EFFECT OF INSULIN ON TRANSKETOLASE ACTIVITY IN THE RAT LIVER

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Injection of insulin increases the transketolase activity in the liver of intact rats. In fasting animals, the activity of the transketolase reaction at first rises and then falls. If the animals are fasted for 6 days and then fed for 1 day, the activity of the enzyme rises above its initial level. Transketolase activity in samples of liver from long-fasting animals returns to normal on preincubation with insulin. In vitro, insulin has no effect on the activity of the transketolase reaction of the liver in intact rats.

Transketolase is a thiamine enzyme concerned in pentose metabolism. Thiamine pyrophosphate (TPP) [18], the coenzyme of transketolase, is formed by transphosphorylation of thiamine with ATP under the influence of thiamine kinase [14]. The activity of this process is highest in the liver [7].

The dependence of activity of the transketolase reaction on the vitamin B_1 intake has received adequate study [5, 9, 16]. In thiamine deficiency, the transketolase activity of the tissues is lowered. Investigation of activity of the enzyme when phosphorylation of thiamine is disturbed is an interesting problem. According to Foa et al. [13], inhibition of thiamine phosphorylation takes place in alloxan diabetes and can be abolished by addition of insulin to the incubation mixture. Experiments with samples of purified thiamine kinase have shown [17] that insulin activates the thiamine kinase of the liver after preincubation with the blood serum of normal or diabetic animals.

The transketolase activity of the liver during fasting has been investigated [8, 15]. The results showed that transketolase activity (estimated from the degree of reduction of NAD), calculated per gram of organ tissue, was unchanged after fasting for 2 days and increased after fasting for 4 days. A decrease in the content of pentoses was found in hemolysates of erythrocytes from cows after injection of insulin [1].

The object of the present investigation was to study transketolase activity in the liver of rats when the insulin level is raised and lowered.

EXPERIMENTAL METHOD

Experiments were carried out on 57 albino rats weighing 175-250 g. In the experiments of series I, the effect of insulin was studied on transketolase activity in the liver of rats receiving a standard caseine and starch diet formulated by the Institute of Nutrition, Academy of Medical Sciences of the USSR. Four groups of animals were used in this series: groups 1 and 2 were controls and groups 3 and 4 experimental. The rats of the control groups received an intramuscular injection of 0.1 ml of 0.85% sodium chloride solution 2.5 h before samples were taken, while the rats of experimental groups 3 and 4 received an injection of insulin (0.04 and 4 units/100 g body weight respectively [1, 6]).

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TABLE 1. Effect of Injection of Insulin on Transketolase Activity in Rat Liver

Experimental conditions	Transketolase activity		
	M ± m	n	P (%)
Control	128.5 ± 10.1	5	
Injection of insulin (0.04 units/100 g)	181.7 ± 4.9	6	99.9
Control	159.3 ± 10.1	5	
Injection of insulin (4 units/100 g)	204.2 ± 21.3	6	90.6

TABLE 2. Transketolase Activity in Liver of Fasting Rats

Experimental conditions	Transketolase activity		
	M ± m	n	P (%)
Animals on standard diet			
Control	79.9 ± 2.5	5	98.2
Fasting for 3 days	93.8 ± 4.0	7	
Control	145.1 ± 2.9	5	99.2
Fasting for 6 days	125.6 ± 4.3	5	
Fasting for 6 days + feeding	177.5 ± 14.7	6	98.7
Animals on ordinary diet			
Control	198.6 ± 5.4	5	99.9
Fasting for 6 days	144.6 ± 8.9	6	

TABLE 3. Effect of Insulin in Vitro on Transketolase Activity in Liver Samples from Satiated and Fasting Rats

Experimental conditions	Transketolase activity		
	M ± m	n	P (%)
Supernatant of liver of satiated animals			
Control	198.6 ± 5.4	5	
Injection of insulin (without preincubation)	193.8 ± 5.0	5	
Injection of insulin (with preincubation)	194.4 ± 13.75	5	
Supernatant of liver of fasting rats			
Control	144.6 ± 8.9	6	
Injection of insulin (without preincubation)	146.0 ± 6.25	6	> 99.9
Injection of insulin (with preincubation)	188.5 ± 4.56	6	

In series II the effect of fasting on transketolase activity was studied. The animals of this series were divided into two subgroups: A and B. Rats of subgroup A were kept on a standard diet for the month preceding the experiment, while rats of subgroup B received the ordinary diet of the animal house. Subgroup A included 5 groups of animals: groups 1 and 2 were controls, group 3 consisted of animals fasting for 3 days, group 4 fasting for 6 days, and group 5 animals fasting for 6 days and then fed on the 12 h before samples were taken (control diet). Subgroup B consisted of 2 groups of animals: 1) control, and 2) animals fasting for 6 days. The experimental animals of this series were deprived of food but could drink ad lib.

In parallel tests the effect of insulin on the transketolase activity of satiated and fasting animals was studied in vitro, with preincubation of the samples for 30 min. Insulin was injected into the incubation mixture in a dose of 0.5 unit/1.5 ml of reaction mixture [2].

The animals were lightly anesthetized with ether for taking the samples. Preparation of the material for analysis and determination of the enzyme activity were carried out by the method of Bruns et al. [10] in the modification of Ostrovskii and Trebukhina [4] for the liver. Activity was expressed in micromoles sedoheptulose-7-phosphate (S-7-P) per gram fresh liver tissue per hour.

EXPERIMENTAL RESULTS

The results are given in Tables 1-3.

They show that injection of insulin into the rats in a dose of 0.04 unit/100 g body weight increased the liver transketolase activity by 40%. A dose of 4 units/100 g body weight proved toxic to the rats: the animals were in a state of profound shock 2.5 h later.

Transketolase activity in the liver of the fasting rats was increased (by 17%) after the 3rd day, and fell significantly toward the 6th day of fasting. Under the influence of feeding, the enzyme activity in the animals which had fasted for 6 days was higher than the corresponding control level (Table 2).

In the tests in vitro, insulin had no effect on enzyme activity of the satiated animals, but increased the transketolase activity of the fasting rats after incubation with supernatant (Table 3).

The stimulant action of insulin on transketolase activity in the liver of intact animals in vivo and the absence of this effect in vitro suggest that under normal conditions the effect of an excessive intake of insulin takes place through systems linked with the pentose cycle. An increase in the glucokinase activity [3] of the liver and in the activity of glucose-6-phosphate (G-6-PDH) and 6-phosphogluconic acid dehydrogenases (6-PGDH) [11] is characteristic of a raised insulin background. The pentose phosphate cycle is evidently the chief alternative glycolytic pathway of carbohydrate metabolism. Under fasting conditions (the 1st period), against the background of inhibition of the systems of glucose phosphorylation [12], the low Michaelis constant of G-6-PDH probably enables the pentose cycle to compete successfully for G-6-P (activation of the transketolase reaction in rats fasting for 3 days). Subsequent inhibition of transketolase in the rats fasting for 6 days was evidently the result of a disturbance of thiamine phosphorylation. This hypothesis is confirmed by results showing the positive effect of insulin on transketolase activity in liver samples from fasting animals (Table 3).

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