DYNAMICS OF SOMATOSTATIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS

P. SCHAUDER, C. McINTOSH, U. PANTEN, J. ARENDS, R. ARNOLD, H. FRERICHS and W. CREUTZFELDT
Department of Medicine, Division of Gastroenterology and Metabolism, University of Göttingen, FRG

Received 27 June 1977

1. Introduction

Isolated islets of Langerhans release somatostatin in response to glucose [1], cAMP [2], α-ketoisocaproic acid, glucagon and theophylline [3]. The aim of the present study was to investigate the interactions that might exist between the secretion of somatostatin and insulin, i.e., a possible D-cell/B-cell interrelationship.

A static incubation system, as previously used [1–3], is of limited help for this purpose because it does not give information on the dynamics of the respective release processes. Therefore, the kinetics of somatostatin and insulin release from isolated rat islets were investigated in a perifusion system.

2. Materials and methods

Fed, male Wistar rats (220–270 g) were used throughout the study. Bovine serum albumin was purchased from Behringwerke A. G., Marburg, FRG; ^{125}I-labeled porcine insulin (spec. act. 150–200 mCi/mg) from Farbwerke Hoechst A. G., Frankfurt, FRG; crystalline rat insulin from Serono, Freiburg, FRG; collagenase from Worthington Biochemical Co., USA.

Islets were isolated from rat pancreas by collagenase 2 h after intraperitoneal administration of 0.6 ml pilocarpine hydrochloride (2% w/v), as previously described [4,5] and perifused for 90 min with Krebs-Ringer bicarbonate buffer (0.2 mg/ml BSA; 1000 KIU/ml Trasylol®, 2 mM or 25 mM glucose with or without 5 mM theophylline, pH 7.4, 37°C), 0.9 ml/min flow rate, 300 islets/perifusion [6]. All perifusions were preceded by a preperifusion period of 30 min with 2 mM glucose.

Insulin release into the medium was determined by radioimmunoassay with rat insulin as reference standard. Insulin release is expressed as ng/300 islets/2 min. Somatostatin was measured by radioimmunoassay as described [3]. The release of somatostatin is expressed as pg/300 islets/2 min. Statistical analysis was by Student's *t*-test based on paired comparisons.

3. Results

Figure 1 shows the dynamics of somatostatin and insulin release from rat islets perifused for 3 periods of 30 min with a medium containing first 2 mM, then 25 mM and thereafter 2 mM glucose. An increase in the concentration of glucose from 2–25 mM was associated with an immediate increase of insulin release. This release was biphasic, with a rapid first peak and a sustained second peak. Somatostatin release likewise increased in response to 25 mM glucose (148 ± 18 pg/300 islets/30 min versus 214 ± 35 pg/300 islets/30 min; $P < 0.0125$). However, this increase was significantly less pronounced than the increase in the release of insulin.

As expected, insulin secretion decreased immediately when the glucose concentration was lowered from 25–2 mM. This decrease was associated with a transient increase of somatostatin secretion (176 ± 34 pg/300 islets from 60–68 min versus 70 ± 14 pg/300 islets during the preceding 8 min; $P < 0.01$).

The effect of theophylline on somatostatin and insulin release is shown in fig.2. Theophylline (5 mM) significantly enhanced the stimulatory effect of 25 mM glucose on the secretion of somatostatin and insulin. In the presence of 5 mM theophylline plus 25 mM glucose 639 ± 115 pg somatostatin/300 islets/30 min

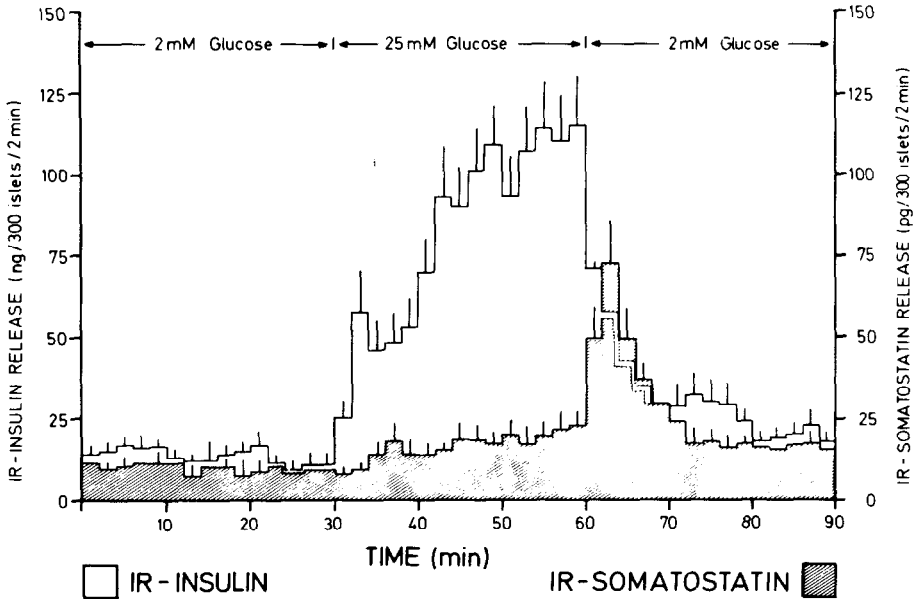
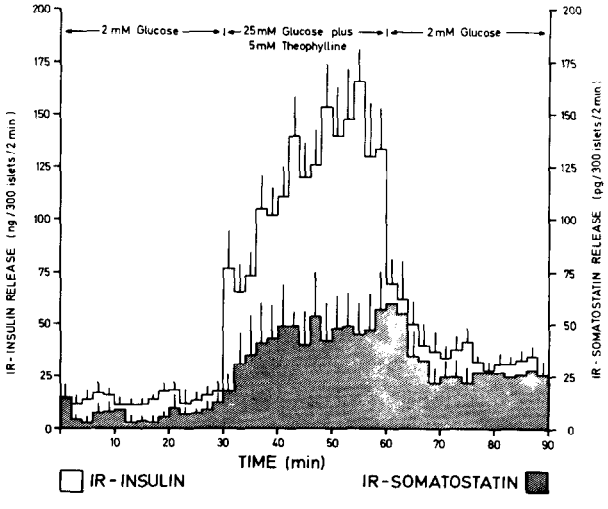


Fig.1. Effect of glucose on the kinetics of somatostatin (shaded area) and insulin release (light area) from isolated perifused rat pancreatic islets. Batches of 300 islets were perifused for 3 periods of 30 min each with Krebs-Ringer bicarbonate buffer containing 2 mM or 25 mM glucose. Two minute fractions are plotted. Mean values ± SEM obtained from 10 experiments are shown. Significantly less somatostatin is released in the presence of 2 mM glucose (1–30 min) than in the presence of 25 mM glucose (31–60 min) ($P < 0.0125$). The sudden decline of the glucose concentration from 25–2 mM is associated with a decrease of insulin but an increase of somatostatin release (60–68 min) versus the preceding 8 min; $P < 0.01$).

were released versus 214 ± 35 pg/300 islets/30 min in the presence of 25 mM glucose without theophylline (fig.1, period 2 versus fig.2, period 2; $P < 0.01$). If 5 mM theophylline and 25 mM glucose in the medium were replaced by 2 mM glucose, insulin secretion declined immediately, but somatostatin release with a lag period of about 4 min (fig.2, period 3).



4. Discussion

Release of somatostatin from isolated rat pancreatic islets by glucose could be demonstrated in a

Fig.2. Effect of theophylline on glucose-induced somatostatin (shaded area) and insulin release (light area) from isolated perifused rat pancreatic islets. Batches of 300 islets were perifused for 3 periods of 30 min each with Krebs-Ringer bicarbonate buffer containing 2 mM glucose or 25 mM glucose plus 5 mM theophylline. Two minute fractions are plotted. Mean values ± SEM obtained from 10 experiments are shown. Theophylline (5 mM) significantly enhanced the stimulatory effect of glucose (25 mM) on the secretion of somatostatin and insulin (period 2 versus period 2 in fig.1; $P < 0.01$).

static incubation system [1,2]. However, it remained undecided whether glucose affects somatostatin release directly or whether its effect is mediated via insulin or other factors. An answer to this question could be expected from studies on the dynamics of somatostatin and insulin release.

The stimulatory effect of glucose on the release of somatostatin has been confirmed (fig.1). Islets perifused first with a medium containing 2 mM glucose increased somatostatin release if the glucose concentration was subsequently raised to 25 mM.

The dynamics of somatostatin and insulin release differed. Insulin release increased immediately in response to 25 mM glucose, and declined rapidly if the glucose concentration was lowered subsequently to 2 mM. Somatostatin release likewise increased in response to 25 mM glucose, although significantly less than the secretion of insulin. However, if the concentration of glucose was lowered from 25–2 mM, a dissociation occurred between the secretion of insulin and somatostatin. While the release of insulin declined immediately, somatostatin release increased for a period of about 8 min (fig.1).

The sudden increase of somatostatin release, despite the decrease in the concentration of glucose suggests the removal of an inhibitory effect on the D-cell's secretory mechanism. It is conceivable that factors associated with a high secretory activity of B-cells, such as insulin itself, ion fluxes, changes in the electrical activity or other phenomena are responsible for the inhibition of somatostatin release. This would explain why insulin and somatostatin release dissociate when the glucose concentration is suddenly decreased and why the stimulatory effect of a high (25 mM) glucose concentration on somatostatin release is only moderate.

Despite reports to the contrary, evidence is accumulating that insulin lowers the availability of cAMP in various tissues (for review see ref. [7,8]). Whether the release of somatostatin is decreased via diminishing the availability of cAMP in D-cells is not known but compatible with the observation that theophylline enhances the effect of glucose on somatostatin release (fig.2).

The replacement of 5 mM theophylline and 25 mM glucose in the medium by 2 mM glucose was associated with an immediate decrease in the release of insulin. Somatostatin release remained high for about 4 min, i.e., did not show the transient increase occurring from islets exposed to glucose without theophylline (fig.1 and 2). This is understandable if the secretory mechanism of the D-cell is maximally stimulated by 5 mM theophylline plus 25 mM glucose. One then would not expect a further increase in somatostatin release, despite the sudden decrease in the secretory activity of the B-cell, i.e., cessation of the inhibitory effect on somatostatin release.

The present data thus suggest that somatostatin release is stimulated by glucose and inhibited by phenomena associated with a high secretory activity of B-cells. Whether the increase of somatostatin release, which is associated with the sudden decrease in the concentration of glucose contributes to the decline of insulin secretion remains to be demonstrated. Such an effect seems possible, because exogenous somatostatin is known to inhibit the secretion of insulin in vitro [9–13] and in vivo [14–17].

Endogenous somatostatin could have a similar effect because this peptide has been suggested to be a candidate paracrine transmitter substance with local action [18]. To what extent secretory profiles of insulin and somatostatin reflect local changes in the concentrations of the hormones is not known. In addition, the interactions between insulin and somatostatin are likely to be influenced by other islet hormones such as glucagon and pancreatic polypeptide. This complicates the interpretation of the results.

References

[1] Schauder, P., McIntosh, C., Arends, J., Arnold, R., Frerichs, H. and Creutzfeldt, W. (1976) FEBS Lett. 68, 225–227.
[2] Barden, N., Alvarado-Urbina, G., Cote, J. P. and Dupont, A. (1976) Biochem. Biophys. Res. Commun. 71, 840–844.
[3] Schauder, P., McIntosh, C., Arends, J., Arnold, R., Frerichs, H. and Creutzfeldt, W. (1977) Biochem. Biophys. Res. Commun. 75, 630–635.
[4] Schauder, P. and Frerichs, H. (1974) Diabetologia 10, 85–97.
[5] Schauder, P., Ebert, R. and Frerichs, H. (1975) Biochem. Biophys. Res. Commun. 67, 701–705.
[6] Panten, U., Ishida, H., Schauder, P., Frerichs, H. and Hasselblatt, A. (1977) Anal. Biochem. in press.
[7] Jungas, R. L. (1975) in: Handbook of Experimental Pharmacology (Hasselblatt, R. Bruchhausen, F. v. eds) Vol XXXII/2, pp. 371–412, Springer, Berlin, Heidelberg, New York.

[8] Volfin, P. and Hanoune, J. (1975) in: Handbook of Experimental Pharmacology (Hasselblatt, A. Bruchhausen, F. v. eds) Vol. XXXII/2, pp. 303–327, Springer, Berlin, Heidelberg, New York.
[9] Effendic, S., Luft, R. and Grill, V. (1974) FEBS Lett. 42, 169–172.
[10] Curry, D. L. and Bennet, L. L. (1974) Biochem. Biophys. Res. Commun. 60, 1015–1019.
[11] Fujimoto, W. Y., Ensick, J. W. and Williams, R. H. (1974) Life Sci. 15, 1999–2004.
[12] Oliver, J. R. and Wagle, S. R. (1974) Biochem. Biophys. Res. Commun. 62, 772–777.
[13] Johnson, D. G., Ensinck, J. W., Koerker, D., Palmer, J. and Goodner, C. J. (1975) Endocrinology 96, 370–374.
[14] Alberti, K. G. M. M., Christensen, N. J., Christensen, S. E., Hansen, A. P., Iversen, J., Lundback, K., Seyer-Hansen, K. and Orskov, H. (1973) Lancet 2, 1299–1301.
[15] Koerker, D. J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C. J., Ensinck, J. W. and Gale, C. C. (1974) Science 184, 482–483.
[16] Peracchi, M., Reschini, E., Cantalamessa, L., Giustina, G., Cavagnini, F., Pinto, M. and Bulgheroni, P. (1974) Metabolism 23, 1009–1015.
[17] Gerich, J. E., Lorenzi, M., Schneider, V. and Forsham, P. H. (1974) J. Clin. Endocrinol. Metab. 39, 1057–1060.
[18] Creutzfeldt, W. (1975) in: Stimulus-Secretion Coupling in the Gastrointestinal Tract. (Case, R. M. and Goebell, H. eds) pp. 415–428, M-T.P. Press Ltd. Lancaster, St. Leonard's House, England.