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TANIGUCHI, Hiroshi

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A ROLE OF ENDOGENOUS SOMATOSTATIN AND GLUCAGON IN INSULIN RELEASE FROM PANCREATIC ISLETS

Hiroshi TANIGUCHI, Keiji MURAKAMI, Akimitsu TSUTOU, Masahiro TAMAGAWA, Kazushige EJIRI, Masako YOSHIOKA, Tetsuo KOBAYASHI, Katsumi TERASHI, Noboru KOBAYASHI, Masafumi UTSUMI, Soichiro MORITA, and Shigeaki BABA

2nd Department of Internal Medicine
Kobe University School of Medicine

INDEXING WORDS

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SYNOPSIS

The present investigation was aimed to clarify whether somatostatin and glucagon secreted from islets influence insulin release. Islets isolated from Wistar male rats were incubated with somatostatin antiserum or glucagon antiserum produced in rabbits for 60 min in various concentration of glucose. In the control study the antiserum was replaced by their corresponding normal rabbit serum. At 3.3 and 8.3mM glucose insulin release from islets treated with somatostatin antiserum and glucagon antiserum was enhanced and suppressed, respectively, compared with islets treated with their corresponding normal rabbit serum. However, at 16.7mM glucose it was not different from the control. These observations suggest that endogenous somatostatin and glucagon might be involved in the regulation of insulin release by the suppressive and potentiating action at supposedly physiologic glucose concentration, respectively, whereas at non-physiologically high glucose concentration their regulation of insulin release would be out of order. Both endogenous somatostatin and glucagon, thus, would play a physiologic role in insulin release.

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Authors' names in Japanese : 谷口 洋 村上啓治 傅 秋光 玉川正博 江尻一成 吉岡正子 小林哲雄 寺師克洋 小林 登 内海正文 森田聰一郎 馬場茂明

INTRODUCTION

It is well known that islets contain and release insulin, glucagon and somatostatin and has been thought that insulin secretion from B cells might be influenced by the somatostatin and glucagon released from the neighbouring cells in the islets, because exogenously administered somatostatin and glucagon suppresses and enhances insulin release, respectively.[24] However, there have been very few reports on the role of endogenous somatostatin and glucagon of the islets in insulin release,[6, 9, 13, 19, 20, 22, 23] and those observations have not been in accordance. The present study was, therefore, carried out to elucidate their role by passive immunization using specific antisera.

MATERIALS AND METHODS

Antisera to cyclized somatostatin (Protein Research Foundation, Osaka, Japan) was produced in rabbits according to the method of Arimura, Sato, Coy, and Schally[2] in our laboratory and porcine glucagon antiserum generated in rabbits were a generous gift from Dr. Y. Kohga, Shimizu Pharmaceutical Co., Ltd., Shizuoka, Japan. The somatostatin antiserum used in the present investigation showed its binding rate with ^{125}I-tyr^{1}-somatostatin of 48% at 1:200 dilution and the glucagon antiserum possessed that with ^{125}I-glucagon of 72% at 1:24,000 dilution by radioimmunoassay. The somatostatin antiserum cross-reacted with an excess concentration of naturally occuring peptides in the islets like rat insulin, porcine glucagon, vasoactive intestinal polypeptide and TRH less than 10^{-3}%, while the glucagon antiserum cross-reacted with an excess concentration of rat insulin, porcine proinsulin, porcine C-peptide, cyclized somatostatin, vasoactive intestinal polypeptide, TRH and human pancreatic polypeptide less than 10^{-3}% as well.

Pancreatic islets were isolated with collagenase (Boehringer Mannheim Corp. Ltd., Germany) according to the method of Lacy and Kostianovsky[10] from Wistar male rats, 250-270g in body weight, fed ad libitum. Krebs-Henseleit bicarbonate buffer adjusted to pH 7.4 (KHBB) containing 3.3mM glucose was injected into the pancreatic duct instead of Hanks' solution for disruption of the acinar tissue.

Islets were incubated in KHBB containing 0.5% bovine serum albumin (Fraction V, Armour Pharmaceutical Co., U.S.A.) and 3.3mM glucose. Thereafter, 10 islets were transferred to a vessel containing a total volume

of 0.7ml, i.e., 0.6ml of standard medium supplemented with 3.3, 8.3 or 16.7mM glucose as final concentration corrected from the glucose content in the serum and 0.1ml of antiserum. The standard medium consisted of KHBB, 0.5% bovine serum albumin and 1,000KIU/ml aprotinin (Bayer, Germany) as final concentration. The last incubation was performed for 60 min at the same condition as in the first under a gas phase of 95% O_2-5% CO_2 at 37°C in an incubator (Taiyo incubator M-1^N type, Taiyo Kagakukogyo Co., Japan) at 80 strokes per min. For the control study normal rabbit serum was applied instead of the antiserum but otherwise the incubation procedure was similar to that in the experimental study.

Insulin released in the medium was radioimmunoassayed [12] with rat insulin (Novo Industri A/S, Denmark) as standard.

For the detection of free hormones in the medium after 60 min incubation with rabbit sera, an aliquot of medium was mixed with an equal volume of 25% polyethylene glycol and kept at 4°C for 45 min. It was centrifuged at 2,000g at 4° C for 45 min, and the supernatant was subjected to the radioimmunoassay for somatostatin [26] and glucagon.[1]

Hormones in the medium were expressed as ng insulin, pg somatostatin or pg glucagon/islet·60 min and in some experiments as per cent against the control value. The data were shown as mean ± S.E.M. and analysed with Student's t-test.

RESULTS

Effect of dilution of antisera on insulin release

The somatostatin antiserum and its control normal serum were diluted for application to incubation medium to 1/7, 1/35 and 1/70 time their original concentration using the standard medium containing 8.3mM glucose. The antiserum-treated islets released much insulin as compared to the normal serum-treated groups, and as both sera were diluted, the enhancement of insulin release by the antiserum treatment became little; the mean ratio of the increase against the control value was 210 ± 6.1 (n=4, $p < 0.001$), 132.7 ± 3.1 (n=4, $p < 0.001$) and 113.0 ± 6.6% (n=4, n.s.) at 1/7, 1/35 and 1/70 time their original concentration in 8.3mM glucose, respectively.

The glucagon antiserum and its control normal rabbit serum were similarly diluted to 1/7 and 1/70 time the original concentration with the standard medium containing 3.3mM glucose. As the sera were diluted, the potency of the antiserum to reduce insulin release decreased against that of the corresponding normal rabbit serum; the decrease against the control

value was 70.0 ± 6.9 (n=5, $p<0.005$) and 91.1 ± 4.2% (n=2, n.s.) at 1/7 and 1/70 time their original concentration in 8.3mM glucose.

The enhancement and the suppression of insulin release by somatostatin and glucagon antiserum was most remarkable at 1/7 time the original concentration, respectively. Therefore, the 1/7 concentration of both original antisera and their control normal sera was employed for the subsequent studies.

Insulin release by somatostatin antiserum treatment

Insulin release from the islets treated by somatostatin antiserum was 2.39 ± 0.35 (n=4), 7.59 ± 0.64 (n=4) and 8.75 ± 1.21ng/islet·60 min (n=4) at 3.3, 8.3 and 16.7mM glucose, respectively, while that by the corresponding normal serum was 1.07 ± 0.18 (n=4), 3.88 ± 0.20 (n=4) and 7.85 ± 0.72ng/islet·60 min (n=4) at these glucose concentrations, respectively.

In comparison with the control groups, insulin release by the somatostatin antiserum was enhanced by 229.1 ± 24.7 (n=4, $p<0.005$) and 262.5 ± 14.7% (n=4, $p<0.001$) at 3.3 and 8.3mM glucose, respectively, whereas no significant difference of insulin release between the two treatments was observed at 16.7mM glucose. The ratios of increment in insulin release at 3.3 and 8.3mM glucose were remarkably higher than the ratio at 16.7mM glucose, i.e., 111.4 ± 11.4% (n=4) of the control (Fig. 1).

Free somatostatin in medium released from islets treated by somatostatin antiserum

Free somatostatin in the medium after incubation with normal rabbit serum was found at three different glucose concentrations utilized and the value was 15.00 ± 0.78 (n=2), 15.97 ± 1.05 (n=4) and 19.89 ± 1.63ng/islet·60 min (n=4) at 3.3, 8.3 and 16.7mM glucose, respectively. These values were not different among them significantly. In contrast, free somatostatin was not detected in the somatostatin antiserum-containing medium after 60 min incubation (Fig. 2).

Insulin release by glucagon antiserum treatment

At 3.3, 8.3 and 16.7mM glucose, insulin release from the islets treated by glucagon antiserum was 0.64 ± 0.03 (n=5), 2.40 ± 0.28 (n=5) and 7.45 ± 0.93ng/islet·60 min (n=5), respectively, while that by normal rabbit serum was 1.16 ± 0.13 (n=5), 3.42 ± 0.23 (n=5) and 7.34 ± 1.06ng/islet·60 min (n=5), respectively.

Insulin release was, thus, suppressed to 57.0 ± 5.6 (n=5, $p<0.001$)

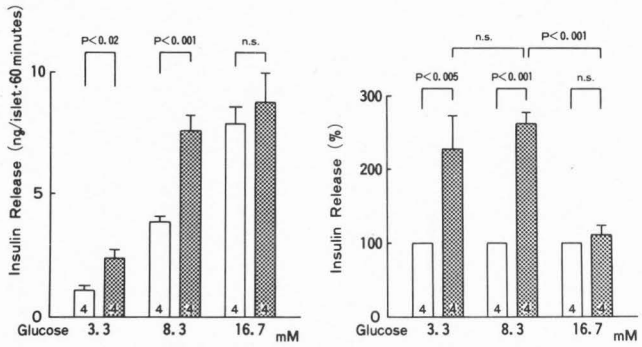

Fig. 1 Insulin release from islets treated with somatostatin antiserum. Adult rat islets were incubated in Krebs-Henseleit bicarbonate buffer containing 3.3, 8.3 and 16.7mM glucose and 1/7 time the original somatostatin antiserum produced in rabbits for 60 min (dotted column). In the control the antiserum was replaced by the normal rabbit serum (open column). In the left panel insulin released from an islet per 60 min is shown against different glucose concentrations in the medium and in the right panel the ratio of insulin release against the control at each glucose concentration is indicated as per cent. The vertical column represents the mean and the vertical bar S.E.M. The number of experiments is shown at the bottom of each column. n.s.: not significant.

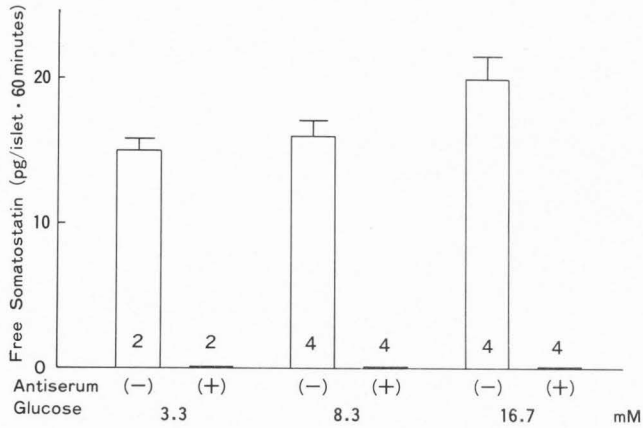

Fig. 2 Free somatostatin in medium after incubation of islets with somatostatin antiserum. Adult rat islets were incubated as mentioned in Fig. 1. An aliquot of the medium after the incubation was mixed with an equal volume of 25% polyethylene glycol and then centrifuged at 2,000g for 45 min. Actual value of somatostatin is indicated on the ordinate. The vertical column represents the mean and the vertical bar S.E.M. The number of experiments is expressed over the abscissa.

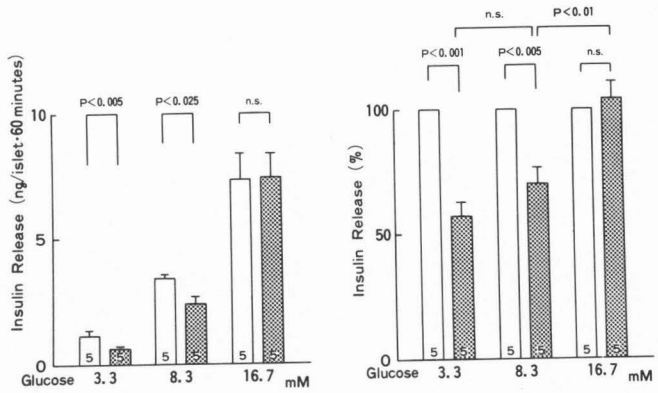

Fig. 3 Insulin release from islets treated with glucagon antiserum. Adult rat islets were incubated in Krebs-Henseleit bicarbonate buffer containing 3.3, 8.3 and 16.7mM glucose and 1/7 time the original glucagon antiserum produced in rabbits for 60 min (dotted column). In the control the antiserum was replaced by the normal rabbit serum (open column). The actual value of insulin released is shown in the left panel and the ratio of insulin release against the control at each glucose concentration is indicated as per cent in the right panel. The vertical column represents the mean and the vertical bar S.E.M. The number of experiments is shown at the bottom of each column.
n.s.: not significant.

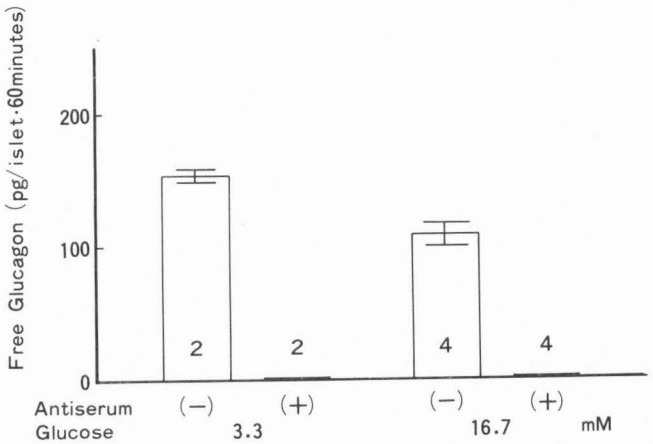

Fig. 4 Free glucagon in medium after incubation of islets with glucagon antiserum. Adult rat islets were incubated as described in Fig. 3 and free glucagon was obtained using polyethylene glycol as explained in Fig. 2. The actual value of free glucagon is indicated on the ordinate. The vertical column signifies the mean and the vertical bar S.E.M. The number of experiments is expressed over the abscissa.

and 70.0 ± 6.5% (n=5, $p<0.005$) of the control at 3.3 and 8.3mM glucose, respectively. These ratios in insulin release were significantly lower than the ratio at 16.7mM glucose, i.e., 104.2 ± 6.9% (n=5) of the control. There was no difference between both treatments at 16.7mM glucose (Fig. 3).

Free glucagon in medium released from islets treated by glucagon antiserum

Free glucagon in the medium after 60 min incubation with glucagon antiserum was not found at both 3.3 and 16.7mM glucose, but that in the control medium was 135.5 ± 4.7 (n=2) and 97.0 ± 8.1pg/islet·60 min (n=4) at 3.3 and 16.7mM glucose, respectively. In the control medium the glucagon level at 3.3mM glucose was significantly higher than that at 16.7mM glucose (Fig. 4).

DISCUSSION

The physiologic significance of somatostatin and glucagon in hormone release has been emphasized by numerous studies. It is based largely on pharmacological investigations with the exogenous application of somatostatin[15] and glucagon,[11] which indicate that in the pancreas somatostatin suppresses and glucagon enhances insulin release. Recently, however, using the method of passive immunization, their physiologic role has been strongly recognized. In the hypothalamopituitary system secretion of growth hormone and thyroid stimulating hormone is enhanced at the basal level as well as under stress conditions after the treatment of somatostatin antiserum.[3, 4, 8]

In the pancreas the present investigations revealed that in the lack of endogenous glucagon and somatostatin in the milieu of islets insulin release was decreased and increased at supposedly physiologic range of glucose concentration, respectively, while it was not influenced at non-physiologically high glucose concentration. These observations would signify that endogenous glucagon and somatostatin stimulates and inhibits insulin release at physiologic glucose concentration, respectively.

The reduction of insulin release by the treatment of glucagon antiserum in the present study is not in accord with the report by Galbo et al.[9] indicating that no change of plasma insulin concentration in rats, but coincides with our previous findings in vivo[20] and those in vitro by Barden et al.[6] The present results with somatostatin antiserum do not agree with the earlier observations in vivo[7, 17, 19, 23] and in vitro[6] of unchanged insulin release, but supported our previous studies,[13, 22]

although our earlier results were obtained by the prior treatment of islets with somatostatin antiserum for the elimination of possible contamination of various factors affecting insulin release directly. These discrepancies might be caused by the different character of the antisera employed and their application methods, but the precise explanation remains to be elucidated.

The suppression of insulin release by endogenous somatostatin would be induced not only through its direct action on B cells but also through its inhibition of endogenous glucagon release, since exogenously applied glucagon potentiates insulin release. [11] In the meanwhile, the enhancement of insulin release by endogenous glucagon would not physiologically be caused through the increase of somatostatin secretion by glucagon in islets, because exogenous administration of somatostatin rather inhibits than elevates insulin release.[15] Besides, the blood stream in the rat islets directs from the periphery, in which A and D cells are chiefly located, to the center of the islet, in which B cells are abundant.[14] The present observations, therefore, would partly be explained by a local influence of A and D cells onto B cells along the direction of the blood stream.

Alternate possible control mechanism, i.e., paracrine regulation of insulin release by endogenous somatostatin and glucagon has been considered electron microscopically. [15, 24] Intercellular direct communications have been documented in a variety of tissues [18] including the islets[15, 24] through the gap junction. Furthermore, the tight junction is found in the islet, in which the outer leaflets of adjacent membranes converge to create a single-fused structure, resulting in compartments of the intercellular spaces. In these compartments hormone concentration seems to be extremely high. These structures represent a morphological basis for paracrine regulation. To date it is not possible to discriminate modes of action of a secreted hormone on the adjoining cells or cells nearby in the islet via local blood vessels, via gap junctions or via intercellular compartments formed by tight junctions.

In the present study using the normal rabbit serum, insulin release increased and glucagon release decreased, as glucose concentration was raised. This was concordant with the earlier observations that high concentration of glucose stimulates insulin release and suppresses glucagon release. [11, 25] However, the somatostatin release was not enhanced by the elevation of glucose concentration. It was not in accord with the findings by Schauder, McIntosch, Arends, Arnold, Frerichs and Creutzfeldt, [16] but compatible with the reports by Barden, Alvarado-Urbina, Côté and

Dupont[5] and Weir, Samols, Loo, Patel and Gabbay.[27] Irrespective of such argument, D cells could be regarded to be less sensitive to glucose than B cells in short-term incubation studies.[21]

From these considerations, therefore, non-physiologically high glucose concentration must have overcome the suppressive action of endogenous somatostatin, which would be functioning at lower glucose concentration, to make no difference in insulin release between the presence and the absence of endogenous somatostatin, since somatostatin level was consistent among various glucose concentrations employed. In the meanwhile, the level of glucagon suppressed by high glucose concentration would be so low that it would not be significant in the local regulation of insulin release, and, thus, insulin release would be similarly enhanced irrespective of the treatment with glucagon antiserum at non-physiologically high glucose concentration.

In summary, in the physiologic range of glucose concentration endogenous glucagon might be involved in the regulation of insulin release by its potentiating action, while endogenous somatostatin by its suppressive influence. However, at non-physiologically high glucose concentration such regulation would be out of order. Both endogenous glucagon and somatostatin, thus, might play a physiologic role in insulin release in islets.

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REFERENCES

1. Aguilar-Parada, E., Eisentraut, A.M., and Unger, R.H. Am. J. Med. Sci. 1969. 257. 415/419. Pancreatic glucagon secretion in normal and diabetic subjects.
2. Arimura, A., Sato, H., Coy, D.H., and Schally, A.V. Proc. Soc. Exp. Biol. Med. 1975. 148. 784/789. Radioimmunoassay for GH-release inhibiting hormone.
3. Arimura, A., and Schally, A.V. Endocrinology 1976. 98. 1069/1073. Increase in basal and thyrotropin-releasing hormone (TRH)-stimulated secretion of thyrotropin (TSH) by passive immunization with antiserum

to somatostatin in rats.

4. Arimura, A., Smith, W.D., and Schally, A.V. Endocrinology 1976. 98. 540/543. Blockade of the stress-induced decrease in blood GH by anti-somatostatin serum in rats.
5. Barden, N., Alvarado-Urbina, G., Côté, J.-P., and Dupont, A. Biochem. Biophys. Res. Commun. 1976. 71. 840/844. Cyclic AMP-dependent stimulation of somatostatin secretion by isolated rat islets of Langerhans.
6. Barden, N., Lavoie, M., Dupont, A., Côté, J., and Côté, J.-P. Endocrinology 1977. 101. 635/638. Stimulation of glucagon release by addition of somatostatin serum to islets of Langerhans in vitro.
7. Brazeau, P., Tannenbaum, G.S., Epelbaum, J., Colle, E., and Martin, J.D. Metabolism 1978. 27 (Suppl. 1). 1263/1267. Dissociation of effects of somatostatin antiserum on growth hormone and insulin secretion.
8. Ferland, L., Labrie, F., Jobin, M., Arimura, A., and Schally, A.V. Biochem. Biophys. Res. Commun. 1976. 68. 149/156. Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion.
9. Galbo, H., Richter, E.A., Holst, J.J., and Christensen, N.J. Pfluegers Arch. 1977. 369. 21/25. Lack of influence of glucagon on glucose homeostasis after prolonged exercise in rats.
10. Lacy, P.E., and Kostianovsky, M. Diabetes 1967. 16. 35/39. Methods for the isolation of intact islets of Langerhans from the rat pancreas.
11. Malaisse, W.J. In : Steiner, D.F., and Freinkel, N., ed., Handbook of Physiology Section 7 : Endocrinology Volume 1 : Endocrine Pancreas (Washington, D.C., American Physiological Society). 1972. 237/260. Hormonal and environmental modification of islet activity.
12. Morgan, C.R., and Lazarow, A. Diabetes 1963. 12. 115/126. Immunoassay of insulin. Two antibody system, plasma insulin levels of normal, subdiabetic and diabetic rats.
13. Murakami, K., Taniguchi, H., Hasegawa, M., Kobayashi, T., Watanabe, Y., Seki, M., Tsutou, A., Kawaguchi, A., Utsumi, M., Makimura, H., Sakoda, M., and Baba, S. Folia Endocrinol. Jap. 1977. 53. 1140/1147. The physiological role of somatostatin in insulin release - Enhanced insulin release from rat islets pre-treated with anti-somatostation serum -.
14. Ohtani, O., and Fujita, T. Biomedical Research 1980. 1. 130/140. Microcirculation of the pancreas with special reference to periductular circulation. A scanning electron microscope study of vascular casts.

15. Schauder, P. In : Creutzfeldt, W., ed., The Entero-Insular Axis (Basel, S. Karger). 1980. 52/64. Somatostatin - Endocrine or paracrine substance?
16. Schauder, P., McIntosch, C., Arends, J., Arnold, R., Frerichs, H., and Creutzfeldt, W. FEBS Lett. 1976. 68. 225/227. Somatostatin and insulin release from isolated rat pancreatic islets stimulated by glucose.
17. Schusdziarra, V. and Rouiller, D., Arimura, A., and Unger, R.H. Endocrinology 1978. 103. 1956/1959. Antisomatostatin serum increases levels of hormones from the pituitary and gut, but not from the pancreas.
18. Staehelin, L.A. Int. Rev. Cytol. 1974. 39. 191/283. Structure and function of intercellular junctions.
19. Steiner, R.A., Stewart, J.K., Barber, J., Koerker, B., Goodner, C.J., Brown, A., Illner, P., and Gale, C.C. Endocrinology 1978. 102. 1587/1594. Somatostatin : A physiological role in the regulation of growth hormone secretion in the adolescent male baboon.
20. Taniguchi, H., Ejiri, K., Tamagawa, M., Yoshioka, M., Tsutou, A., Seki, M., Murakami, K., Kobayashi, T., Utsumi, M., Kawaguchi, A., Morita, S., and Baba, S. Proceedings of the Symposium on Chemical Physiology and Pathology 1978. 18. 87/93. Studies on the cellular interrelationship in the islet of Langerhans (2) - Physiologic role of somatostatin and glucagon in insulin release.
21. Taniguchi, H., Utsumi, M., and Baba, S. In : Creutzfeldt, W., ed., The Entero-Insular Axis (Basel, S. Karger). 1980. 302/303. D cells are less sensitive to glucose than B cells in hormone release.
22. Taniguchi, H., Utsumi, M., Hasegawa, M., Kobayashi, T., Watanabe, Y., Murakami, K., Seki, M., Tsutou, A., Makimura, H., Sakoda, M., and Baba, S. Diabetes 1977. 26. 700/702. Physiologic role of somatostatin. Insulin release from rat islets treated by somatostatin antiserum.
23. Tannenbaum, G.S., Epelbaum, J., Colle, E., Brazeau, P., and Martin, J.B. Endocrinology 1978. 102. 1909/1914. Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion.
24. Unger, R.H., Dobbs, R.E., and Orci, L. Annu. Rev. Physiol. 1978. 40. 307/343. Insulin, glucagon, and somatostatin secretion in the regulation of metabolism.
25. Unger, R.H., and Lefebre, P.J. In : Lefebre, P.J., and Unger, R.H.,

ed., Glucagon. Molecular Physiology, Clinical and Therapeutic Implications (Oxford, Pergamon Press). 1972. 213/244. Glucagon physiology.

26. Utsumi, M., Makimura, H., Sakoda, M., and Baba, S. Folia Endocrinol. Jap. 1977. 53. 221/234. Radioimmunoassay for somatostatin.
27. Weir, G.C., Samols, E., Loo, S., Patel, Y.C., and Gabbay, K.H. Diabetes 1979. 28. 35/40. Somatostatin and pancreatic polypeptide secretion. Effects of glucagon, insulin, and arginine.