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Influence of Magnesium Sulphate on Evoked Activity of Rat Brain after Exposure to Short-term Hypoxia

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Summary

Young Wistar rats (aged 12, 25 and 35 days) were exposed to short-term (60 min) hypobaric hypoxia of 41 kPa. Cortical afterdischarges (ADs) were evoked by repeated direct stimulation of the sensorimotor cortex and the duration of ADs was monitored to examine the influence of magnesium sulphate injection (0.3 g/kg b.w.). In 12-day-old hypoxia-exposed rats, an increase of the mean duration of ADs after the repeated stimulation appeared. This effect was prevented by magnesium administration. In 25- and 35-day-old rats exposed to hypoxia a shortening of ADs was registered but no specific effect of magnesium sulphate pretreatment was observed. The brain susceptibility and ability to terminate evoked seizures is discussed.

Key words

Hypoxia • Magnesium • Excitability • Calcium • Glutamate

Introduction

Hypoxia belongs to the most serious factors that can directly impair the function of metabolic pathways in the animal cell. Excessive intracellular accumulation of calcium (Ca^{2+}) ions is proposed as a "final common pathway" for cell injury (Vannucci *et al.* 2001). Morphological or functional effects depend on the age of the organism undergoing hypoxia, on the duration, intensity and the type of hypoxic stimulus (Benešová *et al.* 2004, Marešová 2004). Some experimental studies confirmed that hypoxia causes overall membrane depolarization and a higher excitability (Nieber 1999, Rubaj *et al.* 2003). The altered membrane potential can lead to a voltage-dependent influx of Ca^{2+} ions into the cell. Another Ca^{2+} influx is mediated by an activation of glutamate receptors. NMDA receptor-mediated excitotoxic injury accompanied by depolari-zation and hyperexcitability results in neurodegeneration and an apoptotic/necrotic process (Martin *et al.* 1998). Magnesium blocks NMDA channels in a voltage-dependent manner (Gathwala 2001). An increase of this activity in a situation of decreased membrane polarization could prevent neurons from damage (Pokorný *et al.* 1989, Nelson *et al.* 2003). Magnesium also has other protective effects (Maulik *et al.* 2001). It is known that its administration in a simulated hypoxic/ischemic damage limits neurological impairment in several animal models (Sameshima and Ikenoue 2001).

The developing brain reaction to hypoxia may differ from that of the adult brain. The aim of this study was to test the influence of a single of magnesium

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sulphate on evoked cortical afterdischarges (ADs) in young rats, which had previously been exposed to short-term hypobaric hypoxia.

Methods

All experiments were approved by the Ethical Committee of the First Faculty of Medicine (Charles University in Prague) and were in agreement with the Guidelines of the Animal Protection Law of the Czech Republic.

Experiments were performed on freely moving 12-, 25- and 35-day-old Wistar rats of our own breed. Six silver electrodes were implanted through the cranium under ether narcosis: over prefrontal cortex (reference electrode), right sensorimotor cortex (two stimulation electrodes), left sensorimotor cortex, left visual and right visual cortex (registration electrodes). All experimental manipulations were carried out after recovery of the uprighting reflex (i.e. approximately 15 min after surgery).

Each of the age groups was divided into four subgroups – control group (neither injection nor hypoxia), hypoxia-exposed rats, PS-group (injection of saline before hypoxia) and Mg^{2+}-group (received injection of magnesium sulphate before the exposure to hypoxia). Each subgroup consisted of 8-10 animals with successful elicitation of the epileptic seizure. Cortical afterdischarges were elicited by stimulation of the right sensorimotor cortex. We used constant current (CC) stimulation (bipolar pulses – pulse period 1 ms; duration of stimulation 15 s; frequency 8 Hz; intensity 3-5 mA). The basic stimulation intensity level was set at 3 mA. In case of no response, another stimulation of 4 mA was used 5 min after the first stimulation. The process was similarly repeated with 5 mA stimulation. Finally, if no epileptic graphoelements appeared after the 5 mA stimulation, the animal was excluded from the experiment. If a distinct response (epileptic graphoelements) was recorded, the stimulation was repeated five times at one-minute intervals (timed from the end of each seizure to the beginning of the next stimulation).

In the Mg^{2+}-groups the magnesium sulphate (0.3 g/kg b.w.) was injected intraperitoneally and in the PS-groups the same volume of saline was injected. A 15-min rest after the injection was followed by exposure to 41 kPa hypobaric hypoxia, i.e. to the simulated altitude of 7000 m, which was reached in 2 min (30 kPa/min) and lasted for 60 min. After returning to air pressure of approximately 101 kPa (30 kPa/min) and after another 15-min period of rest the stimulation paradigm was started. The duration of evoked ADs and shape of evoked graphoelements was monitored. The unpaired t-test and ANOVA in GraphPadPrism were used for evaluation of the results.

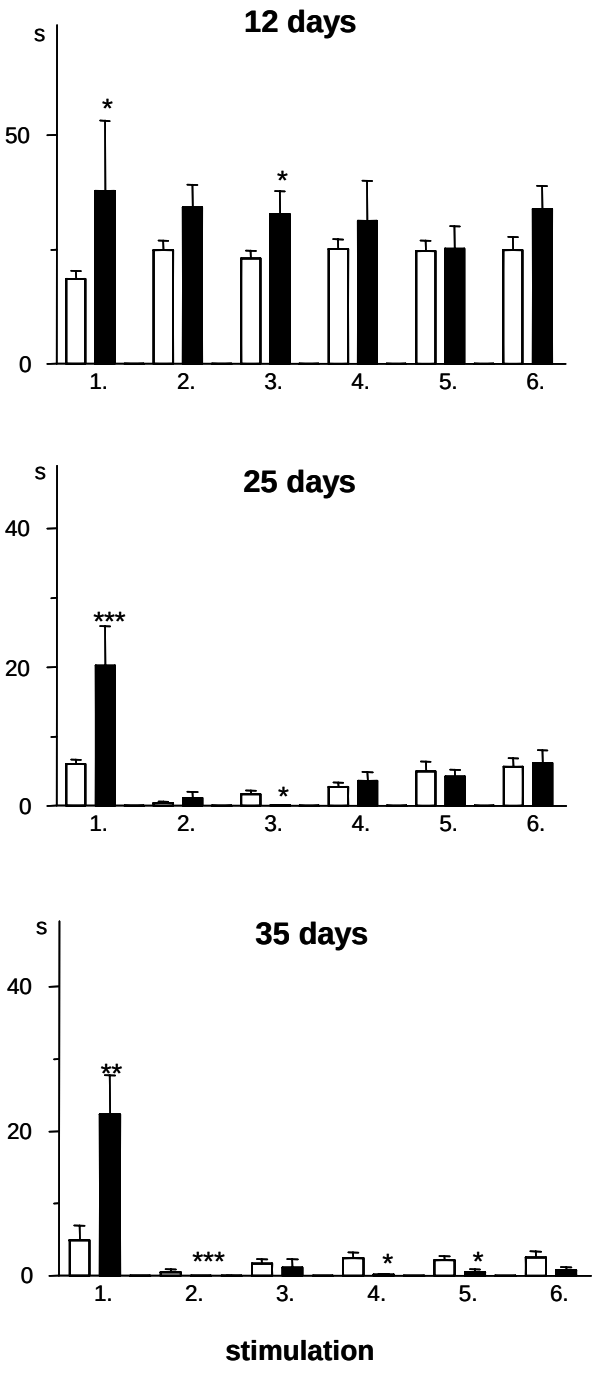

Fig. 1. Afterdischarges duration in control rats and in rats exposed to hypoxia. White columns – rats not exposed to hypoxia. Black columns – rats exposed to hypoxia, * indicates results significant at $p<0.05$, ** $p<0.01$, *** $p<0.001$

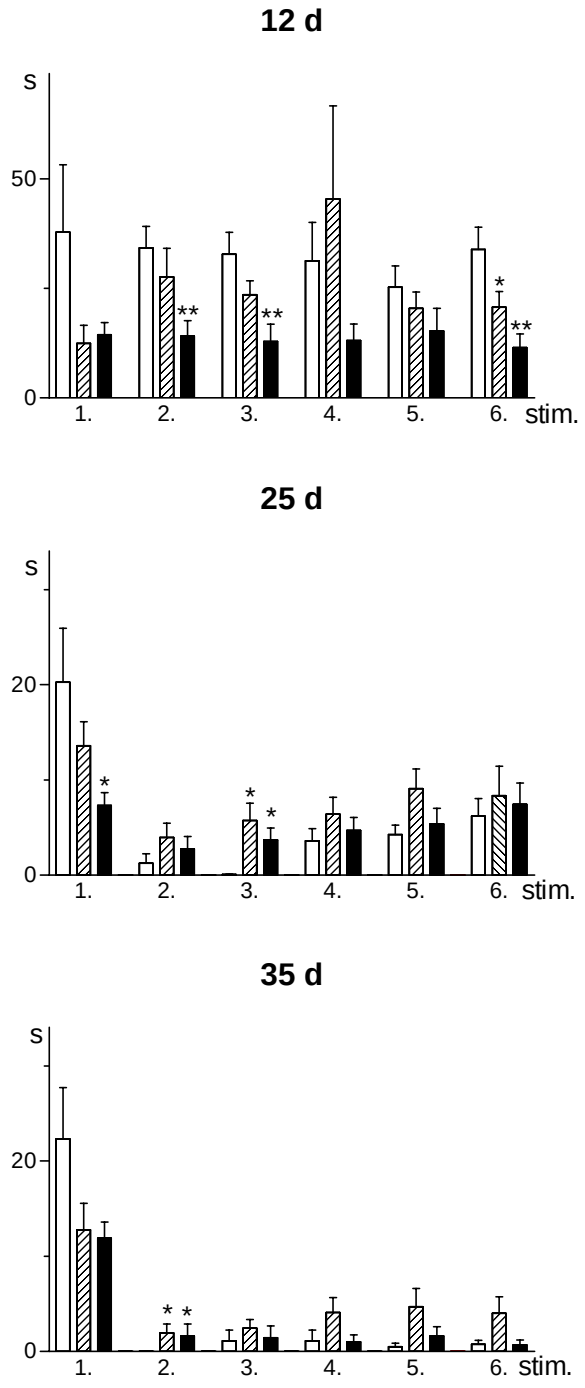

Fig. 2. Afterdischarges duration in rats exposed to hypoxia and after PS and Mg^{2+} pre-treatment. White columns – rats exposed to hypoxia. Hatched columns – rats after PS pretreatment. Black columns – rats after Mg^{2+} pretreatment, * indicates results significant at $p<0.05$, ** $p<0.01$

Results

Short-term exposure to hypobaric hypoxia led to prolongation of the first AD in all age groups in comparison to control rats not exposed to hypoxia. With the stimulation repetition the increase of the mean durations of the ADs in 12-day-old rats after the 3rd stimulation and shortening of ADs in older animals was registered (Fig. 1).

Duration of ADs in the 12-day-old PS-group and control group rats was rising after the first AD. In 12-day-old Mg^{2+}-group no prolongation of seizures after the repeated stimulation appeared. No specific effect of magnesium on the duration of ADs after repeated stimulation in older animals was observed.

The comparison of PS- and Mg^{2+} -groups revealed, that the first ADs in 25-day-old animals were shorter. No significant change of ADs duration in 35-day-old rats was registered. In 12-day-old magnesium treated rats, significantly shorter ADs were found after the 2nd and 3rd stimulation (Fig. 2). No difference was found between the PS- and Mg^{2+}-groups in the CC-stimulation intensity thresholds.

Various types of ECoG graphoelements were observed. In 25-day-old and 35-day-old animals graphoelements consisting of spike-and-waves prevailed. A small amount of biphasic bursts superposed on slow waves appeared. Contrary to this, in most of the 12-day-old rats positive fast sharp waves were observed, while the bursts and spike-and-waves were rarely recorded. No difference in the appearance of graphoelements among all experimental groups was identified.

Discussion

Due to the differences in mean ADs duration between the age groups studied (Fig. 1), the ability to terminate seizure seems to be lower in young animals than in older ones (ANOVA $p<0.001$) (Mareš and Trojan 1990, Marešová *et al.* 2001). This correlates with maturation of the nervous structures and neurotransmitter systems, which influence the excitability of nerve cells (Moshé 1987, Sutor and Luhmann 1995).

In 12-day-old animals we observed the prolongation of the mean AD duration with their repetition. It could signalize the possibility of occurrence of acute kindling before the postictal inhibition caused by activation of inhibitory systems (Heinemann and Eder 1999). Inhibitory systems in 25-day-old and 35-day-old rats were enough developed to prevent the prolongation of ADs after the repeated stimulation – postictal inhibition was registered. Some decrease of excitability could also be supposed in 12-day-old animals, but its basis must differ from that in older animals due to the later development of inhibitory transmitter systems. It is

possible that developmental changes of long posttetanic inhibition may also be involved (Mareš and Marešová 1998). The intracellular Ca^{2+}, plays important role in this form of synaptic plasticity.

Depolarization is well known as an early-onset mechanism that occurs after a short-term oxygen deficit (Balestrino 1995). The excitotoxic hypothesis suggests synaptic glutamate oversecretion to be the primary reason of the depolarization (Choi 1988). The calcium hypothesis considers that the exceeding intracellular Ca^{2+} concentration has the most remarkable effect (Siesjö and Bengtsson 1989, Vannucci *et al.* 2001). Excessive increase in the intracellular Ca^{2+} concentration (so called Ca^{2+} overload) is caused by the decreased extrusion of Ca^{2+} by Ca^{2+}-ATPase due to the diminished ATP level (Kass and Lipton 1986), increased Ca^{2+} influx through voltage-gated Ca^{2+} channels (Choi 1990), increased activity of the Na^+/Ca^{2+} exchanger caused by a prolonged depolarization and Na^+ influx (Frandsen and Schousboe 1993) and increased release of Ca^{2+} from intracellular stores (Frandsen and Schousboe 1991, Nieber 1999). Finally, the metabolic hypothesis is based on the fact that the oxygen deficit leads to a metabolic block in oxidative phosphorylation, decrease in intracellular pH and increase in lactate concentration (Groenendaal *et al.* 1999). It brings ATP shortage with mitochondrial Na^+/K^+-ATPase lesion (Maulik *et al.* 2001) and mitochondrial dysfunction. All of the intracellular mechanisms suffer from inadequate energetic supply and thus the plasma membrane becomes soon short of energy necessary to maintain the ionic gradients.

Magnesium sulphate eliminates the prolongation of ADs after repeated stimulation in the group of youngest animals (enhanced by exposure to short-term hypoxia). At this age AMPA-receptors-related Ca^{2+} inflow may play an important role (Sanchez *et al.* 2001). In contrast, the function of NMDA-receptors remains sufficient in most regions (Hurst *et al.* 2001). Lowering of the Ca^{2+} influx through NMDA channels may cause relatively low activity of Ca^{2+}-dependent protein kinases.

The ability of nervous system to terminate seizure depends on the mechanisms renewing equilibrium between the excitatory and inhibitory systems after the seizure has already started. Susceptibility of the central nervous system to begin seizure is not affected by the magnesium sulphate injection (there was no distinct difference between Mg^{2+}-group and PS-group in the intensity of stimulation necessary to evoke seizures).

In newborns and children, hypoxia-ischemia is a major factor leading to epileptogenesis, and several schemes are proposed to classify, quantify and prevent hypoxic-ischemic encephalopathy (Lombroso and Burchfiel 1987). The beneficial effect of magnesium sulphate can be explained by an increase of blood flow in the cerebral vascular bed (Kemp *et al.* 1999). Magnesium sulphate can be used in obstetrics and neonatology as a prophylactic agent administered before a predicted hypoxic period protecting the fetal brain from hypoxic-ischemic injury and consequently reducing the risk of epilepsy development, as well as in neurosurgery of patients suffering from stroke (Marinov *et al.* 1996). On the basis of our results we can presume an important modulatory role of NMDA receptors in very young animals after the hypoxia. This can elucidate some characteristics of the development of epileptic phenomena influenced by hypoxia.

In conclusion, magnesium sulphate increases the ability of the nervous system to terminate evoked afterdischarges after the exposure to short-term hypobaric hypoxia. Only in the early ontogenesis of rats, it can also eliminate the prolongation of afterdischarges after the repeated stimulation.

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References

BALESTRINO M: Pathophysiology of anoxic depolarization: new findings and a working hypothesis. *J Neurosci Methods* **59**: 99-103, 1995.

BENEŠOVÁ P, LANGMEIER M, BETKA J, TROJAN S: Changes in the number of nitrergic neurons following kainic acid administration and repeated long-term hypoxia. *Physiol Res* **53**: 343-349, 2004.

CHOI DW: Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci* **11**: 465-469, 1988.

CHOI DW: Cerebral hypoxia: some new approaches and unanswered questions. *J Neurosci* **10**: 2493-2501, 1990.

FRANDSEN A, SCHOUSBOE A: Dantrolene prevents glutamate cytotoxicity and Ca^{2+} release from intracellular stores in cultured cerebral cortical neurons. *J Neurochem* **56**: 1075-1078, 1991.

FRANDSEN A, SCHOUSBOE A: Excitatory amino acid-mediated cytotoxicity and calcium homeostasis in cultured neurons. *J Neurochem* **60**: 1202-1211, 1993.

GATHWALA G: Neuronal protection with magnesium. *Indian J Pediatr* **68**: 417-419, 2001.

GROENENDAAL F, DE GRAAF RA, VAN VLIET G, NICOLAY K: Effects of hypoxia-ischemia and inhibition of nitric oxide synthase on cerebral energy metabolism in newborn piglets. *Pediatr Res* **45**: 827-833, 1999.

HEINEMANN U, EDER C: Control of neuronal excitability. In: *Epilepsy: The Comprehensive CD-ROM*, ENGEL J, PEDLEY TA (eds), Lippincott Williams & Wilkins, 1999.

HURST RS, CEPEDA C, SHUMATE LW, LEVINE MS: Delayed postnatal development of NMDA receptor function in medium-sized neurons of the rat striatum. *Dev Neurosci* **23**: 122-134, 2001.

KASS IS, LIPTON P: Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the rat hippocampal slices. *J Physiol Lond* **378**: 313-334, 1986.

KEMP PA, GARDINER SM, MARCH JE, RUBIN PC, BENNETT T: Assessment of the effects of endothelin-1 and magnesium sulphate on regional blood flows in conscious rats, by the coloured microsphere reference technique. *Br J Pharmacol* **126**: 621-626, 1999.

LOMBROSO CT, BURCHFIEL JL: Etiologic and preventive aspects of epilepsy in the child-bridging the gap between laboratory and clinic. *Epilepsia* **28** (Suppl 1): S25-S35, 1987.

MAREŠ J, MAREŠOVÁ D: Postictal depresion in 12-day-old animals. *Cesk Fysiol* **47**: 67-73, 1998.

MAREŠ J, TROJAN S: Effect of the sequelae of acute hypobaric hypoxia on epileptic seizures in laboratory rats. Ontogenic studies. *Sb Lek* **92**: 59-63, 1990.

MAREŠOVÁ D: Changes of evoked epileptic seizures after the short term hypobaric hypoxia in the young rats. Prague. Med Rep105: 381-390, 2004.

MAREŠOVÁ D, VALKOUNOVÁ I, JANDOVÁ K, BORTELOVÁ J, TROJAN S: Excitability changes of cortical neurons during the postnatal period in rats exposed to prenatal hypobaric hypoxia. *Physiol Res* **50**: 215-219, 2001.

MARINOV MB, HARBAUGH KS, HOOPES PJ, PIKUS HJ, HARBAUGH RE: Neuroprotective effects of preischemia intra-arterial magnesium sulfate in reversibile focal cerebral ischemia. *J Neurosurg* **85**: 117-124, 1996.

MARTIN LJ, AL-ABDULLA NA, BRAMBRINK AM, KIRSCH JR, SIEBER FE, PORTERA-CAILLIAU C: Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res Bull* **46**: 281-309, 1998.

MAULIK D, QAYYUM I, POWELL SR, KARANTZA M, MISHRA OP, DELIVORIA-PAPADOPOULOS M: Post-hypoxic magnesium decreases nuclear oxidative damage in the fetal guinea pig brain. *Brain Res* **890**: 130-136, 2001.

MOSHÉ SL: Epileptogenesis and the immature brain. *Epilepsia* **28** (Suppl 1): S3-S15, 1987.

NELSON RM, LAMBERT DG, GREEN RA, HAINSWORTH AH: Pharmacology of ischemia-induced glutamate efflux from rat cerebral cortex in vitro. *Brain Res* **964**: 1-8, 2003.

NIEBER K: Hypoxia and neuronal function under in vitro conditions. *Pharmacol Ther* **82**: 71-86, 1999.

POKORNÝ J, PEČOVÁ Y, TROJAN S, LANGMEIER M: Hypoxia and development of interneurones of the rat hippocampus. *Physiol Bohemoslov* **38**: 215-222, 1989.

RUBAJ A, ZGODZIŃSKI W, SIEKLUCKA-DZIUBA M: The epileptogenic effect of seizures induced by hypoxia. *Pharmacol Biochem Behav* **74**: 303-311, 2003.

SAMESHIMA H, IKENOUE T: Long-term magnesium sulphate treatment as protection against hypoxic-ischemic brain injury in seven-day-old rats. *Am J Obstet Gynecol* **184**: 185-190, 2001.

SANCHEZ RM, KOH S, RIO C, WANG C, LAMPERTI ED, SHARMA D, CORFAS G, JENSEN FE: Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurosci* **21**: 8154-8163, 2001.

SIESJÖ BK, BENGTSSON F: Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J Cereb Blood Flow Metab* **9**: 127-140, 1989.

SUTOR B, LUHMANN HJ: Development of excitatory and inhibitory postsynaptic potentials in the rat neocortex. *Perspect Dev Neurobiol* **2**: 409-419, 1995.

VANNUCCI RC, BRUCKLACHER RM, VANNUCCI SJ: Intracellular calcium accumulation during the evolution of hypoxic-ischemic brain damage in the immature rat. *Dev Brain Res* **126**: 117-120, 2001.

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