Somatostatin Release from the Isolated, Perfused Diabetic Rat Pancreas

Inverse Relationship Between Pancreatic Somatostatin and Insulin

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SUMMARY

The changes in pancreatic somatostatin content and release were studied in streptozotocin (STZ)-diabetic rats. Male Wistar rats were treated with a graded dose of STZ (group I, 0; II, 25; III, 50; IV, 75 mg/kg), which produced various grades of diabetic rats four weeks later. The pancreatic somatostatin content increased in proportion to the dose of STZ (I, 189 ± 31; II, 222 ± 20; III, 343 ± 4; IV, 515 ± 36 ng/g wet wt), while graded reductions of insulin content were observed. Significant increases in glucagon content were found only in groups III and IV. Pancreatic somatostatin release increased during arginine infusion (19.2 mM) dose dependently, (I, 543 ± 36; II, 946 ± 64; III, 1229 ± 55; IV, 2186 ± 150 pg/15 min), and it correlated with the graded decreases of insulin release. The glucagon release induced by arginine, however, did not change significantly.

These results indicate that pancreatic somatostatin content and release increased in STZ-diabetic rats in proportion to the degree of insulin deficiency. DIABETES 29:960–963, December 1980.

Previous studies have shown that pancreatic somatostatin content and the number of somatostatin-containing D cells increased in insulin-deficient forms of diabetes,[1,2] but decreased in hypoinsulinemic forms of diabetes.[3,4] Schusdziarra[5] and Datel[6] subsequently reported a significant reduction of the plasma somatostatin in response to insulin treatment in diabetic animals. Berelowitz et al.[7] also demonstrated a significant fall of the increased pancreatic somatostatin content in the diabetic rat in response to insulin therapy.

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These results raise the possibility that D cell function may be regulated by a direct or an indirect action of insulin.

The present study was designed, therefore, to investigate pancreatic somatostatin content and release in graded severities of diabetes induced by streptozotocin and to provide a more detailed analysis of the relationship between the D and B cells and the D and A cells.

MATERIALS AND METHODS

Nonfasted male Wistar rats, weighing 250–300 g, were used in this experiment. The animals were divided into four groups and were treated with graded doses of streptozotocin (STZ) (group I, 0; II, 25; III, 50; IV, 75 mg/kg) by intravenous injection into the penile vein. The STZ was dissolved in 1 ml of saline immediately before injection. Group I received the 1 ml of saline alone. Four weeks after the STZ treatment, blood glucose and plasma insulin levels were measured and a number of the rats from each group was selected for the perfusion while the remainder were used for the tissue extraction. The pancreases were isolated from overnight-fasted rats and were perfused by the method of Grodsky with minor modifications.[8] The perfusate, composed of 4.6% dextran, 0.25% bovine serum albumin, 5.5 mM glucose, and Krebs-Ringer bicarbonate buffer, was perfused at a constant rate of 2 ml/min via a cannula inserted into the celiac artery. After a 20-min prestimulation period, L-arginine hydrochloride solution was infused for 15 min via a sidearm syringe to obtain a final concentration of 19.2 mM. The portal effluent was collected into chilled tubes containing 1000 U of Trasylol (Bayer Leverkussen, Germany) and 10^{-5} U bacitracin (Sigma Chemical, St. Louis, Missouri) and was stored at −20°C until assayed. Immunoreactive somatostatin was determined by the method described previously.[9] Immunoreactive insulin was measured by radioimmunoassay using polyethylene glycol to separate bound and free hormones with rat insulin as the standard.[10] Immunoreactive glucagon was assayed by the talc adsorption method.[11] Statistical analysis was performed by unpaired Student's *t* test when two means were compared. For comparison of more than two means, Duncan's new multiple-range test[12] was used.

TABLE 1
Effects of various doses of streptozotocin on the body weight, blood glucose, and plasma and tissue insulin (IRI), glucagon (IRG), and somatostatin (SRIF) concentrations

					Plasma		Tissue		
Group	N	STZ (mg/kg)	Body weight (g)	Blood glucose (mg/dl)	IRI (ng/ml)	IRG (pg/ml)	IRI (μg/g wet wt)	IRG (μg/g wet wt)	SRIF (ng/g wet wt)
I	5	0	423 ± 13	130 ± 13	2.16 ± 0.16	45 ± 4	69 ± 3	2.2 ± 0.2	189 ± 31
II	5	25	358 ± 14	166 ± 20	1.56 ± 0.08†	64 ± 7	42 ± 3*	2.4 ± 0.05	272 ± 20
III	5	50	282 ± 11*§	263 ± 43*	0.68 ± 0.16*‡	100 ± 24*§	10 ± 2*‡	4.97 ± 0.69†§	343 ± 4*‡
IV	5	75	228 ± 21*‡¶	535 ± 51*‡‖	0.20 ± 0.08*‡‖	134 ± 4*‡	7 ± 1*‡	5.64 ± 1.3*‡	515 ± 36*‡‖

The values represent the $\bar{x}$ ± SEM of indicated numbers of animals in each group. Statistical differences were estimated by Duncan's new multiple-range test and are represented as follows: *†—significantly different from group I (*$P < 0.01$, †$P < 0.05$); ‡§—significantly different from group II (‡$P < 0.01$, §$P < 0.05$); ‖¶—significantly different from group III (‖$P < 0.01$, ¶$P < 0.05$).

RESULTS

Effects of various doses of STZ on blood glucose, plasma insulin, and glucagon (Table 1). STZ increased blood glucose in a dose-related manner (group I, 130 ± 13; group II, 166 ± 20; group III, 263 ± 43; group IV, 535 ± 51 mg/dl), along with a corresponding reduction of body weight. Plasma insulin decreased with increasing doses of STZ and a concomitant elevation of plasma glucagon. Various degrees of insulinopenic diabetes were produced by changing the dose of STZ.

Tissue somatostatin (SRIF), insulin, and glucagon content (Table 1). Various grades of pancreatic insulin depletion were produced by changing the dose of STZ. Pancreatic SRIF content, on the contrary, increased with increasing doses of STZ; significantly higher values were observed in group III than in the control group (group I vs. II, NS; group I vs. III, $P < 0.05$; group I vs. IV, $P < 0.01$). Pancreatic glucagon content also increased in the higher STZ-treated groups (group I vs. II, NS; group I vs. III, $P < 0.05$; group I vs. IV, $P < 0.01$).

Pancreatic SRIF, insulin, and glucagon release from the isolated, perfused rat pancreas (Figure 1, Table 2). In control animals (group I), at 2 min after arginine infusion, SRIF release reached the peak value of 30 ± 4 pg/ml, which was significantly higher than the basal value of 15 ± 4 pg/ml ($P < 0.05$).

Figure 1. Arginine-induced somatostatin (SRIF), glucagon (IRG), and insulin (IRI) release from isolated, perfused rat pancreas treated by various doses of streptozotocin. Numbers of experiments are as follows: Stz 0=6, Stz 25=5, Stz 50=5, and Stz 75=6. The data are shown $\bar{x}$ ± SEM and are plotted at 1-min intervals during the first 5 min and at 2-min intervals thereafter.

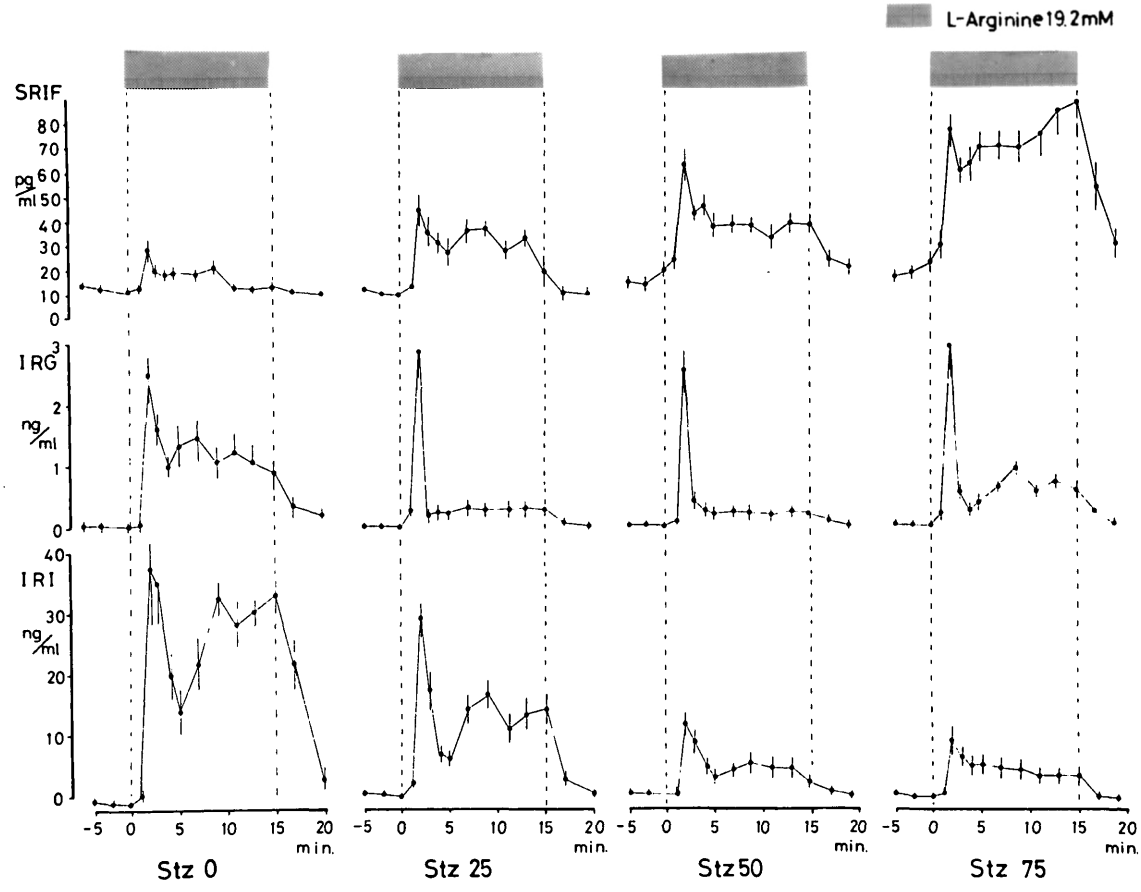

TABLE 2
Integrated amount of insulin, glucagon, and somatostatin secretion during arginine infusion

Group	N	Blood glucose (mg/dl)	Insulin (ng/15min)	Glucagon (ng/15min)	Somatostatin (pg/15min)
I	6	128 ± 10	404 ± 50	32.7 ± 6.1	543 ± 36
II	5	160 ± 15†	368 ± 60	14.7 ± 1.0*	946 ± 64*
III	5	273 ± 30*‡	176 ± 20*‡	13.4 ± 0.7*	1229 ± 55*‡
IV	6	495 ± 10*‡§	84 ± 16*‡	20.6 ± 2.4†	2186 ± 150*‡§

The values represent the $\bar{x}$ ± SEM of the total amount of secretion rates of insulin, glucagon, and somatostatin release during arginine infusion.
Statistical differences were estimated by Duncan's new multiple-range test and are represented as follows: *$P < 0.01$, †$P < 0.05$—significantly different from group I; ‡$P < 0.01$—significantly different from group II; §$P < 0.01$—significantly different from group III.

A typical, biphasic release pattern was observed during arginine infusion. In group II, arginine-stimulated SRIF release was augmented (maximum of the first phase, 45 ± 7 pg/ml; maximum of the second phase, 38 ± 3 pg/ml). In group III, the basal release of SRIF was 23 ± 1 pg/ml, significantly higher than that in group I ($P < 0.05$). After arginine infusion, the SRIF release was even more pronounced (maximum of the first phase, 64 ± 6 pg/ml; maximum of the second phase, 47 ± 6 pg/ml). In group IV, the basal SRIF release was 24 ± 4 pg/ml, significantly higher than that in group I ($P < 0.05$). The arginine-stimulted SRIF release increased markedly in both phases (maximum of the first phase, 78 ± 10 pg/ml; maximum of the second phase, 89 ± 15 pg/ml). On the other hand, the basal glucagon release did not differ significantly among the four groups (group I, 45 ± 16 pg/ml; group II, 33 ± 6 pg/ml; group III, 40 ± 5 pg/ml; group IV, 50 ± 22 pg/ml). The arginine-stimulated glucagon release also did not differ among the four groups in maximum value of the first phase (group I, 2525 ± 322 pg/ml; group II, 2958 ± 441 pg/ml; group III, 2498 ± 148 pg/ml; group IV, 3200 ± 613 pg/ml).

The arginine-stimulated glucagon release in the second phase, on the contrary, decreased significantly with increasing doses of STZ in groups II and III compared with group I (maximum value of the second phase: group I, 1276 ± 340 pg/ml; II, 368 ± 55 pg/ml; III, 328 ± 24 pg/ml; IV, 852 ± 205 pg/ml). The arginine-stimulated insulin secretion was reduced by grades related to the STZ dose in both phases (maximum of the first phase: group I, 38 ± 2.0 ng/ml; II, 29 ± 6.7 ng/ml; III, 12 ± 1.6 ng/ml; IV, 9.3 ± 3.1 ng/ml; maximum of the second phase: group I, 33 ± 3.7 ng/ml; II, 17 ± 1.8 ng/ml; III, 5.9 ± 1.8 ng/ml; IV, 4.7 ± 1.2 ng/ml). The total amount of hormone secreted during the arginine stimulation was then calculated and is presented in Table 2. The total secretion of somatostatin during arginine stimulation increased by grades with increasing doses of STZ, while a graded reduction of the insulin release also was observed.

An inverse relationship was also found between SRIF and insulin release. On the other hand, the total amount of glucagon release was greatest in group I, and increases of the dose of STZ caused significant reductions of glucagon release.

Relationship between SRIF and insulin content and release. The relationship between SRIF and insulin content was investigated in each animal. A significant negative correlation was observed ($r = 0.73$, $P < 0.01$). A similar relationship also was observed between SRIF and insulin release ($r = 0.68$, $P < 0.01$).

Relationship between SRIF and glucagon content and release. The relationships between SRIF and glucagon content and release were also analyzed. A significant positive correlation was found between these hormone contents ($r = 0.67$, $P < 0.01$), although no significant correlation was found between their release.

DISCUSSION

The present study indicates an inverse relationship between the pancreatic somatostatin and insulin contents, since graded increases of pancreatic somatostatin content and release were produced along with the graded insulin depletion induced by the various doses of streptozotocin. The diabetogenic action of streptozotocin was first discovered by Rakieten and Nadkarni,[13] and the morphologic alteration of pancreatic β-cells by this agent was evaluated.[14] They suggested that the intensity of the damage to the β-cells could be graded according to the doses used. Thereafter, Junod[15] reported the possibility that a graded severity of insulinopenic diabetes can be produced by changing the dose of streptozotocin. Previous reports also demonstrated that both the pancreatic somatostatin content[1,2] and the number of somatostatin-producing cells[16] increase in the chronic insulin-deficient state and decrease[3,4] in the hyperinsulinemic state, indicating a mutual relationship between B and D cells. We were able to demonstrate in this study, by analysis of the pancreatic hormones in diabetic rats under various degrees of insulinopenia, that pancreatic somatostatin content and release are both closely related to insulin, also confirming our previous studies[17] describing the close interrelationship between B and D cells, though the former studies were done in diabetics and the latter in normal animals.

It is a well-known fact that somatostatin inhibits insulin secretion. No agreement, however, was obtained on the effect of insulin on pancreatic somatostatin. According to studies in vitro, exogenous insulin failed to affect somatostatin release in the normal rat[17] and canine[18,19] pancreas. Moreover, no alteration of somatostatin release in response to glucagon or theophylline was observed from islets treated with anti-insulin serum.[20] Contrary to the negative results, a suppressive effect of insulin on somatostatin release was reported by Rothman,[21] Rouiller,[22] and Schusdziarra[5] and a significant reduction of pancreatic somatostatin was reported in hyperinsulinemic diabetic mice,[3,4] suggesting that insulin might have an inhibiting action on pancreatic somatostatin.

As shown in this study, on the contrary, hypoinsulinemia may enhance D-cell function. The contribution, however, of other factors, such as hyperglycemia and elevated levels of plasma glucagon, can't be excluded in the increase of pancreatic somatostatin content and release in the diabetic state. Since glucose[17,23–26] and glucagon[17,18] are well-known stimulators of pancreatic somatostatin, it is possible that the elevated levels of these factors in the diabetic state

might stimulate the D cells chronically to produce D-cell hyperfunction. The pathophysiologic significance of the increased pancreatic somatostatin in the diabetic state remains unknown. Unger et al. suggested that somatostatin has a major role in the regulation of carbohydrate metabolism. It is possible that increased somatostatin in the diabetic state may contribute to the reduction of blood glucose by inhibiting the glucose absorption from the gut,[27] glycogenolysis in the liver,[28,29] or glucagon release in the islets.

The relationship between A and D cells presents the second problem. The failure of increase of pancreatic glucagon release along with the increase of tissue glucagon content is, rather, unexpected in view of the increase of the glucagon level in circulating blood. Similar results were reported by other investigators. Pagliara et al.[30] demonstrated the failure of enhancement of arginine-induced glucagon release from the isolated, perfused diabetic rat pancreas in vitro, unlike the enhancement in vivo.[31,32] The reason for this discrepancy between the in vivo and in vitro results is unclear, but other factors, such as neuroendocrine stimulation or decreased renal function, may be responsible for the hyperglucagonemia in the diabetic animal in vivo.[30] They also raised the possibility of the participation of extrapancreatic glucagon in this mechanism, since a marked hyperglucagonemia was observed in totally pancreatectomized dogs, as previously reported.[33,34] According to another rather attractive explanation, the increased somatostatin in the islet causes the inhibition of glucagon locally, but only if a paracrine mechanism is operative between A and D cells, a possibility for which no method of confirmation is yet available. Therefore, the negative correlation between somatostatin and glucagon release observed in this study does not necessarily imply the absence of a close interrelationship between A and D cells.

In summary, both pancreatic somatostatin content and release are increased in the streptozotocin-diabetic rat, with an inverse relationship to insulin. The pancreatic D cell function thus appears to be regulated by the direct or indirect effect of insulin.

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