EFFECT OF ANOXIA ON CHANGES IN PHOSPHOINOSITIDE CONTENT AND SINGLE UNIT ACTIVITY IN THE CAT CEREBRAL CORTEX

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In recent years a tendency has been noted for the role of disturbances of energy metabolism to be overestimated as a factor triggering intracellular responses to disturbances of the oxygen and substrate supply [3, 16, 17]. The attention of research workers has been drawn to the involvement of intracellular regulatory systems in this process (calcium, cyclic nucleotides, polyphosphoinositides — PPI) for their metabolism is quickly modified in response to a disturbance of the cerebral circulation and to hypoxia.

PPI metabolism has been shown to be sensitive to a disturbance of the oxygen and substrate supply [5, 18]. However, no link has been established between changes in the PPI system, disturbances of energy metabolism, and functional disorders (especially electrophysiological parameters).

The aim of this investigation was to study the trend of the phosphoinositide concentration and to correlate it with changes in the spontaneous spike discharge of cortical neurons in the cat brain during anoxia for 5 min.

EXPERIMENTAL METHOD

Experiments were carried out on male cats weighing 2800-3200 g, lightly anesthetized with pentobarbital (30 mg/kg), and subsequently immobilized with tubocurarine and artificially ventilated with the aid of the UIDZh artificial respiration apparatus. Anoxia was created by stopping the artificial respiration at expiration. Biopsy material for determination of the PPI content was taken before anoxia and 50 sec and 2.5 and 5 min after the cessation of artificial respiration. The depth of the sections for taking the biopsy material did not exceed 2 mm, and the weight of the fragments was 20-25 mg. Phosphoinositides were extracted during stirring for 30 min with a mixture of chloroform — methanol — conc. HCl (200:100:1) and washed with 1 N HCl. The film formed on the boundary between the chloroform and aqueous-methanol phases was removed for determination of the protein content. The extract was evaporated to dryness in a current of nitrogen, dissolved in a mixture of chloroform—methanol—conc. HCl (200:100:0.1), applied to formal-treated paper, and chromatographed. Spots corresponding to individual phosphoinositide fractions were cut out and mineralized, and the content of lipid phosphorus determined, and used to calculate molar concentrations of phosphoinositides.

Parallel with the removal of biopsy material, spontaneous spike activity of sensomotor cortical neurons was recorded. Potentials were recorded extracellularly with the aid of metallic microelectrodes, with a resistance of under 10 MΩ and with a tip 3-5 μ in diameter. Potentials amplified by an AC amplifier were recorded on a CRO and photographed with FOR camera.

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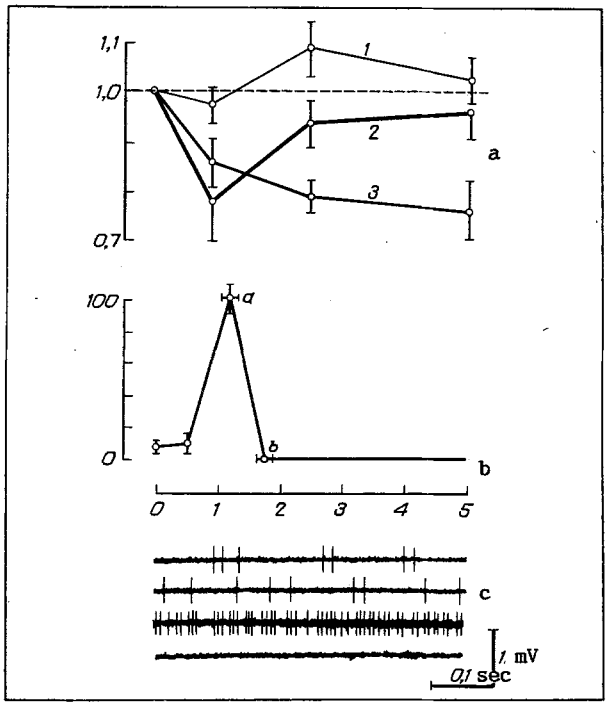

Fig. 1. Time course of phosphoinositide levels and changes in firing pattern of single neurons in cat sensomotor cortex during anoxia: a) time course of concentrations of mono- (1), di- (2), and triphosphoinositides (3) during anoxia (n = 6). Broken line — control level; b) time course of firing rate of single neurons during anoxia (n = 28); a and b denote extrema of anoxic activation and cessation of activity respectively. Values shown are means standard error of the mean. Abscissa, duration of anoxia (in min); ordinate: for a) phosphoinositide concentration (in relative units); for b) firing rate (sec^{-1}); c) typical example of change in firing pattern of a single neuron during anoxia. Top trace shows initial background (normoxia); from top to bottom: anoxia lasting 40 sec, 70 sec, and 120 sec.

EXPERIMENTAL RESULTS

The initial level of triphosphoinositides (TPI) in the cat cerebral cortex was 0.24 ± 0.013 mM (n = 15), the diphosphoinositide (DPI) level 0.279 ± 0.012 mM, and monophosphoinositide (MPI) 2.09 + 0.071 mM. These values were independent of the zone from which the biopsy material was taken. The average frequency of the spontaneously active neurons was 8.2 ± 2.4 sec^{-1}.

The choice of times for taking the biopsy material was determined by the following factors. According to data in the literature [3, 6, 13], during the first 50 sec after the beginning of anoxia the concentrations of high-energy phosphates still remained unchanged, but meanwhile changes had developed in the firing pattern and redox state of the neurons. After 2 min, spike activity usually ceased and changes in the energy potential of the cell were observed. Toward the 5th minute of anoxia, deep depolarization occurred and exhaustion of high-energy compounds took place.

The time course of phosphoinositide levels and spontaneous spike discharge of the cortical neurons after termination of the oxygen supply is illustrated in Fig. 1. After 50 sec the DPI and TPI levels fell sharply to 79.7% and 82.8% of the initial values respectively (Fig. 1A). This lowering of the PPZ concentration took place at a time when the firing pattern did not change significantly (Fig. 1C), and preceded the phase of high-frequency activation of the neurons, which as a rule was observed during the period of 60-90 sec after cessation of artificial ventilation (Fig. 1B). In the subsequent period of anoxia the DPI level returned virtually to the control value, whereas TPI continued to fall slowly. These changes took place against a background of depression of unit activity, which as a rule disappeared after 1.5 min of anoxia. The MPI concentration did not change signifi-

cantly at any time during the period of investigation. Thus acute oxygen starvation leads to rapid changes in PPI metabolism, preceding the development of a phase of high frequency activation of the neurons.

The fall in the PPI concentration during the first minute resembles their breakdown during total ischemia [12]. In the opinion of some workers [10], changes in PPI concentration are connected with disturbances of energy metabolism. However, in the model which we used, by the end of the first minute of anoxia no change was observed in the concentrations of high-energy phosphates or the energy potential of the cell [13]. Meanwhile, by this time definite changes had already appeared in the intracellular redox state, calcium homeostasis, and electrogenesis. It has been shown that early changes in calcium metabolism in response to oxygen insufficiency arise in connection with membrane-bound Ca^{2+} [4, 14]. During the first tens of seconds there is a gradual decrease in the content of membrane-bound Ca^{2+}, evidently in both plasmalemma and endoplasmic reticulum [14], which must lead to serious modifications of calcium-dependent processes in the cell. The calcium and polyphosphoinositide systems are known to be closely connected [9, 15]. Lowering of the PPI concentration, evidence of their increased rate of hydrolysis, may in the opinion of some workers lead to changes in microviscosity of the membrane, its ionic permeability, and its excitability [1, 7, 10]. Another result of this process is evidently the release of part of the Ca^{2+} from the membranes. In addition, one of the products of TPI hydrolysis, namely inositol triphosphate, stimulates Ca^{2+} release from the endoplasmic reticulum [7]. It can be tentatively suggested that the abrupt changes in the PPI concentration observed in response to disturbance of the oxygen supply play an important role in changes in the intracellular distribution of calcium and calcium-dependent processes in structures of the cerebral cortex in the early period of anoxia. It must also be recalled that persistent alterations of K^+ and Ca^{2+}-conductance of the neuronal plasmalemma during anoxia may be connected with activation of protein kinase C by another product of TPI breakdown, namely diacylglycerol [8, 11].

The disappearance of spike activity observed immediately after the activation phase correlates with a sharp rise of the extracellular K+ ion concentration, deep depolarization of the neurons, exhaustion of high-energy compounds, and a marked increase in the level of intracellular reducing equivalents, with consequent acidosis and a further fall in the level of membrane-bound Ca^{2+} [3, 13, 14, 16]. The fall in the TPI concentration at this period of anoxia is due mainly to disturbance of their synthesis on account of exhaustion of the ATP reserves [2].

The results described above, when compared with data in the literature, are evidence of the important role of interconnected polyphosphoinositide and calcium intracellular regulatory systems in the mechanisms of response of structures of the cerebral cortex to a disturbance of the oxygen supply.

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