Role of Endogenous Neuromodulator Peptides in Functional Tolerance Enhancement of Cerebral Neurons to Ischemia

M. O. Samoilov and A. A. Mokrushin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 5, pp. 503-505, May, 1998
Original article submitted December 26, 1996

A short-term hypoxic stress induces synthesis and release of neuromodulator peptides from the olfactory cortex slices of the rat brain, which are protectors against the damage to synaptic transmission caused by long-lasting anoxia.

Key Words: *anoxia; neuromodulator peptides; protection; cycloheximide*

The problem of enhanced resistance of cerebral neurons to hypoxia and ischemia is important, so there is intense search for new pharmacological protective preparations among antihypoxants, antioxidants, calcium channel blockers, glutamate receptor blockers, exogenous peptides, etc. However, a more efficient approach to the problem seems to be the search for the ways to enhance the reserve protection potency of the brain itself. A moderate hypoxic stress is known to enhance tolerance of cerebral neurons to subsequent long-term hypoxia or ischemia [6,8,12]. A similar phenomenon was observed during electrical stimulation of vagus nerve [7] or expression of neurotrophic factors [10,11]. However, little is known on molecular and cell mechanisms that underlie enhancement of cerebral neuron resistance to hypoxia.

Previously, it was found that preliminary short-term anoxia (STA) *in vivo* prevents functional disturbances of feline cerebral neurons caused by long-term anoxia (LTA) [4]. Taking into account the available data, it has been proposed that in addition to intracellular regulator systems, protective effect of STA involves the genome and endogenous neuromodulator peptides synthesized in response to STA [2-4]. Our aim was to check up this hypothesis experimentally.

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg

MATERIALS AND METHODS

The study was carried out on 22 cultured slices of the olfactory cortex of the rat brain. Preincubation and perfusion of the slices with oxygenated medium, the mode of recording of the orthodromic focal potentials evoked by electrical stimulation of the lateral olfactory tract, and the protocol of experiments with donor and recipient slices were described in detail elsewhere [1]. In each experiment, the dynamics of evoked focal postsynaptic potentials was assessed by changes in their area and expressed as percentage relative to the initial values. STA (2 min), LTA (5-10 min), and reoxygenation of the slices were performed by replacing oxygen in the medium by nitrogen and vice versa. At the stage of translation of peptide synthesis, cycloheximide (CH, 100 μm, Sigma) was used as a blocker. The results were statistically analyzed using Student's t test and Wilcoxon—Mann—Whitney's nonparametric *U* test.

RESULTS

Similar to the effect observed in hippocampal slices [5], STA induced long postanoxic potentiation of synaptic transmission in the olfactory cortex during the oxygenation period (90 min), while LTA caused a stable deep inhibition of focal postsynaptic potentials (Fig. 1, *1*). However, when LTA was im-

0007-4888/98/0005-0444$20.00

posed on the same slice 90 min after STA, the LTA-induced depression of synaptic transmission did not occur (Fig. 1, *2*).

Therefore, both *in vivo* [3,4] and *in vitro* slices, STA induces adaptive reaction of cerebral neurons to severe anoxia. The possible molecular and cellular mechanisms of such an adaptive reaction were considered earlier [2-4]. Hypothetically, an important role in this reaction is played by *de novo* synthesized neuromodulator peptides. Some of these peptides are released from cells to produce adaptive effect on these and surrounding cells via modulation of synaptic transmission.

To test this hypothesis, a number of experimental series have been carried out. In series I, the effects of perfusates collected from donor slices were studied in the recipient slices subjected to LTA. The study evaluated effects of so-called early and late perfusates collected 0-20 and 60-90 min of reoxigenation after STA, respectively. The experimental protocol (Fig. 2, *1, 2*) was as follows: the recipient slices were preincubated in the early and late perfusates during 10 min, thereafter they were subjected to LTA with subsequent reoxygenation. The perfusates were applied both during LTA and 10 min after it.

When the late perfusates were applied, LTA did not inhibit focal potentials in the recipient slices (Fig. 2, *1*). The early perfusates did not produce this effect. These data show that after STA cells in the slices release some chemical agents that protect synaptic transmission from damage produced by LTA. Importantly, these agents are released not in the early, but in the late postanoxic period (60-90 min).

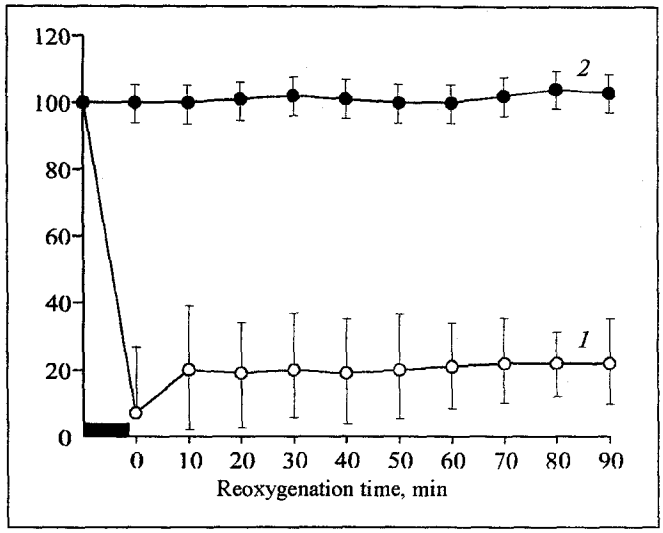

Fig. 1. Variations of the focal potential amplitude in the olfactory cortex slices (*1*) during reoxygenation after long-term anoxia and (*2*) under the same conditions with preceding short-term anoxia. Here and in Fig. 2: ordinate, focal potential amplitude as percent of the initial value; solid bars mark anoxic period.

The second series of experiments was performed with the synthesis blocker CH according to the following protocol (Fig. 2, *3*). Donor slices were preincubated for 60 min in CH-containing medium, and then STA was imposed with subsequent reoxygenation. CH was applied during STA and 60 min after

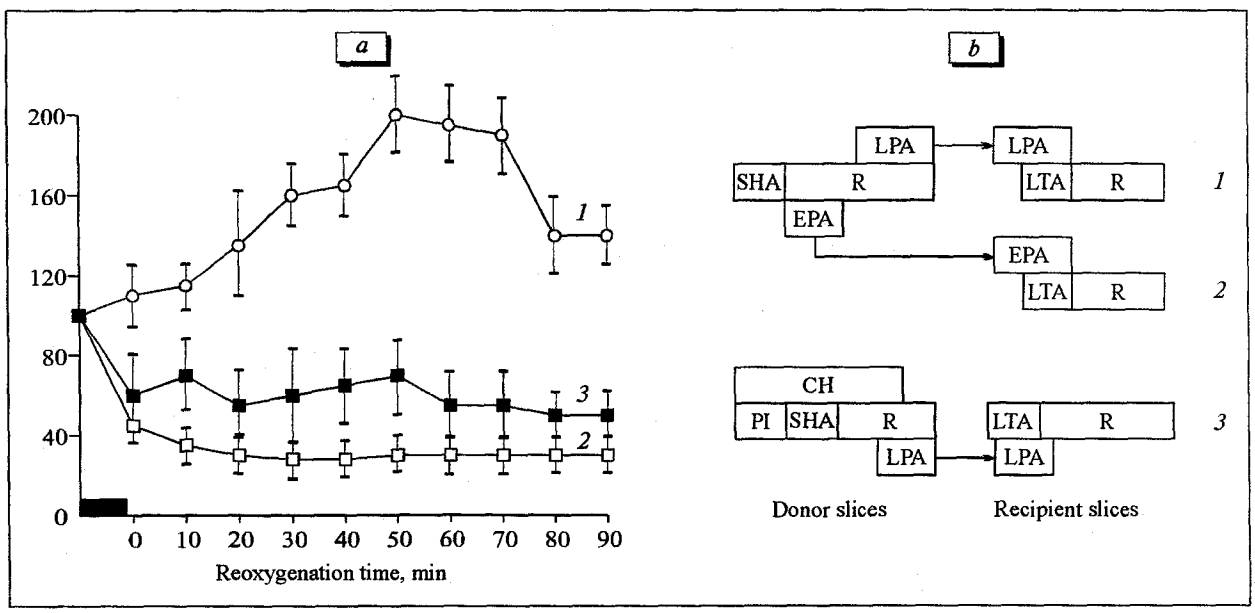

Fig. 2. (*a*) Variations of the focal potential amplitude in the recipient slices subjected to long-term anoxia and treated with the donor slice perfusates collected after short-term anoxia (*1, 2*) without and with (*3*) incubation in the medium containing a peptide synthesis blocker. (*b*) Experimental protocols. *EPA* and *LPA* are, respectively, the early and late postanoxic perfusates; *CH*, cycloheximide; *PI*, preliminary incubation; *SHA* and *LTA* are, respectively, a short- and long-term anoxia; *R*, reoxygenation.

it. After washing the slices from CH, the late perfusates were collected and transferred to the recipient slices that were subjected to LTA. The perfusates produced the effect 10 min prior to, during, and 10 min after LTA.

The late perfusates collected from CH-treated donor slices subjected to STA did not prevent inhibition of synaptic transmission induced by LTA in the recipient slices, although inhibition of focal potentials was less expressed than under the effect of LTA (Fig. 2, *3*). Thus, blockade of peptide synthesis at the stage of translation in the donor slices subjected to adaptive action of STA, notably antagonized the protective effect of chemical agents secreted during the late period (60-90 min) after this adaptive action.

Based on these finding, we conclude that the basic components of such agents are neuromodulator peptides that are *de novo* synthesized in response to STA. Synthesis of these peptides is related to STA-dependent activation of intracellular regulator systems (calcium, polyphosphoinositide, cyclic nucleotide), early genes (*c-fos*, *c-jun*, *ras*, etc.), transcription factors, and phenotype-specific late genes [2-4]. A certain period is necessary for development of this process: by our data, no less than 60 min after the adaptive stimulation.

The targets of newly synthesized neuromodulator peptides are probably the neurotransmitter receptors, and ionic channels of the producing cells themselves and of the surrounding neuronal population. It is supposed that released peptides are the carriers of the "voluminous" adaptive information [2]. Our data are corroborated by the study of the mechanisms of long-term post-tetanic potentiation in hippocampal slices [9]. It was found that 60 min after tetanic stimulation, the cells release newly synthesized peptides. Incubation of the slices in CH prevented long-term potentiation and synthesis of these peptides.

Although the mode of action of the peptides produced and released in response to adaptive stimulation is still unknown, it is evidenced here that in addition to their adaptive and protective abilities, they play an effective regulatory role in synaptic transmission.

This work was supported by the Russian Foundation for Basic Research, project No. 95-04-11516A.

REFERENCES

1. A. A. Mokrushin and I. V. Karpova, *Ros. Fiziol. Zh.*, **80**, No. 3, 8-12 (1994).
2. M. O. Samoilov, *Ibid.*, **81**, No. 8, 3-11 (1995).
3. M. O. Samoilov, D. G. Semenov, E. I. Tyul'kova, and E. A. Bolekhan, *Ibid.*, **80**, No. 11, 37-43 (1994).
4. M. O. Samoilov, D. G. Semenov, E. I. Tyul'kova, and E. A. Bolekhan, *Ibid.*, No. 12, 71-75 (1994).
5. C. Hammond, V. Crepel, H. Gozlan, Y. Ben-Ari, *Trends Neurosci.*, **17**, No. 11, 497-503 (1994).
6. K. Kitagawa, M. Matsumoto, M. Tagaya, *et al.*, *Brain Res.*, **528**, No. 1, 21-24 (1990).
7. T. Masada, T. Itano, M. Fujisawa, *et al.*, *Neuroreport*, **7**, No. 2, 446-448 (1996).
8. K. Miayshita, H. Abe, T. Nakajima, *et al.*, *Ibid.*, **6**, No. 1, 46-48 (1994).
9. S. Otani, M. Roisin-Lallemano, Y. Ben-Ari, *Neuroscience*, **47**, No. 2, 255-272 (1992).
10. P. A. Pechan, T. Goshida, N. Panahian, *et al.*, *Neuroreport*, **6**, No. 4, 669-672 (1995).
11. T. Shigeno, T. Mima, K. Takakura, *et al.*, *J. Neurosci.*, **11**, No. 9, 2914-2919 (1991)
12. A. Shurr, K. H. Reid, M. T. Tseng, *et al.*, *Brain Res.*, **374**, No. 2, 244-248 (1986).