# TURNOVER OF RAT BRAIN PHOSPHOLIPIDS DURING HYPOXIA AND POST-HYPOXIA

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In a study of the metabolic intensity of the phosphate groups of the phospholipids of the rat cerebrum under conditions of oxygen starvation of the organism, induced by a lowering of the barometric pressure, different sensitivities of individual fractions of the phospholipids of brain tissue to different degrees of oxygen insufficiency was detected [2].

It was hypothesized that the different sensitivity of the individual fractions of the rat brain tissue phospholipids to conditions of oxygen starvation of the organism is due to the different sensitivity to hypoxia of enzyme systems catalyzing individual reactions of the phospholipid biosynthesis.

The purpose of this work was to study the turnover of individual fractions of the rat brain phospholipids during hypoxia and in the first hours after severe hypoxia.

## PROCEDURE

The work was conducted on adult white male rats of the Wistar line, weight 180-240 g. The rat (two to three animals at a time) was placed in a pressure chamber, and in a period of 20-25 min, with brief stoppages, the pressure in it was lowered to 200-180 mm Hg, which corresponded to an altitude of 10,000-11,000 meters above sea level; the rats were kept in the pressure chamber for 110 min. The animals received subcutaneous injections of radioactive phosphate in a dose of 5 microcuries/g directly before placement in the pressure chamber and immediately or 2, 4, or 6 h after cessation of the influence. The animals were killed 120 min after the injection of the isotope. Thus, the intensity of the turnover of phosphate groups of the phospholipids was determined during stay in the pressure chamber and during periods of 0-2, 2-4, 4-6, and 6-8 h after the state of hypoxia. The phospholipids were fractionated by the method of adsorption chromatography on a silica gel column [1], which we modified for the analysis of small amount of tissue and for series experimental investigations. Using this method, the phospholipids of the rat cerebrum were separated into five fractions: I) phosphatide acids and polyglycerophosphatides; II) amino-phospholipids, mixture of phosphatidyl ethanolamines and phosphatidylserines; III) phosphoinositides; IV) lecithins (phosphatidylcholines); V) sphingomyelins. In each fraction we determined the phosphorus content in milligrams per g of moist tissue and its specific radioactivity. The rate of renewal of the phosphate groups of individual fractions was judged by the relative specific radioactivity, which represented the percent ratio of the specific radioactivity of the phosphorus of each fraction to the specific radioactivity of the inorganic phosphorus of brain tissue.

## RESULTS

The data obtained are presented in the table.

As can be seen from the table, a 2 h stay of the rats in the pressure chamber at a pressure of 180-200 mm Hg gave rise to a distinct, statistically reliable ($P < 0.001$) decrease in the relative specific radioactivities of all the investigated fractions, but its degree differed for different fractions, just as in the previous experiments [2]. The

Relative Specific Radioactivity of Individual Fractions of Phospholipids of the Rat Cerebrum During Hypoxia and Post-Hypoxia

| Series of experiments | | Index | Phospholipids (total) | Fraction I | II | III | IV | V |
|---|---|---|---|---|---|---|---|---|
| Control | | $\overline{X} \pm S$ | 1,88 ± 0,07 | 5,10 ± 0,26 | 0,79 ± 0,03 | 7,92 ± 0,37 | 1,29 ± 0,06 | 0,35 ± 0,02 |
| Stay in pressure chamber (180-200 mm Hg) | | $\overline{X} \pm S$ | 1,13 ± 0,04 | 3,68 ± 0,14 | 0,53 ± 0,04 | 4,61 ± 0,28 | 0,67 ± 0,05 | 0,23 ± 0,02 |
| | | % of control | 60,2 | 72,2 | 67,8 | 58,3 | 51,9 | 65,7 |
| | | $P$ | < 0,001 | < 0,001 | < 0,001 | < 0,001 | < 0,001 | < 0,001 |
| Post-hypoxic period | 0 — 2 h | $\overline{X} \pm S$ | 1,36 ± 0,07 | 4,12 ± 0,23 | 0,67 ± 0,05 | 6,63 ± 0,37 | 0,71 ± 0,06 | 0,23 ± 0,02 |
| | | % of control | 72,2 | 80,7 | 84,5 | 83,7 | 54,9 | 65,7 |
| | | $P$ | < 0,001 | < 0,01 | = 0,05 | < 0,02 | < 0,001 | < 0,01 |
| | 2 — 4 h | $\overline{X} \pm S$ | 1,93 ± 0,10 | 4,96 ± 0,28 | 0,93 ± 0,05 | 8,81 ± 0,41 | 1,24 ± 0,08 | 0,38 ± 0,04 |
| | | % of control | 102,5 | 97,3 | 117,3 | 111,2 | 95,9 | 109,9 |
| | | $P$ | > 0,1 | > 0,1 | < 0,02 | > 0,1 | > 0,1 | > 0,1 |
| | 4 — 6 h | $\overline{X} \pm S$ | 2,23 ± 0,05 | 4,72 ± 0,19 | 1,00 ± 0,04 | 8,64 ± 0,43 | 1,44 ± 0,06 | 0,36 ± 0,02 |
| | | % of control | 118,4 | 92,5 | 127,0 | 109,1 | 111,3 | 102,5 |
| | | $P$ | < 0,001 | > 0,1 | < 0,001 | > 0,1 | 0,05>$P$>0,1 | > 0,1 |
| | 6 — 8 h | $\overline{X} \pm S$ | 1,98 ± 0,02 | 5,06 ± 0,33 | 0,85 ± 0,02 | 7,34 ± 0,25 | 1,41 ± 0,03 | 0,41 ± 0,01 |
| | | % of control | 105,4 | 99,3 | 107,8 | 92,6 | 109,1 | 118,1 |
| | | $P$ | > 0,1 | > 0,1 | > 0,1 | > 0,1 | 0,05>$P$>0,1 | < 0,02 |

relative specific radioactivity of the fraction of phosphatized acids and polyglycerophosphatides was least reduced (by 27.8%), that of the fractions of aminophospholipids and sphingomyelins to a greater degree (by 32.2 and 34.2%, respectively), and the maximum decrease was observed in the fractions of phosphoinositides (by 41.7%) and phosphatidylcholines (by 48.1%).

The dynamics of the post-hypoxic restoration of the intensity of the turnover of individual fractions, as can be seen from the table, also was found to differ. In the first 2 h after cessation of the influence, the relative specific radioactivity of fractions I, II (aminophospholipids), and III increased; moreover, in fraction II it almost reached the level of the control, while in fractions IV and V it remained practically at the same low level as during hypoxia. The relative specific radioactivities of all the fractions of phospholipids did not reach the control values during this period; the difference is statistically reliable in all fractions with the exception of fraction II, in which the difference from the control lies at the limit of reliability ($P = 0.05$). During the subsequent period of post-hypoxia (2-4 h), there was a restoration of the relative specific radioactivity of all the fractions to the control values, while in fraction II it even somewhat exceeded the level of the control ($P < 0.02$). During the period from 4-6 h, the radioactivity of fraction II continued to rise, exceeding the control level by 27% ($P < 0.001$) and decreased to normal value only during the period of 6-8 h after hypoxia. Against a background of a maximum increase in the relative specific radioactivity of fraction II during the period from 4-6 h, in fraction IV a tendency toward an excess over the control values was detected, maintained during the subsequent period 6-8h, when the radioactivity of fraction II was normalized. The relative specific radioactivity of fraction V in the period from 6-8 h after hypoxia increased gradually, exceeding the level of the control. In fractions I and III it was already normalized 2-4 h after hypoxia, and in the subsequent periods did not differ significantly from the control values.

Thus, a study of the post-hypoxic restoration of the turnover of individual fractions of the rat brain tissue permitted the establishment of an early and rapid restoration of the intensity of the turnover of fraction II, followed by an increase in the rate of renewal of the phosphate group of this fraction above the control values and a delayed restoration of the relative specific radioactivity of fraction IV and V.

It may be that the peculiarities of the dynamics of restoration of the intensity of turnover of individual phospholipid fractions, established in our experiments, reflect to some degree the peculiarities of the biosynthesis of these fractions during the post-hypoxic period. It may be assumed that the early normalization and subsequent increase in the intensity of turnover of fraction II are due to the increasing role of aminophosphatides in the biosynthesis of choline-containing phospholipids, in particular, phosphatidylcholines, under post-hypoxic conditions. A number of investigations [4-8, 11, 14] in vitro and in vivo have shown that the reactions of interconversion of serine and ethanolamine, followed by methylation of ethanolamine to choline, occur in the animal organism not at the level of the free bases, but only after the incorporation of these bases into the corresponding phospholipids. It has been established that this pathway of phosphatidylcholine biosynthesis does not require the presence of specific cofactors (cytidine nucleotides) and energy sources (ATP) and is especially important for those organisms which are capable of endogenous synthesis of choline (such organisms, in which the predominant synthesis of free choline and choline-containing systems proceeds by stepwise methylation of phosphatidylethanolamines to phosphatidyl cholines include rats, in particular) [9, 13]. Consequently, it may be assumed that during post-hypoxia, when the classical pathway of biosynthesis of choline-containing phospholipids in brain tissue is hindered as a result of a deficiency of high-energy compounds under these conditions [3, 10, 12], the reactions of methylation of aminophosphatides to lecithin take on vital importance. It may be that under post-hypoxic conditions the restoration of the intensity of the turnover of individual phospholipid fractions takes the pathway of primary energy supply of the restoration of the turnover of the aminophosphatide fraction, followed by utilization of the material of this fraction for phosphatidylcholine biosynthesis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to cover English translations appears at the back of this issue.

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