Somatostatin Release from Isolated Perfused Rat Pancreas

Possible Role of Endogenous Somatostatin on Insulin Release

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SUMMARY

In order to elucidate the role of endogenous somatostatin in the control of insulin and glucagon secretion, glucagon- or insulin-induced somatostatin release from the isolated perfused rat pancreas was studied.

Immunoreactive somatostatin was persistently released for 60 min in response to perfusion by 5.5 mM glucose at concentrations ranging between 10 and 15 pg/ml. The addition of glucagon (10^{-8}, 10^{-7}, and 10^{-6} M) caused a dose-related increase of somatostatin release. In contrast, insulin release, especially its first phase, was suppressed when concentrations of glucagon were increased. The addition of insulin (10^{-7} M and 10^{-6} M) had no significant effect on somatostatin and glucagon release.

These results raise the possibility that endogenous somatostatin and glucagon together regulate insulin secretion, suggesting a close interrelationship between insulin, glucagon, and somatostatin secretion within the islet. DIABETES *28*:600–603, June 1979.

Somatostatin is known to exert a potent inhibitory action on insulin and glucagon secretion. Immunoreactive somatostatin was recently demonstrated to be located in the D cells, close to both the A and the B cells, within the pancreatic islets. This intimate, mutual relationship[1,2] raises the possibility of a local interaction among A, B, and D cells within the islets.

Although the endogenous somatostatin is released from pancreatic D cells in response to various stimuli,[3–6] the role of endogenous somatostatin on the function of the neighboring A and B cells is poorly understood. In order to elucidate the interrelationship between A, B, and D cells, attempts were made in the present study to evaluate immunoreactive somatostatin release from isolated perfused rat pancreas in response to exogenous glucagon and insulin.

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Received for publication 2 January 1979 and in revised form 8 March 1979.

MATERIALS AND METHODS

Overnight-fasted male Wistar rats weighing from 300 to 350 g were anesthetized by intraperitoneal injection of 30 mg/kg of pentobarbital, and pancreases were isolated and perfused according to the procedure described by Grodsky[7] (with minor modifications). The pancreas was separated from adjacent stomach, spleen, and duodenum and subsequently perfused without recirculation. The perfusate solution, consisting of 0.25% bovine serum albumin, 4.6% dextran, and 5.5 mM glucose in Krebs-Ringer bicarbonate buffer, was kept at 37 °C and pH 7.4 under a gas phase of 95% O_2 and 5% CO_2. After introduction of the solution into the celiac artery, perfusion was carried out by means of a peristaltic pump at a flow rate of 2 ml/min. Effluent was collected every minute from a cannula inserted into the portal vein.

After an equilibrium period of 20 min, porcine glucagon and porcine insulin were infused for 15 min by a sidearm syringe to obtain final concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M. Porcine glucagon (Eli Lilly, Indianapolis, Indiana) and porcine insulin (Novo Industri A/S, Copenhagen, Denmark) were 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 presence of glucagon or insulin. Control experiments were performed by the infusion of the solution with acetic acid alone. The portal effluent was collected into chilled tubes containing 2000 U of Trasylol (Bayer Leverkusen, Germany) and 10^{-5} M bacitracin (Sigma Chemical, St. Louis, Missouri). Tubes were stored at −20 °C until assayed.

Immunoreactive somatostatin was determined by the method described previously.[8] The minimum detectable quantity of the assay was 10 pg/ml. Mean recovery rate of synthetic somatostatin added into the perfusate was 88 ± 2%. Insulin and glucagon did not cross-react. Immunoreactive insulin was measured by radioimmunoassay using polyethylene glycol[9] to separate bound and free hormone and rat insulin as the standard. Immunoreactive glucagon was assayed by the talc adsorption method.[10]

Statistical analysis was performed by unpaired Student's *t* test when two means were compared. For comparison of

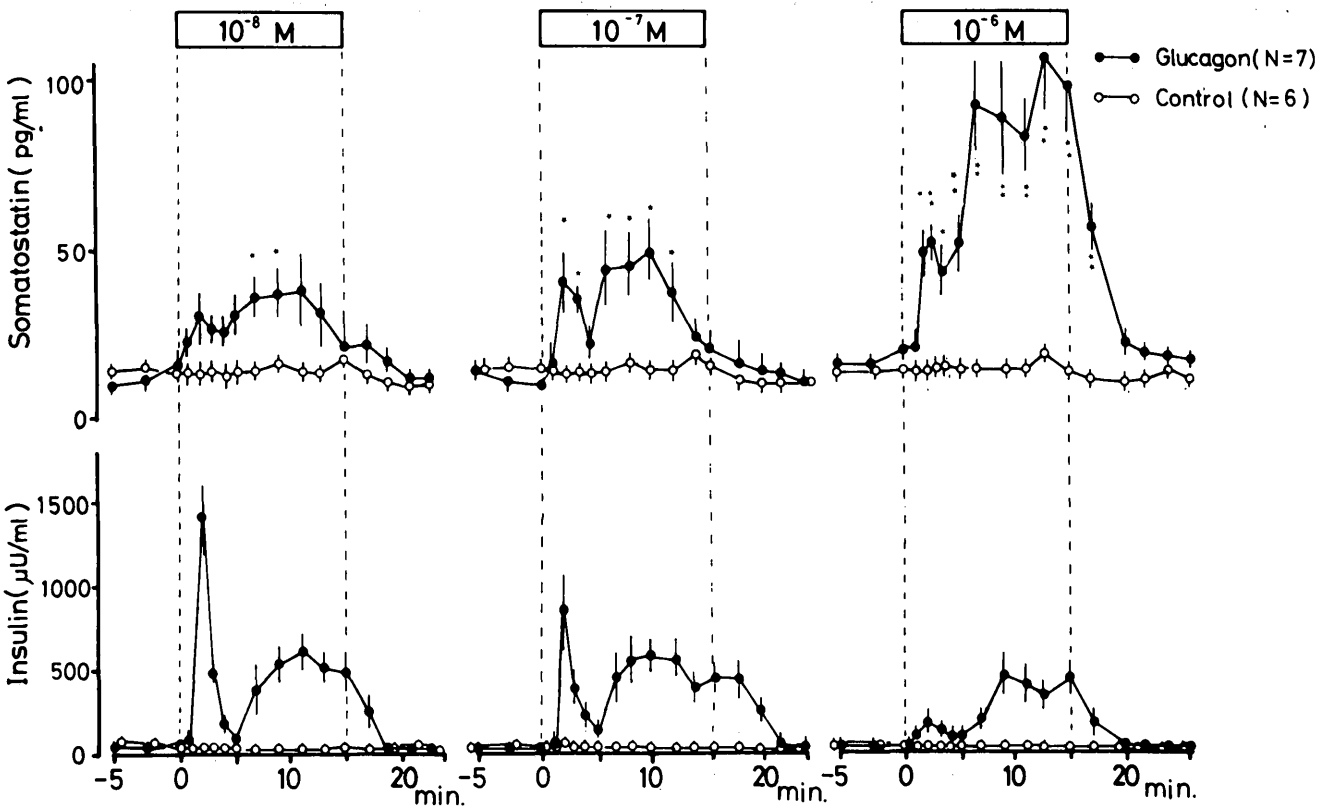

FIGURE 1. Somatostatin and insulin responses during exogenous glucagon infusion (10^{-8} M ~ 10^{-6} M). Means ± SEM of indicated numbers of experiments are shown. The data are plotted at 1-min intervals during the first 5 min and at 2-min intervals thereafter. Statistically significant differences between the values of the glucagon and vehicle experiments at a given time were examined by Student's *t* test and are represented as follows: * $P < 0.05$, ** $P < 0.001$

more than two means, Duncan's new multiple-range test[23] was used.

RESULTS

SOMATOSTATIN AND INSULIN RESPONSES TO EXOGENOUS GLUCAGON (FIGURE 1)

When the pancreas was perfused with 5.5 mM glucose alone, somatostatin levels ranged from 10 to 15 pg/ml through the whole perfusion period.

The addition of 10^{-8} M glucagon induced a gradual increase of somatostatin release through the stimulation period (left upper panel). Somatostatin release elicited by 10^{-7} M glucagon was more pronounced than that after 10^{-8} M glucagon (middle upper panel). When the concentration of glucagon was further increased to 10^{-6} M, the maximal increase of somatostatin release was observed (right upper panel). The infusion of 10^{-8} M glucagon caused a typical biphasic insulin release. In all experiments, the first phase occurred within 5 min after the start of stimulation and was followed by the sustained second phase of insulin release (left lower panel).

Insulin release, however, was attenuated by increasing the glucagon concentration in contrast to a graded increase of somatostatin (middle and right lower panels in Figure 1). The most remarkable decrease of insulin release, especially the first phase, was found at a glucagon concentration of 10^{-6} M, while somatostatin release was strikingly enhanced

TABLE 1
Integrated amount of somatostatin secreted in the venous effluent during the periods corresponding to the first and second phases of insulin release

Exp	Glucagon (M)	N	First phase (t_{0-5}) Insulin μU/5 min	First phase (t_{0-5}) Somatostatin pg/5 min	Second phase (t_5-t_{15}) Insulin μU/10 min	Second phase (t_5-t_{15}) Somatostatin pg/10 min
1	0	6	103 ± 7	140 ± 3	345 ± 12	410 ± 8
2	10^{-8}	7	4221 ± 612	180 ± 30	9513 ± 1146	638 ± 167
3	10^{-7}	7	2645 ± 579*	299 ± 42	7438 ± 1881	895 ± 153
4	10^{-6}	7	917 ± 168*†	430 ± 61*†	7019 ± 1658	1982 ± 334*†

The values represent the mean ± SEM of the integrated secretion rates of somatostatin and insulin release during the indicated time period.

Statistical differences were estimated by Duncan's new multiple-range test and are represented as follows: *significantly different from experiment 2; †significantly different from experiment 3.

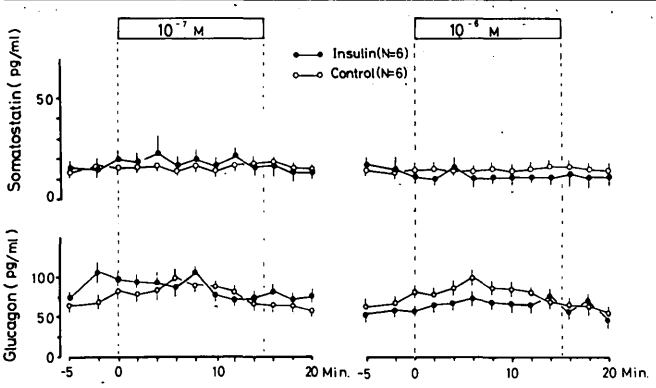

FIGURE 2. Somatostatin and glucagon responses to exogenous insulin (10^{-7} M and 10^{-6} M). Means ± SEM of indicated numbers of experiments are shown. The data are plotted at 2-min intervals.

under the same condition. The typical biphasic insulin release curve disappeared at this glucagon concentration.

The tendency mentioned above was even more clearly demonstrated when the integrated amount of somatostatin secreted during the periods corresponding to the first (0 to 5 min) and second (5 to 15 min) phases of insulin release was calculated (Table 1) as a summation of the hormone released per minute. By increasing the glucagon concentration, the first phase of insulin release but not the second phase was decreased and there was a reciprocal increase of somatostatin released.

SOMATOSTATIN AND GLUCAGON RESPONSES TO EXOGENOUS INSULIN

As shown in Figure 2, the infusion of 10^{-7} M and 10^{-6} M porcine insulin did not affect pancreatic somatostatin and glucagon release.

DISCUSSION

Since the time when somatostatin was identified as a potent inhibitor of insulin and glucagon secretion,[11-14] the pathophysiologic role of the peptide in glucose homeostasis has been intensely investigated. As to the physiologic significance of somatostatin, a role in the local regulation of insulin and glucagon secretion within the pancreatic islets was suggested.[15]

Patton et al.[3] reported a decline of arginine-induced insulin and glucagon release from isolated perfused dog pancreas from its initial peak despite continued perfusion of arginine, while somatostatin release remained high throughout the stimulation period, pointing out the possibility that D cells could well serve to regulate concentrations of insulin and glucagon emerging from the pancreatic islets.

In the present study, a dose-related enhancement of somatostatin released from the rat pancreas by glucagon was demonstrated. Recently, glucagon, a well-known stimulator of insulin secretion, was shown to stimulate somatostatin release.[22,24] However, previous studies failed to find a dose-related increase of somatostatin released by glucagon.

It is noteworthy that insulin release, especially its first phase, was blunted by increasing the dose of glucagon, while somatostatin release was enhanced. As far as we know, this is the first report of the attenuation of insulin release by increasing the glucagon concentration. According to a rather attractive interpretation, endogenously secreted somatostatin might suppress insulin release. Curry et al.[20] reported that the first phase of insulin release is about 25 to 50 times as sensitive to somatostatin inhibition as the second phase. This provides an explanation for the predominant decrease of the first phase of insulin release. It is conceivable that glucagon evokes pancreatic somatostatin release, and endogenously secreted somatostatin, in turn, acts to restrain insulin release, especially its sensitive, first phase. Factors attenuating insulin release other than endogenous somatostatin should also be considered. For example, high concentrations of glucagon per se might attenuate insulin release, because several insulin secretagogues, such as calcium, exhibit a suppressive effect on insulin released at a high concentration.[25]

Since somatostatin content in the islets of chronically insulinopenic diabetes is increased,[21] insulin may exert some kind of action on the function of pancreatic D cells. In this study, exogenous insulin failed to affect somatostatin release, in accordance with the previous report.[22] This, however, does not necessarily indicate that insulin has no effect on somatostatin release. Further studies are required under the conditions of chronic insulinopenia or varying glucose concentrations.

The present study clearly demonstrated a direct glucagon stimulation of somatostatin release with a simultaneous restraint on insulin release, possibly by enhancement of somatostatin release. The findings support an intimate, mutual relationship between pancreatic A, B, and D cells within the islets.

ACKNOWLEDGMENTS

This study was supported in part by a research grant from the Ministry of Education, Science, and Culture, Japan. The authors wish to thank Miss Atsuko Toyonaga for technical assistance and Miss Satomi Yanagihara for secretarial aid.

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