CHANGES IN THE CONTENT AND STRUCTURE OF THE LIVER COENZYME A RESERVES IN DIABETIC (db/db) MICE DURING NICOTINAMIDE THERAPY

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The importance of disturbances of lipid and lipoprotein metabolism in the development of chronic diabetic complications [3, 7] makes the search for effective hypolipodemic agents and the study of the mechanisms of their action in diabetes an urgent task. In this respect vitamin preparations merit attention: pantenol, nicotinic acid, nicotinamide [2, 6, 13]. One way by which nicotinamine inhibits lipogenesis is by increasing the acetyl-CoA/CoA ratio (CoA denotes coenzyme A) in the liver [2]. It is also known that low values of the acetyl-CoA/CoA ratio and of the long-chain acyl-CoA (LCA-CoA) [CoA ratio lead to activation of several enzymes involved in the initial stages of fatty acid biosynthesis: citrate synthetase, ATP-citrate liase, the pyruvate dehydrogenase complex [11, 12], and also acetyl-CoA carboxylase [8], in the liver of control animals and during adaptive hyperlipogenesis. Conversely starvation and alloxan diabetes are characterized by high values of the acetyl-CoA/CoA and LCA-CoA/CoA ratios and, as a result, by inhibition of lipogenesis in the liver [2, 5].

The aim of this investigation was to study the action of the hypolipidemic agent nicotinamide on the content and structure of the CoA reserve in the liver of diabetic (db/db) mice, which are widely used at the present time as a model of insulin-independent diabetes, with accompanying hyperphagia, hyperlipogenesis, and the development of obesity [1, 9].

EXPERIMENTAL METHOD

Experiments were carried out on control C57BL/K_SI mice weighing 14.0 ± 0.5 g and diabetic C57BL/K_SI (db/db) mice weighing 26.0 ± 1.5 g. Some of the diabetic animals received nicotinamide (intramuscularly in a dose of 2.5 mg/100 g body weight) daily for 10 days. The blood glucose level, determined by an automated glucose-oxidase method, was 71 ± 8 mg/dl in the control mice, 179 ± 15 mg/dl in the diabetic mice, and 201 ± 14 mg/dl in diabetic animals receiving a course of nicotinamide. Concentrations of total CoA, free CoA, and LCA-CoA were determined in the liver of the control and experimental animals 24 h after the last

TABLE 1. Effect of Nicotinamide on Structure of CoA Reserves of Mouse Liver (in nmoles/g wet weight of tissue, n = 10)

Parameters studied	Control	Diabetes	Diabetes + nicotinamide
Free CoA	177,55±21,70	283,70±19,46*	138,24±16,93**
Total CoA	318,3±30,88	420,1±21,46*	323,9±18,22**
LCA-CoA	66,33±3,96	64,80±4,70	54,40±2,45
AS-CoA	252,44±35,11	353,8±19,56*	270,73±18,11
SCA-CoA	74,89	70,10	132,48
SCA-CoA/CoA	0,42	0,24	0,95
LCA-CoA/CoA	0,37	0,22	0,39

Legend. *) $p < 0.05$ compared with control; **) the same, compared with diabetic mice not receiving nicotinamide.

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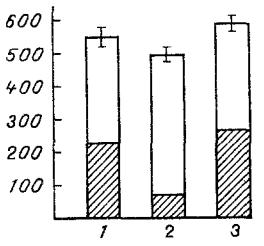

Fig. 1

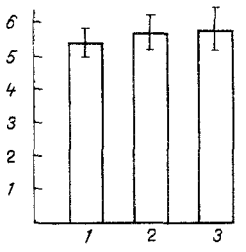

Fig. 2

Fig. 1. Total content of CoA and its precursors (phosphopantothein, dephospho-CoA) in mouse liver, determined by arylamine acetylation reaction (in nmoles/g tissue, n = 10). Shaded part of columns denote content of precursors, unshaded part — content of total CoA. Here and in Fig 2: 1) control; 2) diabetes, 3) diabetes + nicotinamide.

Fig. 2. Pantothenate kinase activity of mouse liver (in nmoles/mg protein/h, n = 10).

injection of nicotinamide, after selective extraction from the tissue sample [15]. The fractions were estimated quantitatively by measuring the quantity of free CoA formed after extraction by an enzymic method, using 2-oxoglutarate dehydrogenase [14]. The total concentration of CoA and its precursors (phosphopanthetein, dephospho-CoA) was estimated by the arylamine acetylation reaction [4]. Pantothenate kinase activity [4] and the protein concentration were determined in the supernatant obtained after centrifugation (105,000g, 1 h) of liver homogenates in 0.25 M sucrose. Levels of acid-soluble CoA (AS-CoA) and short-chain acyl-CoA (SCA-CoA) were obtained by calculation [4].

EXPERIMENTAL RESULTS

The data in Table 1 indicate a marked change in the content and structure of the CoA reserves in the liver of mice with genetically determined insulin-independent diabetes compared with the control. Elevation of the total CoA level (by 32%) took place mainly on account of an increase in concentration of the free CoA fraction (by 60%), whereas the levels of LCA-CoA and SCA-CoA were essentailly unchanged. However, values of LCA-CoA/CoA and SCA-CoA were depressed by 1.4 and 1.75 times, respectively, creating favorable conditions for activation of enzymes involved in the initial stages of lipogenesis, for which free CoA is a positive modulator [2, 11]. The changes of this kind in structure of the CoA reserves also enable the pyruvate flow to switch from carboxylation in the pyruvate-carboxylase reaction to decarboxylation, in the pyruvate-dehydrogenase reaction [11], and also lead to activation of acetyl-CoA carboxylase [8]. The structure of the CoA reserves of the liver in mice with insulin-independent diabetes thus differs significantly from that in animals with alloxan [5] and streptozotocin [10] diabetes, when levels of SCA-CoA and LCA-CoA are raised, and lipogenesis is correspondingly inhibited.

A course of nicotinamide given to mice with insulin-independent diabetes reduced the total CoA concentration by 23%, free CoA by 51%, AC-CoA by 23%, and LCA-CoA by 16%, whereas the SCA-CoA concentration rose sharply (by 1.9 times) compared with values obtained in diabetic animals not receiving nicorinamide. The value of SCA-CoA/CoA in animals receiving nicotinamide was 4 times higher than in diabetic animals, and the value of LCA-CoA was 1.7 times higher. Thus a course of nicotinamide injections created conditions in the liver in diabetic mice than were unfavorable for function of the enzymes of lipogenesis and, in particular, of acetyl-CoA carboxylase and synthetases of fatty acids. Accumulation of SCA-CoA, with some reduction in the LCA-CoA concentration suggests that the main inhibitory effect of nicotinamide on lipogenesis is located at the fatty acid synthetase level. It may be mediated through a change in the redox state of the nicotinamide coenzymes, which determines the rate of NADP-dependent dehydrogenase reaction (glucose-6-phosphate- and 6-phosphogluconate-dehydrogenase, malate-dehydrogenase, isocitrate-dehydrogenase), the principal function of which is to supply coenzyme (NADPH) for fatty acid synthetase.

Analysis of the data on the total content of CoA and its precursors, determined by the acetylation reaction (Fig. 1), indicates a sharp decline in the content of CoA precursors in

the liver of diabetic animals, accompanied by elevation of the total CoA level. This may be evidence of intensification of CoA biosynthesis in insulin-independent diabetes. Administration of nicotinamide restored the normal pool of CoA precursors, while at the same time reducing the total coenzyme concentration. The fact that there was no change in activity of pantothenate kinase, the key enzyme of CoA biosynthesis, in diabetes and in diabetic animals treated with nicotinamide (Fig. 2) suggests that this enzyme is not the factor limiting the reaction velocity under the experimental conditions studied, but that the principal effects are localized at the level of the final mitochondrial stages of biotransformation of the precursors into CoA.

The results of these investigations, together with data in the literature, thus indicate opposite changes in the structure of the CoA reserves in diabetes of insulin-dependent and insulin-independent types, with correspondingly opposite changes in the intensity of lipogenesis; hypolipogenesis in alloxan and streptozotocin diabetes (type I) and hyperlipogenesis in type II diabetes. After a course of injections of the hypolipidemic agent, nicotinamide, to animals with genetically determined insulin-independent diabetes, characteristic changes took place in the structure of the CoA reserves, aimed at maintaining inhibition of reactions of fatty acid biosynthesis. It can be concluded from the facts described above that the structure of the CoA reserves is an integral parameter regulating the intensity of lipogenesis in diabetes.

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