Somatostatin-Containing Cells in the Rat and Horse Pancreatic Islets

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ITO, S., YAMADA, Y., HAYASHI, M. and MATSUBARA, Y. *Somatostatin-Containing Cells in the Rat and Horse Pancreatic Islets*. Tohoku J. exp. Med., 1978, 124 (1), 57-64 — Somatostatin-, glucagon- and insulin-containing cells in the rat and horse pancreatic islets were investigated by an indirect immunofluorescent technique using antibodies to insulin, glucagon and somatostatin. In the rat pancreatic islets, insulin-containing cells were located centrally, and glucagon- and somatostatin- or somatostatin-like substance (SLS)-containing cells were peripherally disposed and glucagon-containing cells were situated more peripherally as compared with distribution of somatostatin-containing cells. On the other hand, in the horse pancreatic islets, insulin-containing cells were found in the peripheral area and glucagon- and somatostatin-containing cells were distributed centrally. Insulin-containing cells were most numerous among the three types of cells in the islets of both the rat and horse. Somatostatin- or SLS-containing cells in the horse were about equal in number to the glucagon-containing cells while those in the rat seemed to be smaller in number than the glucagon-containing cells. The present study clearly demonstrates that the somatostatin- and glucagon-containing cells are located, in relation to the bloodstream, on the upstream side of the insulin-containing cells in the islets of Langerhans of both the rat and the horse though these two animal species are known to have completely reverse bloodstream pattern in the islets. According to the mechanism of glucagon and insulin secretions from the islets, we proposed the hypothesis that the somatostatin-containing cells regulate the neighbouring glucagon- and insulin-containing cells by an intercellular transport mechanism which provides somatostatin through the junctional complex, while other insulin-containing cells which are too remote from the somatostatin-containing cells to be affected by such a mechanism are regulated by a fraction of somatostatin released and carried down by the bloodstream. — somatostatin; glucagon; insulin; local hormone; immunofluorescence

It has been shown that somatostatin, first extracted from the sheep hypothalamus (Brazeau et al. 1973) and later synthesized (Brazeau et al. 1974), inhibits the secretory functions of various cells containing insulin (Alberti et al. 1973; Curry et al. 1974; Gerich et al. 1974a), glucagon (Gerich et al. 1974b; Iversen 1974), gastrin (Bloom et al. 1974), pepsin and hydrochloric acid (Barros D'Sa et al. 1975; Gometz-Pan et al. 1975) as well as that of pituitary cells secreting growth hormone

(Brazeau et al. 1974; Parker et al. 1974). Concerning the distribution of somatostatin-containing cells, it is reported that these cells are located not only in the hypothalamic region (Hökfelt et al. 1974; Pelletier et al. 1974) but also in the pancreatic islets (Luft et al. 1974; Dubois 1975; Polak et al. 1975), gastrointestinal tract (Polak et al. 1975; Rufener et al. 1975; Ito et al. 1977), thyroid (Hökfelt et al. 1975), etc. In view of the results of these studies of the physiological effects of somatostatin and the distribution of somatostatin-containing cells, it seems clear that the somatostatin-containing cells are distributed near secretory cells containing peptide hormones or other chemical substances sensitive to the inhibitory action of somatostatin, though it is not yet sure whether somatostatin is a local hormone which is released by the somatostatin-containing cells to regulate locally the secretory activities of nearby target cells.

If somatostatin is really such a local inhibitor of secretion, it would be more natural that the somatostatin-containing cells are located on the upstream side of the blood flow in relation to the secretory cells containing peptide hormones or other chemical substances that were inhibited by somatostatin since the somatostatin released could then be transported down to the secretory cells.

Among the tissues with a known distribution of somatostatin-containing cells, the islets of Langerhans have been widely studied in relation to their pattern of blood circulation. Particularly, it has been found that the rat and horse are in an entirely reversed relation to each other with respect to the process of blood circulation in the islets (Fujita 1973, 1976). That is, with the rat, blood is first supplied to the peripheral area of the islets, it flows toward their centers and then leaves them; with the horse, the blood stream in the islets starts at their centers, and the blood then reaches the peripheral area and leaves the islets. Therefore, in discussing whether somatostatin is a local inhibitor based on the hypothesis that the secretory cells located upstream of the blood circulation regulate downstream cells, it seems very meaningful to compare the positional relation between the somatostatin-containing cells and insulin- or glucagon-containing cells, which are likely to be inhibited by the former cells, in the islets of the rat and the horse.

This paper reports a study of the distribution of the aforementioned three types of secretory cells in the islets of Langerhans of the rat and horse as revealed by immunofluorescence techniques using antibodies to insulin, glucagon and somatostatin. The results of our study are discussed to see whether or not somatostatin is a local hormone.

MATERIALS AND METHODS

Antisera

Anti-somatostatin antisera: Synthetic somatostatin of a cyclic form supplied by the Peptide Institute Research Foundation, Osaka, Japan, was coupled to bovine serum albumin (BSA, Sigma) using the one step glutaraldehyde method (Ito and Kobayashi 1976). The somatostatin-BSA conjugate thus prepared was emulsified with complete Freund's adjuvant (Difco) and subcutaneously injected monthly into four rabbits. The antisera used in the present study were prepared after six boosters.

Anti-glucagon antisera: The specific antibody to pancreatic glucagon, G-8, was employed in the present study (Ito et al. 1975).

Anti-insulin antisera: Subcutaneous injections of monocomponent insulin (Novo Institute, Denmark) with complete Freund's adjuvant were made monthly in six guinea pigs for four months. After the final booster, serum was pooled and used in the present study.

Tissue

Pieces of the pancreas were obtained from adult male rats and horse. They were fixed in Bouin's fluid for two hours at room temperature. They were then dehydrated through use of a graded alcohol series and embedded in paraffin (M.P. 42°C). Sections of 6 μ m thickness were applied on glass slides coated with a mixture of 0.5% gelatin and 0.05% chromium potassium sulfate.

Immunofluorescence

Sections deparaffinized in xylene and then treated with a descending series of alcohol solution were treated with one of the three kinds of antibodies (anti-insulin, anti-glucagon and anti-somatostatin) in a final dilution of 1:20 in a 0.01 M phosphate saline buffer (pH 7.4) at 37°C for 90 min. Then they were allowed to react with anti-rabbit IgG-FITC or anti-guinea pig IgG-FITC at 37°C for 40 min.

Control study

a) The sections were treated with one of the following three kinds of antibodies; anti-insulin, anti-glucagon and anti-somatostatin. Each of the antibodies had been preincubated with four antigens comprising the excess amounts of synthetic somatostatin, pancreatic glucagon, monocomponent insulin and gastrin. Then they were allowed to react with anti-rabbit IgG-FITC or anti-guinea pig IgG-FITC.

b) The sections were treated with normal rabbit serum and then combined with anti-rabbit IgG-FITC. In order to confirm the reactive specificity of the insulin-containing cells, the sections were incubated with normal guinea pig serum and allowed to react with anti-guinea pig IgG-FITC.

c) The sections were also incubated with one of the three kinds of antibodies or FITC-conjugated IgG serum alone.

RESULTS

The immunofluorescent reactions to the three kinds of antibodies comprising the anti-insulin, anti-glucagon and anti-somatostatin antisera were located in the pancreatic islets. This finding, therefore, clarified that insulin, glucagon and somatostatin or somatostatin-like substance (SLS) are present in the pancreatic islets of both the rat and the horse. The immunofluorescent reactions were homogeneously demonstrated in the cytoplasm but not in the nucleus of these cells.

In the rat pancreatic islets, insulin-containing cells were located centrally and glucagon- and somatostatin- or SLS-containing cells were peripherally disposed, as shown in Fig. 1. Furthermore, in many of islets, glucagon-containing cells were situated more peripherally, as compared with somatostatin-containing cells. On the other hand, in the horse pancreatic islets, insulin-containing cells were found in the peripheral area. Glucagon- and somatostatin- or SLS-containing cells were distributed centrally, as seen in Fig. 2. Insulin-containing cells were most numerous

Figs. 1. *Fluorescent photomicrographs* of the rat pancreatic islets stained by the indirect immunofluorescent technique for insulin (a), glucagon (b) and somatostatin (c). Note that specific immunofluorescence for insulin is located centrally while specific immunofluorescence for glucagon and somatostatin is situated peripherally ($\times 900$, reduced to 3/4).

among the three types of cells in the islets of both the rat and horse. Somatostatin- or SLS-containing cells in the horse were about equal in number to the glucagon-containing cells while those in the rat seemed to be smaller in number than the glucagon-containing cells.

The immunofluorescent reactions to the anti-somatostatin antibody were not inhibited by the addition of insulin, glucagon and gastrin, but there was a reaction of somatostatin itself with the anti-somatostatin antibody.

The intensity of the immunofluorescent reactions in the glucagon-containing

Fig. 2. *Fluorescent photomicrographs* of the horse pancreatic islets stained by the indirect immunofluorescent technique for insulin (a), glucagon (b) and somatostatin (c). Both glucagon- and somatostatin-containing cells are located centrally ($\times 640$, reduced to 3/4).

cells stained by the specific antibody to pancreatic glucagon (G-8) was not different from that in cells stained by the antibody (G-8) which had been preincubated with somatostatin and insulin.

Positive cells were not demonstrated in the specimens incubated with normal rabbit serum followed by treatment with anti-rabbit IgG-FITC, or with normal guinea pig serum followed by treatment with anti-guinea pig IgG-FITC. Moreover, positive cells were not revealed in the specimens which had been treated with FITC conjugated IgG or with the diluted anti-somatostatin antibody, anti-glucagon antibody or anti-insulin antibody alone.

DISCUSSION

The present study clearly demonstrates that the somatostatin- and glucagon-containing cells are located, in relation to the bloodstream, on the upstream side of the insulin-containing cells in the islets of Langerhans of both the rat and the horse though these two animal species are known to have completely reverse bloodstream patterns in the islets. Based on the hypothesis that upstream secretory cells regulate downstream cells, it could be stated that the somatostatin- and glucagon-containing cells are situated so that they may regulate the downstream secretory cells containing insulin.

Many scholars (Samols et al. 1965; Dupre et al. 1966; Floyd et al. 1966) have reported that glucagon has the physiological effect of facilitating insulin secretion.

Further, based on special staining techniques, Fujita (1973) found that glucagon-containing cells are located on the upstream side of the insulin-containing cells in the islets of Langerhans in the horse, and he suggested that it is highly probable that glucagon locally regulates insulin secretion in the islets. The present immunohistological study agrees well with the findings by Fujita (1973), supporting his hypothesis concerning the local regulation of insulin secretion by glucagon.

The hypothesis that somatostatin inhibits insulin secretion has been almost confirmed by several recent reports (Alberti et al. 1974; Curry et al. 1974; Gerich et al. 1974a). Further, it has been elucidated by immunohistological studies that somatostatin-containing cells are present in the islets of Langerhans in many animal species, such as man (Dubois 1975), rat (Dubois 1975), mouse (Luft et al. 1975), dog (Rufener et al. 1975), and pigeon (Orci et al. 1975). However, no report indicates that the somatostatin-containing cells are located on the upstream side of the insulin-containing cells. Results of the present study teleologically indicate a high possibility that somatostatin released from the upstream cells is carried to the downstream insulin-containing cells by bloodstream to inhibit locally their secretion just as glucagon is carried to facilitate locally their insulin secretion.

It is now widely admitted that somatostatin inhibits not only insulin secretion but also glucagon secretion (Gerich et al. 1974b; Iversen 1974). However, based only on the hypothesis that the upstream secretory cells regulate the downstream cells, no possibility could be conceived that somatostatin may locally inhibit glucagon secretion since both the somatostatin- and glucagon-containing cells are located on the upstream side of the blood circulation in the islets of both the rat and the horse. Then, how is it possible for somatostatin to inhibit locally glucagon secretion? On this point, Orci and Unger (1975) recently reported the finding, based on electronmicroscopy, that a so-called junctional complex is formed between adjacent cells in the islets of Langerhans and suggested the possibility that different types of hormone-containing cells may regulate each other through this junctional complex. According to this hypothesis, somatostatin could locally regulate glucagon secretion without any intervention by the bloodstream. In addition, the same hypothesis suggests that a partial population of insulin-containing cells located in the borders adjacent to the somatostatin- or glucagon-containing cells may be affected by the regulation mechanism through the junctional complex. In the light of only the hypothesis of Orci and Unger (1975), however, the major population of insulin-containing cells, which are located in the central region of the islets of the rat and in the peripheral region of the horse are remote from the glucagon- or somatostatin-containing cells, could be regulated in no way.

Thus, neither the hypothesis that the somatostatin-containing cells regulate the secretory activities of the glucagon- and insulin-containing cells through the junctional complex nor the one that the upstream somatostatin-containing cells release somatostatin in the bloodstream to be carried down to the insulin-containing cells for their regulation can alone account for the local regulation of glucagon and insulin secretions by somatostatin in the islets as a whole. Only the

combination of both hypotheses offers a complete mechanism for the regulation of glucagon and insulin secretions throughout the islets. That is, the combined hypothesis we propose is that the somatostatin-containing cells regulate the neighboring glucagon- and insulin-containing cells by an intercellular transport mechanism which provides somatostatin through the junctional complex, while other insulin-containing cells, which are too remote from the somatostatin-containing cells to be affected by such a mechanism, are regulated by a fraction of somatostatin released and carried by the bloodstream.

Further quantitative measurements of somatostatin will be necessary for a final conclusion concerning whether or not somatostatin provides a local control mechanism for the insulin and glucagon secretion. As far as our morphological findings suggest, it seems reasonable to assume that somatostatin is produced in the islets of Langerhans as a local hormone to regulate the glucagon and insulin secretions there.

References

- 1) Albrocci, K.G.M.M., Christensen, N.J., Christensen, S.E., Hansen, A.P., Inversen, J., Lundbeak, K., Seyer-Hansen, K. & Orskov, H.H. (1973) Inhibition of insulin secretion by somatostatin. *Lancet*, **2**, 1299-1301.
- 2) Barros D' Sa, A.A.J., Bloom, S.R., & Baron, J.H. (1975) Direct inhibition of gastric acid by growth hormone release inhibiting hormone in dog. *Lancet*, **1**, 886-888.
- 3) Bloom, S.R., Mortimer, C.H., Thorner, M.O., Besser, G.M., Hall, R.R., Gometzpan, A., Roy, V.M., Russell, R.C.G., Coy, D.H., Kastin, A.J. & Schally, A.V. (1974) Growth hormone release inhibiting hormone; inhibition of gastrin and gastric acid secretion. *Lancet*, **2**, 1106-1109.
- 4) Brazeau, P.W., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. & Guillemin, R. (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, **179**, 77-79.
- 5) Brazeau, P., River, J., Vale, W. & Guillemin, R. (1974) Inhibition of growth hormone secretion in the rat by synthetic somatostatin. *Endocrinology*, **94**, 184-187.
- 6) Curry, D.L., Bennet, L.L. & Li, C.H. (1974) Direct inhibition of insulin secretion by synthetic somatostatin. *Biochem. biophys. Res. Commun.*, **58**, 885-889.
- 7) Dubois, M.P. (1975) Presence of immunoreactive somatostatin in discrete cells of the endocrine pancreas. *Proc. nat. Acad. Sci. (Wash.)*, **72**, 1340-1343.
- 8) Dupre, J., Rojas, L., White, L.T., Unger, R.H. & Beck, J.C. (1966) Effects of secretin on insulin and glucagon in portal and peripheral blood in man. *Lancet*, **2**, 26-27.
- 9) Floyd, J.C., Fajians, S.S., Conn, J.W., Knopf, R.F. & Rull, J. (1966) Insulin secretion in response to protein ingestion. *J. clin. Invest.*, **45**, 1479-1486.
- 10) Fujita, T. (1973) Insulo-acinar portal system in the horse pancreas. *Arch. histol. Jap.*, **35**, 161-171.
- 11) Fujita, T., Yanatori, Y. & Murakami, T. (1976) Insulo-acinar axis, its vascular basis and its functional and morphological changes caused by CCK-PZ and caerulein, In: *Endocrine Gut and Pancreas*, edited by T. Fujita, Elsevier Scientific Publishing Company, Amsterdam, pp. 347-357.
- 12) Gerich, J.E., Lorenzi, M., Schnedier, V. & Forsham, P.H. (1974a) Effect of somatostatin on plasma glucose and insulin responses to glucagon and tolbutamide in man. *J. clin. Endocr.*, **39**, 1057-1060.
- 13) Gerich, J.E., Lorenzi, M., Schneider, V., Karam, J.H., Rivier, J., Guillemin, R. & Forsham, P.H. (1974b) Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus; pathophysiologic and therapeutic implications. *New Engl. J. Med.*, **291**, 544-547.

- 14) Gometz-Pan, A., Reed, S.D., Albinus, M., Shaw, B., Hall, R., Besser, G.M., Coy, D.H., Kastin, A.J. & Schally, A.V. (1975) Direct inhibition of gastric acid and pepsin secretion by growth hormone release inhibiting hormone in cats. *Lancet*, **1**, 888-890.
 - 15) Hökfelt, T., Efendic, S., Johansson, O., Luft, R., & Arimura, A. (1974) Immunohistochemical localization of somatostatin (Growth hormone release-inhibiting factor) in the guinea pig brain. *Brain Res*, **80**, 165-169.
 - 16) Hökfelt, T., Efendic, S., Hellerström, C., Johansson, D., Luft, R. & Arimura, A. (1975) Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A-cells of the pancreatic islets and to the hypothalamus. *Acta. endocr. (Kbh.)*, **80**, Suppl. 200, 1-40.
 - 17) Ito, S. & Kobayashi, S. (1976) Immunohistochemical demonstration of glucagon and GLI-containing cells in the canine gut and pancreas. *Arch. histol. jap.*, **39**, 193-202.
 - 18) Ito, S., Suzuki, T. & Sasaki, H. (1975) Immunohistochemical study on glucagon and GLI producing cells in the canine pancreas and gut. In: *International Symposium of GEP.* at Kyoto, edited by T. Fujita, pp. 209-218.
 - 19) Ito, S., Yamada, Y. & Kobayashi, S. (1977) An immunohistochemical demonstration of somatostatin-positive cells in the canine gut. *Tohoku J. exp. Med.*, **121**, 85-90.
 - 20) Iversen, J. (1974) Inhibition of pancreatic glucagon release by somatostatin; In vitro. *Scand. J. clin. Lab. Invest.*, **33**, 125-129.
 - 21) Luft, R., Efendic, S., Hökfelt, T., Johansson, O. & Arimura, A. (1974) Immunohistochemical evidence for the localization of somatostatin-like immunoreactivity in a cell population of the pancreatic islets. *Med. Biol.*, **52**, 428-430.
 - 22) Orci, L., Baetens, D., Dubois, M.P. & Rufener, C. (1975) Evidence for D-cell of the pancreas secreting somatostatin. *Horm. Metab. Res.*, **7**, 400-402.
 - 23) Orci, L. & Unger, R.H. (1975) Functional subdivision of islets of Langerhans and possible role of D cells. *Lancet*, **2**, 1243-1245.
 - 24) Parker, D.C., Rossman, L.G., Siler, T.M., Rivier, J., Yen, S.S.C. & Guillemin, R. (1974) Inhibition of the sleep-related peak in physiologic human growth hormone release by somatostatin. *J. clin. Endocr.*, **38**, 496-499.
 - 25) Pelletier, G., Larbir, F., Arimura, A. & Schally, A.V. (1974) Electronmicroscopic immunohistochemical localization of growth hormone-release inhibiting hormone (somatostatin) in the rat median eminence. *Amer. J. Anat.*, **140**, 445-450.
 - 26) Polak, J.M., Grimelius, L., Pearse, A.G.E., Bloom, S.R. & Arimura, A. (1975) Growth hormone release-inhibiting hormone in the gastrointestinal and pancreatic D-cells. *Lancet*, **1**, 1220-1222.
 - 27) Rufener, C., Dubois, M.P., Malaisse-Large, F. & Orci, L. (1975) Immunofluorescent reactivity to anti-somatostatin in the gastrointestinal mucosa of the dog. *Diabetologia*, **11**, 1-4.
 - 28) Samols, E., Marri, G. & Marks, V. (1965) Promotion of insulin secretion by glucagon. *Lancet*, **2**, 415-417.
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