Metabolic Alterations in Normal and Streptozotocin-Diabetic Rats in Vivo: Influence of Prolonged Starvation

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Abstract—We studied the influence of prolonged starvation on carbohydrate metabolism in streptozotocin-diabetic rats compared with normal rats. In streptozotocin (STZ)-diabetic rats, the plasma glucose concentration decreased gradually during prolonged starvation, while it did not change in normal rats. In normal rats, glycogen depletion in the liver occurred within 24 hr of starvation, while in STZ-diabetic rats, glycogen content did not change even after 72 hr of starvation. Impaired glucose tolerance and glycogen deposition in response to oral administration of glucose were observed in STZ-diabetic rats compared with normal animals. STZ-diabetic animals generally had lower glycogen synthase and phosphorylase activities compared with normal rats during starvation. In normal animals, there is a significant correlation between the plasma concentration of free fatty acids and 3-hydroxybutyrate. On the basis of these findings, metabolic alterations in chemically-induced diabetic animals were discussed.

Carbohydrate metabolism differs between the fed and starved states. However, the blood glucose concentration remains unchanged. Insulin is thought to play an important role in maintaining the blood glucose level (1-3). Therefore, insulin-deficient animals are a useful model for providing insights into the roles of insulin in the regulation of carbohydrate metabolism. Although a large number of studies have been done, the exact lesion in diabetes has not been established. In our preliminary experiment, we found that diabetic animals had relatively high amounts of liver glycogen during starvation. The purpose of the present study was to investigate the change in carbohydrate metabolism during starvation in diabetic animals induced by streptozotocin (4).

Materials and Methods

Animals: Male Wistar rats weighing about 250-300 g were used. They were housed in a conditioned room with a 12/12-hr light/dark cycle. (The lights were turned on at 7 A.M. and turned off at 7 P.M.) They were given free access to food and water until the start of experiments.

Experimental procedure: Diabetes was induced under pentobarbital anesthesia (45 mg/kg, i.p.) by a single intravenous injection of streptozotocin (45 mg/kg, dissolved in 50 mM citrate buffer, pH 4.5). After 24 hr, the blood glucose concentration was determined to judge the severity of diabetes in the fed state. At this dose of STZ, the blood glucose concentration of the animals was about 300-350 mg/dl. The diabetic animals were used after 24 hr. They were randomly divided into 9 groups. To each group, a different starvation time was assigned. The oral glucose tolerance test was performed on 48 hr-starved rats using a 50% glucose solution at a dose of 250 mg/100 g. At each time of starvation, animals were killed by decapitation. Blood was collected into a centri...
fugation tube and added with 0.1 ml of 5 mM EDTA. It was centrifuged at 3000 rpm for 5 min at 0°C. The supernatant was used for biochemical assay and radioimmunoassay. Also a part of the major lobe of the liver was rapidly excised and frozen in a clamp previously cooled in liquid nitrogen. All samples were stored at −70°C until the biochemical determination.

Assay: Blood glucose was measured by the glucose oxidase method (5). Glycogen content in the liver was determined by the method of Seifter et al. (6). Glycogen synthase and phosphorylase were determined according to the procedure described in detail elsewhere (7). The plasma concentration of free fatty acids was determined by the radiochemical method (8). A protein-free neutralized sample was submitted to the enzymatic analysis of plasma 3-hydroxybutyrate according to the procedure described by Williamson and Mellanby (9). Plasma insulin was determined by radioimmunoassay, employing a dextran-coated charcoal to separate the antibody-bound hormone from the non-bound (free) one (10).

Statistical analysis: Statistical significance was evaluated by Student’s t-test. The linear regression coefficient was calculated by the least squares method.

Drug used: Streptozotocin was a gift from Upjohn Co. (U.S.A.). Sodium pentobarbital was donated by Otsuka Pharmaceutical Co. (Tokushima, Japan). Insulin was a kind gift from Eli Lilly Co. (U.S.A.). Other relevant products were obtained from Sigma Chemical Co. (U.S.A.).

Results

Effect of prolonged starvation on plasma glucose and insulin levels: Figure 1 (top) shows the effect of starvation on the plasma glucose concentration in normal and streptozotocin (STZ)-diabetic rats. In normal rats, plasma glucose concentration slightly decreased, but was almost constant throughout the whole period from the fed state to prolonged starvation. However, the plasma glucose concentration in STZ-diabetic rats gradually fell and returned to values close to those of the normal rats during starvation. An oral glucose load (250 mg/100 g wt.) to STZ-diabetic rats at 48 hr of starvation caused a marked rise in plasma glucose concentration. On the other hand, it caused only a slight rise in plasma glucose level in normal animals. In normal animals, the fasting plasma insulin level was 50±4 μU/ml, and the fed plasma insulin level was 200±15 μU/ml. They were increased slightly during prolonged starvation (data not shown). Plasma insulin levels in STZ-diabetic rats were about 50% those of normal animals. They did not change significantly during 72 hr of starvation.

Effect of prolonged starvation on glycogen content: As shown in Fig. 1 (bottom), there was a significant difference in liver glycogen content between normal and STZ-diabetic rats in the fed state. In normal rats, the liver glycogen content decreased rapidly during
starvation. Oral glucose administration (250 mg/100 g wt.) to the normal and STZ-diabetic rats resulted in an increase in the hepatic glycogen content. However, the increase in the hepatic glycogen contents in normal rats induced by oral glucose administration was appreciably different from that in STZ-diabetic rats. These results have important implications for a diabetic-related defect caused by STZ. Then, we measured the activities of glycogen synthase and glycogen phosphorylase which are the key enzymes for glycogen synthesis and glycogenolysis, respectively.

Effect of prolonged starvation on glycogen synthase and glycogen phosphorylase in normal and diabetic rats: Glycogen synthase was activated (Fig. 2, top) and phosphorylase was inactivated (Fig. 2, bottom) in normal as well as in STZ-diabetic rats starved for 24 hr. There was an inverse correlation between the two enzymes. In general, during starvation glycogen synthase and phosphorylase activities in normal rats were higher than those in STZ-diabetic rats. As to glycogen synthase, diabetic animals maintained a low enzyme activity in spite of the relatively high plasma glucose concentration. Glycogen synthase and glycogen phosphorylase activities in normal rats were significantly higher than those in STZ-diabetic rats following 24 hr starvation. Phosphorylase in normal rats was inactivated by oral glucose load to the level observed with STZ-diabetic rats. From these results, the rates of glycogen synthesis and degradation in normal rats may be higher than that in STZ-diabetic rats. Since glycogen synthesis is limited by the supply of the substrate glucose, net glycogen synthesis seems to be
low in normal rats.

**Effect of starvation on plasma free fatty acid and 3-hydroxybutyrate levels:** In normal animals, the plasma free fatty acid and 3-hydroxybutyrate levels in the circulation were negligible. From the postprandial state to starvation, they increased rapidly and reached a steady state concentration (Fig. 3). In STZ-diabetic rats, however, the levels of free fatty acid and 3-hydroxybutyrate remained high regardless of the metabolic states. The plasma concentration of free fatty acid and 3-hydroxybutyrate was significantly reduced by an oral glucose load. In contrast to normal rats, plasma free acid and 3-hydroxybutyrate did not change 1 hr after the glucose load in STZ-diabetic rats. In normal rats, the effect of glucose to lower plasma free fatty acid and 3-hydroxybutyrate was explainable by the effect of insulin released from the pancreatic B cells. There was a significant correlation between plasma free fatty acid and 3-hydroxybutyrate in normal rats (Fig. 4). The regression line is significant at 95% confident limits. However, there was no correlation between plasma free fatty acid and 3-hydroxybutyrate in STZ-diabetic rats.

**Discussion**

Insulin is now known as a hormone essential for storage and utilization of metabolic fuels. It can potentially affect fuel metabolism in a manner opposite to epinephrine, glucagon, etc. (11). Therefore, insulin deficiency may cause generation of glucose and a decrease in the storage of glycogen and lipid. It has been well documented that regulation of hepatic glycogen synthesis by glucose is defective in the diabetic rat either in vitro or in vivo (12, 13). Kreutner and Goldberg (13) showed that glucose load activated hepatic glycogen synthase in normal rats, but not in alloxan-diabetic rats. In agreement with these results, glycogen contents in the livers of normal rats were significantly higher than that of STZ-diabetic animals in some nutritional states (fed and in oral glucose load). It is attributable to a major degree to an exaggerated stimulation of insulin secretion in normal rats compared with diabetic animals.

However, the present findings also showed that in STZ-diabetic rats, there was relatively high glycogen contents during prolonged starvation. The liver glycogen contents remained unchanged in diabetic rats in contrast to normal animals in which it was rapidly decreased. The differences between normal and diabetic rats with respect to the glycogen contents were statistically significant ($P<0.05$). In STZ-diabetic animals, plasma glucose levels seem to decrease in place of liver glycogen by starvation. Glycogen synthase and phosphorylase activities were generally higher in normal rats than in STZ-diabetic animals (Fig. 2). These results do not seem to be consistent with our previous report (7), but exact comparison is impossible because some experimental conditions (e.g. basal glycogen contents) are different. Our recent studies showed that liver glycogen synthesis in normal rats from $^{14}$C-glutamate was very low when the liver has high amounts of glycogen such as 10–50 mg/g (Yaiima and Ui, unpublished observations). These results indicate that feed-back inhibition of glycogen synthesis by glycogen itself seems to play an important role in the regulation of glycogen synthesis. If such a mechanism is operative in vivo, lower glycogen synthase activity in diabetic rats is largely explainable as a result of the relatively high glycogen contents. Differences in liver glycogen contents between normal and STZ-diabetic rats can
not be explained by the change in glycogen synthase. Therefore, it is conceivable that the relatively high glycogen contents by starvation may be ascribed to the inhibition of phosphorylase activity. Feed-back inhibition of glycogen phosphorylase by glucose has been reported in perfused liver (14) and liver cells (15) of alloxan-diabetic rats. High concentrations of plasma glucose and probably responsible for the inactivation of glycogen phosphorylase in the liver of STZ-diabetic rats during starvation. Further work is needed to determine the sites of the diabetes related defect in hepatic glycogen metabolism. It seems that the results have important implications for metabolic abnormalities associated with STZ-induced diabetes in vivo.

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