ACTION OF NICOTINAMIDE ON THE ADENINE NUCLEOTIDE SYSTEM AND ON MITOCHONDRIAL OXYGENATION AND PHOSPHORYLATION IN THE LIVER OF db/db MICE

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The phosphate potential of adenine nucleotides and the redox state of nicotinamide-adenine dinucleotides in equilibrium with them in the various compartments of hepatocytes perform the role of the principal integrative factors in the regulation of metabolism [1]. Administration of nicotinamide, accompanied by an increase in the $NAD^+/NADH$ and $ATP/ADP \cdot P_i$ ratios of the cytosol [1, 6], can be used to assess the role of these "coenzyme loops" [5] in the regulation of individual processes of carbohydrate and lipid metabolism in various physiological and pathological states. The study of systems of adenine and nicotinamide nucleotide systems on models with different hormonal backgrounds is particularly interesting, because the character and mechanisms of interaction of hormones and factors of nonhormonal nature in the regulation of metabolic processes have been inadequately studied.

The aim of this investigation was to study the system of adenine nucleotides and processes of mitochondrial oxidation and phosphorylation in the liver of db/db mice with genetically determined insulin-independent diabetes and obesity [11]. These animals are characterized by severe hyperinsulinemia and by a marked imbalance between the insulin level and the level of contrainsulin hormones in the blood [11].

EXPERIMENTAL METHOD

Experiments were carried out on $C57Bl/K_sJ$ (db/db) mice weighing 26.0 ± 1.5 g and control $C57Bl/K_sJ$(db/+) and (+/+) mice weighing 14.0 ± 0.5 g. Some of the db/db mice received a 2-week's course of nicotinamide in a dose of 25 mg/kg intramuscularly. The animals were killed by decapitation. Concentrations of ATP, ADP, and AMP in the liver extracts with perchloric acid [9] were determined by kits from "Boehringer–Mannheim" (West Germany), P_i as in [12], and lactate, pyruvate, phosphoenolpyruvate, glyceraldehyde-3-phosphate, and 3-phosphoglycerate as in [9]. The value of the phosphate potential ($ATP/ADP \cdot P_i$) of the cell was calculated from measured concentrations of individual adenine nucleotides and P_i of the cytosol by equation (1) in [2]:

$$\frac{ATP}{ADP \cdot P_i} = \frac{\text{Oyruvate} \times \text{glyceraldehyde 3-phosphate}}{\text{Lactate} \times \text{3-phosphoglycerate}} \times K. \quad (1)$$

The $NAD^+/NADH$ ratio of the cytosol, Atkinson's adenylate charge (AC), and the ratio of the active masses of the combined pyruvate — oxaloacetate — phosphoenolpyruvate reaction (AM_{pop}) were calculated by Eqs. (2), (3), and (4) respectively:

$$\frac{NAD^+}{NAD \cdot H} = \frac{\text{Pyruvate}}{\text{Lactate}} \cdot \frac{1}{1 \cdot 10^{-4}}; \quad (2)$$

$$AC = \frac{1}{2} \cdot \frac{ADP + 2ATP}{AMP + ADP + ATP}; \quad (3)$$

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TABLE 1. Effect of Nicotinamide on Adenine Nucleotide System, on Concentrations of Metabolites of Glycolysis (in μmoles/g tissue) $NAD^+/NADH$ Ratio and AM_{pop} Level in Liver of db/db Mice (M ± m)

Parameter	Control (db/+ and +/+ mice)	db/db Mice	db/db Mice receiving nicotinamide
ATP	2,46±0,23	3,30±0,28*	4,58±0,32**
ADP	1,40±0,05	1,49±0,06	1,55±0,15
AMP	1,33±0,09	2,24±0,09*	2,87±0,26**
ATP/ADP	1,75	2,21	2,95
P_i	6,43±0,36	7,38±0,37	9,21±0,76**
ATP/ADP × P_i in cell	215	309	306
AC	0,58	0,56	0,61
Lactate	6,08±0,72	6,10±0,60	4,53±0,33**
Pyruvate	0 065±0,0064	0,094±0,010*	0,195±0,023**
Glyceraldehyde-3-phosphate	0,049±0,0061	0,025±0,0021*	0,038±0,0034**
3-Phosphoglycerate	1,61±0,143	0,74±0,175*	1,04±0,21
Phosphoenolpyruvate	0,039±0,0038	0,281±0,015*	0,225±0,020**
$NAD^+/NADH$ in cytosol	96	140	390
ATP/ADP × P_i in cytosol	159	260	742
AM_{pop}	1,25	4,50	1,22

Legend. *p < 0.05 compared with control, **p < 0.05 compared with db/db mice not receiving nicotinamide (n = 10).

$$AM_{pop} = (\text{phosphoenolpyruvate } P_i \cdot ADP^2)/(\text{Pyruvate ATP}^2). \quad (4)$$

The intensity of oxidation and phosphorylation in isolated [8] liver mitochondria was studied by measuring the partial pressure of oxygen with an LP-7 polarograph (Czechoslovakia). The incubation medium for the mitochondria contained (in mM): sucrose 150, KH_2PO_4 20, $MgCl_2$ 10, KCl 15, EDTA 1; pH 7.2. The oxidation substrates and inhibitors were added to the polarographic cell in the following concentrations: D,L-palmitoylcarnitine 50 μM; succinate 5 mM; ADP 250 μM; NADH 1 mM; 2,4-dinitrophenol 50 μM; rotenone 5 μM. Only mitochondria with a respiratory control of at least 4 during oxidation of succinate were used in the experiments.

EXPERIMENTAL RESULTS

Higher concentrations of ATP and AMP than in the control were found in db/db mice, but their ADP level was the same (Table 1). An increase in the ATP/ADP ratio and the phosphate potential of the cell and cytosol also was observed, whereas the value of Atkinson's AC was virtually identical in the liver of the control and diabetic animals. These changes in the state of phosphorylation of adenine nucleotides, probably arising as a result of hyperinsulinemia [13], led to activation of energy-dependent stages of gluconeogenesis (reflected in a rise of AM_{pop}), and also of glycolysis [10], lipogenesis, and cholesterol formation [7, 15] in the liver of the db/db mice, and they explain the general intensification of metabolism in these animals with predominance of anabolic processes [11]. An increase in the rate of gluconeogenesis at a time when the $NAD^+/NADH$ ratio during hyperinsulinemia was 45.8% higher than in the control, is a characteristic feature distinguishing the metabolism of db/db mice.

The increase in the total concentration of adenine nucleotides by 38.2% suggested activation of their de novo biosynthesis in the liver of the diabetic mice. That this suggestion is correct is confirmed by data on the velocity of mitochondrial oxidation and phosphorylation (Table 2). For instance, an increase in oxygen consumption in the 1st and 2nd metabolic states and a tendency in the 3rd and 4th states during oxidation of palmitoylcarnitine by mitochondria of diabetic animals are evidence of intensification of β-oxidation of fatty acids. An increase in the rate of free oxidation was observed in the liver mitochondria of db/db mice, uncoupled by 2,4-dinitrophenol, a result which reflects marked functional reorganization of the electron transport chain. The rate of phosphorylation of ADP to ATP under these circumstances was increased by 47.9%, and this explains the increase in concentration of ATP

TABLE 2. Effect of Nicotinamide on Parameters of Mitochondrial Oxidation and Phosphorylation in Liver Mitochondria of db/db Mice (M ± m)

Parameter	Control (db/+ and +/+ mice; n = 6)	db/db Mice (n = 5)	db/db Mice receiving nicotinamide (n = 6)
V_1	20,0±1,08	24,0±1,0*	27,0±1,8
V_2	25,0±0,95	33,0±2,14*	45,0±0,54**
V_3	68,0±6,12	80,0±9,39	96,0±5,5
V_4	33,0±2,31	37,0±2,18	46,0±1,15**
V	64,0±12,47	104,0±11,21*	122,0±6,5
V_p	140,0±8,71	207,0±26,6*	224,0±18,81
RC	1,9±0,077	2,0±0,169	2,0±0,114
ADP/O	2,2±0,15	2,4±0,14	2,3±0,05
V_{NADH}	14,0±2,96	15,6±0,3	19,0±2,5

Legend. V_1, V_2, V_3, V_4) Rate of respiration in Chance's metabolic states. V_{NADH}) Rate of oxidation of exogenous NADH; V) rate of uncoupled respiration after addition of 2,4-dinitrophenol — all parameters shown in nanoatoms O_2/min/mg protein; V_p) rate of phosphorylation of ADP in nanomoles ADP/min/mg protein. RC) Chance's respiratory control.

and of total adenine nucleotides in the liver. Incidentally, Chance's respiratory control coefficient for the mitochondria, as an indicator of coupling of oxidation and phosphorylation, and the ADP/O ratio as an indicator of the efficiency of phosphorylation did not differ significantly in the control and diabetic mice. The functional state of the inner mitochondrial membrane also was similar in the two groups of animals examined, as shown by the values of the rate of oxidation of exogenous NADH.

In db/db mice receiving a course of nicotinamide a tendency was observed for the rate of respiration to increase further in different functional states of the mitochondria, and also for an increase in the rate of phosphorylation of ADP. Despite reports in the literature [14] of gluconeogenesis activation by oxidation products of fatty acids, under the present experimental conditions glucose biosynthesis de novo was inhibited, as shown by a decrease in the value of AM_{pop} and the phosphoenolpyruvate concentration in the liver. The fact that nicotinamide inhibits gluconeogenesis in the liver of db/db mice is in agreement with data obtained by other workers in experiments on control and alloxan-diabetic animals [3, 4].

Inhibition of glucose biosynthesis de novo in the liver of db/db mice following administration of nicotinamide can be explained by appropriate restructuring of the adenine nucleotide system. ATP and AMP levels were raised whereas the ADP level was virtually unchanged. A sharp increase in the phosphate potential of the cytosol (by 2.9 times), a parameter which is in equilibrium with the free NAD^+/NADH ratio in the given compartment, promotes inhibition of glucose biosynthesis de novo, for a sharp increase in the oxidative properties of NAD pairs in the cell cytoplasm (Table 1) after administration of nicotinamide causes a "switch" of metabolism from gluconeogenesis to glycolysis [3]. An increase in the total adenine nucleotide concentration and in the value of the adenylate energy charge in db/db mice receiving nicotinamide is in agreement with data given above on intensification of mitochondrial oxidation and phosphorylation.

The results of this investigation are thus evidence of an increase in the rates of mitochondrial oxidation and phosphorylation in db/db mice with insulin-independent diabetes and obesity and corresponding changes in the adenine nucleotide system, expressed as an increase in the total concentration of these nucleotides, in the ATP level, and in the phosphate potential by aggravation of the changes observed in the total adenine nucleotide content and the phosphate potential of the cytosol; the latter, moreover, is responsible for inhibition of gluconeogenesis in the liver. The increase in the rate of oxidation of palmitoylcarnitine induced by nicotinamide is indirect proof of the intensification of mitochondrial β-oxidation of fatty acids and it limits the potential capacity for their utilization in lipid biosynthesis.

The results can be used to explain mechanisms of inhibition of phosphoacylglycerols and diacylglycerols from pyruvate by nicotinamide in the liver of db/db mice [15]

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SELECTIVE INCORPORATION OF DIETARY ω3 POLYUNSATURATED FATTY ACIDS INTO RAT CEREBELLAR PHOSPHOLIPIDS

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Modern views on the role of polyunsaturated fatty acids (PUFA) of the ω3 family, as essential dietary factors, are associated with their use by the body as plastic material for the synthesis of membrane structures [2, 12]. Whereas the essential nature of α-linoleic acid 18:3ω3 is still in the stage of discussion, the role of its metabolites — icosapentaenic acid 20:5ω3 as a regulator of thrombus formation has been confirmed by numerous investigations [10, 14]. The chief PUFA of the ω3 family, namely docosahexaenic acid 22:6ω3, is an important component of membranes of neurons and the retina, and it determines their fluidity [3]; its mandatory presence in synaptic membranes evidently plays a definite role in the transmission of nervous impulses [5]. The fatty acids (FA) of this family enter the body only by consumption of fish fat, other seafood, and certain vegetable oils [8, 13]. Brain tissues have the highest content of PUFA with predominance of 22:6ω3; the FA composition of the brain lipids, moreover, is maintained fairly constant irrespective of the composition of the fat consumed [6]. If the diet is deficient in fat or if it is excessive, no marked changes have been observed in the composition of FA of the brain phospholipids (PL) [15]. Dependence of the concentration of ω3 PUFA in brain tissues on the character of the dietary fat has been examined only sporadically [4].

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