Hypersecretion of Gastric Somatostatin in Spontaneously Diabetic BB Rats

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SUMMARY

We previously reported that the BB diabetic rat is characterized by a reduction in pancreatic immunoreactive somatostatin (SLI) content, δ-cell mass, and δ-cell secretory reserve. Despite this, portal plasma SLI levels are elevated in diabetic animals and normalized by insulin therapy. These findings comprise indirect evidence for SLI hypersecretion by the gut in untreated BB rats. This study was undertaken with isolated stomach perfusions to investigate directly the secretory status of gastric δ-cells in this diabetic model. Isolated stomachs of three groups of insulin-treated diabetic, untreated diabetic, and nondiabetic control rats were perfused in situ under basal and glucagon-stimulated (5 nM) conditions. Untreated diabetic BB rats exhibited significant enhancement of basal and glucagon-stimulated gastric SLI release. Insulin treatment reduced gastric SLI release to significantly subnormal levels. More than 95% of basal and stimulated SLI released in diabetic BB and normal control rats coeluted with synthetic somatostatin-14 on Sephadex G-50 columns. We conclude that *1*) basal and stimulated gastric SLI release is increased in untreated BB rats and is suppressed with insulin therapy, *2*) gastric δ-cell hyperfunction accounts for portal vein hypersomatostatinemia characteristic of untreated diabetic BB rats, and *3*) somatostatin-14 is the main molecular form of SLI released from normal and diabetic stomachs. *Diabetes* 36:849–52, 1987

The spontaneously diabetic BB Wistar rat develops a characteristic syndrome of autoimmune insulitis, hypoinsulinemia, hyperglycemia, ketoacidosis, and hyperglucagonemia (1–4). We have previously demonstrated that peripheral and portal venous hypersomatostatinemia also characterize this diabetic syndrome and that insulin treatment normalizes the elevated plasma immunoreactive somatostatin (SLI) levels (4). In view of the finding of a marked (66%) reduction in islet δ-cell mass with a concomitant reduction in δ-cell secretory reserve, as assessed by studies with the isolated perfused pancreas, the pancreas has been excluded as a source of the hypersomatostatinemia (5,6). Additionally, we have demonstrated that diabetic BB rats exhibit impaired hepatic clearance of somatostatin-14 (S-14) and somatostatin-28 (S-28), thus establishing diminished hepatic metabolism of somatostatin as an additional contributory factor to the hypersomatostatinemia (7).

The upper gut and pancreas are the principal sources of portal and peripheral plasma SLI concentrations (8,9). Although the stomach is the most probable site for somatostatin overproduction in the diabetic BB rat, there have been no direct studies to document this. Indirect evidence based on tissue SLI (5) or perfusion of abdominal organs en masse (10) has revealed no significant alteration or a reduction of gastrointestinal SLI output in diabetic rats. In this study we directly evaluated the status of SLI secretion from isolated perfused stomachs of control and diabetic BB rats. We quantitated both basal release as well as gastric δ-cell secretory reserve in response to stimulation with exogenous glucagon and examined the effects of in vivo insulin administration on these responses.

MATERIALS AND METHODS

Animals. Male Wistar BB rats were obtained from the Animal Resources Division, Health Protection Branch, Health and Welfare, Ottawa, Canada, and studied in three groups of four each: untreated diabetics, insulin-treated diabetics, and nondiabetic controls. Diabetic rats were identified in diabetes-prone litters by twice-weekly screening for appearance of glycosuria. Control rats were obtained from diabetes-

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Received for publication 19 March 1986 and accepted in revised form 3 February 1987.

free litters and matched for age with diabetic rats. The mean age of diabetic rats was 143 ± 15 days, with a mean duration of diabetes of 66 ± 9 days. The mean daily insulin requirement was 3.6 ± 1.2 U protamine zinc insulin (Connaught-Novo, Ontario, Canada). Animals were fed Purina rat chow ad libitum until they were fasted ~16 h before surgery. Untreated rats were studied 5.6 ± 0.6 days after discontinuation of insulin. Treated animals were studied 14 h after their final insulin injection.

Blood samples. After the administration of pentobarbital sodium (50 mg/kg i.p.) to induce anesthesia, the abdomen was opened via a midline incision, and 2 ml of blood was withdrawn from the inferior vena cava. The plasma was separated immediately in a refrigerated centrifuge and divided into two aliquots; one was frozen pending analysis of glucose, and the other was immediately extracted in acid-ethanol for SLI measurements (4,11).

Isolated stomach perfusions. We used the method of McIntosh et al. (12) with minor modifications (13): isolated stomachs were perfused in situ via the abdominal aorta after ligation of irrelevant vessels emanating from the aorta and celiac and mesenteric arteries. A drainage tube was inserted into the pylorus via the duodenal stump. The effluent was collected from an outflow cannula in the portal vein. Perfusions were carried out at 37°C in an enclosed chamber. The medium consisted of Krebs-Ringer bicarbonate buffer with 5.5 mM glucose and 4 g/dl bovine serum albumin (BSA) fraction V (Sigma, St. Louis, MO). The perfusate was circulated by a roller pump (Gilson, Middleton, WI) at a constant flow rate of 2.5 ml/min and equilibrated with 95% O_2/5% CO_2 in an artificial lung (13), with pH 7.4 and arterial O_2 partial pressure (Pa_{O_2}) 460 mmHg. After a 20-min postcannulation equilibration period, effluent samples were collected every minute via a fraction collector into glass tubes containing 125 μl of 1 M acetic acid. After 8 min of basal perfusion, glucagon (5 nM, Novo, Denmark) was perfused through the isolated stomach for 15 min. Subsequently, for 17 min the stomach was perfused again under basal conditions. On completion of sample collection, the viability of each experimental preparation was confirmed by infusion of methylene blue.

Measurement of glucose and SLI. Glucose concentrations were determined with a Beckman glucose analyzer. SLI in plasma extracts and perfusates was measured by radioimmunoassay with antibody R149 directed toward the central segment of S-14, [^{125}I-Tyr]S-14 radioligand, and synthetic cyclic S-14 as standards (4,8,11).

Gel chromatography. Gel chromatography was used to characterize the molecular forms of SLI released in the stomach perfusates. A Sephadex G-50 (superfine) column (1.2 × 70 cm) was eluted with 6 M urea, 50 mM PO_4–0.1% BSA, pH 7.5 at 4°C. Fractions (1 ml) were collected and assayed for SLI (8).

Statistical analysis. Data are expressed as means ± SE. Group differences were compared by Student's *t* test for unpaired samples; $P < .05$ was considered statistically significant.

RESULTS

Plasma glucose and SLI. Plasma glucose from untreated diabetic rats was significantly elevated (503 ± 56 mg/dl, $P < .01$) compared with that from insulin-treated diabetic rats (153 ± 29 mg/dl) and nondiabetic controls (139 ± 16 mg/dl). The untreated diabetic plasma was also characterized by 4+ ketonemia. Untreated diabetic animals exhibited marked elevation of plasma SLI (181 ± 30 pg/ml, $P < .01$) compared with that of insulin-treated diabetic rats (73 ± 21 pg/ml) and nondiabetic controls (76 ± 16 pg/ml) (Table 1). As previously reported, discontinuation of insulin treatment resulted in a threefold increase in plasma glucagon levels in the untreated group (6).

SLI secretion from perfused stomach. Basal gastric SLI release was significantly elevated in untreated diabetic rats (3.9 ± 0.5 ng/8 min) compared with that in nondiabetic controls (2.6 ± 0.1 ng/8 min, $P < .05$). Insulin treatment diminished basal SLI secretion to subnormal levels (2.1 ± 0.1 ng/8 min). Similarly, glucagon-stimulated SLI release from the stomach preparation was significantly augmented in untreated diabetic rats (15.4 ± 0.6 ng/15 min) compared with that from nondiabetic controls (12.3 ± 0.7 ng/15 min, $P < .05$) (Figs. 1 and 2). The insulin-treated diabetic group exhibited a significant diminution of stimulated SLI secretion (8.7 ± 0.6 ng/15 min) to subnormal levels ($P < .05$) (Figs. 1 and 2). Poststimulus basal gastric SLI secretion was also significantly enhanced in untreated diabetic rats (8.2 ± 0.7 ng/17 min, $P < .05$) and diminished in treated diabetics (4.2 ± 0.3 ng/17 min, $P < .05$) compared with normal controls (6.0 ± 0.5 ng/17 min). As previously reported, there was no change in the SLI content of the stomach of the treated or untreated diabetic rats (4,5). By Sephadex G-50 chromatography, >95% of the basal and stimulated SLI release from both the control and diabetic stomach preparations coeluted with synthetic S-14 ($K_D = 0.78$).

DISCUSSION

Our study demonstrates augmented basal and stimulated release of SLI from the perfused stomach of the insulinopenic diabetic BB rat and provides the first direct proof that enhanced gastric SLI secretion is an important etiological factor in the portal venous hypersomatostatinemia observed in this model. The occurrence of elevated portal venous SLI levels and increased gastric SLI secretion in the face of reduced islet δ-cell mass and δ-cell secretory reserve, as demonstrated earlier (4,5), underscore the importance of the stomach and the relative unimportance of the pancreas as a source of SLI draining into the portal vein (9). The increased secretion of SLI is most probably derived from mucosal gastric δ-cells, the principal somatostatin cell type in the stomach (14). Morphological studies of gastric δ-cells in BB rats are not available. However, our study suggests that gastric δ-cells in this model, unlike their pancreatic counterparts (4), are healthy and indeed hyperfunctional. The main molecular

TABLE 1
Comparison of inferior vena cava plasma SLI values in nondiabetic, untreated diabetic, and insulin-treated diabetic rats

Animal group	Plasma SLI (pg/ml)
Nondiabetic	76 ± 16
Untreated diabetic	181 ± 30*
Treated diabetic	73 ± 21

Values are means ± SE; $n = 4$. SLI, immunoreactive somatostatin. *$P < .01$ compared with values in nondiabetic and treated diabetic rats.

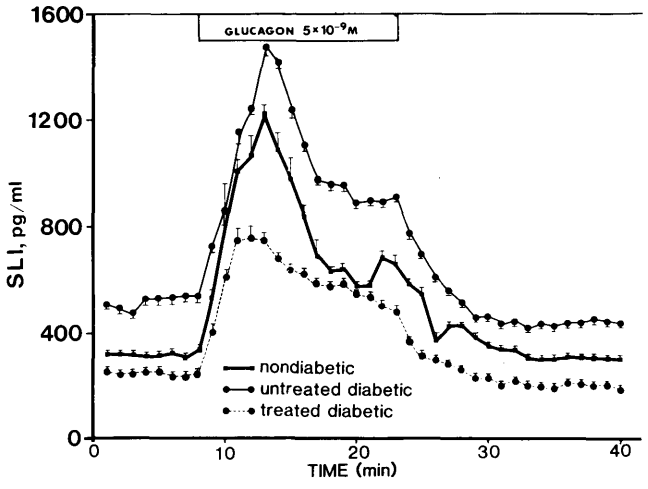

FIG. 1. Patterns of basal and stimulated secretion of immunoreactive somatostatin (SLI) from perfused stomachs of untreated diabetic and insulin-treated diabetic rats compared with nondiabetic controls (means ± SE; *n* = 4).

form of SLI released from the diabetic stomach was S-14, identical to that found in the nondiabetic rat. Because S-14 is the principal storage and releasable form of somatostatin in the normal stomach (8,15), this finding suggests that chronic stimulation of the gastric δ-cells in insulin-deficient diabetes is not accompanied by qualitative alterations in the posttranslational processing of prosomatostatin to products destined for secretion.

A disturbance of gastric SLI secretion in insulinopenic diabetes comes as no surprise, because other insulin-deficient diabetic animal models, e.g., streptozocin-induced diabetic (STZ-D) rats, have been previously shown to exhibit both an increase in the tissue content of SLI as well as enhanced SLI release from the perfused stomach (16,17). Although gastric SLI release is enhanced in both the BB and STZ-D rats, pancreatic SLI release is augmented only in the latter model (21,22) and significantly reduced in the BB group (5,6). This is because islet δ-cells undergo a hyperplastic change in STZ-D as a result of chronic insulinopenia (16),

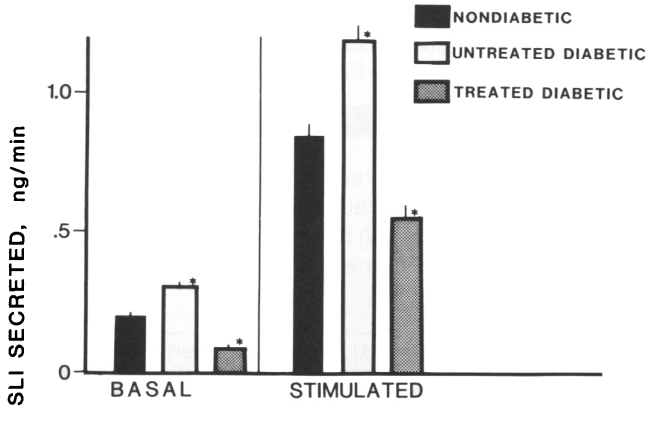

FIG. 2. Comparison of gastric immunoreactive somatostatin (SLI) secretion rates basally and during glucagon stimulation in untreated diabetic, insulin-treated diabetic, and nondiabetic rats. Data derived from perfusion profiles depicted in Fig. 1. *$P < .01$ vs. values in nondiabetic rats.

whereas in the BB diabetic rat there is a loss of islet δ-cells secondary to insulitis (6,6). Our data are at variance with the only other report that attempted to elucidate the pancreatic and gastrointestinal components of SLI secretion in the BB rat (10). In the other study, the pancreas, stomach, duodenum, and spleen were perfused en masse, and the release of SLI was compared with that from the isolated perfused pancreas alone. Gut SLI secretion was decreased, whereas pancreatic SLI secretion was increased. The more stable BB/Phi diabetic rat model was employed in these experiments, and this together with the different perfusion methodology can probably account for the very different results.

Our study suggests that insulin deficiency, acting either directly or through a cascade of metabolic or hormonal derangements, prompts reversible gastric δ-cell hyperfunction. Insulin has long been recognized as a potent inhibitor of both basal and glucagon-stimulated normal gastric SLI secretion (18–20). Our observation that insulin therapy diminishes both basal and glucagon-stimulated secretion of SLI to subnormal levels in BB rats has also been observed in other insulinopenic rat models (19,21–22). Furthermore, we have also previously demonstrated that the diminished pancreatic secretion of SLI in BB rats is further reduced by insulin treatment (6). The mechanism whereby insulin reduces islet and gastric δ-cell function appears to involve both a direct effect as well as indirect influences, e.g., through glucagon. Both the islet and gastric δ-cells are capable of direct suppression by insulin (18,23). In this respect, gastric δ-cells are exquisitely sensitive and appear to be physiologically restrained by circulating levels of insulin (20). The reduction of gastric SLI secretion to subnormal levels can probably be accounted for by overreplacement with exogenous insulin by the single daily injection schedule in the diabetic animals.

Of the indirect effects of insulin deficiency capable of promoting gastric somatostatin secretion, glucagon, both via the circulation as well as locally in the stomach, is a potentially important stimulus in view of the marked hyperglucagonemia and evidence of increased gastrointestinal glucagon production characteristic of the diabetic BB rat (4,5).

The functional significance of gastric SLI overproduction in insulinopenic diabetes remains uncertain. Release of SLI into the circulation, with the attendant hypersomatostatinemia, is certainly capable of exerting a hormonal role on distant target cells, e.g., the pituitary somatotrophs (24,25) and possibly the residual islet cells. Whether gastric δ-cell overactivity by local action influences such gastric functions as acid and gastrin production or motility remains to be explored.

ACKNOWLEDGMENTS

We are grateful to Dr. P. Thibert, Animal Resources Division, Health and Welfare, Canada, for providing the BB rats and to D. Ellen and M. Correia for secretarial assistance.

This work was supported by USPHS Grant AM-21373 and Canadian Medical Research Council Grant MA-6196. Y.C.P. is a Scientist and M.D.R. is a Fellow of the Canadian Medical Research Council.

This work was presented at the International Somatostatin Symposium, Montreal, Quebec, July 1984, and at the Annual Meeting of the Canadian Society for Clinical Investigation, Montreal, September 1984.

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