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EFFECT OF TRIHYDROXYOCTADECADIENE ACIDS FROM *Bryonia alba* L. ON ACTIVITY OF GLYCOGEN METABOLISM ENZYMES IN ALLOXAN DIABETES

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KEY WORDS: diabetes; glycogen phosphorylase; phosphoprotein phosphatase; hexokinase; trihydroxyoctadecadiene acids.

Enzyme systems involved in glycogen synthesis and breakdown are sensitive to the action of insulin and are under the control of cyclic AMP [14], with which is associated the ability of certain prostaglandins (PG) to affect secretion of insulin and glucagon, and also various stages of carbohydrate metabolism that are most vulnerable in diabetes [4, 10]. PGE_1, for instance, which has an insulin-like action, also acts on the cAMP level in various tissues [12], whereas PGE_2 has an antigluconeogenetic action through regulation of the cAMP level [11]. PGE_2 is known to stimulate the pentose cycle and glycolytic pathway of glucose metabolism [15] and PG are also known to have an influence on glycogen synthetase and glycogen phosphorylase in the liver and heart [6, 7]. It is natural to suppose that other oxidation products of polyenic fatty acids and, in particular, metabolites of the lipo-oxygenase pathway, can play a definite role in the processes described. Trihydroxyoctadecadiene acids (THDA), isolated from the root of *Bryonia alba* L. (Cucurbitaceae), which exhibit prostaglandin-like activity [3], are C_{18}-homologs of aliphatic trihydroxy acids (containing a 1,2,5-trihydroxy-trans-3-pentaenic group), and are oxidation products of arachidonic acid, whose physiological role has received little study [5]. Previously, in experiments on albino rats with alloxan diabetes, the writers found that THDA have a hypoglycemic action [2].

The aim of this investigation was to study the effect of THDA on activity of glycogen phosphorylase (the a- and b-forms — GP_a and GP_b respectively), phosphoprotein phosphatase (PPP), and hexokinase (HK) in liver and muscle tissues of albino rats with alloxan diabetes.

EXPERIMENTAL METHOD

Diabetes was produced by the method described previously [1]. From the 7th day of the disease the animals received daily intramuscular injections of an aqueous solution of THDA in a dose of 0.05 mg/kg body weight. GP, PPP, and HK activity was determined by known methods [8, 9, 13]. The animals were killed on the 21st day after injection of alloxan.

EXPERIMENTAL RESULTS

The results (Tables 1 and 2) indicate a marked increase in GP_a activity in the skeletal muscles of albino rats with alloxan diabetes, accompanied by relative stability of total phosphorylase activity measured in the presence of cAMP, and by an increase in the GP_a/GP_b ratio. In the liver of the diabetic animals, GP_a activity was reduced almost by half, GP_b activity was increased, and as a result there was a marked decrease in the GP_a/GP_b ratio.

Under the influence of THDA considerable restoration of GP_a activity and of the GP_a/GP_b ratio was observed in muscle tissue and correlated with an appreciable tendency toward normalization of PPP activity. Total phosphorylase activity and GP_a activity in liver tissue not only were not restored by THDA, but they were considerably higher than the control values. Since under these conditions there were no appreciable shifts of PPP activity it can be postulated that THDA participates in normalization of glycogen metabolism in the liver through

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TABLE 1. Effect of THDA on Glycogen Phosphorylase Activity (μmoles P/h/g wet weight of tissue) in Muscle and Liver Tissues of Rats with Alloxan Diabetes (M ± m, n = 6-8)

Experimental conditions	Control	Diabetes	Diabetes + THDA
Skeletal muscles:			
phosphorylase a	6,8±0,6	11,2±1,4*	7,1±1,0*
total phosphorylase (a + b)	60,6±1,3	62,6±1,9	51,6±1,9*
ratio of activities (a/b)	0,125	0,217	0,158
Liver:			
phosphorylase a	6,1±0,4	3,3±0,5*	8,4±0,8*
total phosphorylase (a + b)	8,1±0,6	6,7±0,9	10,5±1,3
ratio of activities (a/b)	3,0	1,0	3,9

Legend. *P < 0.05.

TABLE 2. Effect of THDA on Phosphoprotein Phosphatase (μmoles P/h/g wet weight of tissue) and Hexokinase (μmoles NADPH/h/g wet weight of tissue) Activity in and Liver Tissues of Rats with Alloxan Diabetes (M ± m, n = 8-10)

Experimental conditions	Control	Diabetes	Diabetes + THDA
Skeletal muscles:			
phosphoprotein phosphatase	8,0±0,2	6,0±0,3*	7,1±0,2*
hexokinase	12,5±1,6	8,3±1,1*	11,6±1,5
Liver:			
phosphoprotein phosphatase	14,2±0,9	12,5±1,1	14,5±1,2
hexokinase	36,0±4,0	24,0±3,6*	41,3±5,2*

Legend as to Table 1.

a cascade system of protein phosphorylation. It is not clear, however, whether THDA acts, like some PG [12], on the level of regulation of protein kinase activity, or whether this effect is realized through the cAMP system.

In alloxan diabetes some depression of HK activity also was found in the tissues studied, in agreement with data in the literature. Under the influence of THDA, HK activity in muscle tissue moved a little toward the normal level, but in liver tissue it exceeded its initial values.

The results thus suggest that one possible mechanism of the hypoglycemic action of THDA is their influence on the stages of carbohydrate metabolism studied.

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COMPARATIVE STUDY OF THE ACTION OF LONG-TERM ADMINISTRATION OF NICOTINIC ACID, NICOTINAMIDE, AND DIETHYLNICOTINAMIDE (NIKETHAMIDE) ON THE RAT LIVER MONO-OXYGENASE SYSTEM

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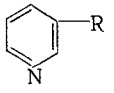
KEY WORDS: liver; microsomal mono-oxygenases; nicotinic acid; nicotinamide; diethylnicotinamide.

Among products of biotransformation of nicotinamide and N-diethylnicotinamide (nikethamide), mainly their N-oxides in the heterocyclic ring are found in the urine of man and animals [2, 3]. Amino acids also are found on incubation of these substances with animal liver microsomes [4]. In different species of animals, formation of the N-oxide of diethylnicotinamide is most stable. For nicotinic acid, oxidation of this type has not been found in microsomes [3, 4]. According to data in the literature [11] nicotinamide can interact with cytochrome P_{450} of liver microsomes to form a type II enzyme–substrate complex.

The data given above may indicate that a cytochrome P_{450}-dependent system participates in biotransformation of nicotinamide and diethylnicotinamide. Meanwhile nicotinamide, like nicotinic acid, is a substrate for synthesis of NADPH [3], essential for its function.

In connection with the facts described above, itwas decided to undertake a comparative study of activity of the mono-oxygenase system of membranes of the endoplasmic reticulum of rat liver cells during repeated injections of nicotinic acid (I), nicotinamide (II), and diethylnicotinamide (III) into the animals:



R: I — $COOH$; II — $CONH_2$; III — $CON(C_2H_5)_2$.

EXPERIMENTAL METHOD

Experiments were carried out in the spring and summer on 56 noninbred male rats weighing 130-170 g. Nicotinic acid, nicotinamide, and diethylnicotinamide were injected into the stomach through a tube once daily for 45 days in equimolar doses: 50, 50, and 73 mg/kg respectively. Control rats received the same volume of water.

The animals were decapitated 24 h after the last injection. The liver microsomal fraction was obtained by the method in [1]. The levels of cytochromes b_5 and P_{450} [9], and activity of NADPH- and NADH-oxidases [5] and oxidoreductases were determined spectrophotometrically on the Specord UV Vis instrument (East Germany). The oxidoreductase function of NADPH-specific flavoprotein (NADPH-ferricytochrome c-oxidoreductase), the middle component of the NADPH-dependent chain of NADH-specific flavoprotein and cytochrome b_5 (NADH-ferricyto-

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