Elevated Portal and Peripheral Blood Concentration of Immunoreactive Somatostatin in Spontaneously Diabetic (BBL) Wistar Rats

Suppression With Insulin

YOGESH C. PATEL, THOMAS WHEATLEY, FRANCINE MALAISSE-LAGAE, AND LELIO ORCI

SUMMARY

Immunoreactive somatostatin (IRS) was measured in extracted plasma obtained from the hepatic portal vein (PV) and inferior vena cava (IVC) of acute, untreated, spontaneously diabetic Wistar rats (BBL), insulin-treated diabetic rats, and nondiabetic controls. Acetic acid extracts of the pancreas and entire gastrointestinal tract were assayed for IRS, and the volume density of pancreatic D-, A-, and B-cells was determined by quantitative morphometry. The concentration of IRS in the PV and IVC of the untreated diabetic rats was significantly elevated compared with controls, with a much greater percent increase in the IVC compared with the PV. Insulin treatment for 4–6 wk restored the elevated PV and IVC levels to control values. The pancreatic content of IRS and the volume density of D-cells was severely reduced in the diabetic groups whereas gut IRS was unchanged. These data suggest that the elevated blood levels are secondary to insulin deficiency and result from altered peripheral metabolism and/or increased secretion of IRS most probably from the gut. The increased peripheral blood concentration of IRS raises the possibility of an endocrine role of circulating somatostatin in diabetes. The reduction in pancreatic IRS found in this model is probably secondary to insulitis and contrasts with the D-cell augmentation reported in streptozotocin-diabetic rats. DIABETES 29:757–761, September 1980.

The peptide somatostatin, originally identified in the central nervous system, is now known to be present in D-cells of the pancreatic islets and throughout the gastrointestinal tract in mucosal cells and in the enteric plexus.[1,2] There is now considerable evidence to suggest a profound disturbance of somatostatin cells in diabetes.[3–5] In insulin-deficient diabetes produced in rats by streptozotocin, there is a marked increase in pancreatic somatostatin concentration due to a hyperplasia of the D-cells accompanied by a parallel increase in gastric somatostatin.[4] These findings provide indirect evidence for increased somatostatin secretion from the pancreas and possibly the gut in diabetes. In the present study we have attempted to correlate the tissue changes with blood somatostatin measurements in a newly recognized model of insulin-deficient diabetes: spontaneously diabetic Wistar rats. These animals develop glycosuria at 4–18 wk of age, require daily insulin injections for survival, and show a pathologic picture of pancreatic insulitis.[6] They therefore closely resemble human juvenile diabetes. We report here that hepatic portal and peripheral plasma levels of immunoreactive somatostatin (IRS) are markedly elevated in the untreated diabetic animals and suppress to normal with insulin treatment.

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From the Fraser Laboratories, McGill University; Departments of Medicine, Neurology and Neurosurgery, Royal Victoria Hospital, Montreal, Canada; and Institute of Histology and Embryology, Geneva Medical School, Geneva, Switzerland.

Address reprint requests to Dr. Y. C. Patel, Room M310, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec H3A 1A1, Canada.

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MATERIALS AND METHODS

Animals. Wistar rats were obtained from the Animal Resources Division, Health Protection Branch, Health and Welfare, Ottawa and studied in three groups: untreated diabetic, insulin-treated diabetic, and nondiabetic control. Litters derived from diabetic parents were screened for glycosuria by twice-weekly testing of spontaneously voided urine. The untreated diabetic group comprised 10 rats (5 ♂, 5 ♀), which were studied within 2–5 days of detection of glycosuria (at 93 ± 7 days of age). A second group of rats (N = 7, ♀) with diabetes of comparable severity was selected for treatment. Mean age of detection of glycosuria in this group was 106 ± 6 days. These animals were treated with subcutaneous injections of protamine-Zn insulin (Connaught Laboratories), 0.2–2.6 U daily for 37 ± 6 days before study. The dose of insulin was determined on the basis of daily urine tests for glucose. Control rats (10, ♀) whose ages were comparable to those of the treated diabetic group at the time they were killed were obtained from breeding pairs that had never produced diabetic offspring.

Blood and tissue samples. The treated diabetic rats were studied approximately 14 h after the last injection of insulin. Animals from all three groups in the fed state were lightly anesthesized with ether, the abdomen was opened, and blood from the hepatic portal vein (PV) and inferior vena cava (IVC) was collected at 0°C and the plasma immediately separated at 4°C and stored at −20°C. The whole pancreas was dissected and divided longitudinally into two halves, both of which were weighed. One of the pieces was then placed in 1 M acetic acid at 0°C for hormone extraction and the other fixed in Bouin's solution for immunofluorescence studies. The stomach, jejunum, ileum, and colon were removed, cleaned of their contents by rinsing in saline, weighed, and placed in 1 M acetic acid at 0°C for extraction.

Plasma and tissue extraction and measurement of glucose, insulin, glucagon, and IRS. Tissue samples were extracted in 1 M acetic acid by sonication and boiling.[7] Plasma for measurement of IRS was extracted with 2 vol of acid-ethanol (95% ethanol; 5% 1 M HCl) in a single step procedure as previously reported.[8,9] Immunoreactive somatostatin was measured by a radioimmunoassay capable of detecting 1 pg IRS or 15 pg IRS/ml plasma.[7–9] Plasma glucose, plasma and pancreatic insulin, and glucagon were determined as previously described.[4] The total pancreatic content of insulin, glucagon, and IRS were calculated from the hormone concentration in the extracted portion of the pancreas and extrapolated to the whole pancreas.

Immunofluorescent-staining techniques and morphometric analysis. Pancreatic tissue of the body and tail of the gland was fixed in Bouin's solution, dehydrated with alcohol, and embedded in paraffin. Sections (5 μM) were cut and stained by the indirect immunofluorescence technique with antisera to insulin, glucagon, and IRS.[3–5] The mean volume density (which represents an indirect estimate of the number and size of individual endocrine cells) of insulin-, glucagon-, and IRS-containing cells was measured in the first 15–20 islets encountered in immunostained sections by the point-counting method described in detail previously.[10] In addition, the volume density of individual islets was also measured by the point-counting method on hemaluneosin-stained sections consecutive to each group of immunofluorescent-stained sections. This double evaluation (see Table 3) permitted an assessment of the variation of the total islet mass in the different pancreases.[10]

Statistical analysis. The values obtained for each group of animals were expressed as mean ± SEM. The unpaired student's *t* test was used in statistical analyses.

RESULTS

Body weight, plasma glucose, insulin, glucagon, and IRS. Treatment of the diabetic group with insulin resulted in mean weight gain from 226 ± 11.4 to 259 ± 5.5 g. The mean body weight of the three groups was comparable at the time of study (Table 1). The untreated diabetic animals were severely insulin-deficient with low plasma insulin (Figure 1) and a fourfold elevation in plasma glucose concentration (Table 1). Mean IRS concentration in the PV of these rats was 486 ± 61 pg/ml, being 160% of the value of 297 ± 24 pg/ml found in the nondiabetic controls (Figure 1). A more striking elevation in the IRS concentration in the IVC of the untreated diabetic animals was found, the mean value of 196 ± 30 pg/ml being 250% of the control value of 77 ± 6 pg/ml (Figure 1). Comparison of paired portal/IVC levels gave a negative transhepatic gradient with a mean value of 434 ± 66% for nondiabetic controls and 291 ± 44% for the untreated diabetic animals. Insulin treatment of the diabetic rats restored both the elevated PV and IVC levels to normal (Figure 1). Almost identical findings were observed for PV and IVC glucagon levels, both of which were elevated threefold in the untreated diabetic animals and suppressed to normal with insulin therapy (Figure 1).

TABLE 1
Body weight and plasma glucose at the time rats were killed

	Nondiabetic	Untreated diabetic	Treated diabetic
Weight (g)	256 ± 10	268 ± 22	259 ± 6
Plasma glucose (mg/dl)	164 ± 9	615 ± 49*	298 ± 78†

* $P < 0.01$ versus nondiabetic.
† $P < 0.025$ versus untreated diabetics.

Pancreatic insulin, glucagon, IRS, and gut IRS. There were no significant differences in the weights of the pancreas and gut tissues among the three groups of animals. Analysis of the pancreas for hormone content by radioimmunoassay (Table 2) showed a severe 98% reduction in insulin and a 60% decrease in IRS in the two diabetic groups. Pancreatic glucagon was reduced by 25%, but this difference was not statistically significant. Measurement of IRS in the gastrointestinal tissues showed no difference in the total gut content of the immunoreactive material in the three groups of animals (Table 2).

FIGURE 1. Comparison of immunoreactive somatostatin (IRS), glucagon, and insulin levels in the portal vein and IVC of control and diabetic rats. IRS and glucagon vary inversely with insulin. *P < 0.01, untreated diabetic versus nondiabetic. **P < 0.01, treated versus untreated diabetic. □ Nondiabetic, ■ untreated diabetic, ▨ treated diabetic rats.

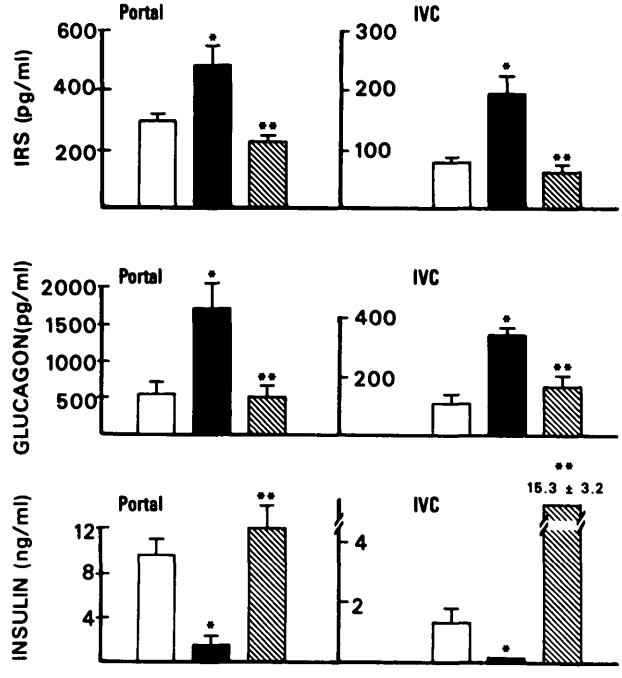

TABLE 2
Insulin, glucagon, and somatostatin content of pancreas, and somatostatin content of the gut of control and diabetic rats*

	Nondiabetic	Untreated diabetic	Treated diabetic
Pancreatic† insulin (μg)	61.8 ± 10.1	0.85 ± 0.41§	0.13 ± 0.11
Pancreatic glucagon (μg)	4.1 ± 0.66	3.1 ± 0.39	3.19 ± 0.58
Pancreatic somatostatin (μg)	0.52 ± 0.06	0.22 ± 0.03§	0.24 ± 0.05
Gut‡ somatostatin (μg)	2.26 ± 0.15	2.11 ± 0.09	2.34 ± 0.16

* Mean data ± SEM.
† Pancreatic hormone content refers to the whole organ.
‡ Gut includes entire stomach, duodenum, jejunum, ileum, and colon.
§ $P < 0.001$.

Morphology. Examination of the islets of the diabetic rats by light microscopy showed disruption of the normal islet architecture and infiltration with large numbers of mononuclear inflammatory cells. The severity of the islet lesions was comparable in the two diabetic groups. The distribution of the islet cells as determined by immunofluorescence is illustrated in Figure 2, and showed in the diabetic rats a virtual absence of insulin-containing B-cells. Glucagon and IRS immunofluorescent cells were scattered throughout the distorted islets in contrast to their peripheral location in the normal islets. Morphometric analysis of the pancreases of the control and diabetic rats (Table 3) showed a significant ($P < 0.01$) 98–100% reduction in the volume density of the insulin cells in both diabetic groups. Likewise, the volume density of the IRS immunofluorescent cells also showed a significant ($P < 0.01$) 66% and 93% reduction, respectively, in the untreated and treated diabetic rats. By contrast, pancreatic glucagon cells were unchanged in the untreated diabetic animals but significantly reduced in the treated diabetic rats.

DISCUSSION

The present data clearly indicate an increase in the PV concentration of IRS in insulin-deficient diabetic rats. This increase could represent increased secretion from the pancreas or gut, or both, although proof for this as well as the relative contribution of the pancreas and gut to the PV levels will require selective arteriovenous sampling. Based on indirect evidence, however, it would appear that the gastrointestinal tract is the major source of PV IRS for several reasons. First, the gut contains at least five times as much IRS as the pancreas (Table 2).[11] Second, a marked increase in gut IRS content has been shown to occur in chronic insulin-deficient diabetes.[4] The lack of alteration in gut IRS in the untreated diabetic rats in the present study is probably due to the fact that the animals were studied acutely, before the tissue IRS changes, which have been shown to be time-dependent, could occur.[4] The marked reduction in pancreatic IRS content in both the untreated and treated Wistar rats in the present study contrasts with the augmentation in pancreatic IRS found in chronic streptozotocin-diabetic rats.[3,4] As shown by morphometry, the reduction is caused by loss of D-cells probably secondary to the islet inflammatory process, as is also found in virus-induced diabetes in mice.[10] It provides additional evidence that the gastrointestinal tract is more important than the pancreas as the source of elevated PV IRS levels in the diabetic animals in this study.

Peripheral blood concentrations of IRS in the normal rat, like those in the PV, appear to be derived mainly from gastrointestinal and pancreatic secretion with perhaps lesser contributions from other IRS-containing tissues, such as the hypothalamus and peripheral nerves.[7,8,12] The present finding of a 2.5-fold increase in the IVC concentration of IRS in the untreated diabetic rats suggests that peripheral plasma IRS levels are strikingly elevated in insulin-deficient diabetes. Peripheral venous IRS concentration has also been reported to be elevated in alloxan diabetic dogs, although not to the same extent as that found in the Wistar rats.[13] The marked negative transhepatic gradient of IRS found in the present study and reported previously[8,14] suggests an important role of the liver in the clearance of IRS. One explanation for the greater percent increase in IRS concentration in the IVC compared with the PV in untreated diabetic rats is diminished hepatic clearance of IRS, as suggested by the smaller transhepatic gradient in these animals, although increased extraportal production or decreased peripheral degradation of IRS are also possible. In practical terms, the present demonstration of elevated IRS levels in the IVC of insulin-deficient animals indicates that peripheral venous

TABLE 3
Volume density of insulin, glucagon, and somatostatin cells in the pancreas of control and diabetic rats

	Nondiabetic (N = 4)	Untreated diabetic (N = 5)	Treated diabetic (N = 5)
Insulin	0.00651 ± 0.00034	0.00012* ± 0.0001	0.0000* ± 0.0000
Glucagon	0.00124 ± 0.00006	0.00137 ± 0.00024	0.00018† ± 0.00005
Somatostatin	0.00028 ± 0.00004	0.00009‡ ± 0.00003	0.00002‡ ± 0.0000

Mean data ± SEM.
* $P < 0.01$ compared with nondiabetic.
† $P < 0.01$ compared with nondiabetic or untreated diabetic.
‡ $P < 0.01$ compared with nondiabetic.

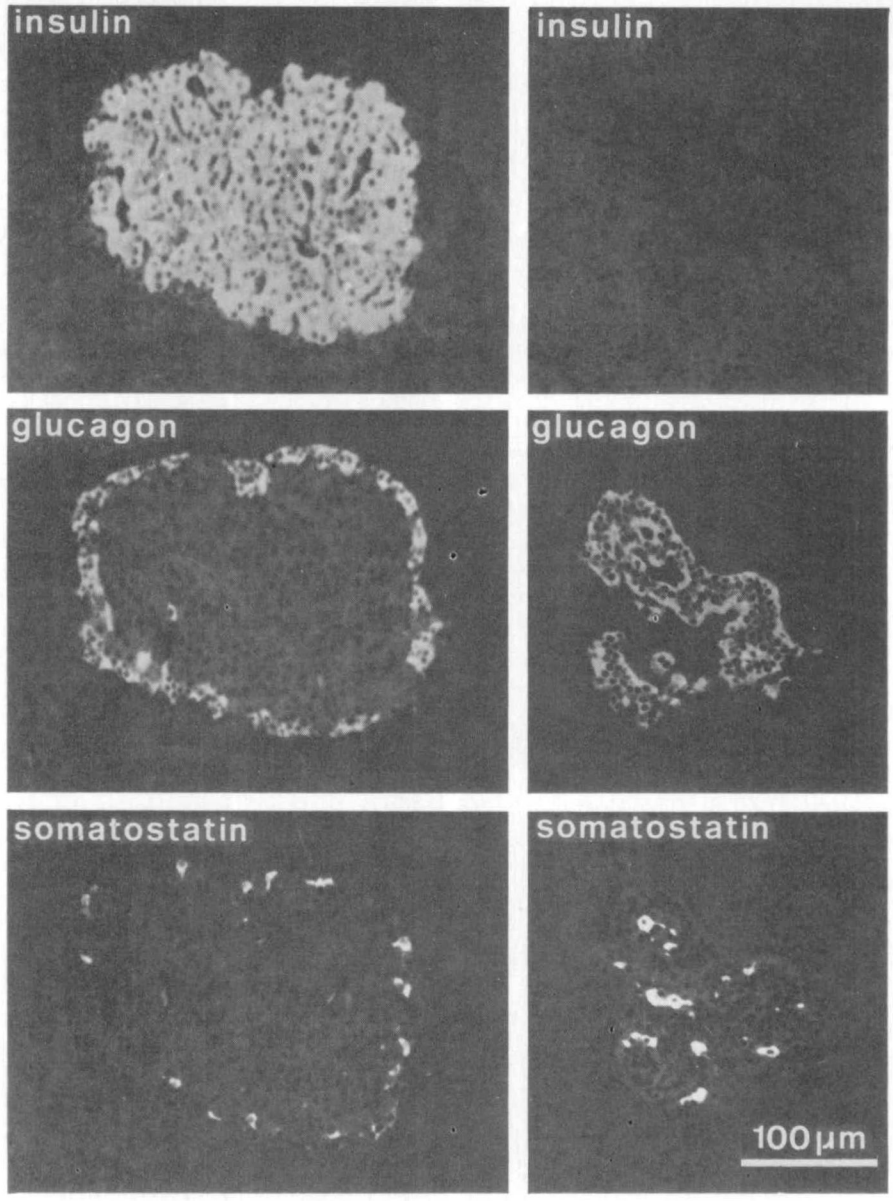

FIGURE 2. Immunofluorescent reactions in serial sections of a control islet (left panel) and of an atrophic islet from an untreated diabetic animal (right panel). The diabetic islet is reduced in size and is devoid of insulin immunofluorescent cells. Glucagon and somatostatin immunofluorescent cells form the bulk of the islet. The distribution of the three cell types was similar in the islets of the treated diabetic animals (not shown).(X180)

sampling for IRS could provide a convenient means for assessing somatostatin function in human diabetes.

Our finding of a reversal of elevated PV and IVC levels of IRS to normal with insulin treatment suggests that the increases result from lack of insulin. It remains to be determined whether this effect is mediated directly by insulin or indirectly through correction of the metabolic and hormone abnormalities of diabetes, some of which (e.g., hyperglucagonemia, increased food intake, dehydration)[15–17] could conceivably influence the secretion and metabolism of somatostatin.

In view of the marked hyperglucagonemia found in the insulin-deficient animals, it is evident that the IRS concentration achieved in the circulation is incapable of suppressing A-cell function completely. This could be due to the fact that the inhibitory effect of somatostatin on A-cells requires a high local concentration of the peptide produced by the juxtaposed D-cells.[18] On the other hand, the elevated IRS levels in the peripheral blood may account for the suppression of spontaneous growth hormone release in diabetic rats,[19] and together with other potential effects on distant targets,[20] raise the possibility of an endocrine role of somatostatin in diabetes.

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