PHARMACOLOGY AND TOXICOLOGY

Impaired Plasticity of Hippocampal Synaptic Transmission in Rats Exposed to Prenatal Hypoxia Is Normalized by Treatment with Nootropic Dipeptides

A. N. Chepkova, S. S. Trofimov,* N. I. Smol'nikova,* T. A. Gudasheva,* R. U. Ostrovskaya,* and V. G. Skrebitskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 12, pp. 592-595, December, 1995
Original article submitted December 23, 1994

Experiments on hippocampal slices from young rats exposed to hypobaric hypoxia during *in utero* development revealed enhanced responsiveness (an increase in a CA1 field response amplitude) and reduced plasticity (a low incidence of field response long-term potentation following high-frequency stimulation) of CA1 pyramidal neurons. Postnatal treatment of animals with piracetam peptide analogs constructed on the basis of pyroglutamate and proline normalized both these physiological indices.

Key Words: *prenatal hypoxia; hippocampus; long-term potentiation; pop-spike; nootropics; L-pyroglutamyl-D-alanine-amide; N-phenylacetyl-L-prolyl-glycine*

Fetal hypoxia is one of the most common forms of human perinatal pathology and its consequences may manifest themselves in severe neurological disorders immediately after birth [4] or later on in mental retardation or cognitive disturbances [5]. A number of perinatal hypoxia models on animals including chronic fetal hypoxia or perinatal asphyxia have been developed to study the mechanisms of hypoxic impairments. Learning and memory disorders have also been revealed after such a relatively moderate exposure as a single episode of prenatal hypobaric hypoxia [2,6]. These functions can be successfully restored by the postnatal treatment of animals with nootropic drugs [6].

The hippocampus is one of the brain structures critically involved in learning and memory processes [1]. Hippocampal synaptic connections are characterized by a marked functional plasticity which manifests itself, among other things, in the phenomenon of long-term potentiation (LTP), persistent enhancement of synaptic efficacy following brief high-frequency stimulation. This phenomenon is considered to be the neurophysiological basis of learning [7]. The hippocampus is also a structure highly sensitive to hypoxia [12]. Recent investigations have shown that similar mechanisms may underlie LTP development and posthypoxic neuronal degeneration in the CA1 area. These mechanisms include the activation of N-methyl-D-aspartate (NMDA) receptors for glutamate coupled to calcium channels, an increase in the intracellular calcium concentration, and the activation of calcium-dependent protein kinases and proteases [7,9,13]. It therefore seemed important to find out whether prenatal exposure to hypoxia affects the functional properties of hippocampal neurons and what effects are produced by postnatal treatment with nootropic drugs. This study was designed to examine the characteristics of long-term potentiation in

Brain Research Institute, Russian Academy of Medical Sciences; *Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0012-1208$12.50

the CA1 hippocampal region in rats prenatally exposed to hypoxia and postnatally treated with highly efficient nootropics synthesized at the Institute of Pharmacology (Moscow) as piracetam peptide analogs on the basis of proline or pyroglutamate [3,14].

MATERIALS AND METHODS

Four groups of male rats aged 5 to 8 weeks (44 in all) were examined. Three groups were formed from the offspring of rats exposed to hypobaric hypoxia on the 16th day of pregnancy using a technique described elsewhere [2,6]: on postnatal days 8-20 pups were subcutaneously injected with L-pyroglutamyl-D-alanine-amide (PGA, 1 mg/kg daily, n=9), phenylacetyl-L-prolyl-glycine (PAPG, 0.1 mg/kg daily, n=11), or an equal volume of physiological saline (n=11). The control group (n=13) comprised pups born to mothers not exposed to hypoxia. These pups were also treated with physiological saline from the 8th to 20th postnatal days. Each experimental group included offspring of 2-3 mothers, tagged with code marks, and used in blind electrophysiological experiments performed on hippocampal slices.

Two to four slices from the right hippocampus were taken for electrophysiological analysis. The slices were allowed to recover for 1.5-2 h before the electrical activity was recorded. Field excitatory postsynaptic potentials (EPSP) and population spikes (PS) evoked by bipolar stratum radiatum stimulation were simultaneously recorded from the CA1 apical dendritic region and stratum pyramidale, respectively, using two microelectrodes filled with 1.5 M NaCl. Single square pulses (0.1 msec, 1-50 V) were used for stimulation.

The following parameters were measured in each slice: threshold stimulus intensities for EPSP and PS, stimulus-response relations (stimulus intensity *vs.* pop-spike amplitude); the maximal PS amplitude, the probability of LTP development after high-frequency stimulation (tetanus, 100 Hz for 1 sec), and LTP magnitude. Any changes in responses during the experiments including those produced by tetanus were assessed by the shifts of a stimulus-response curve and quantified by measuring the areas under the corresponding curves. Tetanic stimulation was applied 2-4 h after slice preparation, the stimulus intensity being adjusted so that the evoked responses were approximately semimaximal. LTP was considered to be present if the area under the linear part of a stimulus-response curve increased by no less than 10% after tetanus and this enhancement lasted at least 60 min. LTP induction was tested in 1-3 slices from each rat and assumed to be present in a given hippocampus if observed in at least one of the slice preparations. The mean LTP magnitude in a group was calculated by taking the maximal LTP value obtained for a given hippocampus.

Field responses were recorded and analyzed on a computer using software developed in the laboratory. The significance of differences in electrophysiological characteristics and LTP properties between the groups was assessed by the Mann-Whitney U test and Fisher's exact test.

RESULTS

The LTP properties in slices prepared from the hippocampus of different rats were evaluated by two criteria: 1) the probability of LTP development following standard tetanus; 2) the magnitude of LTP, that is, the value of the relative increase in responsiveness. High-frequency stimulation produced LTP in all the slices from the control group (100%); its magnitude varied from 10% to 73% and was approximately 37% on average (Table 1). The hippocampal slices from rats prenatally exposed to hypoxia and not treated with nootropics showed LTP with significantly lower probability (54.5%, p=0.01, Fisher's exact test) but its magnitude did not differ from the control value (Table 1). It should be noted that if slices were unable to develop LTP, tetanus also failed to induce short-term (15-30 min) potentiation of responses but often produced their depression.

It is currently believed that the activation of NMDA receptors for glutamate is a necessary, albeit not sufficient condition for LTP induction in the CA1 hippocampal area [7]. These receptors, though not contributing significantly to postsynaptic responses to

TABLE 1. Incidence and Magnitude of Field Response LTP in Hippocampal Slices of Rats Prenatally Exposed to Hypoxia

Group	Number of animals	Number of experiments with/without LTP	LTP magnitude
Control	13	13/0	35.85±5.05 (n=13)
Hypoxia	11	6/5**	37.00±5.36 (n=6)
Hypoxia+PGA	9	7/2	29.00±2.97 (n=7)
Hypoxia+PAPG	11	9/2	20.78±1.94* (n=9)

Note. n = number of slices; *p=0.05 (Mann−Whitney U test), **p=0.01 (Fisher's exact test). Here and Table 2: all means are given±SEM.

TABLE 2. Electrophysiological Characteristics of Responsiveness of CA1 Pyramidal Neurons in Hippocampal Slices of Rats Prenatally Exposed to Hypoxia

Group	Number of slices	Threshold intensity for EPSP, V	Threshold intensity for pop-spikes, V	Pop-spike maximum amplitude, mV
Control	17	7.65±0.71	15.29±1.97	4.49±0.34
Hypoxia	19	6.68±0.84	15.90±2.06	5.48±0.35**
Hypoxia+PGA	13	5.92±0.45	15.00±1.31	4.79±0.50
Hypoxia+PAPG	17	7.18±0.52	16.41±1.62	4.82±0.34

Note. **$p=0.01$ (Mann–Whitney U test).

low-frequency stimulation, become activated at high-frequency stimulation due to membrane depolarization resulting from glutamate interaction with its receptors selectively sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and removing the magnesium blockade of NMDA receptors [10]. High-frequency stimulation may not induce LTP if the stimulus intensity does not provide for membrane depolarization sufficient to activate the NMDA receptors. On the other hand, this failure may be due to alterations in the properties of these very same receptors or intracellular mechanisms regulating their activation.

We resorted to a standard tetanic stimulation, applying a stimulus intensity adjusted to evoke PS of half-maximal amplitude. A comparative analysis of the electrophysiological parameters of slices showed that both control and "hypoxic" slices were similar as regards the stimulus intensities threshold for postsynaptic responses, while the maximal PS amplitude in rats prenatally exposed to hypoxia, far from being lower, was significantly higher than in the control (Table 2).

The PS amplitude is known to correlate with the number of responding neurons and its increase is indicative of an enhanced responsiveness of hippocampal pyramids, which may underlie the lowering of the seizure thresholds in rats prenatally exposed to hypobaric hypoxia [2]. The low probability of LTP induction accompanied by enhanced neuronal responsiveness may indicate that in rats exposed to hypoxia *in utero* the efficacy of excitatory connections is maximal and the LTP induction mechanism is saturated. Such changes may result in learning impairment, as has been shown in experiments with artificial saturation of excitatory hippocampal inputs by intensive stimulation [8,11].

Postnatal treatment of hypoxic offspring with any of the nootropics under study normalized responsiveness (Table 2) and LTP development following tetanus (Table 1). The treatment with PGA increased the probability of LTP development to 78%, while PAPG treatment increased it to 82%. However, the LTP magnitude in both groups under treatment turned out to be lower than in the control: while hypoxic rats treated with PGA revealed only a trend toward a decreased LTP magnitude, in slices from rats treated with PAPG the LTP magnitude was significantly lower than the control ($p=0.05$, Mann-Whitney U test). Thus treatment with nootropic dipeptides normalized the LTP induction process but somehow decreased LTP expression. The mechanisms of action of the nootropics and the causes and functional significance of the LTP magnitude decrease are yet to be established.

This study was partially supported by grants from the Russian Foundation for Basic Research (94-01-11430) and the International Science Foundation (MN 6000).

REFERENCES

1. O. S. Vinogradova, *The Hippocampus and Memory* [in Russian], Moscow (1975).
2. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, *Zh. Nevropatol. Psikhiatr.*, **83**, № 3, 74-79 (1983).
3. R. U. Ostrovskaya, T. A. Gudasheva, S. S. Trofimov, and A. Skoldinov, in: *Pharmacology of Nootropics* [in Russian], Moscow (1989), pp. 26-35.
4. G. M. Savel'eva, *Reanimation and Intensive Care of Newborns* [in Russian], Moscow (1981).
5. I. A. Skvortsov, A. S. Burkova, E. I. Yampol'skaya, *et al., Zh. Nevropatol. Psikhiatr.*, **86**, № 10, 1441-1446 (1986).
6. S. S. Trofimov, R. U. Ostrovskaya, N. M. Smol'nikova, *et al., Eksp. Klin. Farmakol.*, **56**, № 6, 8-11 (1993).
7. T. V. P. Bliss and G. L. Collingridge, *Nature*, **361**, 31-39 (1993).
8. C. A. Castro, L. H. Silbert, B. L. McNaughton, and C. A. Barnes, *Nature*, **342**, 545-548 (1989).
9. D. W. Choi, *Trends Neurosci.*, **11**, 465-469 (1988). 10. G. L. Collingridge, *Exp. Physiol.*, **77**, 771-797 (1992). 11. F. H. Lopes da Silva, J. A. Gorter, and W. J. Wadman, *Neurosci. Lett.*, **63**, 115-120 (1986).
12. W. A. Pulsinelli, *Prog. Brain Res.*, **63**, 29-37 (1985).
13. W. W. Schlaepfer and U.-J. P. Zimmermann, *Prog. Brain Res.*, **63**, 185-196 (1985).
14. S. B. Seredenin, T. A. Voronina, T. A. Gudasheva, *et al.*, USA Pat. 5,439,930, Aug. 5, 1995.