INTENSITY OF RESPIRATION AND PHOSPHOLIPID METABOLISM IN ISOLATED RAT BRAIN TISSUE AT DIFFERENT TEMPERATURES IN THE PRESENCE OF KCN

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The respiration rate and intensity of phospholipid metabolism of a rat brain tissue homogenate was studied during incubation at different temperatures (37, 32, and 27°C) in medium with and without cyanide (0.5 and 1 mM). Both these parameters are directly dependent on incubation temperature between 27 and 37°C. The presence of cyanide in the medium inhibits both processes but phospholipid metabolism more so than respiration. The results indicate that phospholipid metabolism during cyanide poisoning is affected both by the toxic action of the cyanide itself directly, and by temperature, the lowering of which potentiates the toxic action.

KEY WORDS: respiration; phospholipid metabolism; rat brain; cyanides; hypothermia.

Hypoxia considerably depresses the phospholipid (PL) metabolism of the brain. However, the mechanism of the disturbance of PL metabolism depends on the type of hypoxia. Depression of PL metabolism of rat brain tissue in hypoxic hypoxia caused by a decrease in the partial pressure of oxygen in the atmosphere is due mainly to the lowering of the body temperature that accompanies hypoxia, and to a much lesser degree, directly to the fall in the partial pressure of oxygen in the atmosphere [4]. In histotoxic hypoxia, on the other hand, when the oxygen supply to the tissues is normal but their ability to utilize it is disturbed, the effect of two factors is seen on the brain PL metabolism: the direct toxic action of cyanide on the brain tissue on the one hand, and the hypothermia developing as a result of cyanide poisoning on the other hand [1, 2]. All investigations in which the data described above were obtained were carried out on the whole animal. To analyze and interpret the data obtained on the complex system of the whole organism, investigations must also be carried out in vitro, when the influence of the regulatory systems is excluded and it is possible, under model conditions, to create any desired experimental situation, e.g., various oxygen concentrations in the atmosphere, various KCN concentrations, various temperatures, and so on [6, 10, 12].

The object of this investigation was to study the rate of oxygen utilization and the rate of incorporation of P^{32} into PL of a rat brain homogenate kept at different temperatures in the presence of KCN.

The effect of cyanides on metabolism of isolated brain tissue has been studied by several workers. KCN, which blocks cytochrome oxidase, inhibits respiration of isolated tissues, including brain tissue [9, 16]. The presence of cyanides in the incubation medium causes the neurons in the brain sections to swell and reduces the concentration of high-energy compounds in them [12]. In brain tissue poisoned with cyanide in vitro the intensity of incorporation of amino acids into protein is reduced [7, 11] and the incorporation of orthophosphate-P^{32} into PL is inhibited [5, 13-15]. However, the role of temperature in the manifestation of the inhibitory effect of cyanides on isolated brain-tissue metabolism has not previously been investigated.

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TABLE 1. QO_2 and RSR of PL Phosphorus of Rat Brain Homogenate at Different Temperatures and in Different KCN Concentrations

Statistical index	37°					32°					27°				
	QO_2			RSR		QO_2			RSR		QO_2			RSR	
	control	0.5 mM KCN	1 mM KCN	control	0.5 mM KCN	control	0.5 mM KCN	1 mM KCN	control	0.5 mM KCN	control	0.5 mM KCN	1 mM KCN	control	0.5 mM KCN
n	22	6	11	20	10	16	6	17	14	9	12	4	10	16	10
M	1154	1022	537	35,9	22,2	931	793	502	25,0	16,0	631	611	250	17,5	11,3
$\pm m$	35	17	34	3,7	3,6	34	18	29	3,5	3,5	46	16	23	2,7	0,8
% of control at same temperature		88,5	46,5		61,8		85,2	53,8		64,0		97,0	40,0		64,5
P		<0,01	<0,001		<0,02		<0,01	<0,001		<0,1		>0,1	<0,001		<0,05

EXPERIMENTAL METHOD

Adult female Wistar albino rats were used. After decapitation the cerebral hemispheres were carefully freed from meninges and large blood vessels, rinsed with 0.14 M NaCl to remove blood, and homogenized. The tissue homogenate (about 100 mg) was placed in the small container of a Warburg apparatus containing 2.5 ml Krebs–Ringer-phosphate medium with glucose, pH 7.4. Next, $Na_2HP^{32}O_4$ was added to the medium in an amount to give a specific radioactivity (SR) of the medium of about $5 \cdot 10^3$ counts/min/μg orthophosphate phosphorus (SR of the medium was verified in each experiment). The samples were incubated for 1 h at 37, 32, and 27°C. Samples without cyanide (control) and samples containing KCN in final concentrations of 0.5 and 1 mM were incubated simultaneously at each of these temperatures. The rate of uptake of oxygen was expressed in microliters O_2 absorbed per gram brain tissue per hour (QO_2). The methods of extraction of the lipids and of washing the tissue and extract to remove traces of inorganic phosphorus were described previously [3]. The content of lipid phosphorous of brain tissue and the radioactivity were determined in each sample after mineralization and its SR calculated (in counts/min/μg phosphorous); the relative SR (RSR), i.e., the ratio between SR of PL phosphorus and SR of inorganic phosphorus of the incubation medium, multiplied by 10^5, also was calculated. The numerical results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

Figures showing the intensity of respiration of the brain-tissue homogenate at different temperatures and with different KCN concentrations in the medium are given in Table 1. The results show that the rate of absorption of oxygen by the brain-tissue homogenate was directly dependent on the incubation temperature within the range 27-37° both in the control and in the samples with cyanide. The ratios between the values of QO_2 obtained at 37° and at 27° (Q_{10}) were virtually identical in the control and in both concentrations of cyanide, and its mean value was close to 2.

On the other hand, as Table 1 shows, at practically all the temperatures investigated, the rate of oxygen uptake was lower in the media containing KCN than in the control, and it depended on the KCN concentration.

The rate of incorporation of orthophosphate-P^{32} into PL of the brain-tissue homogenate also depended directly on the incubation temperature over the range 27-37°C both in the control and in medium containing 0.5 mM KCN. In these experiments Q_{10} was 2.05 and 1.97, respectively. In medium containing 1 mM KCN, the depression of PL metabolism was very severe (by 80-90 %) and the values of RSR were so low that it was impossible to detect any dependence of them on temperature; for that reason these results are not included in Table 1. At all the temperatures investigated the presence of 0.5 mM cyanide in the medium inhibited the incorporation of orthophosphate-P^{32} into PL practically identically.

Comparison of the data on the effect of cyanide on respiration and PL metabolism of isolated brain tissue shows that, in the concentrations used, KCN had a greater effect on PL metabolism than on respiration: the intensity of PL metabolism in medium con-

taining 0.5 mM KCN was reduced by about 35-38% and the rate of oxygen uptake by 12-15% or less (depending on the incubation temperature), in agreement with results obtained by other workers in investigations carried out at an incubation temperature of 37°C [8].

The results of this investigation, carried out on isolated brain tissue, thus confirm results obtained in vivo [1, 2] and show that during cyanide poisoning two factors influence PL metabolism: first, a direct toxic action of cyanide itself on metabolism of brain tissue is clearly observed, and second, this action is potentiated by lowering the incubation temperature in experiments in vitro or lowering the body temperature in histotoxic hypoxia in experiments in vivo.

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