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Role of Calcium and Phosphoinositide Cell Regulatory System in Adaptation of Neurons in Olfactory Cortex Section to *In Vitro* Hypoxia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 3, pp. 259-262, March, 1998
Original article submitted February 21, 1997

Activities of calcium and phosphoinositide regulatory systems in sections of rat olfactory cortex are analyzed during and after anoxia of different duration. It is shown that short-term anoxia prevents the disturbances in cell regulatory systems induced by long-term anoxia via moderate but sustained rise in neuronal content of second messengers: Ca^{2+} and products of phosphoinositide hydrolysis.

Key Words: *brain; anoxia; calcium; phosphoinositides; adaptation*

The involvement of principal cell regulatory systems (CRS) (calcium, polyphosphoinositide, and cyclic nucleotides) in various physiological and pathological processes in the brain (in particular, pathologies induced by severe hypoxia) is now beyond the question [1,7,9,11]. Cellular mechanisms of adaptation to hypoxia are an important problem of modern medicine and biology. Some investigators demonstrated the role of CRS in adaptive cell mechanisms triggered by both antihypoxic drugs and non-drug antihypoxic influences, in particular, short-term hypoxia, which effectively protects neurons from long-term hypoxia [2,5,8,10]. However, the mechanisms of this protective effect of short-term hypoxia are little studied. In our previous *in vitro* studies we chose experimental conditions for realization of protective effect of preventive hypoxia. These experiments showed that adaptive process is associated with certain shifts in the content of the calcium and phosphoinositide CRS in the brain cortex, which correlate with enhanced bioelectrical neuronal activity [4,5]. The aim of the present study was to elucidate the role of calcium and phosphoinositide CRS in the reaction of anoxia of various duration and in adaptive process induced by short-term hypoxia.

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MATERIALS AND METHODS

Experiments were carried out on 300-400-μ-thick sections of the olfactory cortex from Wistar rats. The sections were placed in a flow chamber and incubated in oxygenated buffer containing (in mM): 124 NaCl, 5 KCl, 2.6 $CaCl_2$, 1.24 KH_2PO_4, 3 Na_2HCO_3, 10 glucose, and 23 Tris-HCl (37°C, pH 7.4). Activity of calcium CRS was assessed by the dynamics of cell content of bound calcium (Ca-c) in different zones of the preparation measured using a chlorotetracycline fluorescent probe [3]. Activity of phosphoinositide CRS was assessed by the content and exchange rate of di- and triphosphoinositides (DPI and TPI). To this end, the section was transferred into a

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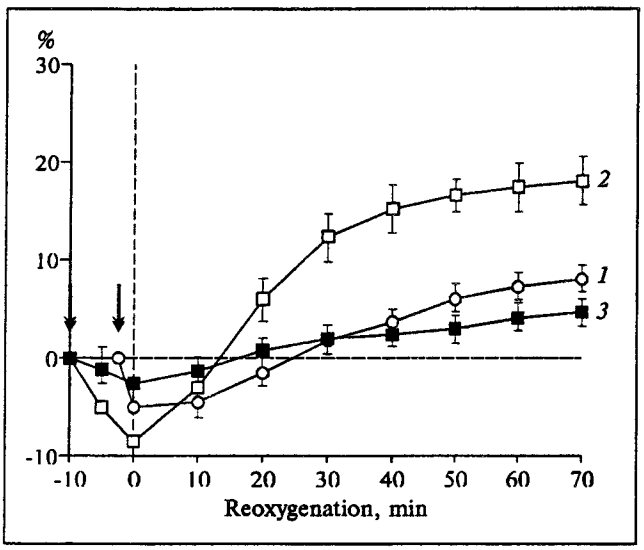

Fig. 1. Content of bound Ca in brain sections during 2-min (*1*) and 10-min (*2*), and 10-min anoxia with preliminary 2-min anoxia (*3*). Each curve is the mean of 6-7 experiments. Here and in Fig. 2: arrows indicate the start of anoxia, vertical dashed line marks the start of reoxygenation, horizontal line shows the initial (before anoxia) level.

medium containing 1 MBq sodium ^{32}P-orthophosphate 15 min before the end of incubation. The reaction was stopped by transferring the section into a chloroform-methanol-concentrated HCl mixture. Phospholipids were extracted and phosphoinositides were separated by chromatography. Radioactivity of DIP and TIP fractions was counted, and the content of lipid phosphorus in the same samples was determined. The data were expressed in cpm/μg P and mg P/μg tissue protein [6].

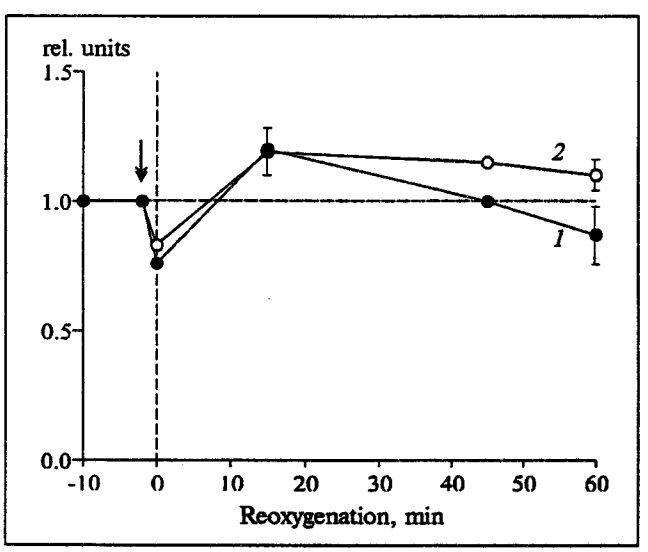

Fig. 2. Dynamics of the content (*1*) and metabolism (*2*) of triphosphoinositides during and after 2-min anoxia (n=7-8 for each curve).

Two- and 10-min anoxia was modeled by replacing the oxygenated incubation medium with that saturated with nitrogen and analogous replacement of gaseous mixture above the incubated section. During reoxygenation, opposite replacements were made.

RESULTS

Anoxia reduced the content of Ca-c: on minutes 2 and 10 it decreased to 95.2±0.7 and 90.7±0.8%, respectively. During reoxygenation this parameter gradually increased and by the 70th min stabilized at the level surpassing the initial one. The rate of calcium binding and the final level of bound calcium depended on the duration of anoxia. After 2-min anoxia, the content of Ca-c gradually returned to normal and starting from the 25th min surpassed the initial values, being 108±1%, while after 10-min anoxia the corresponding parameters were 10 min and 118±1.5% (Fig. 1, *1* and *2*). When 10-min anoxia was preceded (90-100 min before) by short-term anoxia (2 min), changes in the content of Ca-c during anoxia and reoxygenation were much less pronounced (Fig. 1, *3*). The decrease in the content of Ca-c during anoxia was first paralleled by a short-term (2-min) decrease in DPI and TPI to 93±5 and 75±10%, respectively. However, to the 5th min of anoxia the contents of both phosphoinositide subfractions considerably surpassed the initial level and to the 10th min they attained 147±10 and 140±10%, respectively. The intensity of phosphoinositide metabolism decreased during the entire anoxic period, especially after the 5th min, attaining to the 10th min 50±12% (TPI) and 69±10% (DPI) of the initial value. During the first 15 min of reoxygenation after 2-min anoxia, the content and metabolism of polyphosphoinositides (especially TPI) were moderately elevated; TPI metabolism remained enhanced over the 60-min reoxygenation period (Fig. 2). Dramatic changes in the polyphosphoinositide system were seen in the early reoxygenation period after 10-min anoxia: a sharp decrease in the content of TPI and DPI and a marked intensification of their metabolism in comparison with the preanoxia period. These shifts persisted for at least 50 min of reoxygenation (Fig. 3). Short-term preventive anoxia (60-90 min prior to the main session) minimized changes in the content and metabolism of polyphosphoinositides during both anoxia and reoxygenation periods (Fig. 3).

Our previous experiments showed that short-term anoxia led to long-term potentiation, while long-term anoxia suppressed synaptic transmission in sections of the olfactory cortex; preliminary short-term anoxia prevented these functional changes. Our findings suggest that short-term anoxia induces adap-

tive rearrangements of calcium and polyphosphoinositide regulatory systems. In contrast to moderate and sustained adaptive activation of these CRS (after 2-min anoxia), anoxia-induced damage is characterized by deep changes in the content in metabolism of calcium, TPI, and DPI attesting to hyperstimulation of calcium and polyinositide regulatory systems, which is most pronounced during the first 10 min of reoxygenation.

The study was supported by the Russian Foundation for Basic Research (grant 4840F).

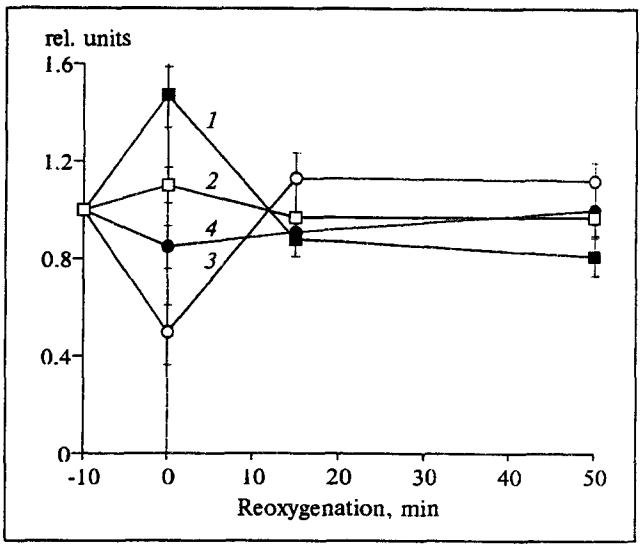

Fig. 3. Dynamics of the content (*1*, *2*) and metabolism (*3*, *4*) of triphosphoinositides during and after 10-min anoxia with (*2*, *4*) and without (*1*, *3*) preliminary anoxia.

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