EFFECT OF ALIMENTARY THIAMINE DEFICIENCY ON THE ACTIVITY OF GLUCONEOGENIC KEY ENZYMES IN RAT LIVER AND KIDNEY

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Activity of the key enzymes of gluconeogenesis under alimentary thiamine deficiency (15 days of dietary treatment) was studied in the liver and kidney of fed and 48 h starved rats. As compared to pair-fed controls vitamin B₁-deficiency was followed by a decrease of glucose 6-phosphatase and fructose 1,6-bisphosphatase activities in both organs; the activity of phosphoenolpyruvate carboxykinase was diminished only in the liver. Starvation of thiamine-deficient rats (as compared to pair-fed starved group) resulted in lower activation of these enzymes. The decrease of the enzyme activities in thiamine-deficient animals indicates that de novo glucose synthesis in the tissues is depressed, though thiamine-requiring enzymes are not directly involved in this process. Possible mechanisms of alterations described are discussed.

Gluconeogenesis is defined as the formation of glucose from noncarbohydrate precursors. The main organs for gluconeogenesis are liver and kidney, which contain high activity of gluconeogenic key enzymes: G6P, FBP and PEPCK. Vitamin B₁ takes an important place in carbohydrate metabolism. The specificity of glucose catabolism due to a thiamine deficiency is well known [2]. However, there are only a few papers about gluconeogenic status in thiamine deficiency, in which the characteristics of the de novo glucose synthesis was described based on indirect data or on the activity of individual gluconeogenic enzymes [3-9]. A considerable group of diseases is known to be accompanied by symptoms of vitamin B₁ deficiency [10]. It was expedient to evaluate the effect of the state of gluconeogenesis in thiamine deficiency on the

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1 Abbreviations used: G6P, glucose 6-phosphatase (EC 3.1.3.9); FBP, fructose 1,6-bisphosphatase (EC 3.1.3.11); PEPCK, phosphoenolpyruvate carboxykinase (EC 4.1.1.32); cAMP, cyclic 3',5'-adenosine monophosphate.
activity of G6P, FBP and PEPCK in animals fed or starved for 48 h, the time by which the activity of the above mentioned enzymes in the liver and kidney achieves maximum values [1].

MATERIALS AND METHODS

Animal model of thiamine deficiency. Male albino rats (150-200 g) were placed on a thiamine-deficient diet [11]. Another group, designated as pair-fed control, was provided the identical diet but supplemented with thiamine, and received weighed amounts of food equivalent to the measured food consumption of the thiamine-deficient rats in the previous 24 h. Coprophagy was prevented by keeping the animals in cages with wire-mesh bottom. Rats were killed after 15 days of dietary treatment, when erythrocyte transketolase activity in thiamine-deficient group dropped to 59.7 ± 7.3% of pair-fed control values; it has been demonstrated that this fall indicates a pronounced thiamine deficiency [2, 12].

Material. In a half of rats of both groups food (but not water) was removed 48 h prior to killing the animals in order to stimulate gluconeogenic enzyme. The remaining animals were starved overnight (12 h). Liver and kidney cortex supernatants were used.

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RESULTS

The activities of G6P and PEPCK (but not FBP) were increased in the liver and kidney of starved control rats (Fig. 1). The activities of G6P and FBP were decreased in both organs in thiamine-deficient rats. Also liver PEPCK activity was lowered in deficient animals, whereas it increased in the kidney. Upon starvation of rats both G6P and PEPCK activities were increased in the liver and kidney in thiamine-deficient rats as well as in pair-fed controls. FBP activity rose in the liver only, but did not change in the kidney of animals fed deficient diet. It may be noted that absolute levels of enzyme activities in starved deficient rats were almost always lower than in starved pair-fed controls, with the exception of kidney PEPCK which remained unchanged.

DISCUSIÓN

Appetite depression and subsequent weight loss of rats fed thiamine-deficient diet began around the 13th day and fell significantly on the 21st day of dietary treatment. Similar observations have been made by other
investigators [7, 9, 16]. Therefore the rats were rendered thiamine-deficient by maintaining them on a thiamine-free diet for 15 days, to minimize the influence of starvation due to vitamin B₁-deficiency and to avoid pronounced metabolic disturbances due to profound avitaminosis.

A drop of the activity of relevant gluconeogenic enzymes in thiamine deficiency indicates that de novo glucose production in the tissues is diminished. A lowered ability of the liver and kidney to produce glucose in the starved thiamine-deficient rats (as compared with the control ones) is reflected by the level of glycaemia during starvation.

In experiments on glucose synthesis from endogenous substrates in rats with alimentary thiamine deficiency (a period of 15 days) a lowering of gluconeogenesis in hepatocytes [7] and in renal cortex slices [8] was observed; that is in good agreement with our results.

Fig. 1. The effect of thiamine-deficiency on the activities of G6P, A; FBP, B and PEPCK, C; in liver, I and kidney, II; of satiated (overnight fasted) or starved for 48 h rats. The animals were killed 15 days after maintaining them on one of the diets consisting of sucrose (63%), vitamin-free casein (22%), salt mixture (4%), corn oil (10%) and vitamin mixture (1%) containing all vitamins or those with exception of thiamine. Liver and kidney cortex were homogenized in specific media: for G6P, in 0.15 M NaCl (subsequent centrifugation at 6000 g for 10 min); for FBP, in 0.05 M Tris containing 0.01 M mercaptoethanol; for PEPCK, in 0.05 M Tris/HCl buffer, pH 7.2 containing 0.05 M KCl (centrifugation in both cases at 18000 g for 10 min). The vertical axis represents values expressed as percentage of the pair-fed control values. The horizontal axis represents group of animals: pair-fed control satiated, 1; or starved, 2; thiamine-deficient rats satiated, 3; or starved, 4. Significant differences are indicated as follows: as compared to satiated, (*) and starved, (△) pair-fed control; as compared to fed thiamine-deficient rats, (●) S. E. is shown by the vertical bars.
In various methods of evoking thiamine deficiency and its different duration the only gluconeogenic enzyme that has been studied by other authors is G6P. Its activity was found to increase [2, 4] or to remain unchanged [3, 5, 6]. These observations differ from ours because they reflect differences in experimental design and are accompanied by other levels of regulatory substances (e.g. hormones, substrates in tissues, etc. [3, 4, 6]).

Gluconeogenic enzymes are not thiamine-requiring. Therefore the data obtained may be attributed to nonspecific effects of thiamine deficiency on the metabolism. At the early stage of thiamine deficiency (the first two weeks of dietary feeding) pyruvate dehydrogenase system, in which thiamine is a coenzyme, is still unaffected [9, 17]. Depression of transketolase activity, due to vitamin B₁ deficiency, appears to be the main biochemical aberration, leading to either a decrease of the intensity of protein and nucleic acid synthesis [18, 19] or disbalance between nitrogenous anabolism and catabolism [19]. The lowered gluconeogenic enzyme activities in both starved and fed thiamine-deficient rats may be due to the enhanced protein break-down, which was shown to occur in such animals [19]. Intensification of amino acid deamination [19] and transamination [6] as well as increase of amine nitrogen excretion with urine [16, 19] point to a change of acid-base balance towards acidosis in thiamine deficiency [19]. In kidney, in contrast to liver, unaltered PEPCK activity (Fig. 1) of starved deficient rats (as compared to starved pair-fed group) and enhanced one in animals fed thiamine-free diet is associated with the fact that PEPCK activity in the former organ [1, 20] but not in the latter one [1, 21] is mainly under the control of the acid-base balance and sharply increases in acidosis. It is also likely that cAMP-dependent mechanism has an influence upon gluconeogenic enzymes in thiamine deficiency. Hormonal stimulation of gluconeogenesis during starvation is mediated by a prior growth of cAMP level [22]. The concentration of this cyclic nucleotide is diminished in alimentary thiamine deprivation [23]. Perhaps the summing up of these two effects results in lower absolute values of enzyme activities observed in deficient starved rats as compared with control starved ones.

Thus the thiamine deficiency clearly causes aberrations in gluconeogenesis in which thiamine-requiring enzymes are not involved.

REFERENCES