EFFECT OF TRYPTOPHAN ON FATTY ACID SYNTHESIS IN RAT LIVER

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Received 26 June 1973

1. Introduction

The hypoglycemic effect of tryptophan is produced by inhibition of hepatic gluconeogenesis at the reaction catalyzed by phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and under the condition, contents of were metabolites of tricarboxylic acid cycle in liver markedly increased [1, 2]. It was shown in the previous study [3] that the intraperitoneal administration of tryptophan to rats produced an increase in the hepatic lipogenesis. The level of acetyl coenzyme A carboxylase (EC 6.4.1.2) which has been considered to play a key role in fatty acid synthesis, was not changed. The content of citrate, a feed-forward allosteric activator, was markedly increased whereas that of long-chain fatty acyl-CoA, a negative feed-back inhibitor, remained unchanged.

In the present work, we found that hepatic pyruvate dehydrogenase (EC 1.2.4.1) was converted to an inactive form by tryptophan administration. The regulation of the enzyme activity is discussed in relation to the alterations of carbohydrate and fatty acid metabolisms.

2. Materials and methods

Male rats of Wistar strain weighing 120–150 g were fasted for 24 hr before the onset of experiments. Animals received, intraperitoneally, either 50 mg of L-tryptophan/100 g of body weight as a suspension in 1 ml of 0.9% NaCl or the saline solution. At appropriate intervals, [1-^{14}C]acetate (62 mCi/mmole, the Radiochemical Centre, Amersham, England; 2.5 μCi in 0.5 ml of the saline solution per 100 g of body weight) was administered intraperitoneally for the determination of fatty acid synthesis. After 30 min, rats were killed by means of a guillotine and exsanguinated. Blood was collected in heparinized test tubes. A portion of blood was deproteinized by addition of $HClO_4$ within 1 min, and the remainder was immediately centrifuged to collect plasma. Livers and other tissues were frozen by the press between two blocks of dry ice within 2 min after sacrifice and stored at $-20°$ until use.

The total fatty acid fraction of the tissue was extracted with *n*-hexane. The hexane layer was transferred into a counting vial and dried under a stream of warm air. The radioactivity was counted in the scintillator solution of Patterson and Greene [4]. Blood glucose, glycerol and triglyceride in plasma were quantified with use of biochemical test combinations (Boehringer, Mannheim, Germany). Lactate and pyruvate were determined enzymatically. Plasma free fatty acid was determined by the method of Itaya and Ui [5]. Pyruvate dehydrogenase activity was measured according to the method of Wieland et al. [6].

3. Results and discussion

The time sequence of ^{14}C incorporation into liver fatty acid was summarized in fig. 1. The incorporation was increased for from 1–4.5 hr after a single injection of tryptophan and lowered to the original level after 6 hr. The lipid content in liver was constant during the experiment, and no lipid accumulation was found under light and electron microscopic analyses. The increased radioactivity is considered as a result of hyperlipogenesis but not of accumulation of lipid.

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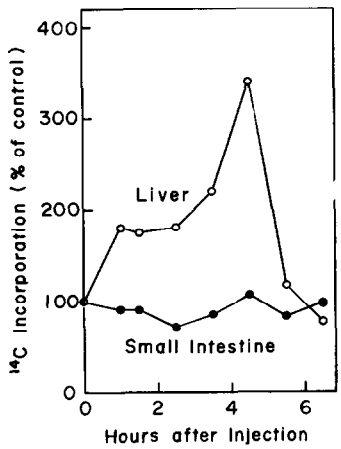

Fig. 1. Effect of tryptophan administration on fatty acid synthesis in liver and small intestine. Rats received tryptophan for varying times before the injection of [^{14}C]acetate. After 30 min, animals were sacrificed. Zero time animals received no tryptophan. Each point represents the average of data from three or more animals. The average ^{14}C incorporation, in cpm/g of tissue together with standard deviations and number of animals in parentheses, from control animals at indicated times from 0–6.5 hr are as follows: Liver, 2732 ± 771 (3); 2759 ± 798 (7); 2700 ± 737 (10); 2371 ± 662 (7); 2110 ± 569 (3); 2302 ± 463 (4); 3000 ± 1227 (3); 2118 ± 885 (4). Small intestine: 5909 ± 1401 (3); 6486 ± 1527 (7); 6297 ± 793 (10); 6035 ± 1186 (7); 5402 ± 1076 (3); 6438 ± 703 (4); 6441 ± 872 (3); 7016 ± 1803 (4).

The enhancement of ^{14}C incorporation into liver fatty acid was also observed by the tryptophan administration per stomach tube. Quinolinate at dosage of equimolar amounts to tryptophan increased ^{14}C incorporation, but alanine did not produce significant effect. The responses to tryptophan and quinolinate were not observed in the small intestine (fig. 1).

It has been reported that the hypoglycemic action of tryptophan was specific to this amino acid, and that the maximum depression of about 20% from the initial blood glucose concentration resulted from subcutaneous injection of tryptophan [7]. The effect of tryptophan on blood glucose in the present experiment was summarized in fig. 2A. The contents of free fatty acid, glycerol and triglyceride in plasma were increased (fig. 2B). The inverse relationship between the level of glucose and those of lipids suggests that the metabolic condition in rat is significantly changed by tryptophan administration.

The levels of lactate and pyruvate in blood were increased about 1.3-fold without changing the ratio. Ray et al. [1] have described that the contents of lactate and pyruvate in liver are markedly increased by tryptophan administration. The elevation of these intermediates suggests the lowered utilization of pyruvate. It has been demonstrated that the activity of pyruvate dehydrogenase is regulated by phosphorylation and dephosphorylation [8–10], and that the activity of the enzyme is inversely related to plasma

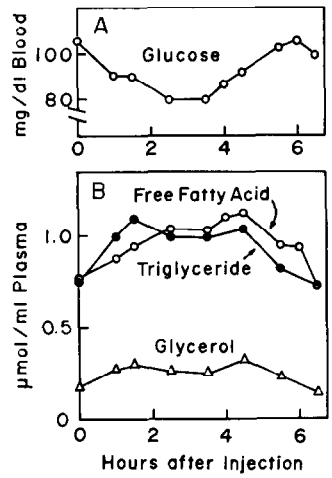

Fig. 2. Effect of tryptophan on blood glucose (A), free fatty acid, glycerol and triglyceride in plasma (B). Each point represents the average of data from two or three animals.

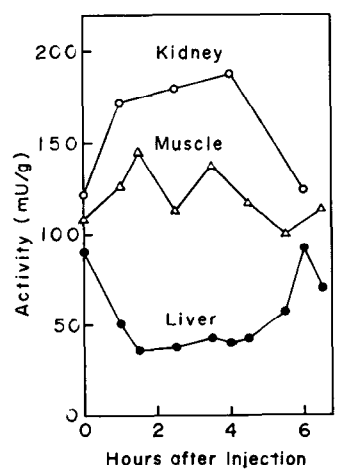

Fig. 3. Effect of tryptophan on pyruvate dehydrogenase activities. Each point represents the average of data from two or three animals.

free fatty acid level [6, 11]. As summarized in fig. 3, liver pyruvate dehydrogenase activity was decreased and then regained to the original level after 6 hr. Kidney enzyme was activated on the contrary and muscle enzyme was not affected. The mechanism of the different responses is unknown at present. The change of liver enzyme activity with time was well correlated with the changes of metabolites in blood. Furthermore the time sequences of these changes are very similar to the accumulation of intermediates of the tricarboxylic acid cycle [1].

The increased consumption of free fatty acid may provide the supply of acetyl-CoA and NADH in mitochondria. It has been discussed that the intramitochondrial NADH is converted to cytoplasmic NADPH via pyruvate carboxylase (EC 6.4.1.1) – malate dehydrogenase (EC 1.1.1.37) – NADP-specific malate dehydrogenase (EC 1.1.1.40) reactions [12–14].

The inhibition of pyruvate dehydrogenase suggests that pyruvate is preferentially converted to oxalacetate. The supply of acetyl-CoA favors the pyruvate carboxylase reaction. The marked increases of citrate and other intermediates of the tricarboxylic acid cycle result from inhibition of gluconeogenesis at the phosphoenolpyruvate carboxykinase reaction [1, 2]. The high level of citrate and an increased supply of acetyl-CoA accelerate the acetyl coenzyme A carboxylase reaction. The cytoplasmic NADPH is utilized by fatty acid synthetase. Therefore fatty acid synthesis in liver is increased even at a high level of plasma free fatty acid.

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