OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA FROM MYOCARDIUM OF RABBITS WITH ALLOXAN DIABETES

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The level of oxidative phosphorylation in mitochondria from the myocardium of rabbits with alloxan diabetes is lowered (in the presence of added creatine) and is restored by the addition of a hexokinase system or by injection of insulin into the animal in vivo. Uncoupling of the level of oxidative phosphorylation in mitochondria from the myocardium of rabbits with alloxan diabetes is evidently secondary and is due to a deficiency of the acceptor (hexokinase) system.

Uncoupling of phosphorylation and respiration has been discovered in mitochondria isolated from the liver of rabbits with alloxan diabetes, and was prevented by the addition of excess of hexokinase to the suspension of mitochondria [6]. If the mitochondria of rats with alloxan diabetes are incubated with hexokinase, uncoupling likewise is not observed [16]. The lowering of the level of oxidative phosphorylation in the liver of animals with diabetes is thus the secondary result of a decrease in hexokinase activity in its cells or, more precisely, as more recent work has shown, the result of depression of synthesis of one of the isoenzymes of hexokinase (type IV) or glucokinase [13, 22]. In the severest form of diabetes found in depancreatized cats, uncoupling in mitochondria from their liver also occurred even after the addition of hexokinase [21]. Uncoupling was present in liver mitochondria from animals with a particularly severe form of alloxan diabetes when damage to structures and a decrease in the number of mitochondria were observed in the liver cells [10, 11]. At the beginning of development of experimental diabetes, the decrease in oxidative phosphorylation in the liver mitochondria is evidently due to a deficiency of the hexokinase acceptor system, and subsequently, as the diabetes becomes more severe, the mitochondria are themselves damaged, so that while they still remain capable of absorbing oxygen, they partially lose their coupling function. A corresponding decrease in the level of oxidative phosphorylation in the liver has also been found in biotin deficiency, when an insulin deficiency in the body, a decrease in hexokinase activity, and, in the late stages of hypovitaminosis, structural damage to the mitochondria are also observed [3]. Whatever the case, ATP generation in the liver of an animal with diabetes is depressed not only as a result of the slowing of reactions of the tricarboxylic acid cycle [2, 19, 20] and of glycolysis [9, 19, 23-25] in the liver, but also as a result of uncoupling of phosphorylation and respiration.

The skeletal muscle and myocardium in diabetes are under more favorable conditions than the liver, because the cells of these tissues can compensate for the slowing of glucose transport to them [14, 15] while fatty acids and ketone bodies are more extensively utilized in their energy metabolism [4, 8, 12, 19]. Functional integrity of both tissues is completely preserved even in states characterized by prolonged and complete insufficiency of insulin, such as in severe diabetic ketoacidosis [17]. However, it was shown as long ago as in 1949 that phosphorylation of creatine is sharply inhibited in myocardial homogenates of rats with alloxan diabetes, if succinate and malate are used as substrates [9]. Katzen [13] has recently found

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TABLE 1. Effect of Insulin in Vivo on Oxidative Phosphorylation in Myocardial Mitochondria of Normal Rabbits and Rabbits with Alloxan Diabetes (acceptor, creatine phosphate)

	Insulin in vivo	O_2 absorption (μatom)	P	P-CP (μatom)	P	P/O	P
Normal	—	7,53 (15)		8,1 (15)		1,07	
"	+	6,56 (6)	<0,05	11,1 (6)	<0,001	1,70	<0,001
Diabetes	—	9,78 (9)	<0,05	3,3 (9)	<0,001	0,36	<0,001
"	+	9,9 (5)		12,3 (5)	<0,001	1,26	<0,001

Note. Number of experiments shown in parentheses.

that the hexokinase activity (the second acceptor system) of the heart muscle is reduced by more than half in diabetes, as a result of the virtually complete inhibition of one of the two hexokinase isoenzymes (type II).

In the present investigation the level of oxidative phosphorylation was compared in mitochondria from the heart muscle of normal rabbits and of rabbits with alloxan diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2.5-3 kg; diabetes was produced by a single intravenous injection of alloxan in a dose of 130 mg/kg body weight. The blood sugar was determined by the Hagedorn–Jensen method. The rabbits were used in the experiments one month after injection of alloxan, when their blood sugar was not below 200 mg %. The mean blood sugar level of the animals with alloxan diabetes was 352 mg%. Insulin (IZS-A) was injected in a daily dose of 2 units/kg body weight for one month, and 1.5 h before sacrifice insulin of ordinary action was injected. Inorganic phosphorus was determined by Sumner's method [18], and creatine phosphate was estimated as phosphorus after precipitation with $CaCl_2$ [5]. Hexokinase activity was estimated from the decrease in glucose. Mitochondria were isolated by Rachev's method [7]. To the reaction mixture, 1 ml of suspension of mitochondria, equivalent to 0.666 g fresh weight of tissue, was added. The composition of the reaction mixture was: glutamic acid 30 μmoles, $MgCl_2 \cdot 6H_2O$ 10 μmoles, ADP 3.5 μmoles, NaF 2.5 μmoles, phosphate buffer 40 μmoles (pH 7.4), cytochrome c 0.022 μmole, glucose 35 μmoles, hexokinase 0.5 μmole. The samples were incubated in a Warburg apparatus in an atmosphere of air at 26°. Absorption of O_2 took place for 21 min, after equalization of the temperature for 10 min. The values of oxygen absorption and decrease in inorganic phosphorus were expressed in μatoms per sample.

EXPERIMENTAL RESULTS

In the experiments of series I, the level of phosphorylation was determined from the increase in content of creatine phosphate when creatine was used as phosphate acceptor. It is clear from Table 1 that the absorption of O_2 by myocardial mitochondria of rabbits with alloxan diabetes was higher than that from normal animals. An increase in the oxygen consumption of slices of liver, brain, kidney, and diaphragm from rats with alloxan diabetes was previously observed by Batunina [1]. Phosphorylation was sharply depressed in alloxan diabetes (8.1 μatoms in the control, 3.3 μatoms P in diabetes, Table 1). Under the influence of insulin injected in vivo, the O_2 absorption in the control rabbits was reduced, but that in diabetes was unchanged. Creatine phosphate formation was increased very slightly in the control animals by the action of insulin (from 8.1 to 11.1 μatoms), but in rabbits with alloxan diabetes it was increased by almost 4 times. Under the action of insulin in vivo, the P/O ratio increased correspondingly both in the control rabbits and rabbits with alloxan diabetes.

In the experiments of series II, when a hexokinase system was used as phosphate acceptor, O_2 absorption by myocardial mitochondria of rabbits with alloxan diabetes also was slightly increased compared with normal (control 6.92 μatoms, experiment 9.9 μatoms). The level of phosphorylation (from the decrease in inorganic phosphorus) was indistinguishable from normal in the myocardial mitochondria of rabbits with diabetes.

Addition of excess of hexokinase to the myocardial mitochondria of rabbits with alloxan diabetes prevented the lowering of their oxidative phosphorylation level. Consequently, the decrease in level of oxidative phosphorylation in the myocardium during diabetes in the experiments of series I (with creatine) was evidently due to depression of hexokinase activity. In fact, its activity in the myocardium of the rabbits with

diabetes was reduced by more than half. Hence, a sharp dissociation of respiration and phosphorylation took place in myocardial mitochondria using creatine as phosphate acceptor from ATP, although the results of experiments in which the powerful hexokinase acceptor system was added to the mitochondria provided evidence in favor of the secondary origin of this fall in the oxidative phosphorylation level. As was mentioned above, creatine phosphate synthesis in the myocardium of animals with alloxan diabetes is retarded [9]. In diabetes both acceptor systems (creatine kinase and hexokinase) are evidently inhibited to a certain degree on the myocardium and are insufficient for maintaining the normal level of oxidative phosphorylation in mitochondria isolated from it. It is also evident that, just as in the liver [6], dissociation in the mitochondria from the myocardium of an animal with diabetes is secondary and is due to a deficiency of acceptor systems and not to primary damage to the coupling system.

LITERATURE CITED

1. V. Ya. Batunina, Arkh. Pat., No. 4, 82 (1955).
2. M. F. Gulyi, Biochemistry of Lipid Metabolism [in Russian], Kiev (1961).
3. O. K. Dokusova and V. S. Il'in, Biokhimiya, No. 5, 854 (1964).
4. V. S. Il'in, Probl. Éndokrinol., No. 1, 54 (1966).
5. D. L. Ferdman, Biochemistry [in Russian], Moscow (1966).
6. M. P. Fomina, in: Annual Report of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, for 1956 [in Russian], Vilnius (1957), p. 305.
7. R. R. Rachev, Probl. Éndokrinol., No. 2, 105 (1966).
8. C.G. Andres and K. L. Zierler, J. Clin. Invest., 35, 671 (1956).
9. E. S. Goranson and S. D. Erulcar, Arch. Biochem., 24, 40 (1949).
10. I. G. Hall, J. Biol. Chem., 235, 6 (1960).
11. L. C. Hall, L. A. Sordal, and P. L. Stefko, J. Biol. Chem., 235, 1536 (1960).
12. V. S. Ylyin, Advances Enzyme Regulat., 2, 151 (1966).
13. H. M. Katzen, Advances Enzyme Regulat., 5, 335 (1967).
14. M. E. Krahl, The Action of Insulin on Cells, New York (1961).
15. C. R. Park, H. E. Morgan, H. Kaji, et al., in: A. B. Eisenstein (editor), The Biochemical Aspects of Hormone Action, London (1964), p. 18.
16. R. E. Parks, J. Adler, and J. H. Copenhaver, J. Biol. Chem., 214, 693 (1955).
17. A. Renold and A. Winegrad, in: Diabetes [Russian translation], Moscow (1964), p. 113.
18. J. B. Sumner and G. F. Somers, Chemistry of Enzymes and Methods of Their Investigation [Russian translation], Moscow (1948), p. 140.
19. M. Siperstein, in: Diabetes [Russian translation], Moscow (1964), p. 90.
20. W. C. Stadie, Physiol. Rev., 34, 52 (1954).
21. J. W. Vester and W. S. Stadie, J. Biol. Chem., 227, 669 (1957).
22. E. Viñuela, M. Salas, and A. Sols, J. Biol. Chem., 238, 1175 (1963).
23. G. Weber, R. L. Singhal, N. B. Stamm, et al., Advances Enzyme Regulat., 4, 59 (1966).
24. G. Weber, M. A. Lea, E. A. Fischer, et al., Enzymol. Biol. Clin., 7, 11 (1966).
25. G. Weber, M. A. Lea, et al., Advances Enzyme Regulat., 5, 257 (1967).