Hepatic Metabolism and Transport in Thiamine Deficiency

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Thiamine deficiency often accompanies alcoholism which causes liver disease, but the role of thiamine depletion per se in modifying hepatic metabolism and function is uncertain. Thiamine is the coenzyme for transketolase and pyruvate decarboxylase in tissues. Decreased pyruvate decarboxylase activity theoretically may impair synthesis of acetyl CoA and then of ATP, the ultimate tissue energy source. In this study, hepatic metabolism, especially ATP stores, and the transport of Rose Bengal across the liver, were assessed in rats with encephalopathy due to dietary thiamine deprivation, and in asymptomatic pair-fed controls. Compared to controls, in the thiamine-deficient rats, (a) hepatic transketolase and pyruvate decarboxylase activity were 89 and 74% lower \((P < 0.01)\) respectively; however, (b) hepatic acetyl CoA and ATP concentrations decreased only 21 and 12% \((P < 0.01)\), hepatic morphology was normal, and transport of Rose Bengal across the liver was unimpaired. Depressed hepatic pyruvate decarboxylase activity and ATP concentration in thiamine-deficient rats in vivo responded to exogenous thiamine much more slowly than it did in brain, heart or kidney. In vitro, however, hepatic pyruvate decarboxylase activity and ATP concentration in thiamine-deficient rats in vivo responded to exogenous thiamine much more slowly than it did in brain, heart or kidney. In vitro, however, hepatic pyruvate decarboxylase activity and ATP concentration in thiamine-deficient rats in vivo responded to exogenous thiamine much more slowly than it did in brain, heart or kidney. In vitro, however, hepatic pyruvate decarboxylase activity and ATP concentration in thiamine-deficient rats in vivo responded to exogenous thiamine much more slowly than it did in brain, heart or kidney. In vitro, however, hepatic pyruvate deca...
acetyl CoA from carbohydrate; in the absence of other substrates for the Krebs cycle, this decreased synthesis could cause impaired formation of adenosine triphosphate (ATP), the ultimate tissue energy source. A decrease in available ATP, in turn, if of sufficient magnitude, could impair transport processes and other reactions dependent on energy. α-Ketoglutarate decarboxylase activity is not decreased much in thiamine deficiency, apparently because the coenzyme is tightly bound to the apoenzyme (6, 8).

This study was designed to investigate the effect of diet-induced, severe thiamine deficiency on hepatic transketolase and pyruvic decarboxylase activity and to determine whether diminished activity of the latter enzyme resulted in decreased concentrations of acetyl CoA and ATP in these organs. Since the thiamine-deficient animals showed decreased hepatic pyruvate decarboxylase activity and acetyl CoA and ATP concentration, hepatic transport function in thiamine deficiency was also investigated.

MATERIALS AND METHODS

Female littermate Sprague-Dawley rats, 60–80 g in weight, were placed in individual metabolism cages. One member of each pair was fed, daily, 20 g of a special synthetic thiamine-deficient diet replete with other nutrients (9, 10), while the second animal (pair-fed control) was pair-fed with the same diet supplemented with thiamine (9, 10). The daily food intake of each pair-fed control animal depended on the measured food intake consumed by the corresponding experimental rat on the previous day. The weight curves of the thiamine-deficient and pair-fed control rats were similar and have been published previously (11). In some of the studies, a third group of female rats of equal weight was fed a regular Purina Chow diet ad libitum and served as normally fed controls. All animals were given free access to water. Pair-fed controls were felt to be the most valid controls for the thiamine-deficient animals.

Thiamine-deficient rats developed signs of neurologic dysfunction (ataxia, incoordination, drowsiness) after about 4–5 weeks of the thiamine-deficient diet, while the pair-fed and normally fed rats showed no abnormal behavior. One intraperitoneal injection of 25 μg of thiamine hydrochloride fully reversed the neurologic signs of the thiamine-deficient rats within 16–36 hours. Two groups of thiamine-deficient rats were studied, one of which had overt neurologic signs; another group of animals were studied after reversal of encephalopathy by three daily injections of 25 μg of thiamine hydrochloride, intraperitoneally. The appropriate pair-fed animals were sacrificed at the same times.

Blood for measurement of transketolase activity, the thiamine pyrophosphate (TPP) effect, lactate, pyruvate, ketone and glucose concentrations, was obtained from the torso of animals decapitated by guillotine without prior anesthesia. Blood transketolase and TPP effect (increase of blood transketolase activity with in vitro addition of thiamine pyrophosphate) were determined by the procedure of Dreyfus (12); lactate and pyruvate were determined by an enzymatic procedure (13, 14), ketones, according to Williamson (15), and plasma glucose, by the glucose oxidase method of Saifer and Gerstenfeldt (16). For measurement of hepatic transketolase and pyruvate decarboxylase activity, the animals were briefly anesthetized with ether, and the unfrozen tissues were assayed for the respective enzymes. For assay of liver ATP and acetyl CoA concentration, the liver in these animals was rapidly excised and frozen immediately in liquid nitrogen. Liver transketolase activity was determined by the procedure of Dreyfus and Moniz (17), pyruvate decarboxylase, by the method of Dreyfus and Hauser (5), ATP, by luciferin-luciferase luminescence (18) and hepatic acetyl CoA, by the method described by Wieland and Weiss (19), and further modified by Pearson (20) and Herrera and Freinkel (21). The first three procedures cited have been previously validated in our laboratory and are described in detail elsewhere (11,22). In carrying out the acetyl CoA assay, it became necessary to run a reagent blank throughout the procedure, which systematically lowered the absolute values as compared to those previously reported. Recovery of weighed amounts of acetyl CoA, added to liver, averaged 91%. Since ether anesthesia is reported to increase the acetyl CoA concentration in liver (21), some groups of animals were not anesthetized before being sacrificed by decapitation, and some were sacrificed after brief ether anesthesia. Hepatic protein concentration was measured as described by Lowry et al (28).

To assess hepatic transport function in thiamine...