Effect of Selenium Pre-treatment on Evoked Cortical Afterdischarges in Young Rats

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Abstract: Prenatal or perinatal hypoxia is among the most frequent pathogenic factors of encephalopaties. It can induce wide-range of morphological, biochemical, energetic and functional alterations. Accordingly we tested changes of excitability of the sensorimotor cortex in 12, 25 and 35-day-old rats exposed and not exposed to short-term (1 hour) hypobaric and normobaric hypoxia. We studied whether sodium selenate (0,26 mg/kg b.w.) can influence character and/or intensity of evoked cortical afterdischarges or if a pre-treatment by selenium changes effects of hypoxia on such seizures. According to the results we can conclude that in our experiment arrangement sodium selenate only partially alters duration of evoked cortical afterdischarges.

Key words: Selenium – Evoked cortical afterdischarges – Hypoxia – Young rats

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Introduction

Hypoxia evokes disturbances of cellular energy metabolism, activation of different transmitters systems in the brain, elevation of catecholamines and corticoids and it can induce many general reaction of organism (circulatory, respiratory). Prenatal or perinatal hypoxic insults are among the most frequent pathogenic factors of encephalopaties accompanied with various forms of morphological and functional changes of the brain [1, 2].

Not only hypoxia itself, but also the period of reperfusion appears to have significant effects in the pathogenesis of encephalopaties. In the reperfusion injury, critical effects have free radicals [3, 4]. Free radicals are also produced by the normal metabolismus and organism has a many natural antioxidant systems. These scavengers can be divided according the mode of their action on enzymatic (superoxiddismutase (SOD), glutathionperoxidase or selenoproteins) and non-enzymatic. Non-enzymatic scavengers are classified according their molecular weight on the high (ferritin, metalothiens) and low molecular weight (ascorbic acid, α - tocopherol). Liquidation of free radicals defends tissues, cells and especially cells membranes [5, 6].

Selenoproteins are characterized by selenocysteine in their primary structure and constitute three groups of selenoenzymes – glutathione peroxidases, iodothyronine deiodinases and thioredoxin reductases [7].

Glutathione peroxidase catalyses reduction of H_2O_2 and organic peroxides and it also takes part as a scavenger of hydroxyl radicals (HO^-). Thyroxine-5'-deiodinase converts hormone thyroxin into biologically most active hormone triiodothyronine. In the NADPH-dependent protein disulphide reduction, thioredoxin reductase (TR) catalyses reduction of oxidized thioredoxin (trx) by NADPH using FAD and its redox-active disulphide.

Inorganic selenium is toxic to several species of mammals in higher doses. On the other hand selenium is trace element necessary for the normal functioning of cells. The human dietary selenium intake is approximately 80 mg per day. Optimum daily dose ranges between 50 to 200 mg per day [8, 9]. Main sources of the selenium are cereals, onion, garlic and meat.

Deficiency of selenium decreases immunity reaction and protection against oxidative stress and causes many disorders [10, 11, 12, 13]. Selenium pre-treatment decreased lipid peroxidation and increased glutathione peroxidase activity after quinolinic acid-induced neurotoxicity in rats [14].

Protective effect of selenium on methamphetamine induced neurotoxicity in the nigrostriatal dopaminergic system [15, 16, 17] as well as protection of lipid peroxidation after vanadium or mercury administration were well documented [18, 19]. Selenium-deficient or selenium-excess diet significantly changes activities of glutathione reductase or glutathione peroxidase in rats [20].

Pathological changes based on alteration of microvascular permeability of different organs are also related to the changes in the selenium level in organism [21].

Epileptic seizures similarly as hypoxia induce short- and long-term changes in the function of the central nervous system. These effects are in many cases related to the intensity and duration of the seizures. Character of epileptic seizures could be influenced by previous hypoxia. The question arose whether selenium can influence character and/or intensity of epileptic afterdischarges evoked by rhythmic electrical stimulation of the cerebral cortex in the rat and or if the selenium pre-treatment changes effects of hypoxia on such seizures.

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 using male rats of the Wistar strain of our breed. Animals were housed under standard temperature and light conditions; they were fed with a complete laboratory diet and water ad libitum.

Rats 12, 25 and 35-day-old were exposed for one hour to:

a) hypobaric hypoxia at a simulated altitude of 7000 m (atmospheric pressure = 307,9 mmHg, pO_2 = 64 mmHg)
b) normobaric hypoxia (PO_2 = 5,2%).

Sodium selenate (0.26 mg/kg b.w.) was administered by intra gastric sonde 15 min or 24 hours before exposition to hypoxia. Control rats (not exposed to hypoxia) received selenium in the same dose 90 min or 24 h before the stimulation of sensorimotor cortex.

Epileptic seizures – cortical afterdischarges (ADs) – were evoked by stimulation of the sensorimotor cortex 15 min after the end of hypoxia or in the same time schedule (after selenium administration) in control rats. Experiments were performed on freely moving animals with implanted silver electrodes [22].

Parameters of stimulation: bipolar pulses, frequency 8 Hz, intensity necessary to evoked cortical afterdischarges ranged from 3 to 5 mA, duration of stimulation was 15 s. Stimulation was repeated 5times, always 1 min after the end of previous cortical afterdischarge.

The duration of cortical afterdischarges and pattern of evoked cortical activity were recorded. Results were analyzed by ANOVA and unpaired t- test by programs of GraphPad Prisma. Significance was settled at 5%.

Results

In controls, cortical afterdischarges induced by stimulation of the sensorimotor cortex (animals not exposed to hypoxia) were in youngest animals (12-day-old rats – 18,5 ± 1.8 s) longer than in older rats (25-day and 35-day-old – 6 ± 0,6 s and 3,5 ± 0,5 s) (Fig. 1a, b, c), ANOVA $p < 0.001$.

With repetition of the stimulation we observed prolongation of cortical ADs in 12-day-old animals and shortening after the 2nd, 3rd, 4th stimulation in older animals ($p < 0,001$, Fig. 1a, b, c).

Exposition to short-term hypoxia of both types prolonged the first ADs in all age groups. In rats exposed to hypobaric hypoxia we observed prolongation of ADs also after the 3rd stimulation in 12-day-old rats and shortening ADs in older animals comparing with controls) (Fig. 1 a, b, c).

In rats after short-term normobaric hypoxia the duration of repeatedly evoked ADs was not changed in 12-day-old rats and in older animals prolongation was registered (Fig. 1a, b, c).

In older animals (25 and 35-day-old) without previous hypoxia the pre-treatment by selenium led to longer first ADs (Fig. 2a, b, c) in comparison with controls. In rats exposed to both types of hypoxia results were unequivocal (Fig. 3a, b, c, 4a, b, c).

Selenium pre-treatment does not change the pattern of afterdischarges typical for the given age – usually sharp waves in 12-day-old rats and 2–3/s spike and waves in older animals.

Discussion

Prolongation of cortical afterdischarges in 12-day-old rats with repetition of the stimulation of the sensorimotor cortex in control (not exposed to hypoxia) rats is a result of increase of susceptibility to seizures induced by different levels of structural maturation and misbalance between excitatory and inhibitory systems of the brain [23, 24, 25]. A key inhibitory neurotransmitter is γ-aminobutyric acid (GABA). GABAergic system is well developed in early stages of development, but electrophysiological studies indicate dynamic changes during development. In young rats (during the embryonic period and the first postnatal week) GABA induces depolarisation and activation of voltage-gated calcium channels (via L-type channels) and thus acts as excitatory neurotransmitter [26]. The excitatory action of GABA is attributed to the activation of postsynaptic $GABA_A$ receptors, which are functional after the second postnatal week [27]. Presynaptic inhibition mediated by $GABA_B$ receptors is also operating at birth [28]. In adults, activation of GABA system induces postsynaptic hyperpolarization [29]. Activation of GABA system also enhances the synthesis of $NO^{\cdot}$, that modifies intracellular second messenger pathways, alters gene expression and take part in plasticity related phenomena in developing rat somatosensory cortex [30, 31].

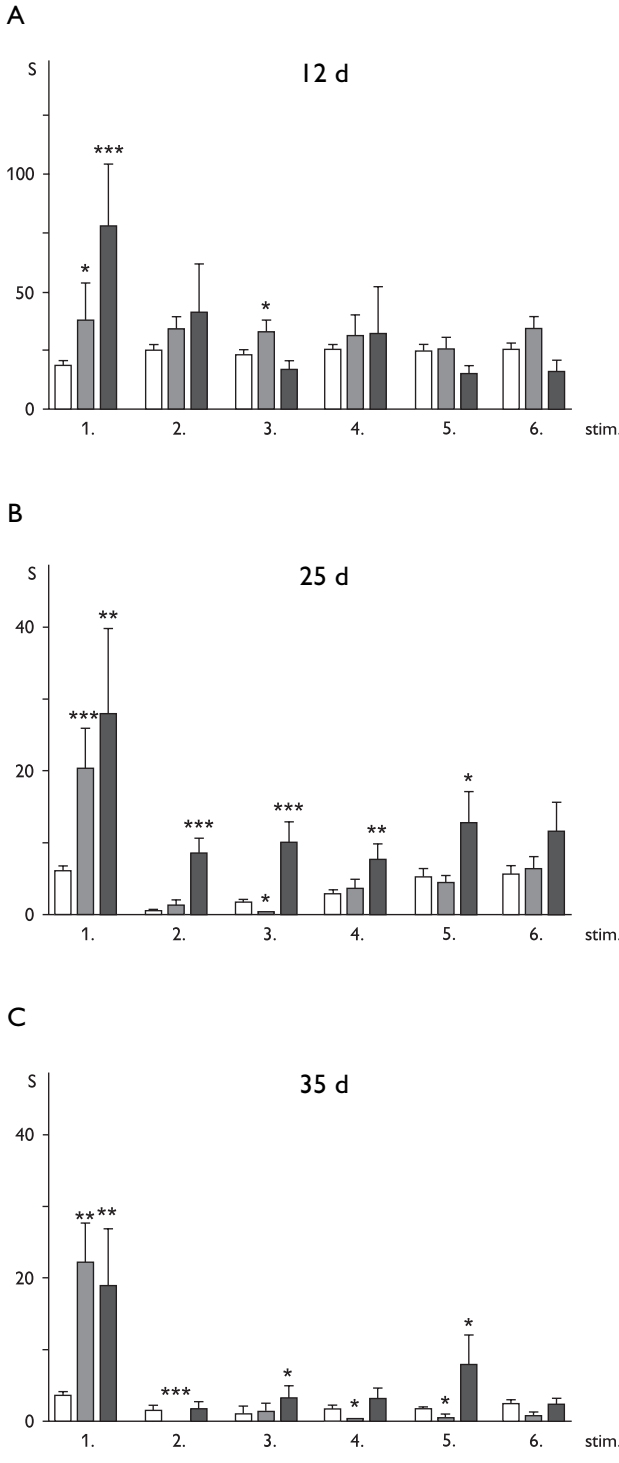

Fig. 1a, b, c – Duration of cortical ADs (in seconds) after the repeated stimulation of sensorimotor cortex (5times) in control 12, 25 and 35-day-old rats and in rats exposed to short-term (1 hour) hypobaric and normobaric hypoxia
White columns – ADs in control rats
Shaded columns – ADs in rats exposed to hypobaric hypoxia
Black columns – ADs in rats exposed to normobaric hypoxia
** indicates results significant at $p < 0.05$*

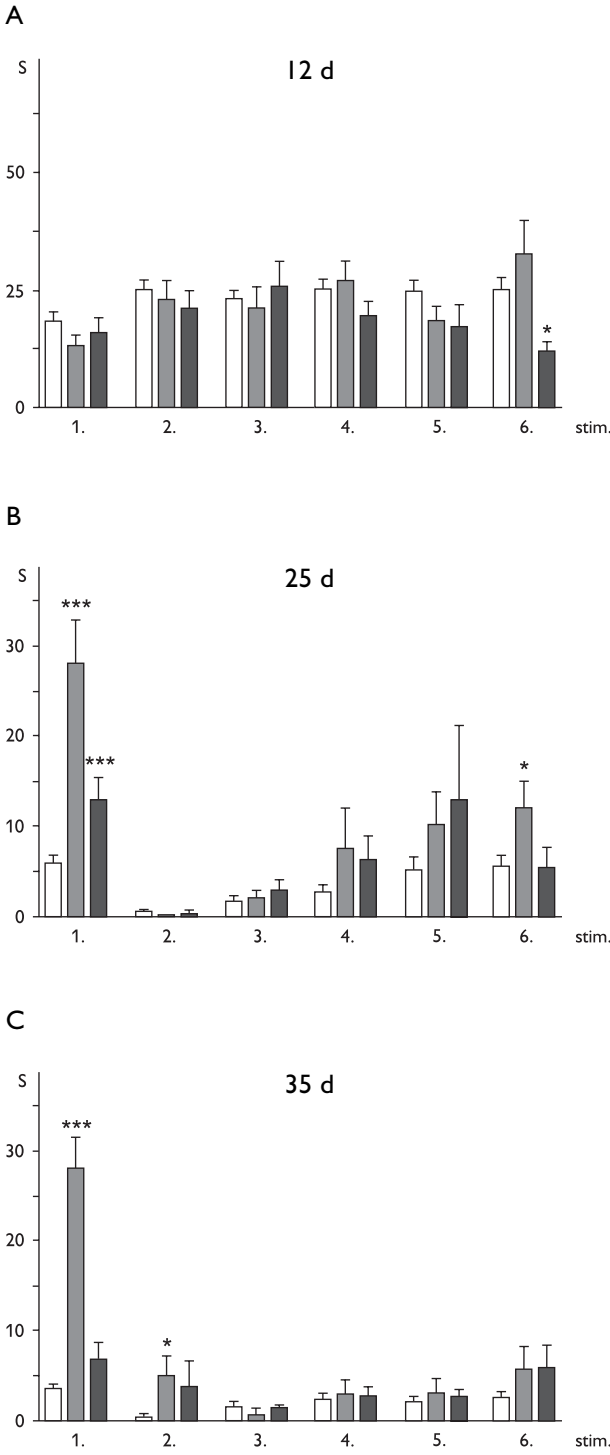

Fig. 2a, b, c – Duration of cortical ADs (in seconds) after the repeated stimulation of sensorimotor cortex (5times) in control 12, 25 and 35-day-old rats and in rats after the selenium pre-treatment
White columns – ADs in control rats
Shaded columns – ADs in rats pre-treated by selenium 90 min before stimulation
Black columns –ADs in rats pre-treated by selenium 24 hours before stimulation
** indicates results significant at $p < 0.05$*

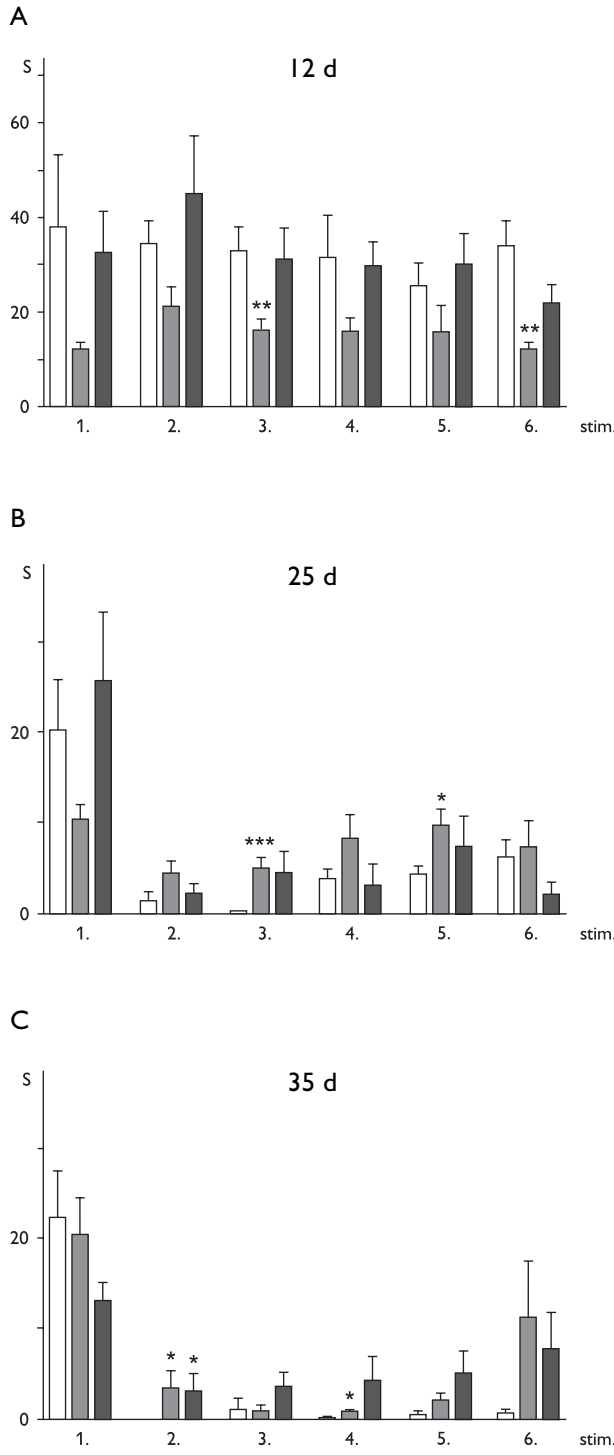

Fig. 3a, b, c – Duration of cortical afterdischarges (in seconds) in 12, 25 and 35-day-old rats exposed to hypobaric hypoxia and in rats after the selenium pre-treatment
White columns – ADs in rats exposed to hypobaric hypoxia
Shaded columns – ADs in rats exposed to hypobaric hypoxia. Selenium administration 15 min before the hypoxia onset
Black columns – rats exposed to hypobaric hypoxia. Selenium administration 24 hours before the hypoxia onset
** indicates results significant at $p < 0.05$*

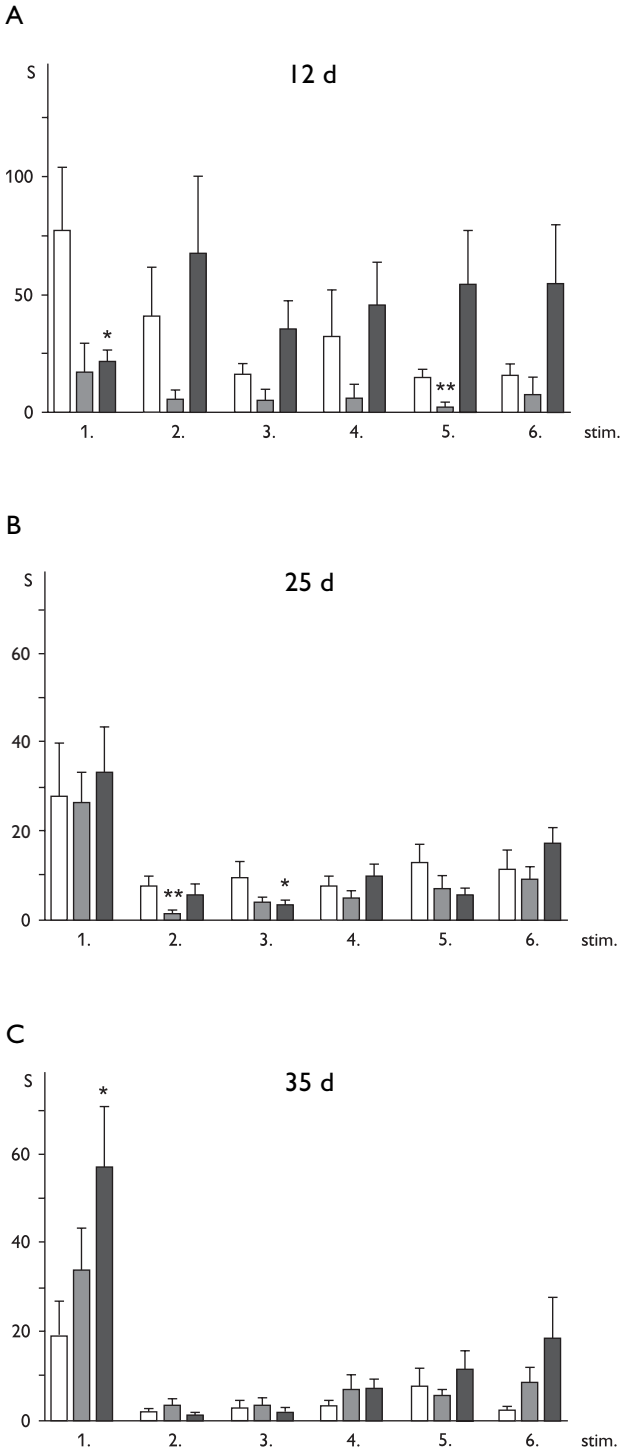

Fig. 4a, b, c – Duration of cortical afterdischarges (in seconds) in 12, 25 and 35-day-old rats exposed to normobaric hypoxia and in rats after the selenium pre-treatment
White columns – ADs in rats exposed to normobaric hypoxia
Shaded columns – ADs in rats exposed to normobaric hypoxia. Selenium administration 15 min before hypoxia onset
Black columns – rats exposed to normobaric hypoxia. Selenium administration 24 hours before hypoxia onset
** indicates results significant at $p < 0.05$*

Two main excitatory amino acids (EAA) – L-glutamate and L-aspartate have been identified. Both EAA acts at tree classes of ionotropic amino acid receptors: NMDA (N-methyl-d-aspartate receptor), KA (kainic acid receptor) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor) and three groups of metabotropic glutamate receptors. In early phases, glutamatergic excitatory transmission is mediated by NMDA receptors. The contribution of AMPA receptors increases to adult level by the end of the second postnatal week [29]. An increase of metabotropic receptors (mGlur1) during the first five weeks after the birth was documented [32].

The repetition of the stimulation in older animals induces a phase of postictal inhibition – the decrease of the duration of evoked ADs. It correlates with maturation of the brain and can prevent the development of seizures after the previous seizure activity.

Different results in rats exposed to short-term hypoxia are due to the different intensity of this insult on brain function. Hypoxia itself induces changes in the bioelectric activity – depolarisation of the neuronal membrane as a consequence of lowering of energetic reserves and membrane ions shifts. Increase intracellular concentration of Na, Cl and Ca is followed by an increase of intracellular water – cytotoxic oedema [33]. Membrane depolarisation activates the glutamate system and resulting intracellular calcium increase can trigger many enzymatic reactions (proteolysis, lipolysis, lysis of nucleotic acid; [34]. Elevated permeability of cerebral microvessel endothelial cells that form the blood-brain barrier, which develops 10 min after the hypoxic insult (6% O_2 for 1 hour) is associated with alterations of the tight junction protein and may also result in oedema [35, 36].

Prolongation of evoked cortical afterdischarges in older rats not exposed to hypoxia after selenium pre-treatment were registered. We presumed that the longer interval between selenium administration and testing stimulation would influence more intensively the duration of evoked seizures. However, effects were more intensive when selenium was administered 90 min before the stimulation than 24 hours before the stimulation. Pre-treatment with selenium increases excitability after the repeated stimulation of the sensorimotor cortex in older rats exposed to hypobaric hypoxia.

The metabolism of selenium in the brain differs from that in other organs. During deficiency of selenium, it is preferentially build up in the brain [37]. More than that, a gender difference in penetration of selenium into the brain exists (it penetrates more easily into brain of female mice than into the male brain) [38].

Conclusion

We can conclude that in our experimental models the pre-treatment with sodium selenate can only partially influence the development of evoked

epileptic seizures. It remains to solve if this effect is connected with the change of function of scavenger proteins elicited by selenium or if it is direct action of selenium on the systems involved in the processes connected with the ADs and their characteristics.

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