# Concentration and Secretion of Gastric Somatostatin in Streptozotocin-diabetic Rats

TSUTOMU CHIBA, SEIZO KADOWAKI, TOMOHIKO TAMINATO, HIROMI ABE, KAZUO CHIHARA, SHIGERU MATSUKURA, YASUO GOTO, YUTAKA SEINO, AND TAKUO FUJITA

**SUMMARY**

**Both the release and the content of gastric somatostatin were investigated in streptozotocin-diabetic rats. Fundic and antral somatostatin contents were both increased in streptozotocin-diabetic rats compared with control animals. Basal somatostatin levels in the perfusates of streptozotocin-treated animals were not significantly different from those of the control animals. However, the peak values of somatostatin release induced by $5 \times 10^{-9}$ M glucagon in the diabetic animals were significantly higher than those of the controls. These results lead us to conclude that a hyperfunctioning state of the gastric D-Cells exists in hypoinsulinemic diabetes. DIABETES 30:188–191, March 1981.**

Recent studies have shown that both somatostatin content and the number of somatostatin-producing D-cells in the islets of Langerhans are significantly increased in certain hypoinsulinemic states such as streptozotocin-treated diabetes in rats[1,2] and juvenile-onset diabetes in humans,[2] while decreased pancreatic somatostatin content has been found in spontaneously diabetic mice with hyperinsulinemia.[3] These observations suggest that pancreatic somatostatin may have a close interrelationship with insulin, playing an important etiologic role in diabetes. Schusdziarra et al.,[4] on the other hand, have reported that gastric somatostatin is released by oral administration of various nutrients. We have also found that gastric somatostatin secretion is influenced by such pancreatic hormones as insulin, glucagon, and pancreatic polypeptide.[5,6] This suggests a possible role for gastric somatostatin in nutrient homeostasis and carbohydrate metabolism. Gastric somatostatin content, furthermore, has been shown to be increased in diabetic rats.[1] Thus, like pancreatic somatostatin, gastric somatostatin may also have pathophysiologic significance in diabetes.

The present study was undertaken to determine the tissue content and the glucagon-induced secretion of gastric somatostatin in relation to the severity of diabetes induced by graded doses of streptozotocin.

## MATERIALS AND METHODS

**Animals.** Male Wistar rats, 10–11 wk old at the time of injection, were used throughout the experiments. After 24-h fasting, the animals were weighed and divided into 4 groups to be given 25, 50, and 75 mg/kg body wt of streptozotocin (Upjohn, Michigan) dissolved in saline immediately before use, by intravenous injection under light ether anesthesia. Control animals received an injection of the same volume of saline alone. The animals were maintained in a temperature-controlled, air-conditioned room under a light-dark cycle, fed Oriental laboratory chow (Oriental Yeast Co., Tokyo, Japan), and given tap water ad libitum. The following experiments were conducted 6 wk later.

**Blood samples.** Twenty-four hours before the experiment, with each animal in a nonfasting state, the right jugular vein was exposed under sodium pentobarbital anesthesia (40 mg/kg, intraperitoneally) while blood sampling (1 ml each) was carried out by the transmuscular puncture technique with an heparinized syringe.[7] The blood was put into a chilled tube containing 0.1 ml (1000 KIU) of Trasylol (Bayer Leverkusen, Germany) and the plasma immediately separated by centrifugation. A small portion of plasma was removed for glucose determination by the orthotoluidine method,[8] and the remaining plasma samples were frozen and kept at −20°C for insulin and glucagon measurements.

**Gastric somatostatin content.** Six animals in each group were used for the extraction study. Extraction was performed by the method described by Arimura et al.[9] After 24-h fasting, the animals were weighed and then decapitated.

From the Third Division, Department of Medicine, Kobe University School of Medicine, Kobe, Department of Medicine, Shimane Medical College, Izumo (Y.G.) and Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto, Japan (Y.S.).

Address reprint requests to Dr. Tsutomu Chiba, Third Division, Department of Medicine, Kobe University School of Medicine, 7 Chome, Kusunoki-cho, Ikuta-ku, Kobe 650, Japan.

Received for publication 20 February 1980 and in revised form 18 September 1980.

The stomach was rapidly exposed through a midline abdominal incision, then resected and divided into the cardia, fundus, and antrum. The tissue samples were weighed and frozen between blocks of dry ice. Each tissue sample was homogenized in 100 ml ice-cold 2 N acetic acid, then heated to boiling for 15 min. The solid material was removed by centrifugation (12,000 rpm, 30 min) and the clear supernatant lyophilized. The residues were dissolved in 0.1% gelatin in phosphate-buffered saline containing 0.025 M ethylenediaminetetraacetic acid, pH 7.0, and assayed for immunoreactive somatostatin. An aliquot of each sample was stored before centrifugation and the protein concentration was determined by the method of Lowry et al.[10]

**Perfusion study.** In the perfusion study, six animals were used for each group. Perfusion of the stomach was performed by adaptation to the rat[5] of the method described by Lefèbvre et al.[11] After an overnight fast, the animals were anesthetized by 40 mg/kg sodium pentobarbital intraperitoneally. After a midline laparotomy, polyethylene cannulae were inserted into the left gastric artery and gastric vein. All other vessels and the pancreas were carefully excluded by ligation. The cardium was ligated and then a catheter was inserted into the stomach through the pyloric ring to drain the gastric juice. All perfusions were performed with 4.6% dextran (mean mol. wt. 70,000) Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose (DKRBG). The perfusate was continuously gassed with 95% $O_2$-5% $CO_2$ and maintained at pH 7.4. Both the perfusate and stomach preparation were kept at 37°C throughout the experiment. Media were perfused into the left gastric artery by means of a peristaltic pump at a flow rate of 2 ml/min without recirculation, and the test material was introduced via a side arm to give an appropriate final concentration. After a 20-min preperfusion of the stomach with DKRBG alone, crystalline beef-pork glucagon (Lilly, Indianapolis, Indiana) to provide a final concentration of $5 \times 10^{-9}$ M was introduced over 15 min. Glucagon was dissolved in 0.1 M acetic acid and then diluted with the perfusate immediately before use. The final pH of the perfusate was found not to be influenced by the acidic glucagon solution. Venous effluents were collected at 1-min intervals into tubes containing a bacitracin (Sigma Chemical Co., St. Louis, Missouri)-Trasylol mixture ($2 \times 10^{-5}$ M and 1000 KIU/ml, respectively), frozen immediately, and stored at −20°C until assayed.

**Assay procedure.** Immunoreactive somatostatin levels were measured by a specific radioimmunoassay, utilizing antiserum RA-823 described previously.[5] Antiserum RA-823 showed no cross-reaction with glucagon, insulin, or many other hormones.[5] The minimum detectable quantity of the assay was 10 pg/ml. The intra- and interassay variations of the assay were 5.4% and 8.5%, respectively.

Plasma insulin was determined by a polyethyleneglycol radioimmunoassay,[12] with rat insulin used as the standard.

Plasma glucagon was measured by a radioimmunoassay in which 30 K (purchased from the University of Texas Southwestern Medical School, Dallas, Texas) was used as antiserum, and the talcum-adsorption method[13] was employed for the separation of free from bound hormones.

**Statistics.** Data are expressed as mean ± SEM, and Duncan's new multiple range test was used in the statistical analysis.[14] $P < 0.05$ indicates a significant difference between two groups.

TABLE 1
Body and stomach weight in normal and streptozotocin-treated rats

| Streptozotocin (mg/kg) | Body weight (g) | Stomach weight (mg) | * Relative stomach to body weight |
|---|---|---|---|
| 0 (Control) | 423 ± 3 | 1796 ± 67 | 0.42 ± 0.01 |
| 25 | 358 ± 14† | 1540 ± 106 | 0.44 ± 0.03 |
| 50 | 282 ± 11†,‡ | 1767 ± 112 | 0.63 ± 0.01†,‡ |
| 75 | 228 ± 21†,§ | 2015 ± 94 | 0.91 ± 0.05†,§ |

N = 6 in each group. Means ± SEM are shown.
* Relative stomach weight is expressed as a ratio of stomach to body weight.
† $P < 0.05$ vs. controls.
‡ $P < 0.05$ vs. 25-mg/kg streptozotocin-treated rats.
§ $P < 0.05$ vs. 50-mg/kg streptozotocin-treated rats.

## RESULTS

**Effect of streptozotocin on body and stomach weight.** Body and stomach weights in control and streptozotocin-treated animals 6 wk after the injection are summarized in Table 1. The body weights of the animals at the time of injection ranged from 280 to 350 g, with a mean value of 313 ± 6 (±SEM) g. During the 6 wk from the time of injection to the experimental period, the control and the 25-mg/kg streptozotocin-treated animals had a weight gain of 111 ± 12 g and 45 ± 5 g, respectively, whereas a weight loss of 31 ± 4 g and 85 ± 10 g was found in the 50-mg/kg and 75-mg/kg streptozotocin-treated animals, respectively. Consequently, the final body weight decreased in proportion to the amount of streptozotocin administered. In contrast to the body weight, there were no significant differences in stomach weight among the four groups (Table 1).

**Effect of streptozotocin on plasma glucose, insulin, and glucagon.** Plasma glucose, insulin, and glucagon levels in the four groups in the nonfasting state 6 wk after the streptozotocin injection are listed in Table 2. The plasma glucose levels in the 50-mg/kg and 75-mg/kg streptozotocin-treated animals were significantly elevated compared with those in the control animals. The administration of graded doses of streptozotocin caused a significant decrease in plasma insulin levels. On the other hand, plasma glucagon was elevated in streptozotocin-treated animals compared with the control animals. With increasing dosages of streptozotocin, plasma glucose and glucagon levels increased proportionally, while graded decreases of plasma insulin levels were noted.

TABLE 2
Plasma glucose, insulin, and glucagon levels in normal and streptozotocin-treated rats

| Streptozotocin (mg/kg) | Plasma glucose (mg/dl) | Plasma insulin (μU/ml) | Plasma glucagon (pg/ml) |
|---|---|---|---|
| 0 (Control) | 156 ± 5 | 54 ± 4 | 45 ± 4 |
| 25 | 201 ± 13 | 39 ± 2* | 64 ± 7 |
| 50 | 302 ± 28*,† | 17 ± 4*,† | 100 ± 24* |
| 75 | 520 ± 42*,‡ | 6 ± 2*,‡ | 134 ± 12*,† |

N = 12 in each group. Means ± SEM are shown.
* $P < 0.05$ vs. controls.
† $P < 0.05$ vs. 25-mg/kg streptozotocin-treated rats.
‡ $P < 0.05$ vs. 50-mg/kg streptozotocin-treated rats.

TABLE 3
Gastric somatostatin content in normal and streptozotocin-treated rats

| Streptozotocin (mg/kg) | Fundic somatostatin | | Antral somatostatin | |
|---|---|---|---|---|
| | ng/g wet weight | ng/mg protein | ng/g wet weight | ng/mg protein |
| 0 (Control) | 711 ± 59 | 3.48 ± 0.25 | 612 ± 41 | 3.60 ± 0.16 |
| 25 | 847 ± 55 | 4.91 ± 0.22* | 743 ± 66 | 4.69 ± 0.26* |
| 50 | 1008 ± 66* | 5.74 ± 0.30* | 823 ± 58* | 5.55 ± 0.30*,† |
| 75 | 1072 ± 80* | 6.34 ± 0.42*,† | 956 ± 66*,† | 6.26 ± 0.35*,† |

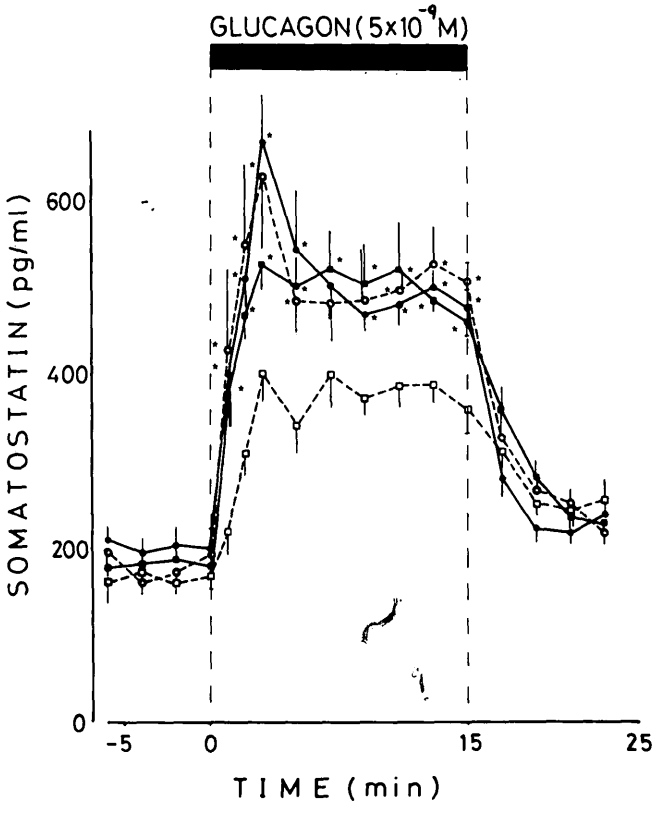
N = 6 in each group. Means ± SEM are shown.
* $P < 0.05$ vs. controls.
† $P < 0.05$ vs. 25-mg/kg streptozotocin-treated rats.

**Effect of streptozotocin on gastric somatostatin content.** Fundic and antral somatostatin content in the four groups is shown in Table 3. Both fundic and antral somatostatin content increased in all groups of streptozotocin-diabetic rats, although in 25-mg/kg streptozotocin-treated animals, significant increases in fundic and antral somatostatin content were observed only when compared on a ng/mg protein basis.

**Effect of streptozotocin on gastric somatostatin release.** Basal perfusate somatostatin concentrations in streptozotocin-treated animals were 181 ± 8 pg/ml (25 mg/kg streptozotocin), 178 ± 14 pg/ml (50 mg/kg streptozotocin), and 206 ± 16 pg/ml (75 mg/kg streptozotocin); none of which was significantly different from the controls (170 ± 10 pg/ml).

On the other hand, $5 \times 10^{-9}$ M glucagon evoked a biphasic increase of gastric somatostatin release in all groups, as shown in Figure 1. The peak values of gastric somatostatin in streptozotocin-treated animals were 552 ± 30 pg/ml (25 mg/kg streptozotocin), 626 ± 82 pg/ml (50 mg/kg streptozotocin), and 668 ± 50 pg/ml (75 mg/kg streptozotocin) occurring 3 min after the start of the glucagon infusion, all of which were significantly higher than the controls (400 ± 21 pg/ml).

**FIGURE 1. Gastric somatostatin release from isolated, perfused stomach in response to $5 \times 10^{-9}$ M glucagon in control (□) and 25-mg/kg (■), 50-mg/kg (○), and 75-mg/kg (●) streptozotocin-treated rats. All values are the mean ± SEM. *P < 0.05 vs. controls.**




## DISCUSSION

In the present study, it has been clearly demonstrated that plasma glucose and glucagon levels are increased while body weight and plasma insulin are decreased with increasing doses of streptozotocin. These changes of body weight, plasma glucose, insulin, and glucagon in streptozotocin-treated rats are in accord with previous observations.[15–18] Rats with varying degrees of hypoinsulinemia were thus obtained by adjusting the dose of streptozotocin.

The primary aim of the present experiment was to investigate changes in both content and release of gastric somatostatin in rats made hypoinsulinemic by graded doses of streptozotocin. Like the pancreatic somatostatin content, the gastric somatostatin content has already been reported to be increased in streptozotocin-treated diabetic rats.[1] In contrast, there have been no available data on the release of gastric somatostatin in streptozotocin-treated rats. In addition to the increase of gastric somatostatin content in both the fundus and antrum, the present experiment clearly shows a remarkable increase in gastric somatostatin release in streptozotocin-treated rats when stimulated by glucagon. The fact that the release as well as the content of gastric somatostatin is increased in streptozotocin-treated rats strongly suggests a hyperfunctioning state of the gastric D-cells in hypoinsulinemic diabetes.

The reason for the increase of gastric somatostatin in streptozotocin-treated rats remains unclear. In the present study, since a significant decrease of plasma insulin level was observed in streptozotocin-treated animals, it is possible that hypoinsulinemia enhances gastric D-cell function. The fact that gastric somatostatin content is decreased in spontaneously diabetic mice with hyperinsulinemia,[21] together with the direct inhibitory effect of insulin on gastric somatostatin release,[6,22] lends support to this possibility. However, the involvement of other factors such as hyperglycemia or the elevated levels of plasma glucagon observed in the present study cannot be ruled out, since both glucose and glucagon are known to stimulate somatostatin release.[5,23,24]

The pathophysiologic role of increased gastric somatostatin in streptozotocin-treated hypoinsulinemic rats is not clear at present. However, since somatostatin is well known to suppress various gastrointestinal functions,[25,26] the gastrointestinal abnormalities found in insulin-deficient diabetes[27] may be due partly to increased gastric somatostatin. Recent studies have suggested that somatostatin inhibits glucose absorption from the intestine[28,29] and glycogenolysis in the liver.[30,31] Therefore, the increase in gastric somatostatin in streptozotocin-treated rats may play a compensatory role, correcting hyperglycemia by reducing glucose absorption or by regulating carbohydrate metabolism.

**ACKNOWLEDGMENTS**

This work was supported in part by grants from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare, Japan.

We wish to thank Dr. D. H. Coy, V.A. Hospital, New Orleans, Louisiana, for a generous gift of N-tyrosyl somatostatin. We are grateful to Prof. H. Imura and Dr. H. Sakurai, Kyoto University School of Medicine, Kyoto, for valuable suggestions.

We also thank A. Toyonaga and S. Yanagihara for technical and secretarial assistance.

**REFERENCES**

[1] Patel, Y. C., Cameron, D. P., Bankier, A., Malaisse-Lagae, F., Ravazzola, M., Studer, P., and Orci, L.: Changes in somatostatin concentration in pancreas and other tissues of streptozotocin diabetic rats. Endocrinology *103*:917–23, 1978.

[2] Orci, L., Baetens, D., Rufener, C., Amherdt, M., Ravazzola, M., Studer, P., Malaisse-Lagae, F., and Unger, R. H.: Hypertrophy and hyperplasia of somatostatin containing D-cells in diabetes. Proc. Natl. Acad. Sci. U.S.A. *73*:1338–42.

[3] Patel, Y. C., Orci, L., Bankier, A., and Cameron, D. P.: Decreased pancreatic somatostatin (SRIF) concentration in spontaneously diabetic mice. Endocrinology 99:1415–18, 1976.

[4] Schusdziarra, V., Harris, V., Conlon, J. M., Arimura, A., and Unger, R. H.: Pancreatic and gastric somatostatin release in response to intragastric and intraduodenal nutrients and HCl in the dog. J. Clin. Invest. *62*:509–18, 1978.

[5] Chiba, T., Seino, Y., Goto, Y., Kadowaki, S., Taminato, T., Abe, H., Kato, Y., Matsukura, S., Nozawa, M., and Imura, H.: Somatostatin release from isolated perfused rat stomach. Biochem. Biophys. Res. Commun. *82*:731–37, 1978.

[6] Chiba, T., Taminato, T., Kadowaki, S., Abe, H., Chihara, K., Matsukura, S., Goto, Y., Seino, Y., and Fujita, T.: Effects of insulin and pancreatic polypeptide on gastric somatostatin release. Diabetes *29*:292–95, 1980.

[7] Kato, Y., Dupre, J., and Beck, J.: Plasma growth hormone in the anesthetized rat. Endocrinology *93*:135–46, 1973.

[8] Hyvärynen, A., and Nikkilä, E. A.: Specific determination of blood glucose with O-toluidine. Clin. Chim. Acta *7*:140–43, 1962.

[9] Arimura, A., Sato, H., Dupont, A., Nishi, N., and Schally, A. V.: Somatostatin: abundance of immunoreactive hormone in rat stomach and pancreas. Science *189*:1007–1009, 1975.

[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin Phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[11] Lefèbvre, P. J., and Luyckx, A. S.: Factors controlling gastric-glucagon release. J. Clin. Invest. *59*:716–22, 1977.

[12] Desbuquois, B., and Aurbach, G. D.: use of polyethylene-glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J. Clin. Endocrinol. Metab. *33*:732–38, 1971.

[13] Sakurai, H., Kuzuya, H., Kurahachi, H., Fukase, M., and Imura, H.: A sensitive radioimmunoassay for glucagon using talc method. Jpn. J. Nucl. Med. *10*:135–36, 1973.

[14] Steele, R. G. D., and Torrie, J. H.: *In* Principles and Procedures of Statistics. New York, McGraw-Hill, 1966, p. 107.

[15] Junod, A., Lambert, A. E., Orci, L., Pectet, R., Gonet, A. E., and Renold, A. E.: Studies of the diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. *126*:201–205, 1967.

[16] Junod, A., Lambert, A. E., Stauffacher, W., and Renold, A. E.: Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J. Clin. Invest. *48*:2129–39, 1969.

[17] Matschinsky, F. M., Pagliara, A. S., Hover, B. A., Page, C. S., Ferrendelli, J. A., and Williams, A.: Hormone secretion and glucose metabolism in islets of Langerhans of isolated perfused pancreas from normal and streptozotocin diabetic rats. J. Biol. Chem. *251*:6053–61, 1976.

[18] Pagliara, A. S., Stillings, S. N., Haymond, M. W., Hover, B. A., and Matschinsky, F. M.: Insulin and glucose as modulators of the amino acid-induced glucagon release in the isolated pancreas of alloxan and streptozotocin diabetic rats. J. Clin. Invest. *55*:244–55, 1975.

[19] Hara, M., Patton, G., and Gerich, J.: Increased somatostatin release from pancreases of alloxan diabetic rats perfused in vitro. Life Sci. *24*:625–28, 1979.

[20] Schusdziarra, V., Rouiller, D., Harris, V., Conlon, J. M., and Unger, R. H.: The response of plasma somatostatin-like immunoreactivity to nutrients in normal and alloxan diabetic dogs. Endocrinology *103*:2264–73, 1978.

[21] Patel, Y. C., Cameron, D. P., Stefan, Y., Malaisse-Lagae, F., and Orci, L.: Somatostatin: widespread abnormality in tissues of spontaneously diabetic mice. Science *198*:930–31, 1977.

[22] Rouiller, D., Schusdziarra, V., and Unger, R. H.: Effect of insulin upon pancreatic and gastric release of somatostatin-like immunoreactivity (SLI). Diabetes *28* (Suppl.):352, 1979.

[23] Patton, G. S., Ipp, E., Dobbs, R. E., Orci, L., Vale, W., and Unger, R. H.: Pancreatic immunoreactive somatostatin release. Proc. Natl. Acad. Sci. U.S.A. *74*:2140–43, 1977.

[24] Honey, R. N., Schwarz, J. A., Mathe, C. J., and Weir, G. C.: Insulin, glucagon, and somatostatin secretion from isolated perfused rat and chicken pancreas-duodenum. Am. J. Physiol. *238*:E150–56, 1980.

[25] Bloom, S. R., Mortimer, C. H., Thorner, M. O., Besser, G. M., Hall, R., Gomez-Pan, A., Roy, V. M., Russel, R. C. G., Coy, D. H., Kastin, A. J., and Schally, A. V.: Inhibition of gastrin and gastric acid secretion by growth hormone release inhibiting hormone. Lancet *2*:1106–1109, 1974.

[26] Boden, G., Sivitz, M. C., and Owen, O. E.: Somatostatin suppresses secretin and pancreatic exocrine secretion. Science *190*:163–65, 1975.

[27] Scapello, J. H. B., and Sladen, G. E.: Diabetes and the gut. Gut *19*:1153–62, 1978.

[28] Wahren, J., and Felig, P.: Influence of somatostatin on carbohydrate disposal and absorption in diabetes mellitus. Lancet *2*:1213–16, 1976.

[29] Unger, R. H., Ipp, E., Schusdziarra, V., and Orci, L.: Hypothesis: physiological role of pancreatic somatostatin and the contribution of D-cell disorders to diabetes mellitus. Life Sci. *20*:2081–86, 1977.

[30] Oliver, J. R., and Wagle, S. R.: Studies on the inhibition of insulin release, glucogenolysis and gluconeogenesis by somatostatin in the islets of Langerhans and isolated hepatocytes. Biochem. Biophys. Res. Commun. *62*:772–77, 1975.

[31] Sacks, H., Waligora, K., Mattews, J., and Pimstone, B.: Inhibition by somatostatin on glucagon-induced glucose release from isolated perfused rat liver. Endocrinology *101*:1751–59, 1977.