# Somatostatin Concentration Responds to Arginine in Portal Plasma

## Effects of Fasting, Streptozotocin Diabetes, and Insulin Administration in Diabetic Rats

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**SUMMARY**

**Changes of somatostatin concentration in response to a single i.v. injection of arginine (400 mg/kg body weight) were examined in extracted portal plasma of normal and diabetic rats in the fully fed state and after 24 h of fasting, as well as in diabetic rats treated with insulin for one week. In both normal and diabetic animals fasted for 24 h, the basal level of somatostatin declined but the magnitude of the arginine-induced elevation of somatostatin was not affected, suggesting a physiologic role of the tetradecapeptide in nutrient homeostasis.**

**When compared with intact rats, diabetic animals were shown to have increased levels of somatostatin before and after arginine administration, both of which were attenuated by insulin replacement therapy. These findings suggest that alterations of D cell function in streptozotocin diabetes may be related to either insulin deficiency or its metabolic consequences.**

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Increases in somatostatin-containing D cells[1] and in the somatostatin content of the pancreatic islets and the stomach[2] have been found in rats with insulin-deficient diabetes. Recent studies[3,4] revealed that circulating levels of somatostatin were elevated in dogs with alloxan diabetes of long duration. On the other hand, it is well known that fasting results in changes of pancreatic hormone secretion.[5] Therefore, the present studies were undertaken to determine changes of somatostatin levels in response to arginine in portal plasma of normal and streptozotocin-diabetic rats in the fed and fasted state. In addition, effects of insulin treatment on somatostatin concentration were also examined in diabetic rats.

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### MATERIALS AND METHODS

Male Wistar rats (310–390 g) were used throughout the study. Diabetes was induced by a single i.v. injection of streptozotocin (Upjohn, Kalamazoo, Michigan), 40mg/kg body weight, after overnight fasting. (The diabetogenic activity of streptozotocin is enhanced in older rats.[6]) Four days after injection, rats developed hyperglycemia in excess of 400 mg/dl in the fed state, though they did not develop ketosis. They had free access to water and were fed ad libitum on rat chow pellets, except during periods of fasting.

Two weeks after streptozotocin treatment, rats in the fully fed state and after being fasted for 24 h were laparotomized under pentobarbital anesthesia. Blood (2 ml each) was taken from the portal vein by syringes containing 1.25 mg of EDTA and 1000 KIU of Trasylol in each milliliter of whole blood, before and after a single injection of L-arginine (Taisho Seiyaku, Osaka, Japan), 400 mg/kg body weight in a 20% solution, into the jugular vein. Blood was withdrawn 2 min after arginine injection, as preliminary studies in intact rats revealed that an identical arginine load resulted in a prompt and significant rise of somatostatin in portal plasma, which peaked at 2 min and was followed by a decline thereafter towards baseline levels. Normal rats were subjected to arginine-loading tests in the same manner. In another group of diabetic rats, 4–6 U of NPH insulin was injected subcutaneously at 6 p.m. for four consecutive days beginning 7 days after streptozotocin treatment, after which 3–4 U was administered for an additional 3 days. Identical arginine loading was performed in these animals in the fed state 15 h after the last insulin injection. Diabetic rats given saline served as controls. All experiments were performed between 9 and 11 a.m.

Blood was centrifuged immediately after each sampling. Plasma (0.5 ml each) was extracted by sonification in 4 vol of acid-acetone for somatostatin determinations as previously reported.[7] Plasma and its extracts were kept at −20°C until assayed.

Glucose and insulin were measured by the glucose-oxidase method using commercially available kits (Green Cross Corporation, Osaka, Japan) and by radioimmunoas-

say (RIA[8]), with rat insulin used as a standard, respectively. Somatostatin was determined by a double antibody RIA.[7]

Data are expressed as mean ± SEM and statistical analysis was performed by the nonpaired Student's *t* test.

## RESULTS

Figure 1 shows the effects of arginine on glucose, insulin, and somatostatin in portal plasma of normal and diabetic rats in the fully fed state and after 24 h of being fasted. Streptozotocin administration produced diabetes characterized by hyperglycemia associated with hypoinsulinemia. Somatostatin levels in diabetic rats were significantly elevated when compared with those of normal animals in both the fasted (235 ± 23 vs. 151 ± 12 pg/ml, $P < 0.005$) and fed state (401 ± 43 vs. 201 ± 10 pg/ml, $P < 0.001$). Somatostatin (151 ± 12 vs. 201 ± 10 pg/ml, $P < 0.02$) as well as glucose (113 ± 4 vs. 153 ± 4 mg/dl, $P < 0.001$) and insulin (3.85 ± 0.35 vs. 6.83 ± 0.79 ng/ml, $P < 0.005$) were significantly lower in normal, fasted rats as compared with fed controls. In diabetic animals as in normal rats, fasting resulted in a marked decrease in both glucose (459 ± 15 vs. 175 ± 12 mg/dl, $P < 0.001$) and somatostatin levels (401 ± 43 vs. 235 ± 23 pg/ml, $P < 0.005$), although there was no significant change in insulin values.

Arginine injection resulted in a significant elevation of insulin and somatostatin levels in all animals used in this study. Normal rats, regardless of the nutrient state, showed 1.3-fold increases of somatostatin in response to arginine. However, the mean somatostatin level in portal plasma of diabetic animals rose from 401 ± 43 to 726 ± 103 pg/ml in the fed state and from 235 ± 23 to 395 ± 30 pg/ml in the fasted state. Thus, arginine administration produced an approximately twofold elevation of somatostatin in diabetic rats. Arginine loading resulted in threefold increases of insulin in diabetics. By contrast, intact rats showed 4.5-fold and 3.7-fold elevations of insulin in the fed (from 6.83 ± 0.79 to 30.99 ± 4.17 ng/ml) and fasted state (from 3.85 ± 0.35 to 14.16 ± 1.56 ng/ml), respectively.

Plasma glucose was significantly lower in the insulin-treated rats, which showed steady weight gain, than in saline-treated diabetic animals, though insulin-treated rats continued to show moderate hyperglycemia (Figure 2). Mean somatostatin levels before and after arginine injection were markedly lower in the insulin-treated group than in the saline-treated group (157 ± 16 vs. 338 ± 31 pg/ml at 0 min, $P < 0.01$; 280 ± 32 vs. 737 ± 63 pg/ml at 2 min, $P < 0.001$). They returned to the levels found in normal rats in the fed state (see Figure 1). Arginine administration resulted in a 1.8-fold elevation of somatostatin in the insulin-treated group, whereas it produced a 2.2-fold elevation of the tetradecapeptide in saline-treated diabetic rats.

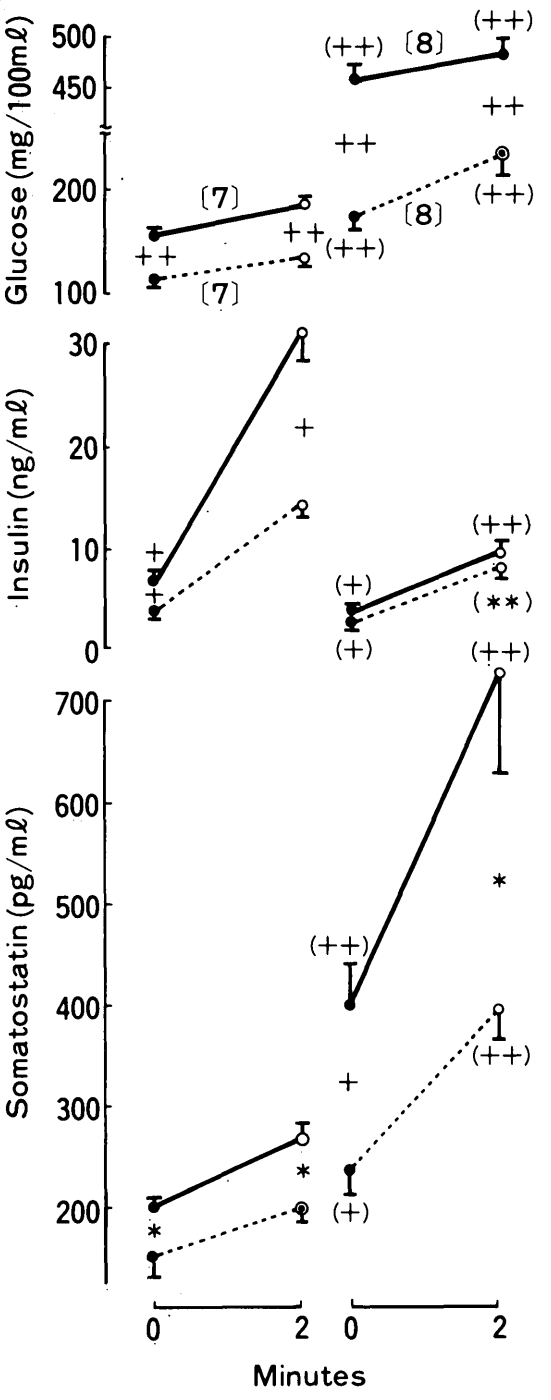


**FIGURE 1. Effects of a single i.v. injection of arginine, 400 mg/kg body weight, on portal plasma glucose, insulin, and somatostatin in normal (left panel) and diabetic rats (right panel) in the fully fed state (solid lines) and after 24 h of fasting (dotted lines). The number of animals is in square brackets. Mean data ± SEM. Significance of difference between fasted and fed states: *$P < 0.05$, **$P < 0.01$, +$P < 0.005$, ++$P < 0.001$. Symbols in parentheses indicate differences between normal and diabetic rats in the fed and fasted states, respectively, at each corresponding time period. ⊙ and ○ indicate significant differences vs. zero values ($P < 0.05$ or 0.01, and $P < 0.005$ or less, respectively).**

## DISCUSSION

The present study demonstrates that rats with streptozotocin diabetes of 2-wk duration have increased levels of somatostatin in portal plasma. A similar result was obtained from the in vitro study[9] using perfused pancreases from alloxan-diabetic rats that had had the disease for a short time. Furthermore, in the present study, insulin replacement therapy for one week significantly lowered the increased levels of tetradecapeptide before and after arginine administration. A recent study[10] in streptozotocin-diabetic rats revealed that insulin therapy lowered the increased pancreatic somatostatin content but did not affect that of the stomach and colon. These findings suggest that alterations in islet D-cell function may be related to either insulin deficiency or its metabolic consequences and that the reduced portal so-

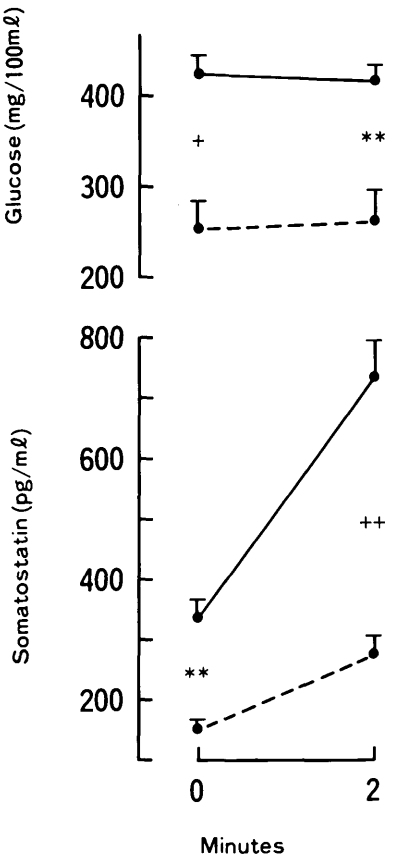


**FIGURE 2. Effects of insulin replacement therapy for 1 wk on arginine-induced elevation of somatostatin in portal plasma of streptozotocin-diabetic rats. Dotted and solid lines represent data from insulin-treated (N = 7) and saline-treated (N = 7) diabetic rats, respectively. Mean ± SEM. For significance of difference between the two groups, see legend for Figure 1.**

matostatin response to insulin therapy found in the present study is of pancreatic origin.

These results in intact rats confirm the previous finding[11] that fasting results in a decrease in portal somatostatin, which appears to reflect its release from the pancreas and gastrointestinal tract. These two studies are not consistent with the recent study[12] using perifused rat islets, which indicated that fasting produced a high secretory activity of D cells. The reason for the discrepancy between in vivo and in vitro results is unclear but may be a result of the different experimental designs employed. Though the effect of arginine on somatostatin's release from the rat stomach is obscure to us, an elevation of portal somatostatin in response to arginine appears to be consistent with the observations[13,14] that arginine can stimulate somatostatin's release from the rat pancreas.

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