BIOPHYSICS AND BIOCHEMISTRY

Individual Peculiarities of Cerebral Energy Metabolism during Local Ischemia. *In Vivo* Nuclear Magnetic Resonance Spectroscopy Data

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The cerebral content of lactate and phosphate metabolites and intracellular pH were studied in intact rats 3 and 8 days after ligation of the middle cerebral artery. Each rat exhibited accumulation of lactate and individual changes in creatinine phosphate, inorganic phosphorus, and monophosphate esters, while the level of ATP, ADP, and pH were constant. Regulatory changes in the system of cerebral energy metabolism are characterized by the correlation analysis of these parameters.

Key Words: *nuclear magnetic resonance in vivo; brain; local ischemia; energy metabolism*

Disturbances in energy metabolism are a key factor underlying damage to the nervous tissue during cerebral ischemia.

Our aim was to characterize the disturbances in cerebral energy metabolism *in vivo* in a new model of local ischemia produced by proximal ligation of the middle cerebral artery.

Local disturbances in cerebral circulation result in individual disturbances in the phosphate metabolism [1,7], which disappear after statistical averaging. Therefore, this paper focuses on variability of individual indices of cerebral energy metabolism in the studied model and assesses general regularities characterizing cerebral energy metabolism during local disturbances in cerebral blood flow.

MATERIALS AND METHODS

The study was carried out on 7 mature male Wistar rats weighing 250-280 g. Local ischemia was produced by proximal ligation of the left middle cerebral artery [8]. This reduces blood flow in the parietal cortical region by 85% [2].

Lactate was measured in intact animals and on ischemia days 3 and 8. Phosphate metabolites and intracellular pH in the brain were determined prior to surgery and on postoperation days 3 and 8 seven times per day with 1-h interval between measurements.

The measurements were made by *in vivo* nuclear magnetic resonance (NMR) on ^{1}H and ^{31}P nuclei using an AM-400 WB NMR spectrometer (Bruker) with a 9.4-T vertical magnet. The scalp was removed in narcotized rats (50 mg/kg chloral hydrate intraperitoneally) fixed in a stereotaxic apparatus of an NMR transducer equipped with two surface coils: a single-turn 10×12 mm coil tuned to ^{1}H and a double-turn 14×14 mm coil tuned to ^{31}P (161.98 MHz). The center of the skull was located in the center of the coil turn plane. Homogeneity of the magnetic field within the sensitive volume was assessed by signal of water protons. To recorder the series of ^{31}P NMR spectra in individual brain in intact and operated rats (ischemic day 3 and 8), the rats fixed in stereotaxic apparatus

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were taken out from the transducer after each measurement. To obtain the lactate signal, spectral correction was used, which summed the alternate spin-echo pulse sequences 1-3-3-1-T-180-T and 1-3-3-1-T-2-6-6-2-T (T=68 msec) with the excitation maximum at CH_3-lactate group resonance frequency (chemical shift δ=1.32 ppm). The EXOR-CYCLE was used for refocusing [9]. The spectrum of a 5-kHz width was obtained by accumulation of 32 scans with a 2-sec relaxation delay. The *in vivo* ^{31}P NMR cerebral spectra were recorded with pulse sequence [3]: $\pi/2$-τ-$\pi[\pm x,\pm y]$-2τ-$\pi[\pm x,\pm y]$-τ, where τ=50 msec and $\pi/2$=35 msec in the middle of sensitive volume. The spectra were obtained by accumulation of 80 scans with 4.41 sec relaxation delay. The width of spectral range was 10 kHz. NMR spectra of ^{1}H and ^{31}P were stored in 16K and 8K operative memory of a mini-computer in the spectrometer.

The integral intensity of phosphate signals was expressed in percentage of the sum of all ^{31}P peaks of the NMR spectrum. Intracellular pH was determined by the formula: $pH = lg[\delta P_i\text{-}3.29)/(5.68\text{-}\delta P_i)$, where δP_i is chemical shift of inorganic phosphorus (P_i) relatively creatinine phosphate (δCP=0) [5]. The data for each rat obtained during a day were averaged and the spectra obtained for a group of rats were averaged. The results were statistically analyzed using two-tail Student's *t* test.

The changes in dispersion were determined for the mean group values and then analyzed by Fisher's test.

A series of individual spectra obtained before surgery and on ischemia days 3 and 8 were used to calculate 7 variables: integral signal intensity (1 to 6 values) and intracellular pH. Each variable assumed 7 values corresponding to the number of spectra in a series. Calculation of paired linear correlation coefficients for these variables yielded 7 correlation matrices for examined states.

RESULTS

Figure 1 shows ^{31}P and ^{1}H-NMR spectra for the brain before and 3 and 8 days after surgery (Fig. 1). All proton spectra recorded after surgery demonstrated a lactate peak, which was absent in the spectra of intact brain and confirms circulatory disturbances in all operated rats on ischemia days 3 and 8. There were no overt changes in ^{31}P-NMR spectra in ischemic brain. The mean values of phosphates and pH in intact and ischemic brain were also similar.

Accumulation of lactate against the background of virtually normal indices of phosphate metabolites and intracellular pH corresponds to the data on cerebral energy metabolism during chronic stage of local ischemia [6].

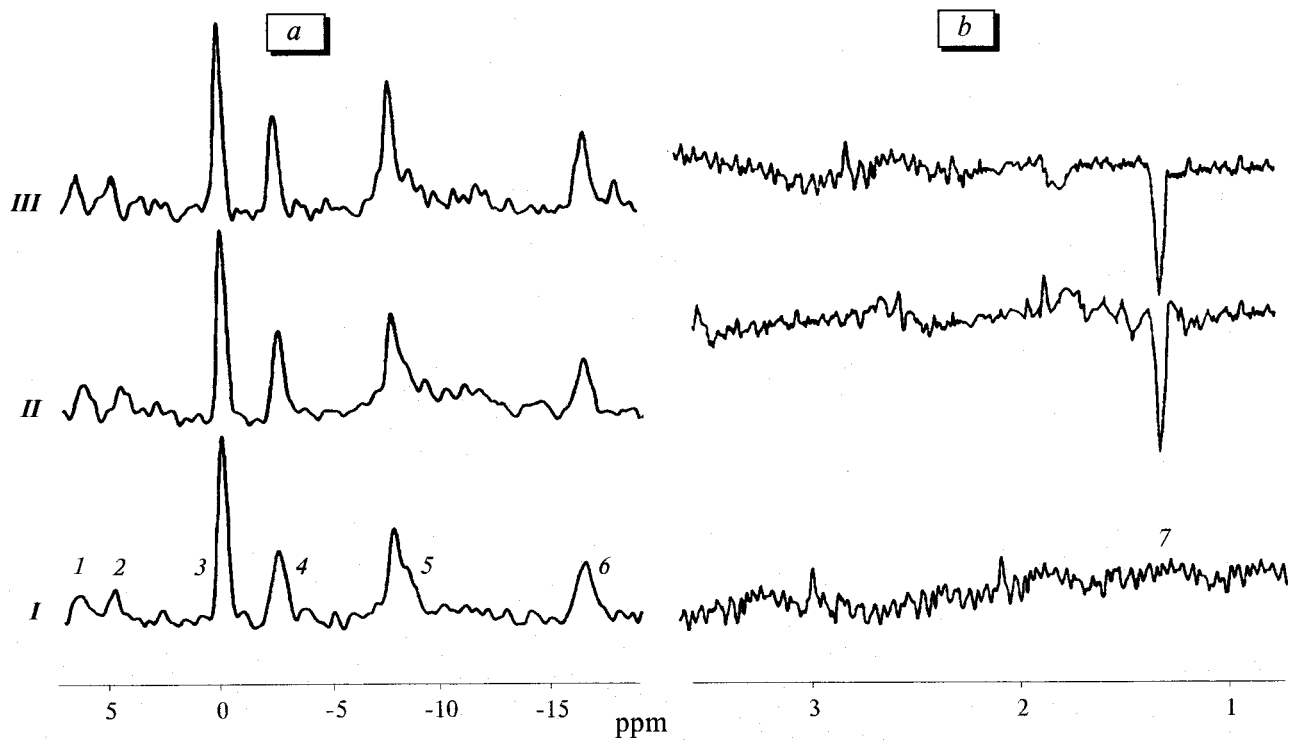

Fig. 1. *In vivo* ^{31}P (*a*) and ^{1}H (*b*) nuclear magnetic resonance spectra of rat brain. Spectra in (*b*) were corrected for lactate. *I*) intact brain; 3 (*II*) and 8 (*III*) days after ligation of the middle cerebral artery. Peaks correspond to: *1*) monophosphate esters (predominantly phosphatidylethanolamine and phosphatidylcholine); *2*) inorganic phosphorus; *3*) γ-ATP+β-ADP; *4*) creatine phosphate; *5*) α-ATP+α-ADP+NAD/NADH$^+$+uridine diphosphosugars; *6*) β-ATP; *7*) lactate.

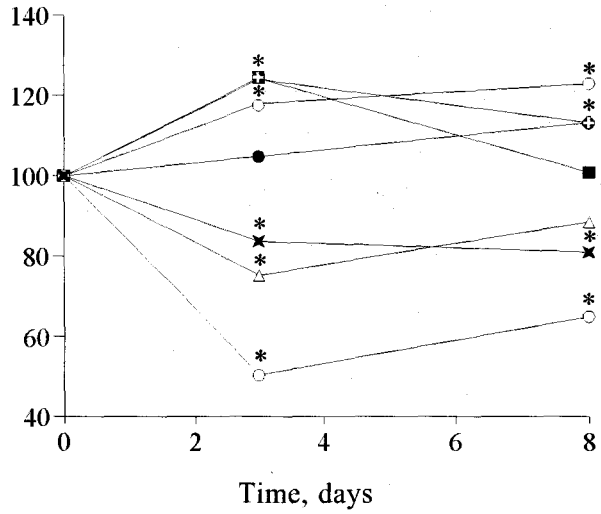

Fig. 2. Changes in individual creatine phosphate level (% of the control) in rat brain after ligation of the middle cerebral artery.

Comparison of dispersions of individual mean values averaged in control and test groups revealed a significant change in dispersions ($p<0.05$) for 2 of 7 indices (the signals *1* and *5*) after surgery, hence ischemia modifies intrapopulation variability. This observation is supported by multidirectional dynamics of the mean indices in each rat. For example, during ischemia CP increased in 3 rats, decreased in 3 rats, and remained unchanged in one rat (Fig. 2). Signal 5 decreased in 3 rats and increased in 1 rat. Two rats demonstrated simultaneous increase in the level of monophosphate esters and P_i.

These shifts are significant ($p<0.05$) but weak (less than 35%). The common feature of all rats was stability of ATP, ADP, and pH levels.

Probably, the observed multidirectional changes in parameters of cerebral phosphate metabolism provoked by local circulatory disturbance result from individual peculiarities of collateral circulation. The morphological assessment of localization and size of ischemic damage on day 8 after occlusion showed that about 50% cells of the ischemic cerebral hemisphere were damaged, but the area and localization of the ischemic foci varied in different rats.

Evidently, the detected individual variations in the dynamics of phosphate metabolites explain why the averaged data in the test group revealed no disturbances in phosphate metabolism.

Individual dynamics of phosphates can result from regulatory changes in cerebral energy metabolism directed to maintainance of ATP homeostasis against the background of local disturbances in oxygen and energy supply. Regulation of energy metabolism modifies interrelation between phosphates involved in this process, as evidenced by *in vivo* ^{31}P-NMR data [1,7]. Analysis of individual correlation matrices revealed lack of repeated significant correlations in the matrices for intact brain ($p<0.05$). By contrast, a stable negative correlation between variables 2 and 5 was observed on ischemic days 3 and 8 (correspondingly, in 5-6 of 7 rats). It implies the appearance of a functional dependence between variables 2 and 5. The intensity of signal *5* is the sum of the signals of α-phosphate groups in ATP, ADP, NAD/NADH$^+$, and uridine diphosphosugars. Since no functional dependence was found between variables 6 (ATP) and 2 (P_i), and between 4 (ATP+ADP) and 2 (P_i), and since the concentration of uridine diphosphosugars in the brain is 25 times lower than that of ATP [4], the considered correlation is determined by the dependence between relative concentrations of NAD/NADH$^+$ and P_i: the higher NAD/NADH$^+$, the lower P_i and *vice versa*. Therefore, P_i, a product of ATP hydrolysis, and NAD/NADH$^+$ involved in oxidative stages of ATP synthesis become functionally interdependent as a result of ischemia. Since NMR detects only extramitochondrial NAD/NADH$^+$ pool, the observed dependence attests to involvement of redox processes in the cytosol in the regulation of cerebral ATP during local ischemia.

Thus, local ischemia induced by ligation of the middle cerebral artery is characterized by lactate accumulation against the background of stable levels of ATP, ADP, and intracellular pH. Various dynamics of CP, P_i, and NAD/NADH$^+$ levels observed in individual rats probably results from individual features of their vascular network. Opposite changes in parameters of energy metabolism make analysis of their population mean values inefficient. During local ischemia, the concentrations of ATP and ADP in the brain are maintained at a constant level. The revealed functional dependence between the relative concentrations of P_i and NAD/NADH$^+$ indicates that extramitochondrial oxidation takes a part in the regulation of cerebral ATP level during local circulatory disturbances in the brain.

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