Hypoxia-induced changes of seizure susceptibility in immature rats are modified by vigabatrin

Hana Kubová, Pavel Mareš

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

ABSTRACT – The effects of hypoxia on susceptibility to pentylenetetrazol (PTZ)-induced seizures were assessed in juvenile rats. Animals at postnatal (P) day 25 were exposed to hypobaric hypoxia (simulated altitude of 7 000 m) for 8 h PTZ in a dose of 100 mg/kg was injected 1, 3 or 7 days later and latency, pattern and severity of seizures were registered. Mortality due to seizures was also evaluated. Two seizure types were evaluated: minimal mostly clonic seizures (mMS) and generalized tonic clonic seizures (GTCS). To study protective effects of vigabatrin (600 mg/kg, i.p.), drug was injected either 24 h before or immediately after hypoxia exposure. Non-hypoxic animals of corresponding age were used for comparison as controls. In non-hypoxic controls, administration of vigabatrin had pro-convulsive effects in intervals from 3 days up to 1 week – incidence of GTCS increased by 56-57%. Hypoxia exposure resulted to increased seizure susceptibility three days later, incidence of generalized tonic clonic seizures increased by 60% and latency to both seizure types shorter than in non-hypoxic controls. Also, mortality due to seizures was higher (by 58%). In other intervals, there was no difference between hypoxic and non-hypoxic animals. Vigabatrin administered 24 h before hypoxia led to significant decrease of seizure-induced mortality in intervals 1 and 3 days. Administration of vigabatrin immediately after hypoxia exposure resulted in decreased seizure severity when assessed 3 days later. Our data suggest that hypobaric hypoxia transiently increases seizure susceptibility. This effect is partially abolished by vigabatrin administered after hypoxia exposure.

Key words: immature brain, hypobaric hypoxia, vigabatrin, pentylenetetrazol-induced seizures

Hypoxia is one of the most important causes of seizure development and impairment of brain functions in the immature brain (Calvert and Zhang 2005). Some experimental studies suggest that hypoxia or hypoxia/ischemia leads to seizure development or to increased seizure susceptibility. In other studies, protective effects of hypoxia preconditioning have been documented. Patterns of changes of seizure susceptibility depend on many variables (Kelly 2002). Severity and duration of insult as well as the model used of seizures are the most important factors affecting outcome. The level of the brain maturation at the time of the hypoxic/ischemic insult is also another key factor which affects seizure susceptibility. In immature rats, there are periods of increased seizure susceptibility after exposure to hypoxia or hypoxia/ischemia. The exact age

Correspondence:
H. Kubová
Institute of Physiology,
Academy of Sciences of the Czech
Republic,
Vídeňská 1083, Prague 4, CZ-142 20,
Czech Republic
<kubova@biomed.cas.cz>

when such "developmental windows of increased seizure susceptibility" occurs seems to be related to the model of insult used. Exposure to global hypoxia (15-20 min, 3-4% O_2) induces seizure activity in rat pups aged 10-12 days (P10-12) but not younger or older (Jensen and Wang 1996). In contrast, increased excitability of sensorimotor cortex was observed in P12, P25 as well as P35 rats exposed to hypobaric hypoxia (stimulated attitude of 7 000 m lasted for 1h) and stimulated 15 min after the end of hypoxia exposure (Kalinčik and Marešová 2005). There are however no data on the time-course of this proconvulsant effect of hypobaric hypoxia in immature rats. Sensitivity to chemoconvulsants has not yet been tested in this model.

The evidence from experimental studies suggests that enhancing the function of the major inhibitory neurotransmitter (GABAergic) system can result in neuroprotection. Green *et al.* (2000) have suggested three reasons why increasing GABA function might be a beneficial therapeutic approach to hypoxia/ischemia. Firstly, biochemical studies have demonstrated that despite an increase of exracellular GABA level in hypoxic tissue, GABA synthesis and release is impaired during an ischemic insult. Secondly, increased glutamatergic activity might itself decrease GABAergic functions. Thirdly, enhancement of GABAergic function is neuroprotective and can prevent or reduce neuronal death. In addition, an increase of GABA inhibition suppresses epileptic activity (Rho and Sankar 1999) and thus treatment with GABAergic drugs might prevent the development of hypoxia-induced seizures or increased excitability. Experimental data on these possible effects are however rare.

We designed a series of experiments to address the following questions: 1) does exposure to hypoxia change susceptibility to chemically induced seizures in immature rats and what is time-course of these changes? 2) Can a vigabatrin-induced increase of GABA level before or immediately after hypoxia exposure prevent changes of seizure susceptibility, seizure pattern and/or seizure outcome at different time-points after hypoxia?

Methods

Experiments were performed in male Wistar albino rats on postnatal day 25 (P25; n = 190). Experimental groups consisted from 8 to 17 animals. The day of birth was defined as day 0. Rats were housed in a controlled environment (temperature 22 ± 1°C, humidity 50-60%, lights on 0600 – 1800 h) with free access to food and water. Experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic. Animal care and experimental procedures were conducted in accordance with the guidelines of the European Community Council directive 86/609/EEC.

The effects of vigabatrin (VGB, gamma-vinyl-GABA; generous gift from Aventis, former Hoechst Marion Roussell) on hypoxia-induced changes of seizure susceptibility were studied in a model of pentylenetetrazol-induced convulsions in two experimental schedules: VGB, freshly dissolved in physiological saline (300 mg/mL), was injected in a dose of 600 mg/kg i.p. before and/or after hypoxia exposure.

Vigabatrin pretreatment

Experimental animals (n = 68) received VGB at P24, controls (n = 75) were injected with a corresponding volume of physiological saline (2 mL/kg). Twenty four hours later (at P25) animals from both control and experimental group were randomly divided into two subgroups and one control and one experimental subgroup was exposed to hypobaric hypoxia (simulated altitude of 7 000 m) for 8 h. Decompression and compression took approximately 12 minutes, during which temperature of the hypobaric chamber was maintained at 22-23°C. During hypoxia exposure, the behavior of animals was monitored by an experienced observer. Other 2 subgroups were kept in the animal room at the same temperature and served as non-hypoxic controls.

To induce convulsions, animals from all four groups were injected with pentylenetetrazol (Sigma, 80 mg/kg s.c.) one, 3 or 7 days later, *i.e.* at P26, P28 or P32. After PTZ injection, animals were individually placed in a plexiglas cages and their behavior was monitored for 30 minutes. Incidence and latency of two types of convulsive seizures, minimal (mMS) *i.e.* predominantly clonic convulsions involving head and forelimb muscles with preserved righting reflexes, and generalized tonic-clonic seizures (GTCS), starting with short running phase and accompanied by a loss of righting reflexes were registered. The incidence of complete tonic phase involving all four limbs or partial tonic phase involving only forelimbs was registered separately. To assess severity of epileptic phenomena animals were assigned a score for the most severe behavioral characteristics as follows (Pohl and Mareš 1987):

0 = no changes,

0.5 = abnormal behavior (*e.g.* automatisms, increased orienting reaction),

1 = isolated myoclonic jerks,

2 = atypical or incomplete minimal seizures,

3 = minimal seizures,

4 = generalized seizures without the tonic phase,

5 complete generalized tonic-clonic seizures.

Mortality was also registered and evaluated.

Vigabatrin postreatment

Another group of animals was exposed to 8 h hypobaric hypoxia at P25 (n = 23). After the end of hypoxic insult, animals were randomly divided into 2 subgroups. Animals from one of them were injected with vigabatrin

H. Kubová, P. Mareš

(600 mg/kg i.p.), control subgroup received saline in a corresponding volume. Non-hypoxic controls (n = 24) were treated in the same way. Three days later (P28), all animals received PTZ according to the protocol described before.

Statistical evaluation

Data are presented as mean ± standard error of the mean (SEM) in the text. There was no difference between animals receiving saline 24 h before hypoxia or immediately after the end of hypoxia exposure in any parameter of PTZ seizures induced 3 days later. Therefore, these two groups were pooled together for statistical evaluation. Statistical analysis for comparison between two groups was performed using Mann-Whitney's Rank Sum Test. The incidence of seizures, tonic phase of GTCS and lethal outcome were compared with appropriate controls by means of Fisher's exact test. Parameters were analyzed using SigmaStat® (SPSS Inc., Chicago, IL) software. A p value of less than 0.05 was considered to be significant.

Results

Behavior and mortality during hypoxia exposure

No signs of motor seizures were observed in any animal during hypoxia exposure. Locomotor activity was lower in VGB treated animals than in controls. Animals from both control and VGB group exhibited pronounced hyperventilation. No animal died.

Effects of hypoxia on pentylenetetrazol-induced seizures

There was a tendency to increase severity of seizures in both 1 and 7 days but the difference was not statistically significant (4.39 ± 0.27 *vs* 3.40 ± 0.63, and 3.46 ± 0.42, *vs* 2.44 ± 0.42, respectively). There were no differences in other seizure parameters between hypoxic and non-hypoxic animals in these intervals. In contrast, hypoxia exposure led to increased seizure sensitivity 3 days later (*i.e.* at P28). Seizure severity expressed as score was higher in hypoxic animals compared to non-hypoxic controls (5.00 ± 0.00 *vs* 3.71 ± 0.34; p = 0.024) and also latencies of both minimal seizures and GTCS were shorter in hypoxic rats than in non-hypoxic controls (for minimal seizures 106 ± 11 s *vs* 222 ± 29 s; p = 0.007; for GTCS 335 ± 40 s *vs* 596 ± 126 s; p = 0.028). The incidence of GTCS was higher in hypoxic than in non-hypoxic animals (100 *vs* 43%; p = 0.018), and complete tonic phase was more frequent in hypoxic animals (87.5 *vs* 36% in non-hypoxic animals; p = 0.031) *(figure 1).*
Exposure to hypoxia also led to increased mortality during PTZ seizures (87.5 *vs* 29% in non-hypoxic animals; p = 0.012) three days later but not at other intervals *(figure 2).*

Effects of vigabatrin in non-hypoxic rats

In general, VGB pretreatment increased sensitivity to PTZ-induced seizures. Seizure severity was significantly higher in rats exposed to PTZ at P28 (score 5 ± 0 *vs* 3.7 ± 0.3; p = 0.02) and at P32 (*i.e.* 8 days after VGB injection; score 4.5 ± 0.5 *vs* 2.4 ± 0.4; p = 0.019). VGB injected 2, 3, 4, or 8 days before PTZ increased incidence of GTCS compared to corresponding saline treated controls by 56-57% (p < 0.01 [*figure 1*]). Incidence of complete tonic phase was higher in animals receiving VGB at P24 and PTZ 4 or 8 days later (increase by 44% and 68%, respectively; p < 0.05). Minimal, clonic seizures were not significantly affected by VGB and mortality remained unchanged in all groups *(figure 2).*

Effects of vigabatrin in rats exposed to hypoxia

Effects of pre-treatment

There was no difference between VGB pretreated and non-treated hypoxic animals in score expressing seizure severity. Pretreatment with VGB 24 h before hypoxia exposure prolonged latencies of minimal seizures one and 3 days after hypoxia. Incidence of GTCS was not affected by VGB pretreatment at any interval, but incidence of complete tonic phase was significantly lower in animals tested one day after hypoxia (P25 + 1) compared to corresponding controls (8% *vs* 69%; p < 0.0001 [*figure 1A*]). Mortality was abolished by VGB in animals tested one day after hypoxia (group 25 + 1, 0% *vs* 46% in controls; p = 0.007) and decreased in animals tested 3 days after the hypoxic insult compared to controls (25 + 3, 25% *vs* 87.5%; p = 0.02 [*figure 1A*]).

Effects of post-treatment

VGB injected immediately after hypoxia exposure prolonged latencies of minimal seizures (106 ± 11 s *vs* 319 ± 49 s; p = 0.009). Neither latency nor incidence of GTCS were affected by VGB post-treatment, but the incidence of complete tonic phase was significantly lower in animals with VGB than in corresponding controls (7% *vs* 87.5%; p < 0.001 [*figure 1B*]). In addition, VGB post-treatment tended to suppress mortality during PTZ-induced seizures (47% *vs* 87.5% in controls; p = 0.06 [*figure 1B*]).

Discussion

The data reported here demonstrate a transient increase of susceptibility to PTZ-induced seizures in P25 rats exposed to hypobaric hypoxia. Previously published studies suggested that episodes of brain hypoxia/ischemia may alter seizure susceptibility in a different way and both seizure protection and proconvulsive effects have been documented. The detailed condition necessary to confer decrease or increase of seizure susceptibility are not yet
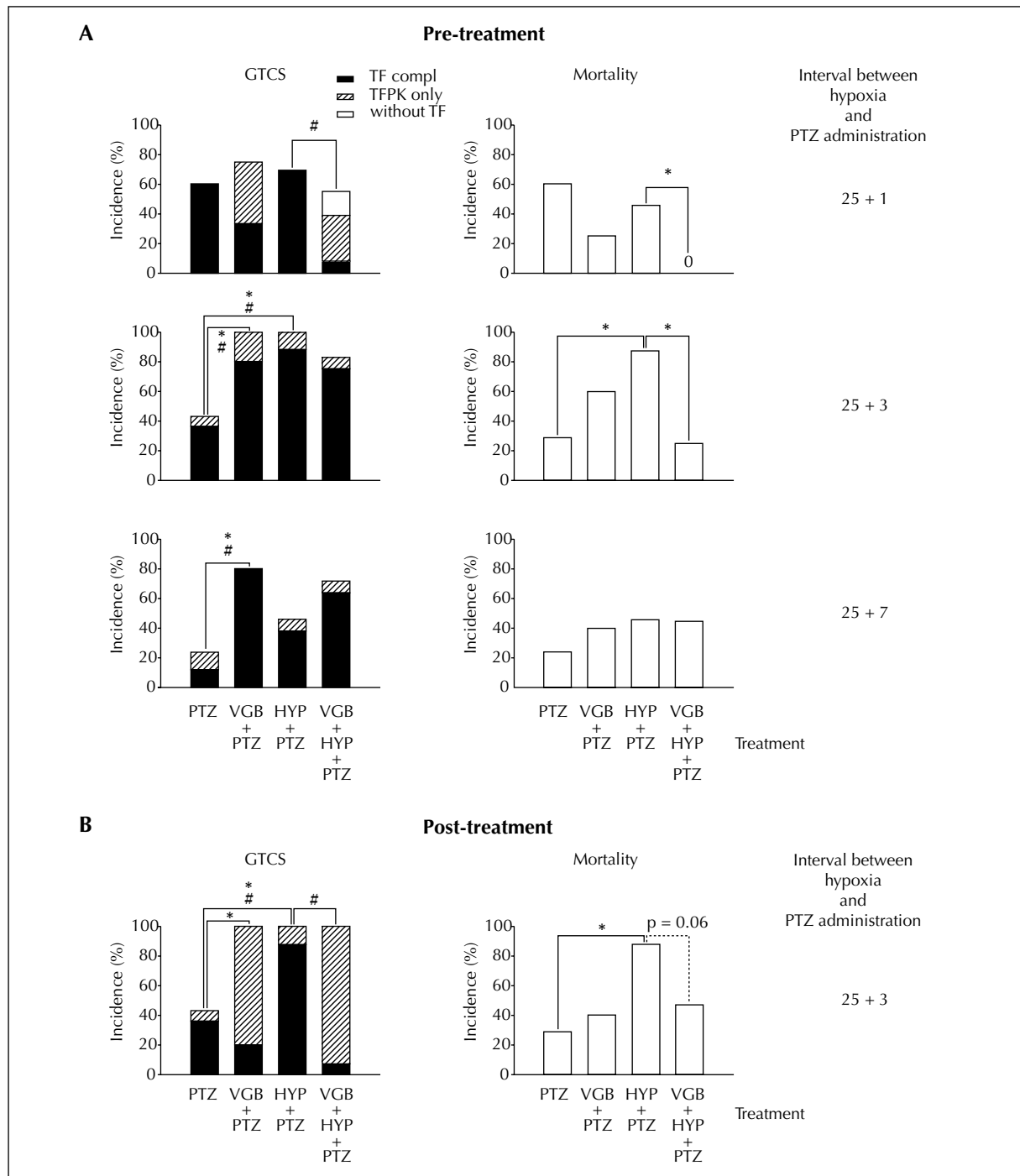
Figure 1. Incidence of generalized tonic-clonic seizures (left graphs; black bars - GTCS with complete tonic phase; striped bars - GTCS with tonic phase restricted to forelimbs; white bars - generalized clonic seizures) and mortality (right graphs) after PTZ injection (80 mg/kg sc).
A) Animals pretreated with vigabatrin in a single dose of 600 mg/kg 24 h before exposure to hypobaric hypoxia and corresponding controls. From top to bottom: rats 1, 3 and 7 days after hypoxia. **B**) Animals injected with vigabatrin (600 mg/kg) immediately after the exposure to hypoxia and injected with PTZ 3 days after hypoxia and corresponding controls. Incidence of generalized seizures in left graphs, incidence of lethal outcome in right graphs.
PTZ: non-hypoxic controls receiving only PTZ; VGB+PTZ: non-hypoxic animals receiving vigabatrin at P24 and PTZ at different intervals; HYP+PTZ: rats exposed to hypoxia and given PTZ 1, 3 and/or 7 days later; VGB+HYP+PTZ: rats pretreated with VGB at P24, exposed to hypoxia at P25 and given PTZ 1, 3 or 7 days after hypoxia. * (p < 0.05) denotes difference in the incidence of seizures and/or mortality; # denotes difference in the incidence of complete tonic phase involving both forelimbs and hindlimbs (left side of the panels) compared to corresponding controls; Student's t-test.
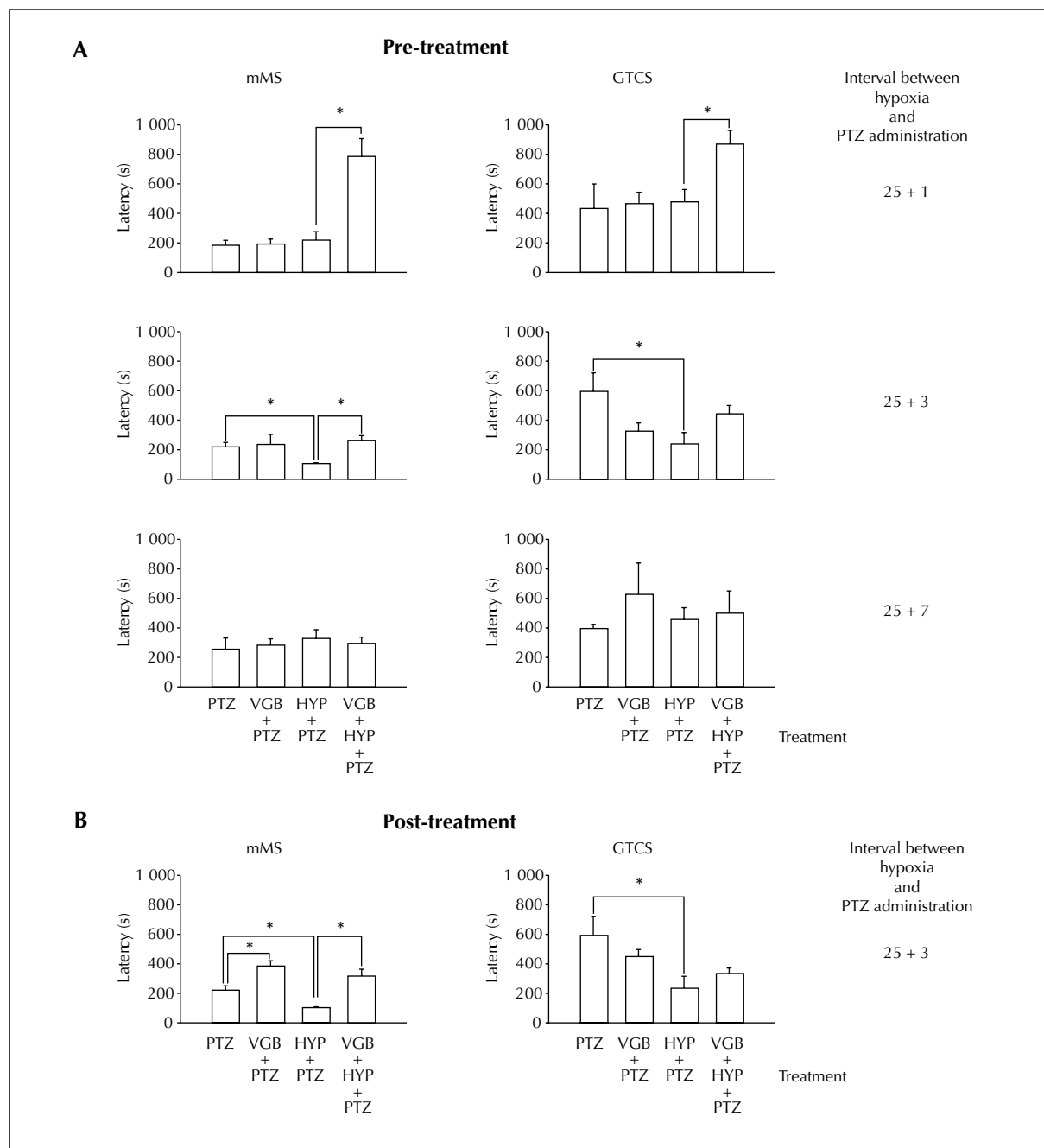
Figure 2. Latencies of two types of seizures elicited by pentylenetetrazol (mean ± S.E.M.). On the left side of panels there are latencies of minimal clonic seizures (mMS), the right side of the panels demonstrates latencies of generalized tonic-clonic seizures (GTCS). Abscissae: the 4 groups as in *figure 1*; ordinates: latencies to seizure onset in seconds.

known. Duration and severity of ischemic/hypoxic insult, chosen model and presence of morphological damage seem to play important role in the outcome of the insult (Vanický *et al.* 1997; Rubaj *et al.* 2003). Mechanisms used to induce seizures after hypoxia/ischemia are also important. In a model of forebrain ischemia, decreased sensitivity to lidocaine- and PTZ-induced seizures was observed

shortly after insult, whereas threshold for NMDA-induced seizures decreased in the same interval (Kim and Todd 1999). In present study increased severity of PTZ-induced seizures and mortality due to seizures was observed three days after hypoxia exposure. There is however some controversy between our and previously published data which show an increase of threshold for PTZ-induced

seizures in adult rats exposed to ischemia/hypoxia insult (Rubaj *et al.* 2000; Kim and Todd 1999). Such differences may be explained by the different intervals between the insult and injection of PTZ (6 h – 1 week) and also by insults used. Both above mentioned studies used models of more severe hypoxia/ischemia accompanied either by seizures during hypoxia exposure (Rubaj *et al.* 2003) or by a neuronal loss (Kim and Todd 1999). In contrast, exposure to hypobaric hypoxia did not lead to seizures and previously published studies did not report significant neuronal loss in this model (Fischer *et al.* 1974). Also, the level of brain maturation at the time of insult (adults *vs* prepubescent animals) can play crucial role in changes of seizure susceptibility. Jensen *et al.* (1991) reported highly age-related hyperexcitability in model of global hypoxia in immature rats. In their study model, seizure activity and permanent hyperexcitability were seen in rats exposed to hypoxia at P10-12 but not younger (P5) or older (P60) rats (Jensen *et al.* 1996). On the other hand, in a model of hypobaric hypoxia, increased susceptibility to electrically-induced seizures was observed immediately after the insult in P12 -35 rats (Kalinčik and Marešová 2005) suggesting that these changes of seizure susceptibility may be less age-specific. Present data demonstrate hypobaric hypoxia-induced changes in susceptibility to PTZ-induced seizures only in one group of immature rats. Therefore we cannot exclude the possibility that even small changes in sensitivity to PTZ with age play a role. This possibility requires further study.

PTZ induces two different types of convulsive seizures and various seizure parameters can be evaluated in individual animals (Velíšek *et al.* 1992). Minimal clonic and generalized tonic-clonic seizures are generated in different structures. Minimal, predominantly clonic seizures originate in the forebrain whereas generalized tonic-clonic seizures in the brainstem and/or spinal cord (Browning and Nelson 1986; Mareš 2006). Shortening of latencies in both minimal and generalized tonic-clonic seizures in hypoxic animals indicate involvement of different brain structures.

In non-hypoxic animals, pre-treatment with vigabatrin increased severity of PTZ-induced seizures in intervals longer than 48 hours after administration. In intervals from 3 to 8 days, the incidence of generalized seizures and mortality due to seizures were significantly higher compared to untreated controls. In our previous study, vigabatrin in doses 300-1200 mg/kg decreased incidence of complete tonic phase of PTZ-induced seizures in P25 rats 4 and 24 h after administration (Haugvicová *et al.* 2002). This is in contrast to data from other laboratories where anticonvulsant effects of vigabatrin seem to be related to the used model, dose and interval after administration (Dalby and Nielsen 1997; Vinogradova *et al.* 2005). In addition, some reports showed that vigabatrin possesses both anti- and proconvulsant properties in various models of epileptic seizures; proconvulsant effects are however more frequently seen at shorter intervals after

vigabatrin administration and/or after doses higher than those used in present study (Löscher *et al.* 1989; Mareš and Šlamberová 2004, Stuchlík *et al.* 2001; Luszczki and Czuczwar 2007). As demonstrated both *in vivo* and *in vitro*, vigabatrin is an irreversible inhibitor of brain 4-aminobutyrate-2-ketoglutarate aminotransferase (GABA-T; Lippert *et al.* 1977; Sarup *et al.* 2003). After a single dose of vigabatrin (200 mg/kg) in adult rats, GABA increase was maintained until 48h with partial recovery at 3 days after drug injection (Valdizan *et al.* 1999). There is however no direct correlation between GABA level in the brain and seizure protection (Bernasconi *et al.* 1988; Löscher *et al.* 1989), because substantially increased brain GABA levels were found already one or two hours after vigabatrin administration when the seizure protection is low. On the other hand, exposure of various brain structures to increased GABA levels may result in "GABA withdrawal syndrome" on cessation. Previously studies have shown that the withdrawal of GABA after brief but sustained administration leads to increased seizure susceptibility or seizure development (Brailowski *et al.* 1987). In adult rats, withdrawal of GABA infusion lasting for 6 h was sufficient to elicit epileptiform activity (Brailowski *et al.* 1988). The recovery period, *i.e.* the disappearance of epileptiform activity and seizures, lasted from 1 to 10 days (Brailowsky *et al.* 1990). The increased seizure severity seen three and more days after the vigabatrin administration in our study might be therefore explained as the rebound of excitability.

Partially protective effects of vigabatrin post-treatment against hypoxia-induced increase of seizure susceptibility and mortality are difficult to understand. Neuroprotective effects of vigabatrin, expressed as decreased severity of the damage in various brain areas, were documented in ischemia-induced brain damage 1 week after injury (Shuaib *et al.* 1996). As mentioned above, quantitative changes in the cytoarchitectonics of the brain were not described in a model used in our study, but some quantitative changes of the microstructure of the hippocampus and sensorimotor cortex occurred after hypobaric hypoxia (Fischer *et al.* 1980a, b; Pokorný *et al.* 1982). However, it is not clear whether these changes may play a role in hypoxia-induced seizure susceptibility and whether they can be affected by vigabatrin.

At the same time point when protective effects of vigabatrin were observed in hypoxic animals, increased seizure severity (proconvulsant effects) and seizure-induced mortality occurred in non-hypoxic animals. Explanation for this effect is purely speculative. Cerebral ischemia was found to result in an increase of GABA concentration with concomitant inhibition of GABA synthesis and release. Following hypoxia, GABA function can therefore decrease and this may participate in changes of seizure susceptibility. Effects of vigabatrin on GABA levels might therefore normalize hypoxia-induced disbalance of the inhibitory system.

In conclusion, our results demonstrated protective effects of vigabatrin administered after mild hypoxia against hypoxia-induced changes of seizure susceptibility in juvenile rats. Mechanisms responsible for these protective effects remain to be analyzed. □

References

Bernasconi R, Klein M, Martin P, *et al.* Gamma-vinyl GABA: comparison of neurochemical and anticonvulsant effects in mice. *J Neural Transm* 1988; 72: 213-33.

Brailowsky S, Menini C, Silva-Barrat C, Naquet R. Epileptogenic gamma-aminobutyric acid-withdrawal syndrome after chronic, intracortical infusion in baboons. *Neurosci Lett* 1987; 74: 75-80.

Brailowsky S, Kunimoto M, Menini C, Silva-Barrat C, Riche D, Naquet R. The GABA-withdrawal syndrome: a new model of focal epileptogenesis. *Brain Res* 1988; 442: 175-9.

Brailowsky S, Kunimoto M, Silva-Barrat C, Menini C, Naquet R. Electroencephalographic study of the GABA-withdrawal syndrome in rats. *Epilepsia* 1990; 31: 369-77.

Browning RA, Nelson DK. Modification of electroshock and pentylenetetrazol seizure patterns in rats after precollicular transections. *Exp Neurol* 1986; 93: 546-56.

Calvert JW, Zhang JH. Pathophysiology of an hypoxic-ischemic insult during the perinatal period. *Neurol Res* 2005; 27: 246-60.

Dalby NO, Nielsen EB. Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. *Epilepsy Res* 1997; 28: 63-72.

Fischer J, Jilek L, Trojan S. Qualitative and quantitative neurohistological changes produced in the rat brain by prolonged aerogenic hypoxia in early ontogeny. *Physiol Bohemoslov* 1974; 23: 211-9.

Fischer J, Langmeier M, Trojan S. Changes in the length and width of the postsynaptic density, the width of the intersynaptic density and the synaptic cleft in the cerebral cortex synapses of rats exposed to prolonged aerogenic hypoxia during early ontogenesis. An electron microscopic morphometric study. *Physiol Bohemoslov* 1980a; 29: 561-7.

Fischer J, Langmeier M, Trojan S. Shortening of presynaptic dense projections produced in the synapses of rat cerebral cortex by prolonged repeated hypoxia in early ontogenesis. *Physiol Bohemoslov* 1980b; 29: 93-8.

Green AR, Hainsworth AH, Jackson DM. GABA potentiation: a logical pharmacological approach for the treatment of acute ischaemic stroke. *Neuropharmacology* 2000; 39: 1483-94.

Haugvicová R, Kubová H, Mareš P. Does vigabatrin possess an anticonvulsant action against pentylenetetrazol-induced seizures in developing rats? *Physiol Res* 2002; 51: 363-70.

Jensen FE, Applegate C, Burchfiel J, Lombroso CT. Differential effects of perinatal hypoxia and anoxia on long term seizure susceptibility in the rat. *Life Sci* 1991; 49: 399-407.

Jensen FE, Wang C. Hypoxia-induced hyperexcitability *in vivo* and *in vitro* in the immature hippocampus. *Epilepsy Res* 1996; 26: 131-40.

Kalinčik T, Marešová D. Influence of magnesium sulphate on evoked activity of rat brain after exposure to short-term hypoxia. *Physiol Res* 2005; 54: 229-34.

Kelly KM. Poststroke Seizures and Epilepsy: Clinical Studies and Animal Models. *Epilepsy Curr* 2002; 2: 173-7.

Kim DC, Todd MM. Forebrain ischemia: effect on pharmacologically induced seizure thresholds in the rat. *Brain Res* 1999; 83: 131-9.

Lippert B, Metcalf BW, Jung MJ, Casara P. 4-amino-hex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric-acid aminotransferase in mammalian brain. *Eur J Biochem* 1977; 74: 441-5.

Löscher W, Jackel R, Muller F. Anticonvulsant and proconvulsant effects of inhibitors of GABA degradation in the amygdala-kindling model. *Eur J Pharmacol* 1989; 163: 1-14.

Luszczki JJ, Czuczwar SJ. Isobolographic characterization of interactions between vigabatrin and tiagabine in two experimental models of epilepsy. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 3: 529-38.

Mareš P. Generalized seizures in rodents – only two patterns? In: Hirsch E, Andermann F, Chauvel P, Engel J, Lopes da Silva F, Luders H, eds. *Generalized Seizures: From clinical phenomenology to underlying systems and networks.* Paris: John Libbey Eurotext, 2006: 70-2.

Mareš P, Šlamberová R. Biphasic action of vigabatrin on cortical epileptic after-discharges in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2004; 369: 305-11.

Pohl M, Mareš P. Effects of flunarizine on Metrazol-induced seizures in developing rats. *Epilepsy Res* 1987; 1: 302-5.

Pokorný J, Trojan S, Fischer J. Changes in the structure of the rat hippocampus after prolonged postnatal hypoxia. *Physiol Bohemoslov* 1982; 3: 193-202.

Rho JM, Sankar R. The pharmacologic basis of antiepileptic drug action. *Epilepsia* 1999; 40: 1471-83.

Rubaj A, Gustaw K, Zgodzinski W, Kleinrok Z, Sieklucka-Dziuba M. The role of opioid receptors in hypoxic preconditioning against seizures in brain. *Pharmacol Biochem Behav* 2000; 67: 65-70.

Rubaj A, Zgodzinski W, Sieklucka-Dziuba M. The epileptogenic effect of seizures induced by hypoxia: the role of NMDA and AMPA/KA antagonists. *Pharmacol Biochem Behav* 2003; 74: 303-11.

Sarup A, Larsson OM, Schousboe A. GABA transporters and GABA-transaminase as drug targets. *Curr Drug Targets CNS Neurol Disord* 2003; 2: 269-77.

Shuaib A, Murabit MA, Kanthan R, Howlett W, Wishart T. The neuroprotective effects of gamma-vinyl GABA in transient global ischemia: a morphological study with early and delayed evaluations. *Neurosci Lett* 1996; 204: 1-4.

Stuchlík A, Kubová H, Mareš P. Single systemic dose of vigabatrin induces early proconvulsant and later anticonvulsant effect in rats. *Neurosci Lett* 2001; 312: 37-40.

Valdizan EM, Garcia AP, Armijo JA. Time course of the GABAergic effects of vigabatrin: is the time course of brain GABA related to platelet GABA-transaminase inhibition? *Epilepsia* 1999; 40: 1062-9.

Vanický I, Čizková D, Prosbová T, Maršala M. Audiogenic seizures after neck tourniquet-induced cerebral ischemia in the rat. *Brain Res* 1997; 766: 262-5.

Velíšek L, Kubová H, Pohl M, Staňková L, Mareš P, Schickerová R. Pentylenetetrazol-induced seizures in rats: an ontogenetic study. *Naunyn Schmiedebergs Arch Pharmacol* 1992; 346: 588-91.

Vinogradova LV, Kuznetsova GD, Shatskova AB, van Rijn CM. Vigabatrin in low doses selectively suppresses the clonic component of audiogenically kindled seizures in rats. *Epilepsia* 2005; 46: 800-10.