INHIBITION BY SOMATOSTATIN OF INSULIN RELEASE FROM ISOLATED PANCREATIC ISLETS

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1. Introduction

Since the finding of somatostatin, the hypothalamic inhibitor of pituitary growth hormone, many experimental data showing the inhibiting activity of somatostatin on the secretion of other hormones have been presented [1]. As for the insulin secretion, somatostatin was reported to suppress a glucose-induced rise of plasma insulin in humans [2] and to inhibit insulin secretion by perfused pancreases [2–4]. However, concerning its direct effect on insulin release from the pancreatic islet of Langerhans there have so far been two controversial results; Although Efendic et al. [3] failed to demonstrate the inhibitory effect of somatostatin on insulin release from the isolated islet, Oliver and Wagle [5] have quite recently reported that the inhibition of insulin release from the islet was observed in experiments with incubation media containing somatostatin concentrations ranging from 0.2 to 100 μg/ml.

Recently, we have isolated rat pancreatic islets under mild conditions of collagenase treatment, and studied the effect of somatostatin on glucose-induced insulin release by the islet. The purpose of present paper is to show that somatostatin, in such a low concentration as 10 ng/ml, did inhibit insulin release from the islet and that the somatostatin inhibition of insulin release was completely reversed by increasing Ca^{++} concentration in the incubation medium.

2. Methods and materials

Pancreatic islets of Langerhans were isolated from 1–2 male Wistar rats weighing 250 g by collagenase treatment [6] with the following modifications: a) the pancreatic pieces were treated in Hanks' solution containing 25 mg collagenase/5 ml and 100 mg bovine serum albumin/5 ml; b) the collagenase treatment was carried out for 10 min at 37°C using a metabolic shaker (approx. 200 oscillations/min). The incubation system for insulin release studies was as described previously [7,8] with the addition of a protease inhibitor, Aprotinin (1000 KIU/ml). The islets were preincubated for 30 min at 37° C in the presence of 3.3 mM glucose and then incubated for 90 min at 37°C in the presence of 20 mM glucose and various concentrations of somatostatin. The insulin content of the incubated media was measured by the double antibody redio-immunoassay technique [9]. Collagenase (CLS IV, Lot No. 44K151, 190 U/mg) was purchased from Worthington Biochemical Corporation and bovine serum albumin (essentially fatty acid free) from Sigma. Aprotinin (Antagosan) was a generous gift of Hoechst Japan, Ltd. Somatostatin (cyclized) was obtained from Bachem Fine Chemicals, Inc., Calif., USA.

3. Results

Fig. 1 shows effective inhibition of glucose-induced insulin release from the isolated rat pancreatic islets by somatostatin. A continuous increase in insulin release in the presence of 20 mM glucose occurred during the period of 90 min after incubation (control). The increase in insulin release by the islets exposed to somatostatin (10 ng/ml) was approx. 40% less than the comparative increase in the control during the same 90 min period.

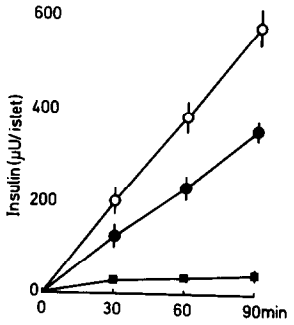

Fig.1. Time course study of insulin release. Incubation medium as described in Methods, supplemented with 20 mM glucose (○) or 20 mM glucose plus 10 ng/ml somatostatin (●). An experiment with low glucose (3.3 mM) was run parallel (■), The points at the different time intervals for each curve are the mean ± S.E. in μU of insulin released in 4 incubation flasks.

Table 1
Effect of Ca^{++} concentrations on somatostatin inhibition of glucose-induced insulin release.

	Ca^{++} concentration			
	5 mEq/l		10 mEq/l	
		p		*p*
Glucose (20 mM)	582 ± 39		592 ± 40	
Glucose (20 mM) + somatostatin (10 ng/ml)	374 ± 19	<0.01	552 ± 63	N.S.

Conditions as in fig.1, except that Ca^{++} concentration in the incubation medium elevated from its 5 mEq/l control level to 10 mEq/l. Results are expressed as the mean ± S.E. in μU of insulin released for 90 min per islet in 4 incubation flasks. *p* Values refer to the significance of difference of experiments with and without somatostatin.

Fig.2 shows the results of experiments in which the relation between the somatostatin concentration and the inhibition of glucose-induced insulin release was studied. Increasing the concentration of somatostatin from 1 to 10 ng/ml decreased the insulin release. Little or no further decrease in insulin release was caused with somatostatin concentrations up to 1000 ng/ml. In control experiments with low glucose (3.3 mM), it was observed that addition of somatostatin (10–1000 ng/ml) had no significant effect on basal insulin release.

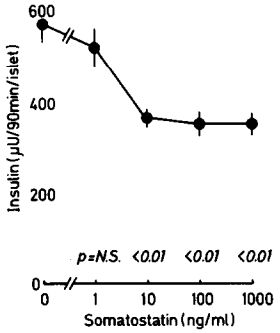

Fig.2. Effect of different concentrations of somatostatin on glucose-induced insulin release. Conditions as in fig.1, supplemented with somatostatin at the concentration indicated. Results represent the mean ± S.E. in μU of insulin released in 4 incubation flasks. *p* Values refer to the significance of difference of experiments with and without somatostatin.

Curry and Bennett [10], using perfused rat pancreases, have reported that increased Ca^{++} concentration reversed somatostatin inhibition of glucose-induced insulin secretion. In the present experiments carried out with isolated Langerhans islets of rats, we also observed reversal of the inhibitory effect of somatostatin by increasing the Ca^{++} concentration (table 1). When the Ca^{++} concentration in the incubation medium was raised from 5 to 10 mEq/l, the inhibitory effect of somatostatin (10 ng/ml) on insulin release was not significant.

4. Discussion

In the present study data were presented showing that somatostatin has a potent activity of inhibiting the glucose-induced insulin release from the isolated rat pancreatic islet in a concentration as low as 10 ng/ml of incubation medium. Further, the somatostatin inhibition of insulin release was almost completely reversed by increasing Ca^{++} concentration. These results strongly suggest that the hypothalamic hormone itself exerts a direct action on insulin release from the β-cell of pancreatic islets.

Additionally, it should be mentioned here that while the amount of insulin released by 20 mM glucose was 570–600 μU/islet/90 min in the present system, that of insulin released by 20 mM glucose from the

islets, prepared under the conditions of 60 mg collagenase per 5 ml and a 20 min digestion time, was less than 150 μU/islet/90 min. Further, the islets were found to be almost insensitive to somatostatin in the latter experimental system*. These suggest the possible occurrence of damage of some receptor sites for glucose as well as for somatostatin on the β-cell membranes during the course of preparation of islet samples.

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* Okamoto, H., Noto, Y., and Miyamoto, S., unpublished data.

References

[1] Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. and Guillemin, R. (1973) Science 179, 77–79.
[2] Alberti, K. G. M. M., Christensen, N. J., Christensen, S. E., Hansen, A. P., Iversen, J., Lundbaek, K., Seyer-Hansen, K., and Orskov, H. (1973) Lancet (December, 7841) 1299–1301.
[3] Efendic, S., Luft, R., and Grill, V. (1974) FEBS Lett. 42, 169–172.
[4] Curry, D. L., Bennett, L. L., and Li, C. H. (1974) Biochem. Biophys. Res. Commun. 58, 885–889.
[5] Oliver, J. R., and Wagle, S. R. (1975) Biochem. Biophys. Res. Commun. 62, 772–777.
[6] Lacy, P. E., and Kostianovsky, M. (1967) Diabetes 16, 35–39.
[7] Okamoto, H., Miyamoto, S., Mabuchi, H., Yoneyama, Y. and Takeda, R. (1973) Biochem. Biophys. Res. Commun. 53, 1297–1303.
[8] Okamoto, H., Miyamoto, S., Mabuchi, H., and Takeda, R. (1974) Biochem. Biophys. Res. Commun. 59, 623–629.
[9] Morgan, C. R. and Lazarow, A. (1963) Diabetes 12, 115–126.
[10] Curry, D. L. and Bennett, L. L. (1974) Biochem. Biophys. Res. Commun. 60, 1015–1019.