Abnormalities of Pancreatic Somatostatin Secretion Corrected by In Vivo Insulin Treatment of Streptozotocin-Diabetic Rats

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SUMMARY

In islets removed from untreated streptozotocin-diabetic rats, the somatostatin-containing cells were unresponsive to changes in glucose concentration, while they did respond to raised cyclic AMP levels. By contrast, in vivo insulin treatment restored the glucose sensitivity of the somatostatin secreting cells. In addition, in vivo insulin treatment resulted in a lowering of the high basal somatostatin secretion rate found in islets from untreated diabetic rats. These changes were made without any alteration of islet insulin content or enhancement of the in vitro insulin secretion. These results indicate that there is not a basic defect in the glucoreceptor of the somatostatin cell in diabetes. DIABETES *30*:865–867, October 1981.

Although somatostatin has a widespread distribution in body tissues, its very short half-life in blood[1,2] would suggest that its major target tissues should be close to the somatostatin secretory cell. If this is so, then it is probable that local factors play a modulatory role in somatostatin secretion. Since the secretion of pancreatic somatostatin has been reported to be abnormal in insulinopenic diabetes,[3–6] the question arises as to whether this could be due to the lack of insulin. It has previously been reported that the glucose sensitivity of the pancreatic D-cell, which is absent in alloxan-induced diabetes in dogs, cannot be restored by the acute addition of exogenous insulin in vitro to pancreases isolated from such animals.[6] We have therefore investigated whether in vivo insulin treatment of insulinopenic diabetic rats has any effect on the characteristics of pancreatic somatostatin secretion subsequently studied in vitro.

MATERIALS AND METHODS

Male Wistar rats were made diabetic with streptozotocin (50 mg/kg i.v.). Studies on somatostatin and insulin secretion were carried out 2–4 mo later. Two separate batches of diabetic and nondiabetic rats were used. The diabetic rats were randomly divided into two groups. One group received no antidiabetic treatment while the other received daily insulin injections (range 4–8 U semilente plus 4–16 U lente insulin per day) (Novo Industries, Copenhagen, Denmark) for 10–21 days before being killed for experimental use. Urine volumes of treated diabetic rats (during the last 3 days before they were killed) were 18 ± 6, 13 ± 1, and 9 ± 1 ml/24 h; for untreated diabetic rats 114 ± 8 ml/24 h; for nondiabetic rats 2–8 ml/24 h. Plasma glucose concentrations at the time of death were 101 ± 51 mg/dl, 623 ± 42 mg/dl, and 131 ± 6 mg/dl for the same groups, respectively.

Animals were killed in the fed state between 8 a.m. and 9 a.m. Insulin-treated rats received the last injection 20 h previously. Islets were isolated by a modification of the collagenase technique,[7] and were preincubated for 30 min at 37°C with 2.8 mM glucose in a modified Krebs-Ringer bicarbonate solution (KRB-Hepes) containing 10 mM N-2-hydroxyethylpiperazine-N′-ethane sulfonic acid (Hepes) and 250 KIU/ml aprotinin (Trasylol). After preincubation the islets were rinsed twice in KRB/Hepes containing 2.8 mM glucose and then incubated in groups of 10 islets for 30 min in 1 ml KRB-Hepes containing the test substances. After incubation, the buffer was removed and stored for hormone assay, the islets were carefully washed, and the remaining insulin and somatostatin content was extracted as previously described in detail.[8]

Insulin and somatostatin were measured by radioimmunoassay, using the charcoal separation technique.[9] Rat insulin standard and guinea-pig anti-pork insulin serum were used in the insulin assay. Cyclic somatostatin was used as standard in the somatostatin assay. N-tyrosylated somatostatin, iodinated by the chloramine-T method[10] and purified on CM 52 cellulose, was used as tracer. The somatostatin antiserum was grown in rabbits and showed no cross-reactivity wth insulin, glucagon, pancreatic polypeptide, gastric

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inhibitory polypeptide, vasoactive intestinal polypeptide, gastrin, motilin, secretin, or cholecystokinin-pancreozymin. The minimal sensitivity of the insulin assay was 50 pg/ml and of the somatostatin assay 3–4 pg/ml.

Results are given as mean ± SEM. Student's two-tailed unpaired *t* test was used to evaluate differences between groups.

Source of materials. Streptozotocin: Dr. W. Dulin, Upjohn Company (Kalamazoo, Michigan). Collagenase: Serva GmbH (Heidelberg, Germany). Three-isobutyl-methylxanthine (IBMX): Sigma Chemical Company (St. Louis, Missouri). Aprotinin (Trasylol): Prof. G. L. Haberland, Bayer, A. G. (Wuppertal, Germany). Rat insulin standard: Dr. J. Schlichtkrull, Novo Research Institute (Bagsvaerd, Denmark). Guinea-pig, anti-pork insulin serum: Dr. H. H. Schöne, Fabwerke Hoechst (Frankfurt, Germany). Somatostatin antiserum: Dr. F. Rohner-Jeanrenaud, Laboratoires de recherches métaboliques, Université de Genève (Geneva, Switzerland).

RESULTS

The somatostatin secretion of islets from untreated diabetic rats was not stimulated by 16.7 mM glucose but showed a marked response to the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) in the presence of both 2.8 and 16.7 mM glucose (Table 1.) Insulin release from islets of diabetic rats, and somatostatin and insulin release from islets of nondiabetic rats, responded to glucose and IBMX stimulation (Table 1).

Islets from diabetic rats that had received insulin treatment in vivo showed a significant somatostatin response to glucose (Figure 1). This was achieved at extremely low levels of insulin secretion. In the presence of 2.8 mM glucose, the insulin secretion rate from islets of untreated diabetic rats was 0.65 ± 0.15 ng/10 islets/30 min. In the presence of 16.7 mM glucose, it was 1.41 ± 0.21 ng/10 islets/30 min, while the insulin secretion rates from islets of insulin-treated rats were 0.14 ± 0.03 ng/10 islets/30 min and 0.37 ± 0.09 ng/10 islets/30 min, respectively (Figure 1).

The diabetic rats, untreated or treated with insulin and used for experiments shown in Figure 1, originated from the same groups of streptozotocin-diabetic rats. As can be seen from Table 2, the somatostatin and insulin contents of the islets from the two subgroups of diabetic animals were similar. The somatostatin content of islets from diabetic rats was 130% and the insulin content was less than 1% of those values found for islets of nondiabetic rats.

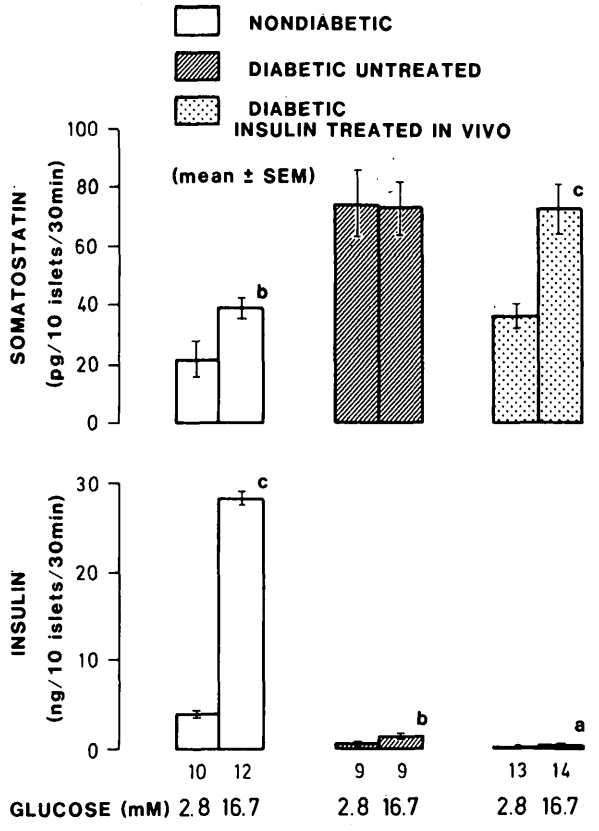

FIGURE 1. Effect of glucose on somatostatin and insulin secretion from freshly isolated islets of nondiabetic and streptozotocin-diabetic rats (mean ± SEM). Diabetic rats receiving insulin treatment in vivo were injected with insulin for 10–21 days before being killed. (a) P < 0.05, (b) P < 0.025, (c) P < 0.001 compared with 2.8 mM glucose control.

DISCUSSION

Glucose stimulation of pancreatic somatostatin secretion was absent in islets freshly isolated from rats with untreated streptozotocin-induced diabetes, while the sensitivity of the somatostatin cells to raised cyclic AMP levels was retained. These results are consistent with the previous report of glucose insensitivity of pancreatic somatostatin cells in experimental insulinopenic diabetes, while sensitivities to other stimuli such as isoproterenol, calcium, and arginine are retained.[6]

In vivo insulin treatment led to restoration of glucose sensitivity in pancreatic D-cells. This result, achieved without any enhancement of either in vitro insulin secretion or in-

TABLE 1
Effect of 3-isobutyl-1-methylxanthine (IBMX) on somatostatin and insulin secretion by freshly isolated pancreatic islets from nondiabetic and untreated streptozotocin-diabetic rats (mean ± SEM)

Glucose (mM)	IBMX (mM)	Somatostatin (pg/10 islets/30 min)		Insulin (ng/10 islets/30 min)	
		Nondiabetic	Untreated diabetic	Nondiabetic	Untreated diabetic
2.8	—	26 ± 3 (12)	86 ± 14 (13)	2.7 ± 0.6 (12)	0.15 ± 0.02 (13)
2.8	1	62 ± 7 (11)*	342 ± 19 (12)*	6.0 ± 0.6 (12)*	1.53 ± 0.39 (13)*
16.7	—	52 ± 5 (10)†	109 ± 18 (13)	36.4 ± 2.4 (10)†	0.43 ± 0.05 (13)†
16.7	1	217 ± 20 (12)*	486 ± 36 (14)*	78.9 ± 6.5 (11)*	2.03 ± 0.46 (14)*

* P < 0.005 compared with glucose control.
† P < 0.001 compared with 2.8 mM glucose and zero IBMX.

TABLE 2
Somatostatin and insulin contents of islets* isolated from nondiabetic and streptozotocin-diabetic rats (mean ± SEM)

	Somatostatin (ng/10 islets)	Insulin (ng/10 islets)
Nondiabetic	3.8 ± 0.2	813 ± 31
Diabetic		
Untreated	5.3 ± 0.5	6.3 ± 1.0
Insulin treated	5.4 ± 0.5	6.3 ± 0.6

* See Figure 1 for insulin and somatostatin secretion rates.

traislet insulin content, suggests that the metabolic state of the somatostatin cell, rather than high intraislet insulin concentrations, is more important for glucose-induced pancreatic somatostatin release. In addition, it has been shown previously that the glucose sensitivity of the pancreatic D-cell cannot be restored merely by the acute addition of even large amounts of insulin to the perfusate of pancreases isolated from alloxan-diabetic dogs.[6]

Basal secretion rates of somatostatin were higher in islets freshly isolated from untreated diabetic rats than in islets isolated from the group of diabetic rats that received insulin treatment in vivo. (Insulin and somatostatin contents were similar.) A tendency to high basal somatostatin secretion rates has previously been described in experimental insulinopenic diabetes;[4] insulin treatment was reported to cause a drop in circulating somatostatin secretion levels to that found in nondiabetic animals.[5,11] It has been suggested that increased secretion from the pancreas may be a major cause of the hypersomatostatinemia of untreated diabetes.[12] If this is so, and in accordance with what has been discussed above, it would appear that the insulin concentration within the pancreas is not the critical factor in causing hypersomatostatinemia. In this respect, it is interesting that in the diabetic state, there is also increased somatostatin secretion from the stomach[13] where, under normal conditions, the somatostatin-containing cells are at a distance from the source of insulin secretion. This would support the idea that a certain level of insulin lower than that found within the normal pancreas may be required to regulate the metabolic state of gastric and pancreatic D-cells. Plasma glucagon concentrations are usually abnormally high in untreated experimental insulinopenic diabetes, but the levels can be reduced by in vivo treatment either with insulin injections[14] or by pancreatic islet transplantation.[15,16] Glucagon stimulates pancreatic somatostatin release[17] and could, therefore, be a secondary reason for the high basal secretion rates found in untreated diabetes. On the other hand, plasma free fatty acids, which are an extremely potent stimulus to somatostatin secretion,[18] are elevated in untreated diabetes and may be the cause of the high basal levels of somatostatin secretion. In this context, consider the obese Zucker rat, in which moderate glucose intolerance occurs in the presence of hyperinsulinemia and very high levels of plasma free fatty acids. Pair feeding of such rats with lean controls has little or no effect on plasma free fatty acid levels.[19] While pair feeding of obese rats with lean controls causes a reduction in insulin secretion, it has been reported to have no effect on pancreatic somatostatin secretion.[8]

The results of this study demonstrate that the glucose sensitivity of the pancreatic somatostatin cell is not dependent on high concentrations of insulin within the pancreas, either in the basal or glucose-stimulated state. The restoration of glucose sensitivity to the pancreatic D-cell by insulin treatment in streptozotocin diabetes may occur mainly as a result of insulin's effect in normalizing the metabolic state of the animal, with correction of circulating levels of metabolites such as free fatty acids or of other hormones such as glucagon.

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