SOME ASPECTS OF CARBOHYDRATE AND PHOSPHORUS METABOLISM IN PYROGENAL PYREXIA

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UDC 612.57:[612.015.32+612.015.31:546.18

Glucose assimilation by rat diaphragm and by rabbit myelokaryocytes is reduced in pyrogenal pyrexia. Hexokinase activity of homogenates of myelokaryocytes is inhibited during pyrexia and in the postpyrexial period, while hexokinase activity of the diaphragm is inhibited only in the postpyrexial period. The content of high-energy phosphorus compounds in myelokaryocytes is unchanged during pyrexia, while in the diaphragm it is reduced in the stages of steady and falling temperatures. Inhibition of hexokinase is accompanied by maintenance of the concentration of high-energy compounds at almost the original level.

In pyrexia and in the postpyrexial period, activity of the enzyme hexokinase, concerned with glucose metabolism, is inhibited [1, 2, 4, 5]. Nevertheless, the character of glucose assimilation by the tissues dur-

TABLE 1. Glucose Absorption by Diaphragms, Hexokinase Activity and Their Homogenates (in mg glucose/g fresh tissue/h) and Their Content of Fractions of Acid-Soluble Phosphorus (in μg P/g fresh tissue) during Pyrogenal Pyrexia

Index studied	Rising temperature	Stages of pyrexia: rising temperature, normal	rising temperature, exptl.	steady temperature, normal	steady temperature, exptl.	falling temperature, normal	falling temperature, exptl.	Postpyrexial period, normal	Postpyrexial period, exptl.
Absorption of glucose by diaphragm	M	1,55	1,03	1,37	0,65	1,46	0,84	1,33	0,69
	$\pm m$	0,18	0,18	0,14	0,15	0,19	0,12	0,14	0,13
	n	21	19	18	27	20	22	27	21
	P	<0,05		<0,001		0,007		<0,001	
Hexokinase activity of homogenates	M	12,85	11,90	12,44	9,65	10,77	8,93	11,88	7,87
	$\pm m$	1,39	2,06	1,50	1,25	0,97	1,63	0,84	0,91
	n	8	8	11	13	12	11	11	9
	P	0,70		0,12		0,38		0,006	
Readily hydrolyzed phosphorus of high-energy compounds	M	252	251	277	166	244	161	221	204
	$\pm m$	20	24	14	12	15	16	15	17
	n	19	19	22	24	24	26	20	18
	P	—		<0,001		<0,001		0,49	
Inorganic phosphorus	M	845	854	805	747	717	645	664	615
	$\pm m$	55	47	46	33	39	41	36	31
	n	18	20	23	24	24	26	26	20
	P	—		0,32		0,20		0,32	

Pathophysiological Laboratory, Kirov Blood Transfusion Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Il'in.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 11, pp. 58-60, November, 1970. Original article submitted March 30, 1970.

TABLE 2. Absorption of Glucose by Myelokaryocytes, Hexokinase Activity of Their Homogenates (in μg glucose/mg protein/h), and Their Content of Fractions of Acid-Soluble Phosphorus (in μg/10 mg protein of myelokaryocytes) during Pyrogenal Pyrexia

Index studied	Statistical index	Normal	Stages of pyrexia			Day of postpyrexial period		
			rising temperature	steady temperature	falling temperature	1st	2nd	7th
Absorption of glucose by myelokaryocytes (incubation in Tyrode solution)	M	210	117.5	131.5	80	152	174	261
	± m	27	34	28	28	37	30	55
	n	15	12	14	13	11	9	9
	P	—	< 0.05	0.011	0.003	0.21	—	—
Absorption of glucose by myelokaryocytes treated with NaF (incubation by Long's method)	M	74	70	47.5	39	55	65	71
	± m	5.2	13.5	9.1	7.8	9.1	9.9	8
	n	23	10	20	21	21	14	19
	P	—	—	0.02	0.001	0.09	—	—
Hexokinase activity of homogenates of these same myelokaryocytes	M	101	93	69.6	52	57	64	94
	± m	4.8	8.7	8.3	8.5	10	10	9.7
	n	22	10	20	20	19	14	20
	P	—	—	0.001	< 0.001	< 0.001	0.007	
	P_1	0.001	—	0.1	0.32	—	—	< 0.05
Readily hydrolyzed acid-soluble phosphorus of high-energy compounds	M	18	18.2	16.2	16	20	22.8	20.7
	± m	1.4	2	1.7	1.7	3.2	2.4	1.6
	n	16	12	15	15	11	11	11
	P	—	—	—	—	—	0.15	—
Inorganic phosphorus	M	44	29	50	50	46	58	59
	± m	5.3	4.3	10.3	10.3	6	14.7	10.7
	n	14	12	15	15	11	11	11
	P	—	0.04	—	—	—	—	—

ing pyrexia still remains unexplained, since this process depends not only on tissue hexokinase, but also on permeability of the cell membranes to glucose. No direct investigations have been made of the rate of glucose transport into the cells or its utilization by the tissues during pyrexia, and the indirect data [3, 4, 7, 13] are contradictory.

The object of the present investigation was to study glucose assimilation during pyrexia by the rat diaphragm and by bone marrow cells of rabbits, and to compare its changes with concurrently determined hexokinase activity and concentration of high-energy phosphorus compounds.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 150-250 g and on rabbits weighing 2.5-3.6 kg. Pyrexia was produced by injection of pyrogenal: 10μg/100 g body weight intramuscularly for rats and 5μg/kg body weight intravenously for rabbits. The rats were decapitated at one of the stages of pyrexia or in the postpyrexial period. Intact rats were sacrificed at the same times (control experiments). Half of the excised diaphragm was incubated for 1 h at 37°C in Tyrode solution containing 80 mg% glucose and buffered to pH 7.3. The other half of the diaphragm was homogenized for determination of its hexokinase content by Long's method [10]. To investigate fractions of acid-soluble phosphorus, diaphragms were taken from other experimental animals. Bone marrow was obtained by puncture of the femoral and tibial epiphyses and the iliac crests of rabbits before injection of pyrogenal, during pyrexia, and in the postpyrexial period. Myelokaryocytes were isolated by the hemolytic method [6]. Some of the cells were used for determination of their glucose absorption in Tyrode solution and their content of fractions of acid-soluble phosphorus. The rest of the myelokaryocytes were suspended, not in physiological saline, but in a mixture of borate buffer, pH 8.4, with 0.5 M NaF solution (7 :3), and their glucose absorption was investigated in Long's medium [10], and homogenates were prepared from them by freezing and thawing them four times, followed by homogenization. Hexokinase activity was determined in the homogenates [10]. Its activity was assessed from

the decrease in glucose content per milligram protein of myelokaryocytes or per gram fresh weight of diaphragms. The glucose content was determined by Nelson's method [12, 15], and the protein content by Lowry's method [11]. Proteins were precipitated by Somogyi's method [14]. Inorganic phosphorus was determined in TCA supernatants of myelokaryocytes and diaphragm tissues triturated after cooling with carbon dioxide [9], and phosphorus of high-energy compounds was estimated after hydrolysis for 10 min in 1 N HCl at 100°C. Statistical analysis of the data was carried out by the method of indirect differences [8].

EXPERIMENTAL RESULTS

Glucose absorption by the rat diaphragm was reduced at all stages of pyrexia by 33-50%. In the postpyrexial period a marked decrease in glucose absorption by the diaphragm also was observed. Hexokinase activity of diaphragm homogenates was not substantially changed during pyrexia. A significant decrease in hexokinase activity was found only in the postpyrexial period (Table 1). Glucose absorption by rabbit myelokaryocytes at all stages of pyrexia was 33-50% lower than initially, while in the postpyrexial period its difference from the initial value was not statistically significant. Hexokinase activity of homogenates of myelokaryocytes was reduced at the stages of steady and falling temperature and in the postpyrexial period (Table 2).

During incubation of myelokaryocytes treated with NaF in Long's medium, the effect of permeability of the cell membranes on the intensity of the hexokinase reaction is observed. Under these conditions the velocity of the hexokinase reaction in the cells was lower than in homogenates obtained from them, because of restrictions imposed upon it by the permeability of the cell membrane. In pyrexia (stages II and III) absorption of glucose by myelokaryocytes treated with NaF was reduced. The difference between the quantity of glucose absorbed by the cells and by phosphorylated homogenates from them was no longer significant. This evidently means that in pyrexia the permeability of the myelokaryocyte membranes no longer restricts the velocity of the hexokinase reaction which is already reduced by inhibition of hexokinase. In the postpyrexial period, the barrier role of the cell membranes relative to glucose was restored (Table 2).

The content of phosphorus of high-energy compounds in pyrexia and in the postpyrexial period was unchanged. In the rat diaphragm it was reduced in the stages of steady and falling temperature. The concentration of inorganic phosphorus, which was unchanged in the diaphragm, was reduced in the myelokaryocytes in stage I of pyrexia (Tables 1 and 2). Analysis of the results shows that the concentration of high-energy compounds was unchanged during pyrexia, if hexokinase activity was reduced in the test objects. It can be assumed that inhibition of the hexokinase reaction helps to ensure maintenance of a constant concentration of high-energy compounds during pyrexia.

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