# PHYSIOLOGY

# Effect of Prenatal Hypobaric Hypoxia on Glutamatergic Signal Transduction in Rat Brain

**E. I. Tyul'kova, D. G. Semenov, L. A. Vataeva, A. V. Belyakov, and M. O. Samoilov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 3, pp. 244-247, March, 2011
Original article submitted July 15, 2009

$Ca^{2+}$-mediated signal transduction of group I metabotropic glutamate receptors (ImGluR) was studied in the brain of young (15 days) and old rats (90 days) exposed to severe hypobaric hypoxia on gestation days 14-16. Changes in the concentration of bound intracellular $Ca^{2+}$ ($Ca^{2+}$ response) were evaluated after repeated application of a selective ImGluR agonist 3,5-dihydroxyphenylglycine (DHPG) to cultured brain slices. Primary application of DHPG for 2 min induced a negative $Ca^{2+}$ response in slices from 15-day-old intact animals, while repeated application caused a positive response. In slices from 90-day-old control animals, both responses were negative. In slices from rats of both age groups subjected to severe prenatal hypobaric hypoxia, both responses were mainly positive, but short-term negative components were present in adult animals. Our results suggest that severe hypobaric hypoxia changes the balance between the two constitutive signal pathways triggered by ImGluR (inosine triphosphate and diacylglycerol pathways). This procedure is followed by the increased influx of extracellular $Ca^{2+}$ (as compared to $Ca^{2+}$ release from the intracellular stores). This imbalance is particularly pronounced at the early stage of ontogeny.

**Key Words:** *calcium; brain slices; metabotropic glutamate receptors; prenatal hypobaric hypoxia; 3,5-dihydroxyphenylglycine (DHPG)*

Prenatal hypoxia is one of the adverse environmental factors that violate normal growth and development of the body during early ontogeny. Prenatal hypoxia can cause severe damage to the brain. Neurotic symptoms of these changes are often observed during postnatal ontogeny. Our previous studies showed that the sensitivity of rats to hypoxia increases sharply at the beginning of the third trimester of gestation (days 14-16), which leads to severe abnormalities of the somatic and neurological development. Hypoxic exposures significantly increase the risk of learning impairment during postnatal ontogeny [2]. We found that the last week of gestation is characterized by pronounced heterogeneity in the effect of hypoxic stress on the behavioral pattern during postnatal ontogeny [1,2].

The phosphoinositide and calcium regulatory systems play a key role in the regulation of functional activity of nerve cells [3,4]. These systems play the major role in the proliferation, migration, and differentiation of brain cells during ontogeny [9,11]. In our recent experiment we measured the content of polyphosphoinositides (phosphatidylinositol-5-phosphates

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia. ***Address for correspondence:*** anoxia@pavlov.infran.ru. E. I. Tyul'kova

and phosphatidyl-4,5-diphosphates) in the cerebral cortex of 15-day-old and 90-day-old rats. We also studied the phosphoinositide response coupled with the glutamatergic receptor system in the brain of the offspring from rats exposed to hypoxia in various periods of pregnancy. Hypoxic exposure on days 14-16 of gestation (but not on days 18-20 of gestation) was followed by significant changes in activity of intracellular signal transduction mediated by the phosphoinositide system [4]. Glutamate triggers hydrolysis of polyphosphoinositides in brain cells via activation of group I metabotropic glutamate receptors (ImGluR), whose constitutive activity is formed during the prenatal period. It can be suggested that these receptors play a role in the formation and/or postnatal maintenance of prenatal hypoxia-induced changes.

Here we studied the effect of prenatal hypoxia on functional activity of calcium regulatory system in rats of two age groups. $Ca^{2+}$ signaling mediated by the excitation of ImGluR was evaluated in cultured brain slices.

## MATERIALS AND METHODS

The research was performed according to the rules of studies with experimental animals (Guidelines for the Use of Animals in Neuroscience Research, Membership Directory of the Society, 1992). The study was conducted on cultured brain slices from male Wistar rats after prenatal hypoxia at gestational age of 14-16 days. Pregnant rats were exposed to severe hypobaric hypoxia (SHH) in a flow altitude chamber at 160-180 mm Hg. Daily hypobaric exposure (3 h) was repeated three times. Newborn males from treated animals were decapitated at the age of 15 and 90 days. The brains were placed in ice-cold incubation medium saturated with oxygen and containing 124 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1.24 mM $KH_2PO_4$, 1.3 mM $MgSO_4$, 3 mM $NaHCO_3$, 10 mM glucose, and 24 mM Tris-HCl (pH 7.4). Two tangential slices (400 μ) of the piriform cortex from both hemispheres were prepared on an EMS-4000 vibrotome (Electron Microscopy Sciences). Both slices from each animal were preincubated in the same medium at 37°C for 2.5 h (continuous flow, 1.2 ml/min).

Relative changes in the concentration of $Ca^{2+}$ bound to hydrophobic (lipid and protein) intracellular domains were estimated spectrophotometrically with a calcium-sensitive fluorescence indicator chlortetracycline [13]. Fluorescence in the spectral range with a maximum of 522 nm was measured in several microregions of each slice (100 μ in diameter) using an AvaSpec-2048 fiber-optic spectrometer (Avantes B.V.) connected to a LYuMAM-K contact microscope (LOMO). Fluorescence was excited with a light-emitting lamp (L2523UVC, Kingbright; peak wavelength 400 nm). The duration of fluorescence excitation did not exceed 10 sec. The measurements were repeated at an interval of 5 min. The incubation solution contained chlortetracycline in a concentration of 50 μM throughout the experiment. ImGluR were stimulated by repeated application of selective ImGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG). DHPG was added to the incubation solution. The mean concentration of DHPG was 100 μM over 2 min. The repeated application of DHPG was performed 40 min after washing. The results were analyzed by analysis of variance (Dunnett's test).

## RESULTS

Repeated application of DHPG was followed by various changes in the concentration of bound $Ca^{2+}$ ($Ca^{2+}$ response) in brain slices from 15-day-old and 90-day-old rats of the control group. The first application of this agonist caused a slight, but significant and sustained decrease in $Ca^{2+}$ concentration in slices from 15-day-old rats. After repeated treatment with DHPG, $Ca^{2+}$ concentration increased significantly and exceeded the baseline level (Fig. 1, curve *1*). In slices from 90-day-old animals, the $Ca^{2+}$ responses were negative (Fig. 2, curve *1*).

In animals exposed to prenatal SHH, the $Ca^{2+}$ responses were different and differ from that in control specimens. As differentiated from control animals, both responses were positive in 15-day-old rats of the treatment group (Fig. 1, curve *2*). Increasing the age of animals was accompanied by the appearance

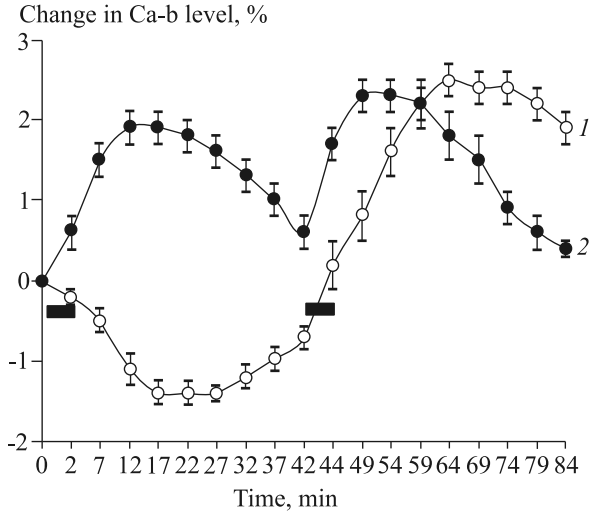


**Fig. 1.** Calcium responses to repeated application of DHPG (100 μM, 2 min) in slices from 15-day-old rats: control (*1*) and exposure to prenatal SHH (*2*). Here and in Fig. 2: Ca-b, bound $Ca^{2+}$. This figure shows changes in the mean values and standard errors of the means for each measurement (5-min interval). Black rectangles: application of DHPG.

of a short-term negative component at the beginning of each response (Fig. 2, curve *2*). These data suggest that $Ca^{2+}$ responses to DHPG are associated with the coexisting opposite changes (decrease and increase in the level of bound $Ca^{2+}$). $Ca^{2+}$ binding tended to dominate after prenatal hypoxia, but a tendency to the release of $Ca^{2+}$ becomes more pronounced with age in animals from the control and treatment groups.

Specific stimulation of ImGluR initiates various signal pathways, including the two following phospholipase C-dependent (PLC) processes: inositol triphosphate-mediated (IP3) release of $Ca^{2+}$ from the endoplasmic reticulum and diacylglycerol/protein kinase C-mediated (DG/PKC) potentiation of $Ca^{2+}$ conductance in inotropic glutamate receptors [7,8,14]. These pathways specifically modulate the concentration of cytosolic $Ca^{2+}$ in brain neurons. The mGluR5-induced potentiation of NMDA receptors and, probably, of AMPA receptors in neurons of the striatum, hippocampus, and neocortex contributes to the increased influx of $Ca^{2+}$ [6,7,10]. The IP3-mediated pathway induces a decrease in $Ca^{2+}$ concentration in the endoplasmic reticulum, but increases the level of calcium ions in the cytosol. These changes determine the $Ca^{2+}$-dependent inhibition of NMDA channels [12].

The negative shift in the $Ca^{2+}$ response to DHPG application (primary response in 15-day-old control animals, both responses in 90-day-old control animals, and initial stages of both responses in adult animals of the treatment group) observed in our experiments reflect predominance of the IP3-mediated signal pathway, which results in $Ca^{2+}$ release from the endoplasmic reticulum. The positive $Ca^{2+}$ responses (secondary response in 15-day-old control animals and both responses in treated animals) reflect the prevalence of the DG/PKC-mediated signal pathway. This pathway characterizes a positive modulation of ionotropic glutamate receptors by ImGluR and results in influx of extracellular $Ca^{2+}$ followed by calcium binding to buffer systems.

Thus, considering $Ca^{2+}$ responses to stimulation of ImGluR as a result of interference between the two major constitutive signal pathways of these receptors we can hypothesize that the signal balance in the brain of prenatally hypoxic animals is shifted to facilitation of $Ca^{2+}$ entry. This modification can be evaluated as a risk factor, which contributes to the development of $Ca^{2+}$-induced excitotoxicity. This state is typical of extreme conditions, including hypoxia/ischemia and posttraumatic stress. Various components of the calcium and phosphoinositide regulatory systems involved in the reaction to prenatal hypoxia, as well as the dynamics of hypoxia-induced modifications in signal pathways should be subjected to detailed studies.

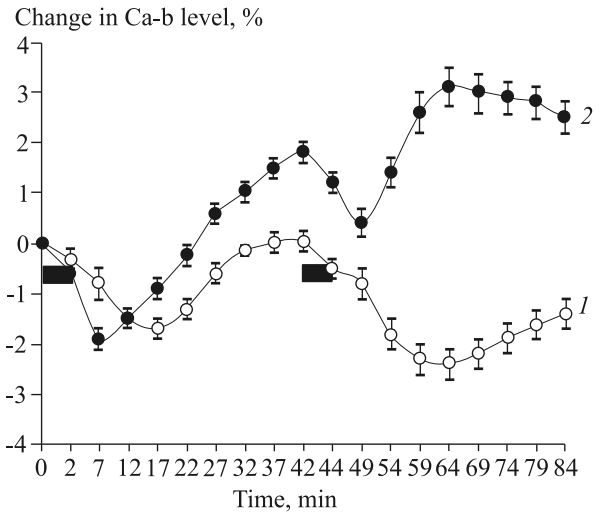


**Fig. 2.** Calcium responses to repeated application of DHPG (100 μM, 2 min) in slices from 90-day-old rats: control (*1*) and exposure to prenatal SHH (*2*).

This work was supported by the Russian Foundation for Basic Research (grant No. 08-04-00655).

## REFERENCES

1. L. A. Vataeva, V. B. Kostkin, G. V. Makukhina, *et al*., *Doklady Akad. Nauk*, **380**, No. 1, 125-127 (2001).
2. L. A. Vataeva, E. I. Tyul'kova, L. I. Khozhai, *et al*., *Zh. Evolyuts. Biokhim. Fiziol.,* **41**, No. 6, 532-535 (2005).
3. M. O. Samoilov, *Brain and Adaptation (Molecular and Cellular Mechanisms)* [in Russian], St. Petersburg (1999).
4. E. I. Tyul'kova, D. G. Semenov, L. A. Vataeva, and A. V. Belyakov, *Proceedings of the Russian-Poland Symposium "Hypoxic and Ischemic Preconditioning of the Brain"*, St. Petersburg (2008), pp. 97-101.
5. F. Ango, L. Prezeau, T. Miller, *et al*., *Nature*, **411**, 962-965 (2001).
6. A. E. Bandrowski, A. V. Aramakis, S. L. Moore, and J. H. Ashe, *Exp. Brain Res.*, **136**, No. 1, 25-40 (2001).
7. P. Benquet, C. E. Gee, and U. Gerber, *J. Neurosci*., **22**, No. 22, 9679-9686 (2002).
8. P. J. Conn and J. P. Pin, *Annu. Rev. Pharmacol. Toxicol*., **37**, 205-237 (1997).
9. M. L. Gonzales and R. A. Anderson, *J. Cell. Biochem*., **97**, No. 2, 252-260 (2006).
10. S. Kotecha, M. Jackson, A. Al-Mahrouki, *et al*., *J. Biol. Chem*., **278**, No. 30, 27,742-27,749 (2003).
11. A. M. Martelli, L. Manzoli, and L. Cocco, *Pharmacol. Ther*., **101**, No. 1, 47-64 (2004).
12. C. Rosenmund, A. Feltz, and G. L. Westbrook, *J. Neurosci*., **15**, No. 4, 2788-2795 (1995).
13. D. G. Semenov, M. O. Samoilov, and J. W. Lazarewicz, *Neurosignals*, **11**, No. 6, 329-335 (2002).
14. O. Valenti, P. Conn, and M. Marino, *J. Cell. Physiol*., **191**, No. 2, 125-137 (2002).