Effects of Somatostatin on Liver Glycogen and Fat Metabolism in Vivo

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Abstract—Effect of somatostatin on liver glycogen metabolism and lipid metabolism were studied in rats in vivo. Somatostatin infused at a rate of 100 ng/min/100 g wt. into the femoral vein resulted in a marked decrease in the blood glucose concentration. The content of glycogen in the liver and the concentration of insulin in the portal vein were also decreased during somatostatin infusion. Glucose was infused at a constant rate of 1.25 mg/min/100 g wt. in combination with somatostatin to prevent the somatostatin-induced hypoglycemia. Under this condition, significant increase in liver glycogen was observed without significant changes in the blood glucose level. The liver glycogen synthase activity did not change significantly during infusion of somatostatin and/or glucose. In contrast, the glycogen phosphorylase activity was markedly inhibited by infusion of somatostatin plus glucose. Liver glycogen phosphorylase was inversely correlated with the blood glucose level. However, there was no correlation between the phosphorylase activity and blood glucose concentration during somatostatin infusion. Infusion of somatostatin alone caused an increase in the blood free fatty acid and a marked decrease in the blood ketone bodies. Glucose-induced decrease in the blood free fatty acids and ketone bodies were partially overcome by the simultaneous infusion of somatostatin. On the basis of these findings, possible physiological roles of somatostatin in regulation of carbohydrate metabolism were discussed.

Somatostatin, the hypothalamic inhibitor, has been shown to inhibit the secretion of a number of hormones such as glucagon, growth hormone and insulin (1, 2). Recent studies have shown that somatostatin, which is secreted from the D cells in the pancreatic islets and the gut, may contribute to the regulation of metabolic fuel homeostasis (3). Furthermore, it was reported that somatostatin caused a moderate fall in the fasting blood glucose level in normal humans (4), baboons (5) and dogs (6). On the other hand, the peptide could potentially affect fuel metabolism by suppression of hepatic glucose-neogenesis or glycogenesis (7). With respect to the effect of glucagon on hepatic carbohydrate metabolism, its in vivo roles in normal and diabetic subjects are still unclear. Metabolic alteration induced by somatostatin might be mediated through a decrease in the plasma glucagon level. Therefore, the present work was undertaken to study the influence of glucagon deficiency induced by somatostatin on fuel metabolism. Such experiments may contribute to the understanding of the possible physiological actions of somatostatin as well as glucagon.

Materials and Methods

Animals: Male Wistar rats weighing about 200 g were used. They were fasted for 24 hr to deplete liver glycogen and divided into 9 groups.

Experimental procedures: Rats were anesthetized with pentobarbital (4.5 mg/100 g wt., i.p.). A polyethylene catheter was
inserted into the left or right femoral vein. Animals were infused with somatostatin through an intravenous canula at a constant rate of 100 ng/min/100 g by a constant infusion pump (Natsume Instrument Co., Ltd.). Glucose alone (1.25 mg/min/100 g) or in combination with somatostatin was infused into the femoral vein from 0 to 30 min. Somatostatin was dissolved in 2 M glycine buffer (pH 3.0) and diluted with saline before use. In another series of experiments, insulin was used at a constant rate of 5 mU/min/100 g.

At each time of infusion, the abdomen was incised and a small part of the liver was quickly removed. The sample was rapidly frozen with a clamp previously cooled in liquid nitrogen. Blood samples were also withdrawn from the portal vein at 10 min intervals. They were transferred to tubes containing 10 mM EDTA-saline (0.2 ml). After centrifugation (3500 rpm, 5 min, 0°C), all samples were cooled on ice and were frozen for storage at −70°C until assayed. For the glucagon assay, samples of the portal blood were transferred to tubes containing 500 KIU aprotinin plus 1.2 mg EDTA (0.1 ml). They were cooled on ice until the next step of the experiments.

Assay: Liver glycogen were determined by the method of Seifter et al. (8). The liver glycogen synthase and phosphorylase were determined by the method described in detail elsewhere (9). Plasma glucose was determined by the glucose oxidase method (10). Plasma free fatty acids and ketone bodies were determined by a sensitive radiochemical method previously described in detail elsewhere (11, 12). Plasma insulin was determined by radioimmunoassay with the polyethyleneglycol technique (13). Plasma glucagon was determined by radioimmunoassay using 30 K antiserum and 125I-labeled glucagon using porcine glucagon as standard (14).

Drugs: Somatostatin was obtained from the Protein Research Foundation (Osaka, Japan). 125I-glucagon and 30 K antiserum were kindly provided by Hoechst Japan Co., Ltd. Insulin was a gift from Eli Lilly and Company (U.S.A.). Other chemicals were purchased from Sigma Chemical Co. (U.S.A.).

Statistics: Statistical significance was evaluated using Student's t-test. Regression lines were drawn by the least squares method.

Results

Effect of somatostatin with or without glucose on the blood glucose concentration: Changes in blood glucose after infusion of somatostatin with or without glucose are shown in Fig. 1. Blood glucose levels decreased rapidly after somatostatin infusion alone. Hypoglycemia induced by somatostatin was overcome by simultaneous infusion of glucose. Blood glucose levels became significant after the infusion of glucose alone or glucose plus somatostatin at 20 and 30 min.

Effect of somatostatin on the portal vein glucagon and insulin level: As shown in Fig. 2, infusion of somatostatin alone (100 ng/min/100 g) resulted in a significant reduction in the portal vein glucagon concentration. Portal insulin levels were also decreased by somatostatin alone (Fig. 3). Portal vein insulin levels were significantly increased by the infusion of glucose alone (1.25 mg/min/100 g). Increase in the portal vein insulin induced by glucose infusion was significantly suppressed by simultaneous infusion of somatostatin.

Effect of somatostatin and/or glucose on liver glycogen metabolism: Liver glycogen
Contents were decreased by somatostatin infusion (Fig. 4). Glucose infusion alone gradually increased liver glycogen. Somatostatin in combination with glucose markedly increased liver glycogen content at 10 and 20 min after the infusion. Under this condition, liver glycogen synthase and phosphorylase activity were measured. As shown in Fig. 5, glycogen synthase did not change significantly during the infusion of somatostatin alone. Infusion of glucose produced a mild increase in glycogen synthase. However, there was no significant differences in glycogen synthase between the infusion of glucose alone and glucose plus somatostatin. Therefore, the increase of glycogen content should be explained by the change in glycogen phosphorylase activity. As shown in Fig. 6, glycogen phosphorylase activity was slightly increased by somatostatin infusion. Infusion of glucose alone gradually decreased phosphorylase activity.
Fig. 6. Effects of somatostatin and/or glucose infusion on liver glycogen phosphorylase activity. Other experimental conditions are the same as shown in Fig. 1. Somatostatin plus glucose: right column. *, **: significantly different from the corresponding control at P<0.05, P<0.01, respectively.

Fig. 7. Correlation between portal glucose and liver glycogen phosphorylase activities. (a): glucose plus insulin (5 mU/min/100 g wt.) infusion, (b): glucose (1.25 mg/mg/100 g wt.) infusion, (c): somatostatin (100 ng/min/100 g wt.) infusion. Number of observations are shown in parentheses.

Fig. 8. Effects of somatostatin and/or glucose infusion on portal free fatty acid levels. Other experimental conditions are the same as shown in Fig. 1. Number of observations are shown in parentheses. Somatostatin: left column, glucose: middle column, somatostatin plus glucose: right column. *: Significantly different from the pre-infusion level at P<0.05.

Fig. 9. Effects of somatostatin and/or glucose infusion on portal total ketone bodies levels. Other experimental conditions are the same as shown in Fig. 1. Number of observations are shown in parentheses. Somatostatin: left column, glucose: middle column, somatostatin plus glucose: right column. *, **: Significantly different from the pre-infusion level at P<0.05, P<0.01, respectively.

The current explanation for phosphorylase inhibition by glucose is activation of phosphorylase phosphatase by glucose. Decrease in phosphorylase activity by glucose was potentiated in the presence of somatostatin. Possible explanations for this most interesting result of the present study are discussed later.

Correlation between the portal vein glucose level and liver phosphorylase activity: As shown in Fig. 7, hyperglycemia and/or hypoglycemia induced by glucose alone and glucose plus insulin infusion vs. liver glycogen phosphorylase were plotted. These results showed that phosphorylase activities were inversely correlated with the blood glucose level. Each regression line showed a 99% and 95% confidence limit, respectively. In contrast, hypoglycemia by somatostatin infusion showed no significant correlation between the blood glucose and glycogen...
phosphorylase activity.

Effects of somatostatin and/or glucose infusion on the blood free fatty acid (FFA) and ketone body levels: As shown in Fig. 8, constant infusion of somatostatin alone increased the blood free fatty acids at 10 min and 20 min. Reduction in the blood free fatty acid induced by glucose infusion was recovered nearly to the basal value in the presence of somatostatin. Figure 9 shows that the blood ketone body levels were decreased by somatostatin infusion. Glucose-induced reduction in the blood ketone bodies was antagonized by the simultaneous infusion of somatostatin.

Discussion

The roles of somatostatin in the regulation of the blood glucose concentration and hepatic glucose production (gluconeogenesis, glycogenolysis) have generally been ascribed to suppression of glucagon and insulin secretions from the pancreatic A and B cells, respectively (15). Somatostatin has been reported to have no direct effects on basal glucose production in isolated liver cells (16). Similarly, in vivo studies in conscious dogs have demonstrated that somatostatin does not alter the basal glucose production rate when the levels of insulin and glucagon are maintained (17).

As illustrated in Figs. 2 and 3, the portal vein insulin and glucagon levels were significantly decreased by somatostatin infusion. These hormonal changes were associated with a significant decrease in glucose levels in the portal circulation. The hypoglycemia in response to somatostatin infusion was not due to selective insulin deficiency because insulin deficiency must increase the blood glucose concentration markedly. Therefore, it seems that hypoglycemia induced by somatostatin is associated with a significant decrease in the portal vein glucagon levels. Furthermore, we have attempted to determine the influence of somatostatin on hepatic glycogen metabolism. Since hypoglycemia by itself potentially affects hepatic glycogen metabolism in vivo, glucose was infused to maintain the basal glucose level. As shown in Fig. 5, increase in liver glycogen content by somatostatin plus glucose infusion was ascribed to the inhibition of glycogen phosphorylase. According to the above argument, the potential decrease in glycogen phosphorylase compared with glucose alone was possibly ascribed to the fall in the portal vein glucagon concentration. However, with respect to the effect of somatostatin on glucagon-stimulated glucose production in isolated perfused liver, studies have yielded conflicting results (17). According to Oliver and Zagle (18), somatostatin directly suppresses glucagon but not epinephrine stimulated glucose release from rat liver in vitro in a dose-dependent manner, probably by a mechanism affecting cyclic AMP action rather than its production. In addition, α-receptor-mediated phosphorylase activation was caused by Ca uptake in the liver (19). If somatostatin directly acts on the liver glycogen phosphorylase system, the following explanation could be possible concerning the potential decrease in phosphorylase. Somatostatin might inhibit phosphorylase kinase by decreasing Ca availability (20) besides glucose increased phosphorylase phosphatase (21). Thus, the conversion of phosphorylase b to a is potentially suppressed. The present results do not necessarily rule out such mechanisms of phosphorylase inactivation.

As shown in Fig. 9, somatostatin infusion by itself increased blood free fatty acid. It is not explained by deficient lipolytic action of glucagon. In contrast, the decrease in blood ketone bodies by somatostatin infusion was presumably associated with the deficient action of glucagon on ketogenesis (22). A significant decrease in blood free fatty acids and ketone bodies by glucose infusion may be the indirect effect of insulin secreted during the glucose infusion since these decreases were partially reversed by somatostatin. The present work is far from being complete in elucidating the possible physiological roles of somatostatin in fuel metabolism. Further investigation on the role of somatostatin in fuel metabolism is required.

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