) and after magnesium administration (H + Mg).

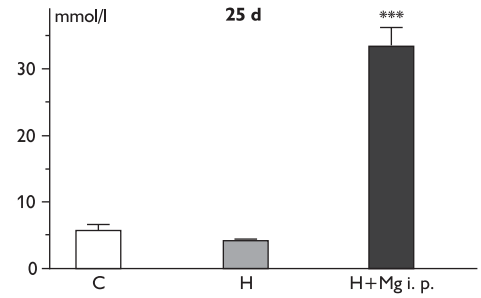

*Figure 10 – Concentration of the magnesium in red blood cells in 25-day-old rats. White column – rats not exposed to hypoxia, shaded column – rats exposed to hypoxia, black column – rats exposed to hypoxia after magnesium administration. *** significance level of differences at $p < 0.05 – 0.001$*

Discussion

Repeated exposition of newborn rats together with theirs mothers to the intermittent hypoxia retarded their somatic development. It may result both from the hypoxia effect on the young animals and from its effect on the maternal organism [3].

Hypoxia alters seizure susceptibility [2, 9, 10]. There are several possible explanations of such increased of excitability in 25-day-old rats, eight days after the last exposition of hypoxia. Effect can result from the alternation of neurotransmitter concentration, receptor densities and sensitivities or from an imbalance between inhibitory and excitatory systems. Green and collaborators described inhibition of GABA synthesis and release as a consequence of the cerebral hypoxia/ischemia and convulsions [19]. Several studies have implicated the role of the excitatory amino acid glutamate in hypoxic/ischemic conditions [20, 21, 22]. Epileptogenic effect of hypoxia may be induced by activation NMDA

and AMPA/KA glutamate receptors [23]. Repeated exposition of animals to hypoxia increases production of lactic acid and decreases the extracellular pH. The decline of pH down-regulates NMDA receptors and enhances AMPA mediated excitotoxicity [24]. Another possibility of the higher excitability after hypoxia is the excess of glutamate during the reperfusion phase. Increase of glutamate level can last several hours after the hypoxia insult [21].

Magnesium pre-treatment partly abolished the phase of postictal depression in control animals, but this effect was not seen in animals exposed to repeated hypoxia. The pro-convulsive effect was probably induced by the influence of magnesium on NMDA glutamate receptors.

Nitric oxide is a source of nitric oxide free radical ($NO^{\cdot}$). $NO^{\cdot}$ acts as a unconventional neurotransmitter and in addition it plays an important role in apoptotic processes [25]. Changes in the density of NADPH-diaphorase positive neurones (an assessment activity of the nitric oxid synthase) in the hilus of the dentate gyrus occurred immediately after the last exposure to hypoxia [18]. In our study, hypoxia did not change the number of NADPH-diaphorase positive neurones in CA1 and CA3 regions of the hippocampus, but induced changes of these neurons in the dentate gyrus (decrease in the ventral and dorsal blade) and in the hilus (increase). Hypothetical explanation is based on the sprouting mechanism in the part of the hilus as a reaction to the loss of NADPH-d positive neurons in the ventral and dorsal parts of the dentate gyrus.

Pre-treatment with magnesium of rats exposed to intermittent hypobaric hypoxia brought about the decrease of the number of NADPH-diaphorase positive neurones in all studied areas. Possible protective effect of the magnesium administration can be explained by the regulation of NMDA receptor activation and by the decrease of calcium influx and subsequent lower production of NO [26].

Conclusion

Described functional and structural changes of the brain of young animals demonstrate the effect of intermittent hypobaric hypoxia. Experimental elevation of magnesium concentration had pro-convulsion effect in hypoxia not exposed rats and it practically did not influence the excitability of cortical neurones in rats exposed to intermittent hypoxia. Magnesium administration caused reduction of the number of NADPH-diaphorase neurones. It cannot be excluded that the NO synthesis inhibition represents a protective mechanism against the possible free radical impairment.

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