Changes of Evoked Epileptic Seizures after the Short Term Hypobaric Hypoxia in the Young Rats

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Received December 16, 2004, Accepted December 29, 2004

Abstract: Exposition to the acute intensive hypobaric hypoxia blocks the triggering mechanism of epileptic seizures only within a short time interval after the end of hypoxia period and it does not influence the progressive epileptogenesis in 12-day-old rats. In 25-day-old rats' hypobaric hypoxia suppresses the postictal depression elicited by the repeated stimulation in short interstimulus intervals and it increases the duration of epileptic seizures evoked by electrical stimulation of the sensorimotor cortex.

Key words: Hypobaric hypoxia – Epileptic seizures – Young rats – Postictal depression

Work was supported by grant UK 45/2004 and MSM 111100001.

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Introduction

Hypoxia insult evokes a broad spectrum of morphological and functional changes in the organism. These changes depend on the type, duration, and intensity of hypoxia as well as on the conditions of oxygen supply after the end of hypoxia. Oxygen deficiency during foetal or perinatal periods is considered to be one of the most important etiological factors of cerebral disturbances e.g. epileptic seizures [1, 2]. The clinical studies have demonstrated that 27% of babies with severe neonatal asphyxia developed recurrent seizures [3].

From experimental studies is known that the toleration of neonates to hypoxia is high and late consequences of hypoxia depend on the severity and the duration of hypoxic insults [4, 5, 6, 7, 8, 9 10]. Experimental approach to the relationship between hypoxia and epileptic phenomena is oriented mainly on the comparison of some models of epilepsy in controls and in animals after miscellaneous types of hypoxia and anoxia. Many acute and semiacute effects of hypoxia are closely related to the increased excitatory amino acids (EAA) outflow and excessive activation of glutamate receptors [11, 12, 13, 14, 15, 16] or to the alteration of GABA mediated inhibition [17]. Cascades of metabolic events as consequence of altered ion homeostasis (an increase of the cytosolic calcium ions) represent major source of disturbances that led to morphological and functional changes of the brain [18, 19].

Changes in the electroencephalogram and evoked potentials in young rats one and two days after the exposition to the acute intensive hypoxia are accompanied by morphological changes and similar findings resulted from a less intensive chronic hypoxia [20, 21, 22, 23].

We decided to study the influence of hypobaric hypoxia on epileptic seizures as they are revealed in cortical afterdischarges (Ad) elicited by electrical stimulation of the sensorimotor cortex in young rats. We tested the effects of a short, but intensive hypoxia on the genesis and termination of epileptic seizures, on the progressive epileptogenesis during the ontogenesis and on the postictal depression.

Materials and Methods

1. Evoked epileptic seizures – cortical afterdischarges

Cortical afterdischarges were elicited by electrical stimulation of the right sensorimotor cortex in the freely moving young male rats 12, 18 and 25-day-old. The stimulation and registration electrodes were implanted under ether anaesthesia. The stimulation electrodes were placed over right sensorimotor cortex; recording electrodes were implanted over the left sensorimotor and visual areas of both hemispheres (a correlation activity between two cortical areas). An indifferent electrode was placed on the nasal bone. Experiments were performed after the recovery period of about one hour. Degree of recovery was tested by righting, placing and suckling reflexes. The minimal number of animals in experimental groups was eight.

Sensorimotor cortex was stimulated with 15 second long series of rectangular bipolar pulses of 1 ms duration and of 8 Hz frequency. Intensity of the stimulating current ranged from 3 to 5mA.

In the first part of experiments the stimulation was repeated five times at 1-min intervals between the end of the afterdischarge and the beginning of the subsequent stimulation. In second part of experiments, the repeated stimulation was three times with 10-min intervals between the end of Ad and the next stimulation.

EEG was continuously recorded before and after the stimulation. We measured duration of afterdischarges, their shape and changes of behaviour in controls and in animals exposed to hypobaric hypoxia. The electrical stimulation began 2, 15 minutes and 1 hour after the end of hypobaric hypoxia. 12-day-old rats were examined also six days after the exposure to hypoxia.

Only those animals were tested that after the first stimulation already developed the cortical afterdischarges. Results were statistically evaluated by standard t-tests of PrismaGraphPad. Level of significance was set at 1%.

2. The hypobaric hypoxia

The basic condition in our experiments with the hypoxia was the utilization of the same intensity of hypobaric hypoxia (expressed as simulated altitude) for all age groups. Animals were exposed to hypobaric conditions for one hour. The verification of the intensity of the hypoxia was by LD_{10} (*dosis lethalis* 10 %) (Tab. 1).

3. Behavioral changes

We monitored the spontaneous motor activity, changes during the stay in hypobaric chamber and during the electrical stimulation and cortical afterdischarges by using the Racine scale [24].

Results

a) Cortical afterdischarges in control animals

The stimulation of the sensorimotor cortex in 12-day-old rats elicited cortical afterdischarge of the duration 18, 9 ± 1, 8 s (M ± S.E.M.). With the repetition of the stimulation (the interval between the end of the cortical afterdischarge and the next stimulation was 1 min) the change of the duration became significant after the 2nd and 4th stimulation (Fig. 1. A).

Duration of the first cortical afterdischarge in 18-day-old rats was 8,4 ± 1,3 s and the repetition of stimulations did not change the duration.

Table 1 – Relation between age of animals and simulated altitude

Age of animals	Simulated altitude
12 days	9 700 m
18 days	9 500 m
25 days	9 000 m

In the group of 25-day-old rats the duration of the first cortical afterdischarge was 6,0 ± 0,6 s. Next cortical stimulation was practically without effect (only 4 of 29 animals developed Ad), the duration the third and fourth Ad was significantly shorter, 5th and 6th Ad was the same as the control (first) afterdischarge (Fig. 2. A).

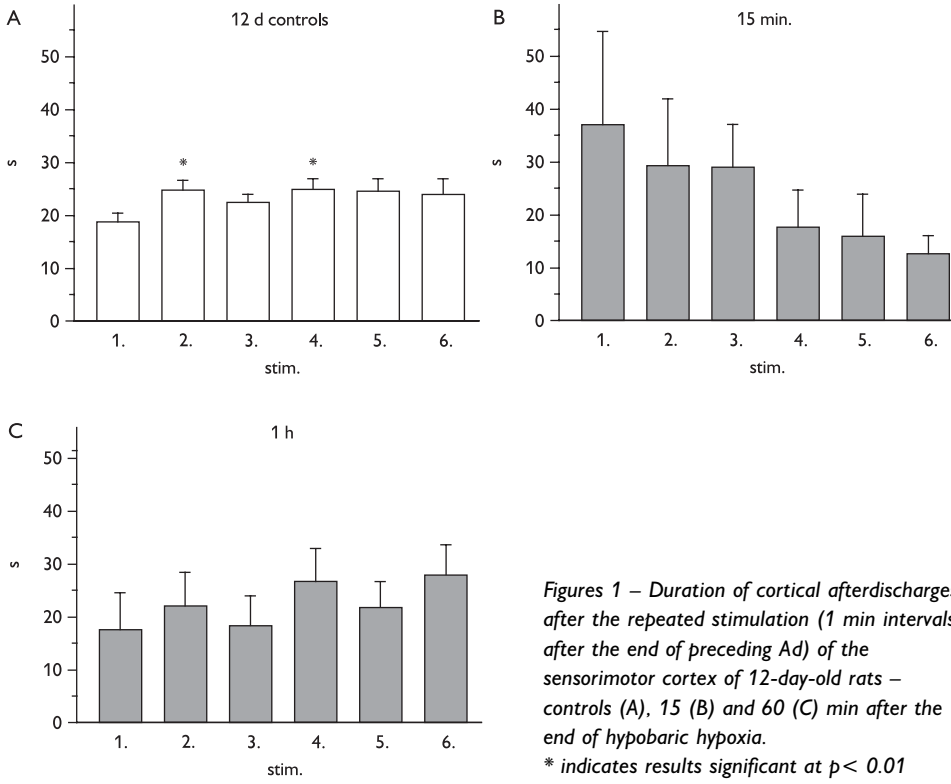
Using the longer interval (10 min between the end of the Ad and the next stimulation) resulted in a prolongation of Ads in 12-day-old rats (Fig. 3. A) and had no effect on the duration of Ads in 18-day-old rats or in 25-day-old rats (Fig 4. A).

b) Influence of hypobaric hypoxia

Electrical stimulation of the sensorimotor cortex in the interval of two minutes after the exposition to hypobaric hypoxia had no effect in 12-day-old rats (the Ads were elicited only in 2 of 28 animals) in both stimulation protocols.

In comparison to the controls animals, the duration of the cortical afterdischarges in 18-day-old rats did not change.

In 25-day-old rats, 2 min after the hypobaric hypoxia the firsts three cortical Ads were significantly longer (in stimulation scheme of 1 min – Fig .2. B) and the firsts two cortical afterdischarges were longer in the second stimulation scheme (interval 10 min – Fig. 4. B).

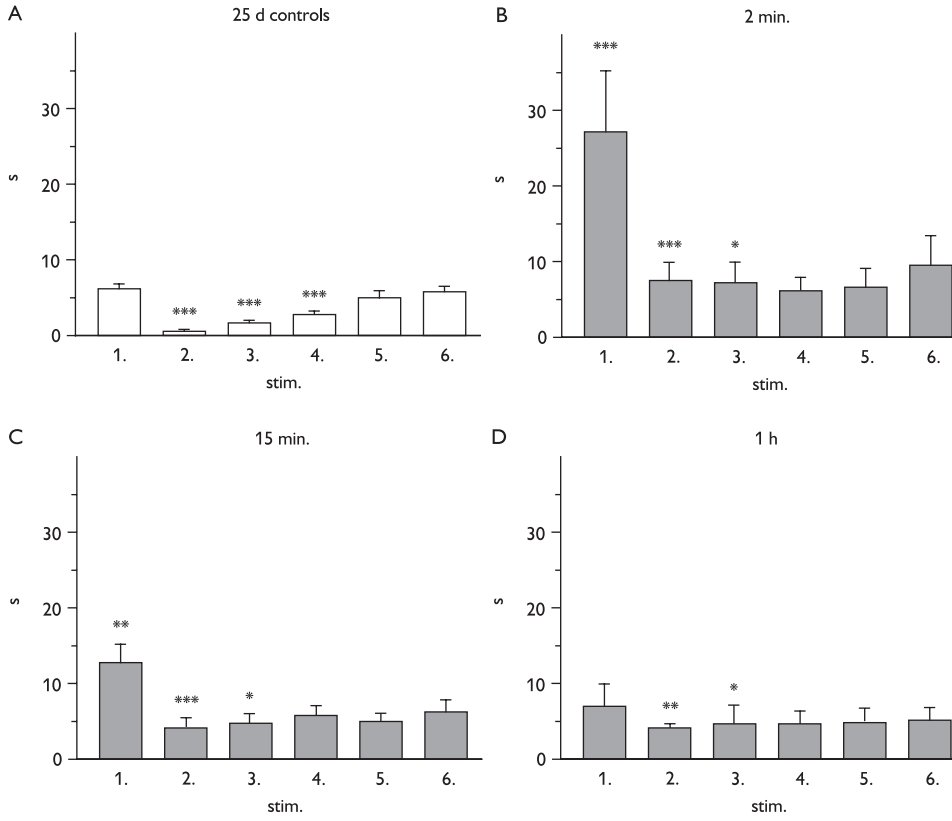


Figures 1 – Duration of cortical afterdischarges after the repeated stimulation (1 min intervals after the end of preceding Ad) of the sensorimotor cortex of 12-day-old rats – controls (A), 15 (B) and 60 (C) min after the end of hypobaric hypoxia.
** indicates results significant at $p < 0.01$*

The impossibility to elicit cortical Ads in 12-day-old rats led us to an experiment where the animals were exposed to stimulation of the sensorimotor cortex six days after the end of the hypobaric hypoxia that means at the 18th day of life. These results were not different from results of control 18-day-old rats.

In 12-day-old rats the longer interval between the end of the hypobaric hypoxia and the stimulation (15 min and 60 min.) corresponded with the duration of Ads in control 12-day-old animals (Fig. 3. B, C).

The proconvulsive effect of hypobaric hypoxia in 25-day-old rats continued also 15 and 60 min. after the end of hypoxia (the inter-stimulation interval was 1 minute – Fig. 2. C, D). With the inter-stimulation interval 10 minutes, only the first cortical Ad was prolonged 15 min after hypoxia, after 60 min the results were the same as in control animals (Fig. 4. C, D).



Figures 2 – Duration of cortical afterdischarges after the repeated stimulation (1 min intervals after the end of preceding Ad) in the sensorimotor cortex of 25-day-old rats - controls (A), 2 (B), 15 (C) and 60 (D) min after the end of hypobaric hypoxia.

** indicates results significant at $p < 0.01$*

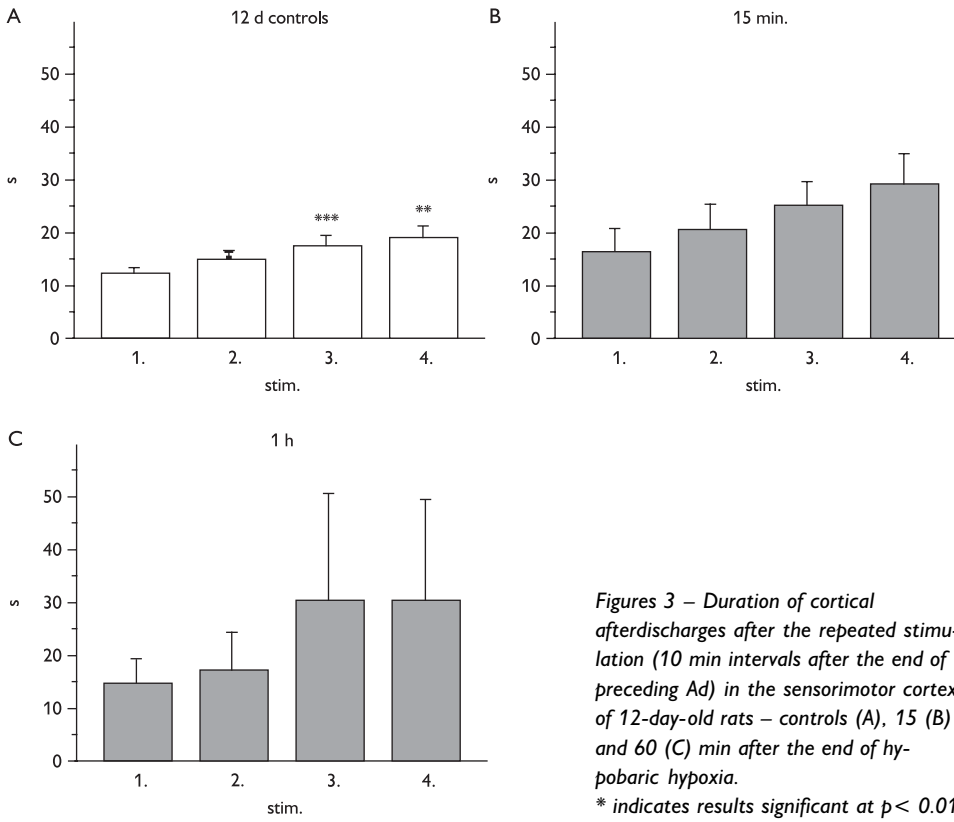
c) The electrocorticogram (ECoG)

The spontaneous electrocorticogram reflected the age of animals. Cortical afterdischarges in 12-day-old rats were composed of sharp waves or spikes with the frequency 1 to 2 Hz. Basic shape of the cortical Ads in older animals (18 and 25 days) consisted of spikes and waves. In some cases we registered the limbic character of ECoG (slow waves with a superposed rapid activity).

The voltage depression of the ECoG after hypobaric hypoxia was expressed only in 12-day-old rats and the hypobaric hypoxia had no effect on ECoG in older animals.

d) Behavioral changes

In the first few minutes after the beginning of hypobaric hypoxia the hyperventilation and the motor excitation of animals (running, rearing) was observed, than hyperventilation and motor hyperactivity disappeared and animals moved only rarely in hypobaric chamber.

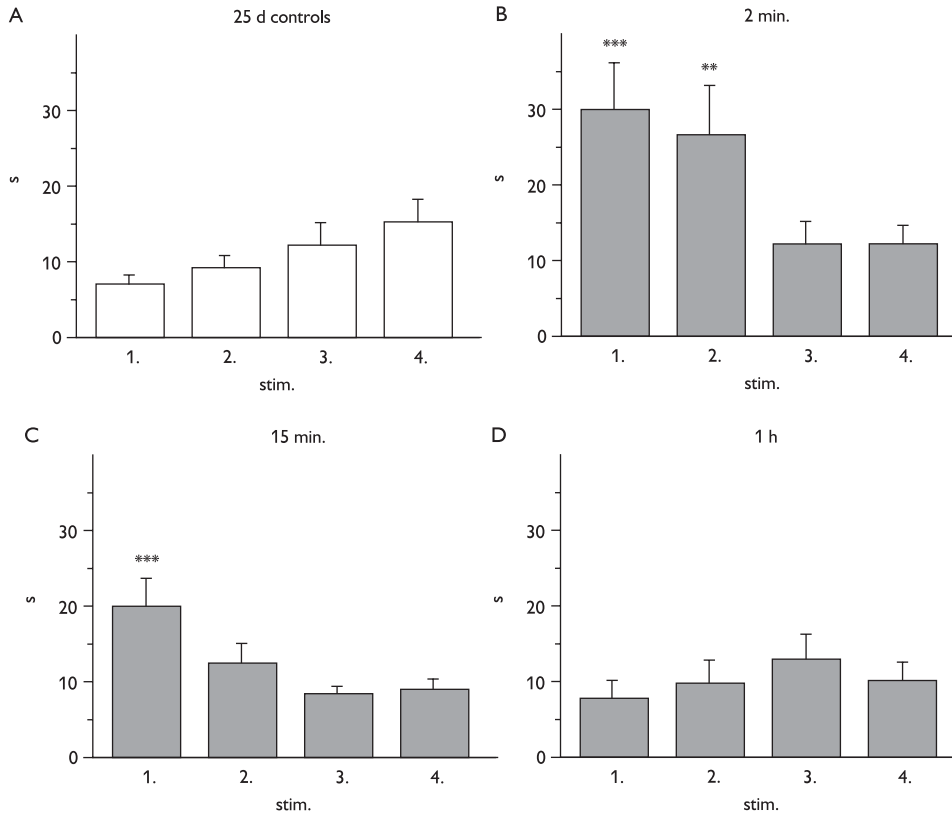


Figures 3 – Duration of cortical afterdischarges after the repeated stimulation (10 min intervals after the end of preceding Ad) in the sensorimotor cortex of 12-day-old rats – controls (A), 15 (B) and 60 (C) min after the end of hypobaric hypoxia.
** indicates results significant at $p < 0.01$*

Motor manifestation during the stimulation and cortical afterdischarges could be evaluated as the stadium 3 and 4 of the Racine's scale [24].

Discussion

Practically all neuronal circuits of the central nervous system have the capacity to trigger some pathological activity e.g., an epileptic seizure activity after the electrical stimulation. The threshold of the stimulation, the shape and duration of these evoked seizures are structure and age depended. The duration of seizures in younger animals is longer as it is in the older ones. The prolongation of epileptic seizures in young animals correlates with the mechanisms of arresting seizures – this active mechanism could be observed also after the cessation of seizure activity as a postictal depression. In rats it is usually demonstrated at the end of the second



Figures 4 – Duration of cortical afterdischarges after the repeated stimulation (10 min intervals after the end of preceding Ad) in the sensorimotor cortex of 25-day-old rats – controls (A), 2 (B), 15 (C) and 60 (D) min after the end of hypobaric hypoxia.
** indicates results significant at $p < 0.01$*

week of the postnatal life [25, 26]. This prolonged excitation coincides with the morphological and functional maturation of cells of the central nervous system and with the easily disrupted equilibration between intrinsic inhibition and excitation mechanisms [27].

The postictal depression was observed after the second stimulation in 25-day-old rats with short interstimulation interval (1 min) but was not present in the younger ones. Shortening of the duration of the next Ads in longer interstimulation interval (10 min) permits to conclude, that in short interstimulation interval, the phase of the excitation coincides with the intrinsic mechanisms of inhibition. Mechanism of such inhibition is probably based on the temporary local effects of GABAergic or excitatory amino acids system on the voltage dependent ionic channels with possible interaction of other mediatory or modulatory systems.

The predominance of the excitation was completely blocked by the influence of the hypobaric hypoxia in 12-days-old rats 2 min. after the end of hypobaric hypoxia but this blockade finished in 15 min. Impossibility to trigger cortical afterdischarges shortly after the end of hypoxia can be based on changes of ions as is K^+ efflux by ATP sensitive K^+ channels [28, 29], on the calcium accumulation [30, 31] or on changes of the metabolism of neurons during hypoxia with rapid depletion of energetic substrates [32].

Another situation was in 25-day-old rats, where hypoxic insult influenced the duration of cortical afterdischarges and the postictal depression (elicited by a stimulation with short interstimulus interval) was present as long as 1 hour after the end of hypoxia. Predominance of the excitation can be based on the hypoxia-induced reduction of postsynaptic GABAergic mechanisms leading to the manifestation of hyperexcitability [17] or by the influence of glutamate on EAA receptors [33]. The involvement of another mediator system (e.g. catecholaminergic, opioid) is also possible [34]. Prolonged hyperexcitability effect in 25-day-old rats may also results from changes in the neuronal membrane as a consequence of oxygen- free radicals generated by xanthine oxidase on membrane phospholipids [35].

Other changes in the excitability of cortical neurons after the hypobaric hypoxia can be evoked by the influence of the pressure on cerebral circulation. Cerebral blood flow in rat is 0.8 – 1.1 ml/min per one gram of the wet weight of the tissue with regional blood flow variations. Vascular autoregulation processes can dampen alterations that occur in response to major decrease of systemic blood pressure. However, another alteration of the cerebral blood flow can develop during the reperfusion period. Fall of the oxygen pressure influence activities of enzymes, as are xanthine oxidase and nitric oxide synthase. The production of free radicals especially during period of reperfusion can induce lipid peroxidation, damages of membranes, activity changes of membrane-bound enzymes and receptors. Limitation in ATP generation and reduction in the ATP level are probably the most significant bioenergetic changes caused by hypoxia [36].

Conclusion

Short but intensive hypobaric hypoxia increases excitability of cortical neurones, prolongs the duration of evoked epileptic seizures in older animals (25-day-old) and suppresses period of the postictal depression. This prolongation lasts up to 1 hour after the end of hypoxia when a short post-stimulus interval was used. In younger age group (12-day-old) 2 minutes after the end of hypoxia, epileptic seizures were impossible to elicit but this situation terminated within 15 minutes and when the duration of Ads did not differ from the controls values.

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