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Excitability Changes of Cortical Neurons during the Postnatal Period in Rats Exposed to Prenatal Hypobaric Hypoxia

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Summary

Pregnant rats were exposed to intermittent hypobaric hypoxia (at a simulated altitude of 7000 m or 5000 m) and the excitability of cortical neurons of their pups was tested. Stimulation of the sensorimotor cortex of rats prenatally exposed to hypoxia shortened the duration of cortical afterdischarges in 12-day-old rats, but did not change the excitability in 25-day-old animals. Shortening of the first afterdischarge in 35-day-old rats but the prolongation of the first afterdischarge in adult rats (as compared to the duration of cortical afterdischarges in rats not exposed to prenatal hypoxia) were registered. The possible mechanisms of different excitability of cortical neurons in rats prenatally exposed to hypobaric hypoxia are discussed.

Key words
Hypoxia • Development • Excitability of neurons • Rats

Introduction

Hypoxic brain damage can be induced by oxygen deprivation during cardiovascular and respiratory insufficiency or by the oxygen radicals formed after reperfusion and reoxygenation (Akaneya *et al.* 1994, Li *et al.* 1996, Joseph *et al.* 2000). Effects of the hypoxia insult are related to the age of animals, to the intensity of hypoxia, and to the spreading of alterations in brain structures (Langmeier *et al.* 1989, Kelly and Richards 1998, Sborová *et al.* 1999). The high tolerance of the immature central nervous system to hypoxia has been confirmed by many authors (Barbashova and Grigorieva 1968, Jílek 1970, Trojan and Šťastný 1988).

Serious consequences of hypoxia have been described in various clinical or experimental models: alterations of membrane potential and ion distribution (Somjen *et al.* 1993), activation of neurotransmitter systems (Olson *et al.* 1983, McDonald and Johnston 1990, Gentile and McIntosh 1993, Huang *et al.* 1994), metabolic effects (Harkness *et al.* 1982) and expression of hypoxia-inducible genes (Prabhakar *et al.* 1996, Soulier *et al.* 1997). The adaptive reactions of nervous cells to hypoxia depend on the mutual relation of all the processes involved (Yun *et al.* 1997, Trojan and Pokorný 1999). A change of the excitability and enhanced tendency to hypersynchrony and seizures can be one of the functional manifestations of the reaction to hypoxia (Trojan 1978, Marešová and Mareš 1999, Valkounová *et al.* 1999, 2000).

To analyze the postnatal changes in excitability of cortical neurons we decided to use the model of prenatal exposure to hypobaric hypoxia and its postnatal effect on the duration of evoked cortical afterdischarges.

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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 Wistar strain rats of our own breed. Pregnant rats were exposed to hypoxia 13 times for 8 hours a day in special pressure chambers, in which the atmospheric pressure was reduced to a simulated altitude of 7000 m (first group, n = 14 rats, barometric pressure = 405 mbar) or 5000 m (second group, n = 12 rats, barometric pressure = 535 mbar). The internal environment of the chamber was maintained at a constant temperature of 24±1 °C. The exposure to hypobaric hypoxia was discontinued in both groups 5 days before delivery. We monitored the number and sex of the newborn pups.

Electrophysiological experiments were done on freely moving male rats prenatally exposed to hypobaric hypoxia *in utero* at a simulated altitude of 5000 m. The animals were studied 12, 25 and 35 days *post partum* and in adulthood (90 days old) and compared with rats of the same age not exposed to hypoxia. Under general inhalation anesthesia, stimulation silver electrodes were placed at the right sensorimotor cortex and registration electrodes were placed at the left sensorimotor cortex and bilaterally at the visual areas. The indifferent electrode was placed on the nasal bone. Experiments were performed after at least one hour of recovery, when righting and placing reflexes were tested and animals were fed with 5 % sucrose.

Stimulation of the right sensorimotor cortex was performed with rectangular bipolar pulses of 0.5 ms duration in 15 s series at a frequency of 8 Hz, intensity of 3-5 mA. The stimulation was repeated five times, always 1 min after the end of the previous cortical afterdischarge (AD). Unipolar and bipolar registration of electrocorticographic activity was performed during the whole experiment. The duration and shape of the cortical afterdischarges together with behavioral changes accompanying the stimulation and evoked epileptic seizures were also monitored.

Unpaired t-test and ANOVA in GraphPadPrism were used for evaluation of the results. The level of significance was set at 5 %.

Results

Two out of the 14 inseminated rats kept at the simulated altitude of 7000 m gave birth to 7 pups (1 male, 6 females), but these pups died during the next 5 days. Control section of the uterus in the other rats confirmed the absence of any fetuses.

All 12 female rats kept at the simulated altitude of 5000 m delivered 94 pups (36 males, 58 females). They had in average 7.8 pups/litter and out of these 38 % were male pups. On the contrary, female rats not exposed to hypoxia had 9.4 pups/litter and out of these 47 % were male pups.

Stimulation of the sensorimotor cortex in rats elicited cortical afterdischarges (AD). The duration of AD is related to the age (ANOVA, $p<0.001$) (Table 1, Fig. 1). In younger animals isolated spikes or low amplitude sharp waves were recorded, in older rats (aged 25 or 35 days) and in adult rats spike and wave pattern of ADs was registered.

Table 1. The duration of the first cortical afterdischarge

	Duration of the first cortical AD (s)	
Age of rats	**Rats not exposed to hypoxia**	**Rats prenatally exposed to hypoxia**
12 days	18.8±1.9	10.1±2.6
25 days	6.0±0.6	4.0±1.4
35 days	4.5±1.1	2.2±0.16*
90 days	6.6±1.3	17.6±7.3*

Data are means ± S.E.M. Significant difference $p<0.05$ between rats not exposed to hypoxia and rats prenatally exposed to hypoxia*

Repeated stimulation of the sensorimotor cortex led to prolongation of cortical afterdischarges in 12-day-old rats after the 2nd and 4th stimulation. In 25-day-old rats and in adult animals the duration of AD was shorter after the 2nd, 3rd and 4th stimulation, and in 35-day-old rats after the 3rd and 5th stimulation in relation to the first evoked cortical afterdischarge (Fig. 1).

In 12-day-old rats prenatally exposed to hypobaric hypoxia, cortical afterdischarges were shorter after the 2nd, 3rd and 4th stimulation in comparison with rats not exposed to hypoxia. The duration of the first cortical AD in 35-day-old young rats was shorter and in 90-day-old rats it was longer than in non-exposed rats. In

25-day-old rats the prenatal exposure to hypoxia had no effect on the duration of elicited cortical ADs (Fig. 1).

During stimulation or during elicited cortical afterdischarges, synchronous movements of the head and/or forelimbs with stimulation were considered as degree 2 and 3 according to scale of Racine (Racine 1972).

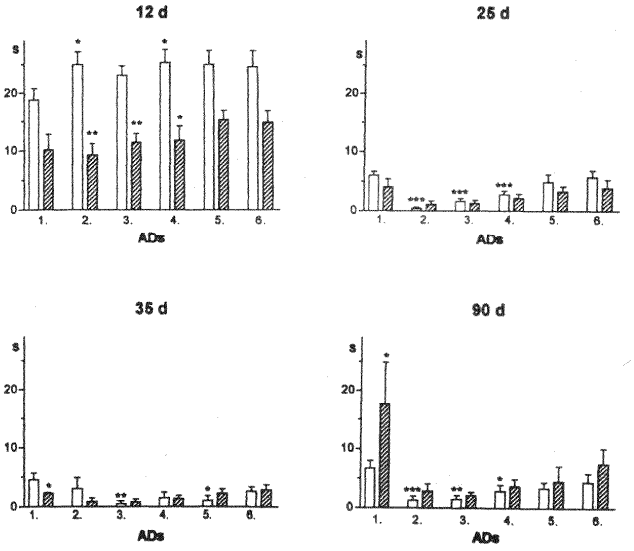

Fig. 1. *The duration of cortical afterdischarges in 12-, 25- and 35-day-old rats and in adult animals after repeated stimulation (five times) of the right sensorimotor cortex. Open columns – rats not exposed to hypoxia. Hatched columns – rats exposed to prenatal hypoxia. Significant difference in the duration of ADs in rats non-exposed and exposed to prenatal hypoxia:* $*p<0.05$, $**p<0.01$, $***p<0.001$.

Discussion

Pregnant rats survived exposure to intermittent hypobaric hypoxia at the simulated altitude of 7000 m or 5000 m, but the number of pups born on term was smaller than in non-exposed rats. The altered ratio in the number of male and female newborns confirms the gender difference in the reaction to low oxygen supply (Pequignot *et al.* 1997). No signs of abortion were registered in fertilized rats exposed to higher simulated altitude (7000 m) and we thus presume the intrauterine fetus resorption.

The suprathreshold stimulation of the sensorimotor cortex evoked epileptic seizures, and the duration of AD shortened with the age of animals. This correlates with the maturation of the nervous structure and neurotransmitter systems, which actively influence the excitability of nerve cells (Moshé 1987). Repeated stimulation at short interstimulus intervals (1 min) prolonged the duration of epileptic seizures in younger animals (12-day-old), with different effects of individual cortical stimulations. The mechanisms involved in arresting the seizures led to shortening of ADs especially from the third week of life, when a postictal depression was registered (Moshé *et al.* 1996).

The prenatal exposure to hypoxia shortened the duration of cortical afterdischarges after repeated stimulation in the younger group of animals. Such a decrease of excitability of cortical neurons induced by hypoxia (functional acceleration) were also seen after handling or acoustic stimulation (Nováková 1976). Activation of N-methyl-D-aspartate (NMDA) receptors by hypoxia or more effective GABAergic inhibition are also possible. The functional acceleration expressed by shortening of ADs duration, which was seen in 12-day-old rats, was not found in 25-day-old rats. The significant shortening of AD after the first stimulation in 35-day-old rats and the prolongation of the first cortical AD in adult rats prenatally exposed to hypobaric hypoxia may be a sign of light brain dysfunction in the stress reaction, but without effect on the basic seizure-arresting mechanisms.

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