Partial Effect of Nimodipine on Evoked Epileptic Seizures in Young Rats

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Abstract: The present work studied the effect of calcium channel blocker (nimodipine) on epileptic seizures elicited by electrical stimulation of somatosensory cortex in young rats exposed to hypoxia. Rats were exposed to different patterns of short term hypoxia (hypobaric or normobaric) and reactions of cortical neurones were registered. In the youngest animals (12-day-old), the effect was minimal, in older rats prolongation or shortening of evoked epileptic seizures were registered after two types of hypoxia. The decrease of the duration of evoked epileptic seizures in control 12-day-old rats and any effect in older animals (in 25- and 35-day-old) after nimodipine administration was observed. In older rats (25- and 35-day-old) exposed to hypobaric hypoxia after pre-treatment with nimodipine (L-type calcium channel antagonist) the duration of epileptic seizures after repeated stimulation was increased.

Introduction

Extracellular and intracellular calcium (Ca^{2+}) metabolism is precisely regulated in all tissues of the organism. Different types of calcium channels (voltage and ligand gated channels) participate on distinct biological effect of calcium. Practically all types of calcium channels are located in the central nervous system and biological effect of calcium includes mechanisms of transmitter release from presynaptic nerve terminals to bursting mode or rhythmic action (pacemaker) of neurones [1, 2, 3]. The contribution of L-type calcium channels on excitatory actions of GABA in immature brain was described [4]. Many studies showed vessel dependent protective effects of dihydropyridine – calcium channel antagonist – nimodipine (blocks the voltage dependent L-type calcium channels) on cerebral artery spasm and regional cerebral blood flow [5] or vessel independent neuroprotective effect during hypoxic condition [6, 7, 8].

Experience with T-type calcium channel blockers confirms its role in epileptogenesis [9] and involvement of L-type calcium channels were confirmed by protection from seizures activity induced by picrotoxin [10, 11]. Other authors described neuroprotective effect of nimodipine on pilocarpine-induced seizures not via blockade of calcium channels, but due to lipid peroxidation [12].

Seizures, cerebral palsy and mental disorders may be consequences of perinatal hypoxia. One of the causal mechanisms in those clinical disorders is the change of intracellular Ca^{2+}. Hypoxic injury activates multiple mechanisms of the accumulation of free calcium in the cytosol: entry of calcium from extracellular compartment by voltage and ligand – gated channels or/and from cellular organelles [13] and influx of calcium through the N-methyl-D-aspartate receptor [14]. The cascade of next enzymatic and non-enzymatic reactions leads to structural and functional changes in tissue cells [15, 16].

In the current study we tested the influence of nimodipine, the L-type calcium channel antagonist, on the excitability changes in young rats exposed to short-term normo- and hypobaric hypoxia.

Materials and methods

The experimental animals were 12, 25 and 35-day-old male Wistar rats of our own breed housed under standard temperature and light conditions and fed a complete laboratory diet and water ad libitum. All experiments were done in agreement with guidelines of the Animal Protection Law of the Czech Republic.

Changes of excitability were studied in freely moving rats with implanted cortical electrodes – two stimulation electrodes on the right sensorimotor cortex and registration electrodes on the left sensorimotor and bilaterally on visual cortex [17]. The stimulation of 8 Hz, bipolar pulses, and duration of the stimulation 15 s and intensity of 3–5 mA was used. Repeated stimulation (five times) of the sensorimotor cortex with 1 min interval between the end of evoked epileptic seizures and the next stimulation were applied. Experimental animals were then exposed to 1 hour to normobaric (5.2% of oxygen) or hypobaric hypoxia at the simulated altitude of 7 000 m (barometric pressure = 405 mbar). Animals were administered with nimodipine in the dose of 10 or 5 mg /kg i.p. or with the solvent drug (methanol) in the same volume always 15 min before exposition to hypoxia. Control animals were not exposed to hypoxia and nimodipine or methanol was administered in the same dose as in experimental animals 90 min before the first stimulation. All experimental groups consisted of at least 8 animals.

The duration and shape of cortical evoked epileptic seizures were analysed. Results were statistically evaluated by t-test and ANOVA (using GraphPad Prism program). Level of significance was set at 5%.

Results

Changes of the excitability of cortical neurones after the exposure to different types of hypoxia depend on the age of animals. The decrease of excitability of cortical neurones during the postnatal development was registered (ANOVA $p < 0.001$). The prolongation of evoked epileptic seizures after the first stimulation was observed in all age groups exposed to both types of hypoxia (Fig. 1). After the repeated stimulation a minimal effect of hypoxia was registered in youngest animals (12-day-old, $p < 0.05$). Hypobaric hypoxia did not influence the duration of epileptic seizures in 25-day-old rats. Seizure shortening was recorded in 35-day-old animals ($p < 0.001$). Normobaric hypoxia prolonged the duration of seizures in 25 and 35-day-old animals ($p < 0.001$, Fig. 1).

The application of nimodipine in the dose of 10 mg/kg i.p. to control rats led to the death of animals caused by heart and ventilation arrest (6 rats from 8 in all age groups) or to the severe ataxia. Lower dose of nimodipine (the 5 mg/kg i.p.) had no peripheral effect (on circulation, ventilation or motor function) and did not change the duration of evoked epileptic seizures in 25- and 35-day-old rats. In the youngest control group (12-day-old rats, $p < 0.001$), shortening of epileptic seizures after the stimulation of sensorimotor cortex was observed

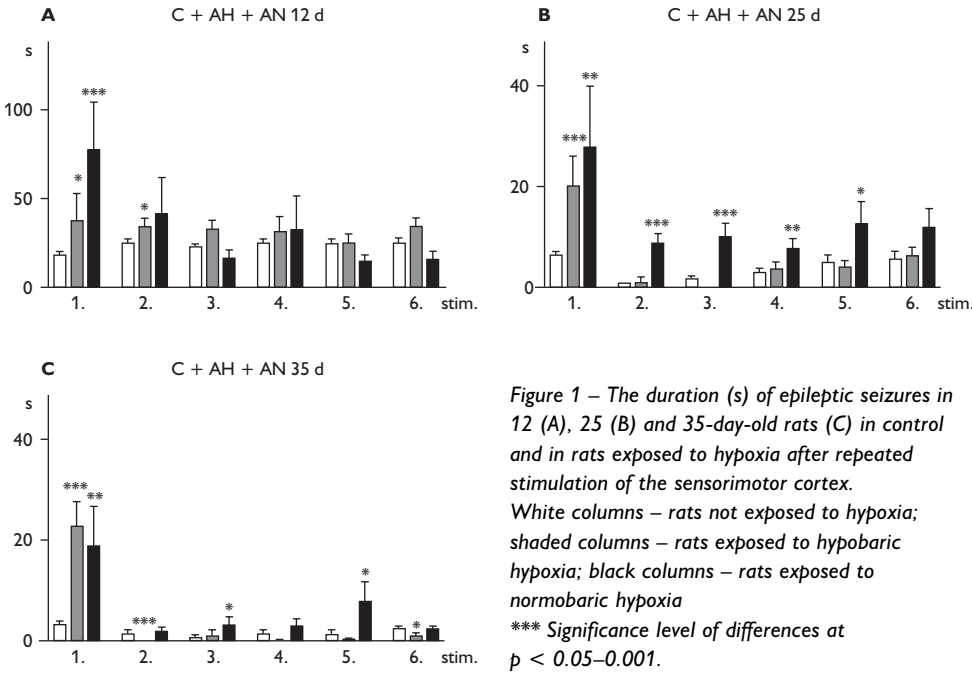

Figure 1 – The duration (s) of epileptic seizures in 12 (A), 25 (B) and 35-day-old rats (C) in control and in rats exposed to hypoxia after repeated stimulation of the sensorimotor cortex.
White columns – rats not exposed to hypoxia; shaded columns – rats exposed to hypobaric hypoxia; black columns – rats exposed to normobaric hypoxia
**** Significance level of differences at $p < 0.05–0.001$.*

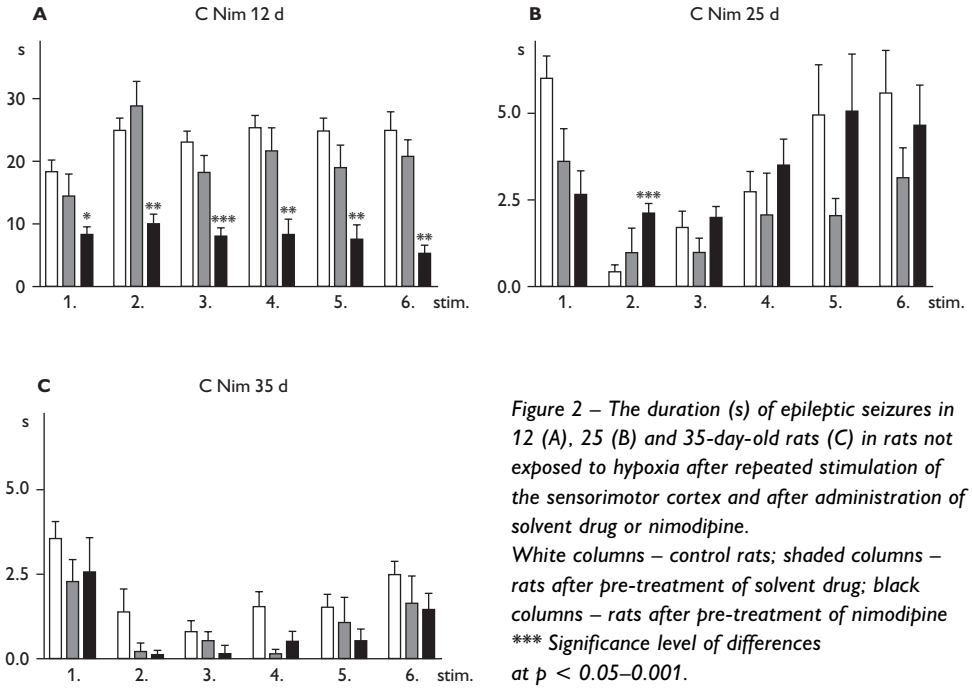

Figure 2 – The duration (s) of epileptic seizures in 12 (A), 25 (B) and 35-day-old rats (C) in rats not exposed to hypoxia after repeated stimulation of the sensorimotor cortex and after administration of solvent drug or nimodipine.
White columns – control rats; shaded columns – rats after pre-treatment of solvent drug; black columns – rats after pre-treatment of nimodipine
**** Significance level of differences at $p < 0.05–0.001$.*

(Fig. 2). The administration of the solvent drug did not influence the duration of evoked epileptic seizures.

Seizures evoked after the repeated stimulation and the administration of nimodipine or the solvent drug in 12-day-old rats exposed to short- term hypobaric hypoxia at the simulated altitude of 7 000 m were shorter. Minimal effect on evoked epileptic seizures of nimodipine was expressed in 25 and 35 day-old rats. Shortening of the first evoked seizure and prolongation after 3rd stimulation in 25-day-old rats and after 4th and 5th stimulation in 35-day-old rats was found. With exception of the first evoked seizure the solvent drug does not influence the seizure duration in 25 and 35-day-old rats (Fig. 3).

In rats exposed to normobaric hypoxia (5.2 % of oxygen), nimodipine administration shortened the seizure duration after the first and the 5th stimulation in 12-day-old rats but applications of solvent drug absolutely inhibited epileptic seizures. In older animals, neither nimodipine nor solvent drug changed the duration of evoked seizures (Fig. 4).

Discussion

Excessive calcium influx as a causal mechanism in the pathophysiology of ischemic brain damage is implicated [18]. Both neuronal and vascular calcium channels may contribute to the different reaction of the central nervous system to hypoxia and to the repeated electrical stimulation [19].

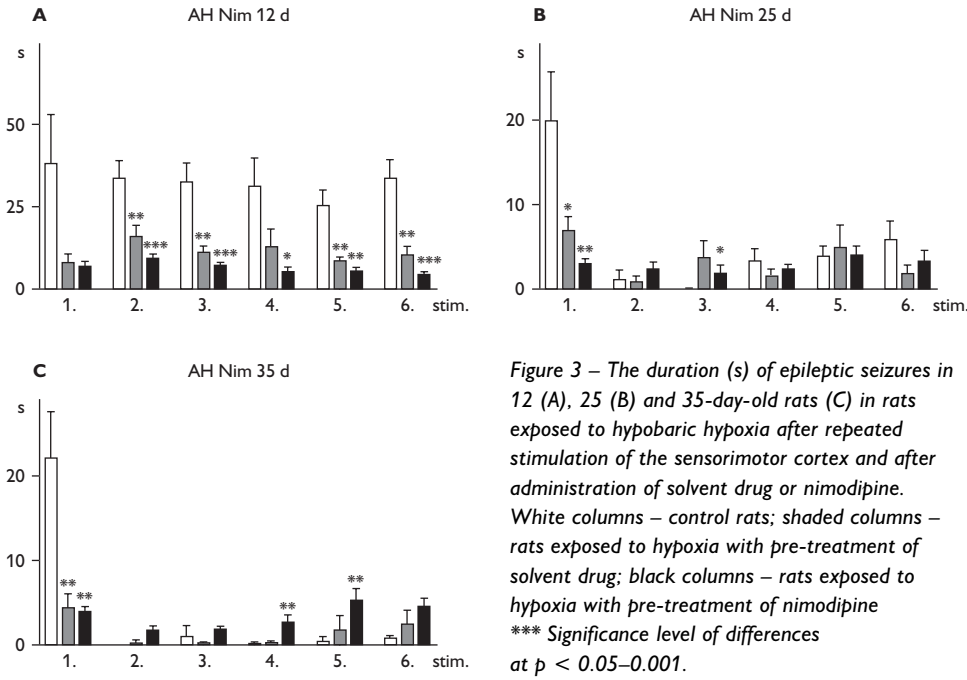

*Figure 3 – The duration (s) of epileptic seizures in 12 (A), 25 (B) and 35-day-old rats (C) in rats exposed to hypobaric hypoxia after repeated stimulation of the sensorimotor cortex and after administration of solvent drug or nimodipine. White columns – control rats; shaded columns – rats exposed to hypoxia with pre-treatment of solvent drug; black columns – rats exposed to hypoxia with pre-treatment of nimodipine *** Significance level of differences at $p < 0.05–0.001$.*

Calcium antagonists interfere with the neuronal excitability, particularly through L-type calcium channels [20]. The different response of cortical neurones to stimulation and short term hypoxia depends on age of animals and type and the intensity of hypoxia. Namely normobaric hypoxia augmented excitability of cortical neurones in older animals contrary to hypobaric hypoxia which decreased the excitability of cortical neurones [21]. Unequivocal effect of nimodipine was observed in 12-day-old control rats. The decrease of excitability but not the impossibility to elicit of epileptic seizures confirmed the crucial role of L-type of calcium channels on functional changes of cortical neurones in immature brain. The lower dose of nimodipine (5 mg/kg i.p.) in older control animals was practically without any effect that can be explained by other excitability regulatory mechanisms.

In our experimental arrangement the influence of nimodipine on the duration of epileptic seizures was minimal. Maximal effect was observed in the youngest rats exposed to normobaric hypoxia after the application of solvent drug. The stimulation of cortical neurones in sensorimotor area does not allow induction of epileptic seizures. Solvent drug and nimodipine was without any effect in older animals (25 and 35-day-old).

Non- specific shortening of the duration of epileptic seizures in 12-day-old rats exposed to short-term hypobaric hypoxia was observed. In older animals, the

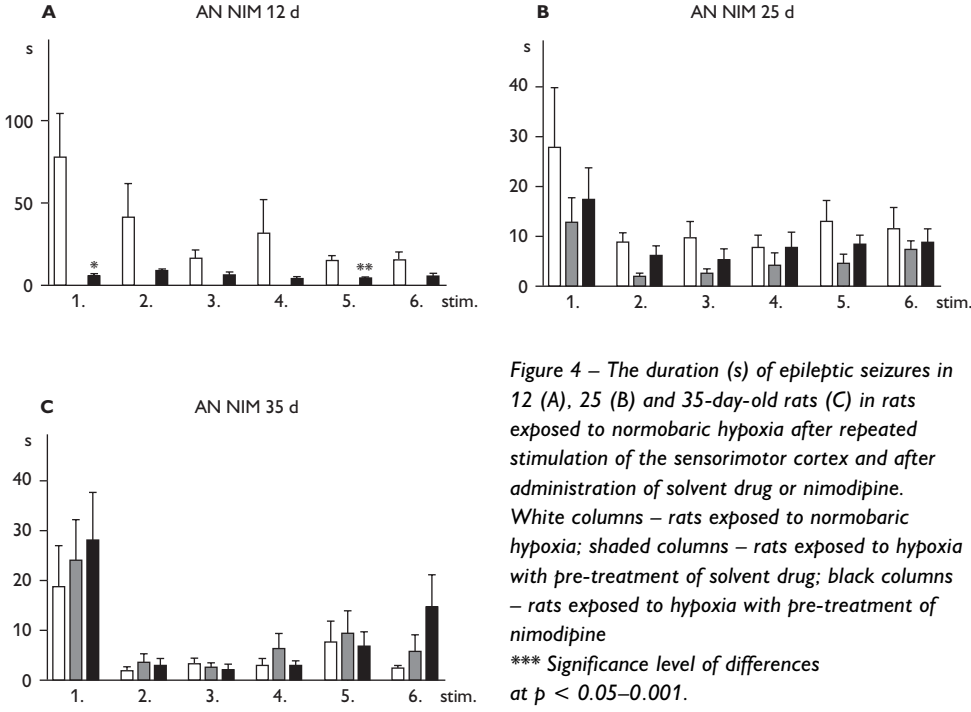

Figure 4 – The duration (s) of epileptic seizures in 12 (A), 25 (B) and 35-day-old rats (C) in rats exposed to normobaric hypoxia after repeated stimulation of the sensorimotor cortex and after administration of solvent drug or nimodipine. White columns – rats exposed to normobaric hypoxia; shaded columns – rats exposed to hypoxia with pre-treatment of solvent drug; black columns – rats exposed to hypoxia with pre-treatment of nimodipine
**** Significance level of differences at $p < 0.05–0.001$.*

administration of L-calcium channels antagonist prolonged the duration of evoked seizures after the repeated stimulation. Complex insult like the short-term hypobaric hypoxia affects excitatory/inhibitory balance in older animals. Although repeated electrical stimulation had inhibitory effect in these animals, administration of nimodipine potentiated excitatory mechanisms.

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