Levels and Metabolism of Phosphoinositides in the Cerebral Cortex of Rats During Anoxia and in the Early Period of Reoxygenation

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During anoxia lasting for 50 sec, the levels of triphosphoinositide and diphosphoinositide in the rat cerebral cortex declined while the metabolic rate of their phosphate groups sharply increased. Thirty minutes after the resumption of oxygen supply their levels rose above the control values, while by the 60th min of the postanoxic period a tendency toward normalization of their metabolism was observed. No significant changes were noted either in levels or metabolism of monophosphoinositide and total phospholipids under the experimental conditions used.

Key Words: *cerebral cortex; anoxia; phosphoinositides*

The phosphoinositide (PI) system is involved in the mechanisms of signal transduction of a number of hormones and transmitters and in the formation of second messengers [6,7,9,14]. Under conditions of oxygen deficiency, the rates at which neurotransmitters are synthesized and released are altered [10,11, 13]. Hence the great interest in the study of metabolic changes which components of the PI system undergo in various hypoxic states. The relevant data are, however, scarce, and most of them have been obtained in animals subjected to ischemia. Lowered levels of phosphatidylinosite-4-phosphate (diphosphoinositide, DPI) and phosphatidylinosite-4,5-diphosphate (triphosphoinositide, TPI) were recorded in brain ischemia [8,16,17] and in experiments using an anoxia model *in vivo* [5,15]. Even less attention has been paid to the rates at which PI are metabolized under these conditions. TPI and DPI have been shown to be metabolized at lowered rates in the cerebral hemispheres of rats with incomplete ischemia of varying duration [4]. Previously, we observed phasic changes in the level and metabolism of PI during anoxia (lasting up to 5 min) and after the resumption of oxygen supply in rat cerebral cortex sections *in vitro* [2]. To gain a better understanding of the intracellular mechanisms by which hypoxic reactions are initiated it is desirable to study PI metabolism early during anoxia - before the content of high-energy compounds begins to change, which in rats occurs 1.5 min after the cessation of oxygen supply [3].

The purpose of these *in vivo* experiments was to measure the levels of, and metabolic rates of phosphate groups in, monophosphoinositide (MPI), DPI, and TPI in the cerebral cortex of rats during short-term anoxia and soon after the resumption of oxygen supply.

MATERIALS AND METHODS

The experiments were carried out in springtime on adult male Sprague-Dawley rats. Anoxia was produced by blowing a gaseous mixture of 95% N_2 and 5% O_2 for 50 sec through the chamber into which the rats had been placed. For the postanoxic study, the rats were removed from the chamber to fresh

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air following the anoxia. To measure the metabolic rates of the PI phosphate groups, rats were injected with ^{32}P-orthophosphate intraperitoneally in a dose of 300 MBq/kg for 30 min. For a study of metabolism during anoxia, the isotope was injected 29 minutes before the onset of anoxia. For the postanoxic study, it was injected either immediately after the anoxia to study the early postanoxic period (0-30 min) or at minute 30 after the anoxia to study the later postanoxic period (30-60 min). Exactly 30 min postinjection, the control and test animals were decapitated, and lipids were extracted from their cerebral cortices with a mixture of chloroform, methanol, and concentrated hydrochloric acid in a 200::100:1 ratio. The extracted lipids were washed free of nonlipid impurities with 1 N HCl and a chloroform:methanol:0.2 N HCl mixture in a 3:47:50 ratio. The washed extract was concentrated in a current of nitrogen and chromatographed on formol-treated paper as described elsewhere [1]. The spots corresponding to MPI, DPI, and TPI were cut out. These fractions were assayed for the content of lipid phosphorus (after mineralization) and its radioactivity (in a Mark-3 scintillation counter); specific radioactivity (SR) of the lipid phosphorus was then calculated in counts per min per μg P. The measure of the metabolic rate of PI was the relative SR (RSR) calculated as the ratio (×100) of the SR of the PI phosphorus to that of inorganic phosphorus. The SR of the latter was calculated as the ratio of radioactivity to the amount of phosphorus in the fraction obtained from the washing out of the lipid extract with 1 N HCl. The results were treated statistically by Student-Fisher's method.

RESULTS

In a series of preliminary tests, levels and metabolic rates of PI and total phospholipids (PL) were measured in cerebral cortices from control rats as no relevant data for the Sprague-Dawley strain were available. The results obtained (Table 1) are in accord with the data reported in the literature for other rat strains [1,4,12].

Data on PI and total PL levels under anoxic and postanoxic conditions are presented in Fig. 1. By the 50th second of anoxia, TPI and DPI levels had decreased by 19% and 22%, respectively. At minute 30 of the postanoxic period, the level of DPI and particularly that of TPI exceeded the control values (by 131% in the case of TPI). By minute 60 of this period, the levels of both TPI and DPI had declined. The contents of MPI and total PL did not undergo significant changes under the experimental conditions used.

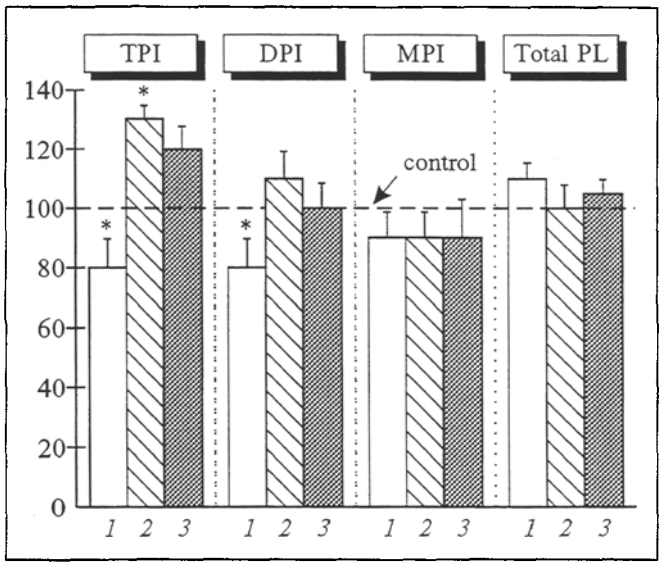

Fig. 1. Levels of TPI, DPI, MPI, and total PL under anoxic and postanoxic conditions (the values shown are percentages relative to control values). The asterisk denotes a significant difference from the control at $p<0.05$. 1) anoxia; 2) at min 30 of the postanoxic period; 3) at min 60 of the postanoxic period

The results of measuring metabolic rates under anoxic and reoxygenation conditions are summarized in Fig. 2. During the 50-second anoxia, the rates of phosphorus metabolism in the PI fractions increased, especially that of DPI, which rose to reach 136% of the control value. In the postanoxic period, there occurred phasic changes in the rates of TPI and DPI metabolism. The rate of phosphorus metabolism in total PL remained essentially unchanged.

It should be kept in mind that Fig. 2 shows the RSR values obtained for a 30-minute period

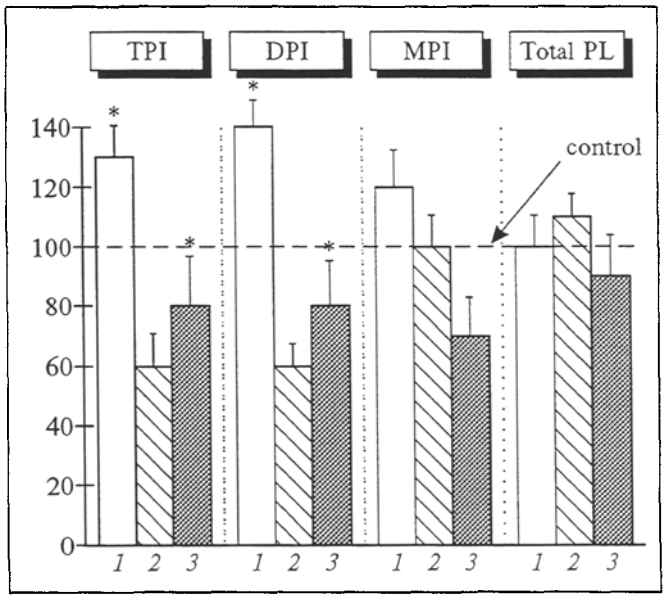

Fig. 2. Metabolic rates estimated for TPI, DPI, MPI, and total PL under anoxic and postanoxic conditions (the values shown are percentages relative to control values). Same designations as in Fig. 1.

TABLE 1. Levels and Relative Specific Radioactivity (RSR) of TPI, DPI, MPI, and Total PL in the Cerebral Cortex of Control Rats ($M \pm m$)

Compound	Level			RSR, 30-min exposure to ^{32}P-orthophosphate
	μg P/g tissue	μmol/g tissue	% of total PL	
TPI	18.4±0.4	0.198±0.005 (9)	1.05±0.03	37.4±3.3 (7)
DPI	12.8±1.6	0.206±0.025 (9)	0.730±0.090	35.7±4.9 (7)
MPI	118±6.0	3.806±0.193 (8)	6.69±0.34	4.02±0.39 (6)
Total PL	1763±60.0	(9)	100%	1.13±0.10 (7)

Note. Figures in parentheses are the numbers of rats.

of exposure to ^{32}P-orthophosphate. During anoxia, the RSR value is in fact the sum of the RSR corresponding to the metabolic rate during the 29 minutes of normoxia and the RSR during the 50 seconds of anoxia. This means that the actual increase in the metabolic rate during the 50-second anoxic period was considerably greater than that indicated in Fig. 2. Calculated RSR values for PI over 50 sec under normoxic and anoxic conditions are given in Table 2.

As can be seen in Table 2, during the 50-second anoxic period the rates of phosphorus metabolism increased by factors of 8.76, 12.8, and 6.43 in TPI, DPI, and MPI, respectively, but much less (1.29-fold) in total PL.

Thus, as follows from our results, during the early anoxic period, which corresponds to the initial alterations in bioelectrical activity not associated with impairment of energy supply to the cell [3], TPI and DPI levels in the rat cerebral cortex declined (approximately by 20%) with concomitant sharp increases in the metabolic rates of their phosphate groups.

Such metabolic changes indicate that the enzymes degrading PI are activated to a greater extent than those which catalyze their synthesis. The breakdown of TPI, mediated by receptors of neurotransmitters, appears to occur primarily at the diester rather than the monoester bond, given that no rise in MPI and DPI levels relative to the lowered TPI level was observed. A consequence of this process is the accumulation in neurons of TPI breakdown products (inositol phosphates and diacyl glycerol), which are secondary mediators involved in the mechanisms of intracellular signal transduction. The rise in TPI over the control level observed early during the postanoxic reoxygenation when the rate of their phosphorus metabolism was lowered is probably attributable to a stable inhibition of the mechanism by which TPI is hydrolyzed. The phasic shifts in PI metabolism induced by the short-term hypoxic stress appear to have contributed to changes in the processes of neuronal excitation and of synaptic transmission not only in response to the anoxia itself but also during the early reoxygenation period.

Taken together, the results of this study suggest that there occurs a prompt and long-lasting involvement of the PI system in the adaptive mechanisms of signal transduction in the central nervous system in response to a short-term interruption and the subsequent resumption of oxygen supply.

TABLE 2. Calculated Values of Relative Specific Radioactivity (RSR) of TPI, DPI, MPI, and Total PL with 50-Second Exposure to ^{32}P-Orthophosphate under Anoxic and Normoxic Conditions

Compound	RSR over 50 sec		
	normoxia	anoxia	% of normal value
TPI	1.039	9.140	876
DPI	0.992	12.7	1280
MPI	0.112	0.720	643
Total PL	0.031	0.040	129

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Effect of Chorionic Gonadotropin on Normalization of Lipid Peroxidation

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A study is performed of the effect of chorionic gonadotropin on lipid peroxidation in the liver and myocardium of white rats injected subcutaneously with tetrachloromethane for a long time. Chorionic gonadotropin is shown to reduce the content of diene conjugates and Schiff bases, which suggests an antioxidant effect of the hormone.

Key Words: *chorionic gonadotropin; lipid peroxidation; liver; heart*

Activation of lipid peroxidation (LPO) arising in diverse injuries in different cells of the organism aggravates various diseases and complicates their treatment [1,4]. Therefore, inhibition of LPO represents an important component in the pathogenetic treatment of many diseases. Established antioxidants usually exert an insufficient effect on LPO [8].

Chorionic gonadotropin (CG) is known as the main specific hormone of pregnancy [2] and as a medicinal preparation used in endocrine disorders [8].

Later investigations revealed the ability of CG to stimulate regeneration of pathologically altered liver and to normalize its structure and function together with the correction of homeostasis [11]. On the basis of this CG was proposed as an agent in regenerative therapy of chronic diffuse liver diseases [11], whose beneficial effect has been demonstrated in both treatment [3,7,10] and surgical [6,9,13] clinics.

MATERIALS AND METHODS

The present study explores the effect of CG on the state of LPO in liver tissue and myocardium of 50 unbred white rats which had for a long time been receiving subcutaneous injections of tetrachloromethane (CCl_4, 0.3 ml 65% solution in vegetable oil, four times per week). The state of LPO in the liver and heart was assessed by the content of diene conjugates (DC) and Schiff bases (SB),

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