Plasma Somatostatin Response to an Oral Test Meal in Liver Transplant Patients

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Ten liver transplant patients were studied in basal conditions and after ingestion of a standard mixed test meal. Control groups included 10 normal subjects, 10 patients with nonalcoholic liver cirrhosis, and seven kidney transplant patients. Plasma somatostatin, blood glucose, and plasma insulin, C-peptide, and glucagon were determined before and 15, 30, 45, 60, 90, 120, and 180 minutes after the start of the meal. In liver transplant patients, basal somatostatin and insulin levels were significantly lower than in cirrhotics and were comparable to those recorded in controls and in kidney transplant patients. The time course of the somatostatin secretory response after the meal was similar in any group, but the increase, evaluated as the incremental area above baseline, was significantly higher in liver transplant patients than in controls and cirrhotics and comparable to that recorded in kidney transplant patients. Insulin incremental areas were also lower than in cirrhotics and comparable to those recorded in controls and kidney transplant patients. The data suggest that in liver transplant patients an increased somatostatin response to a meal may be related to a relative β-cell secretory defect, which in turn seems consequent to immunosuppressive treatment.

IT IS KNOWN THAT somatostatin plays an important role in modulating pancreatic islet secretory function.[1] In particular, somatostatin exerts a potent inhibitory effect on both insulin and glucagon secretion, whereas its own secretion is stimulated by glucagon and inhibited by insulin.[2] Studies regarding postoperative glucose metabolism in patients who have undergone orthotopic liver transplantation (OLT) have shown abnormalities of glucose metabolism, with significantly elevated fasting glucose, immunoreactive insulin, and C-peptide concentrations, suggesting tissue insulin resistance.[3,4] This impaired glucose metabolism has been ascribed to immunosuppressive treatment with cyclosporine and prednisone.[5,6] However, some investigations indicate that long-term cyclosporine treatment does not explain the adverse effects on glucose homeostasis and insulin secretion in nontransplant patients.[7,8] Data are presently unavailable regarding somatostatin secretion and its possible relationship with abnormalities of glucose metabolism observed in OLT patients. In the present study, we evaluated fasting and meal-stimulated plasma somatostatin concentrations in a group of OLT patients. Insulin, C-peptide, and glucagon secretion have also been evaluated. Furthermore, since abnormalities of somatostatin secretion and glucose metabolism have been reported in liver cirrhosis,[9,10] results from the liver transplant recipients were compared with those obtained in a group of nondiabetic cirrhotic patients. Finally, to evaluate the role of immunosuppressive treatment on the glycemic and hormonal response to meal ingestion, the results were also compared with those obtained in a group of nondiabetic kidney transplant patients.

SUBJECTS AND METHODS

The protocol was approved by the Ethics Committee, and informed consent was obtained from all participants before testing.

Subjects

Ten patients who had received cadaveric OLT for cirrhosis due to viral hepatitis type C were studied. Although the metabolic status of these patients before transplantation was not formally assessed, overt diabetes was not identified in any patient during pretransplantation evaluation. Metabolic studies were performed a median of 10 months (range, 8 to 14) postoperatively, when patients were clinically stable. Age- and sex-matched control groups consisted of 10 normal subjects (five men and five women, aged 21 to 47) and 10 nondiabetic patients with compensated nonalcoholic liver cirrhosis. A further control group of seven nondiabetic kidney transplant recipients without a family history of diabetes was selected to match the immunosuppressive treatment. In both liver and kidney transplant patients, the immunosuppressive treatment consisted of prednisone (range, 5 to 15 mg/d), cyclosporine (range, 230 to 300 mg/d), and azathioprine (range, 50 to 100 mg/d). Routine biochemical markers of hepatic function were measured in each patient at the time of study, as were blood cyclosporine concentrations in transplanted patients. Clinical and laboratory features of the patients studied are reported in Table 1. Before testing, subjects were instructed to maintain for at least 3 days a standard hospital diet of approximately 2,500 cal containing 45% carbohydrate, 15% protein, and 40% fat by weight.

Experimental Procedure

Tests were begun at 8 AM following an overnight fast. Subjects consumed a standard test meal of 2 slices of buttered white bread with ham and cheese and 120 mL orange juice (protein 17 g, carbohydrate 67 g, and lipid 20 g) in 15 minutes.[11] The meal tolerance test was preferred to the more usual oral glucose tolerance test (OGTT) because it constitutes a more physiologic stimulus and, unlike the OGTT, is able to stimulate somatostatin secretion. Venous blood was drawn through a catheter inserted into an antecubital vein in basal conditions and 15, 30, 45, 60, 90, 120, and 180 minutes after the start of the meal. These blood samples were collected in ice-chilled polypropylene tubes containing 2.4 mg EDTA and 500 kallikrein inhibitory units of aprotinin (Trasylol; Bayer, Milan, Italy) and immediately centrifuged at 4°C for 15 minutes. The samples obtained were stored at −40°C until assayed.

Assay

Blood glucose concentrations were measured by the glucose oxidase method. Plasma somatostatin concentrations were measured by a radioimmunoassay (RIA) method. Cyclic somatostatin, rabbit antisomatostatin serum, and [^{125}I-Tyr^{1}] somatostatin were supplied as a kit by Immunonuclear (Stillwater, MN). The antiserum used in this assay was

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Table 1. Clinical and Laboratory Characteristics of the Patients Studied

Characteristic	Liver Transplant		Liver Cirrhosis		Kidney Transplant		Normal Range
	Median	Range	Median	Range	Median	Range	
Age (yr)	48.0	30-58	61.5	45-69	41.0	28-59	
Sex ratio (male/female)	6/4		6/4		4/3		
Body mass index	24.0	18-29	23.2	18-27	24.0	18-28	20-24
Creatinine (mg/dL)	1.3	0.9-1.6	1.1	0.9-1.3	1.4	0.9-2.2	0.6-1.4
Albumin (g/L)	42.0	38-45	32.5	28-40	41.0	35-46	35-50
γ-Globulins (g/L)	10.0	8-13	21.0	18-25	11.0	9-12	7-17
Bilirubin (mg/dL)	1.3	0.9-1.8	1.8	0.4-3.8	0.9	0.6-1.0	0.1-1.4
AST (U/L)	25.0	18-34	98.0	13-331	24.0	12-35	0-40
ALT (U/L)	25.0	10-57	83.0	18-205	26.0	15-42	0-53
Hepato-quick (%)	95.0	77-100	62.0	18-205	97.0	90-100	80-120
Cyclosporine (ng/mL)	200.0	120-290			230	170-270	100-300

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.

a highly specific rabbit serum that interacts with the Phe7-Trp8-Lys9 residue of somatostatin-14 (S-14), cross-reacts 45% with somatostatin-28 (S-28), and does not cross-react with vasoactive intestinal polypeptide, insulin, glucagon, secretin, and gastrin. Before assay, somatostatin was extracted (1 mL plasma with 2 mL cold acetone). The extract was further purified with ether extraction followed by air-drying. The mean recovery of standard quantities of synthetic S-14 added to plasma was 93%. Sensitivity of the assay was 3.0 pmol/L; intraassay and interassay coefficients of variation were 7% and 10%, respectively. Plasma insulin, C-peptide, and glucagon levels were also determined by an RIA method using reagents supplied as a kit by Diagnostic Products (Los Angeles, CA). Sensitivities of the assays were 0.007 nmol/L, 0.03 nmol/L, and 3.7 pmol/L, respectively. Intraassay and interassay coefficients of variation were all less than 10%. The antiserum used for glucagon assay does not cross-react with glucagon-like peptides.

S-14 and S-28

To separate the S-28 and S-14 molecular forms, chromatographic analysis was performed on pooled extracts obtained from 1 mL baseline and 1 mL peak plasma from each normal subject and patient of each group. Eight pooled extracts (corresponding to 10 or 7 mL original plasma) were reconstituted with 1 mL eluting buffer and gel-filtrated by fast-performance liquid chromatography (FPLC) on Superdex-peptide HR 10/30 (Pharmacia, Milan, Italy) equilibrated in 0.05 mol/L sodium phosphate-0.15 mol/L NaCl, pH 7.4. Samples were eluted at 0.25 mL/min, and fractions were collected at 1-minute intervals. The column was calibrated with markers of known molecular weight (mol wt): bovine serum albumin (void volume), insulin-like growth factor-II (mol wt 7,411; GroPep, Adelaide, Australia), S-28 (mol wt 3,149; Sigma-Aldrich, Milan, Italy), and S-28 fragment 1-14 (mol wt 1,529; Sigma-Aldrich). Recovery of standard somatostatin and plasma extracts after gel filtration was 85% ± 7.8%. Immunoreactive S-28 and S-14 were evaluated in fractions pooled at 0.05-Kav intervals. In these conditions, S-28 elutes at 0.46 to 0.64 Kav and S-14 elutes at 0.76 to 0.89 Kav. Results are expressed as total picomoles recovered in the appropriate mol wt region for S-28 and S-14.

Statistical Analysis

Incremental somatostatin, glucose, insulin, C-peptide, and glucagon responses (areas under the curve [AUCs] above basal) were calculated using the trapezoidal rule. Results from the four groups were compared by ANOVA followed by the Tukey-Kramer multiple-comparison test. Values for the single variables recorded at each time point of the test were also compared with baseline levels by ANOVA followed by the Tukey-Kramer multiple-comparison test.

RESULTS

Clinical and Biochemical Characteristics of the Patients

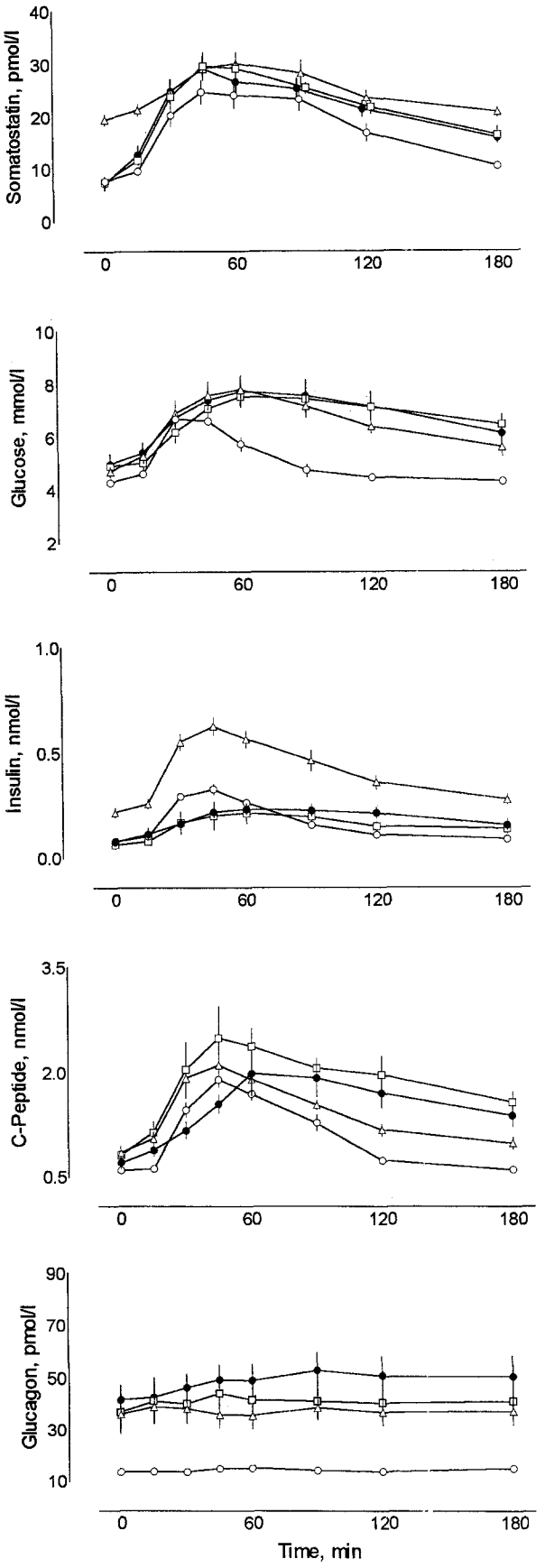
The four groups were well matched for clinical characteristics, with no significant differences ($P > .05$) in age, sex ratio, or body mass index (Table 1). Biochemical liver function test results were slightly greater than the upper limit of laboratory references in two of 14 patients in the liver transplant group. Serum creatinine concentration was greater than the upper limit of the normal reference (>1.4 mg/dL) in two liver and three kidney transplant patients.

Basal Hormone and Glucose Concentrations

In liver transplant patients, fasting mean plasma somatostatin and insulin concentrations were comparable to those recorded in normal subjects and in kidney transplant patients, whereas they were significantly ($P < .05$) lower than in cirrhotics. Furthermore, mean glucagon levels were significantly ($P < .05$) higher than in controls and comparable to those recorded in cirrhotics and in kidney transplant patients. Mean fasting blood glucose and plasma C-peptide levels were comparable in all groups (Fig 1, Table 2).

Meal Test

In liver transplant patients, the dynamics of the somatostatin secretory response was similar to that recorded in the other groups, with peak values occurring between 45 and 60 minutes. In all groups, blood glucose, plasma insulin, and C-peptide increased gradually after the meal. However, in both liver transplant patients and kidney transplant patients, blood glucose and plasma somatostatin, insulin, and C-peptide levels remained higher with respect to baseline for 120 to 180 minutes. Plasma glucagon levels showed no significant meal-induced variations in any group. In liver transplant patients, mean somatostatin secretory areas were significantly ($P < .05$) higher than in controls and cirrhotics, and were comparable to those recorded in kidney transplant patients; glucose and glucagon AUCs were higher than in controls and similar to those recorded in cirrhotics and in kidney transplant patients; and insulin AUCs were lower than in cirrhotics and comparable to those recorded in controls. Finally, in liver transplant patients, C-peptide AUCs were comparable to those recorded in controls, cirrhotics, and kidney transplant patients (Fig 1, Table 2).



Somatostatin Chromatographic Profiles

In controls and patients, S-28 was the predominant molecular form of circulating somatostatin both in the fasting state and after meal ingestion. The S-28/S-14 ratio recorded in basal conditions did not show significant variation after meal ingestion in all groups (Table 3).

DISCUSSION

The study shows that in OLT patients, basal plasma somatostatin values are lower than those recorded in cirrhotics and comparable to those recorded in normal subjects, whereas the somatostatin secretory response to mixed meal ingestion is higher than in controls and cirrhotics. Previous studies in humans have demonstrated that both S-28 and S-14 increase after mixed meal ingestion, with most of the increase attributed to S-28.[12-14] In our OLT patients, somatostatin chromatographic profiles show that S-28 was the predominant circulating molecular form, and that both S-28 and S-14 were released in response to meal ingestion, as in the other groups studied. This would indicate that also in OLT patients, both gut and pancreatic D cells are involved in the increased meal-induced somatostatin release. Somatostatin hyperresponsiveness in OLT patients is not easy to explain. Studies on pancreatic islet hormone interactions have demonstrated that somatostatin secretion is stimulated by glucagon and inhibited by insulin and somatostatin itself.[1-2] In particular, it has been shown that insulin can inhibit somatostatin secretion via the systemic circulation[15-17] and/or via a short-loop intraislet directed portal microcirculation.[18,19] In our OLT patients, the insulin response to the test meal was sluggish and sustained as compared with values in the normal subjects, even though secretory areas were comparable. Furthermore, glucose AUCs were higher than in controls and similar to those recorded in cirrhotics. Conversely, in cirrhotic patients, insulin AUCs were higher than in controls and OLT patients, whereas somatostatin AUCs were lower. On this basis, a relative insulin deficiency state conditioning the meal-induced somatostatin secretion may be suggested in OLT patients.

Experimental studies indicate that the liver is one of the major sites of somatostatin, insulin, and glucagon degradation.[20-22] Therefore, the possibility that the hormonal responses recorded in our patients may be to some extent influenced by a loss of hepatic hormone-degrading activity cannot be ruled out. However, in patients with chronic liver disease, a normal metabolic clearance rate of somatostatin has been found.[14,23-24] Moreover, unaffected insulin and glucagon degradation in hepatic tissue from well-compensated cirrhotic patients has also been reported.[25] Liver function tests recorded in our liver transplant patients were nearly normal. Thus, it seems unlikely that the meal-induced changes in hormone plasma concentrations observed in our patients primarily reflect decreased hormonal liver clearance.

Since similar hormone and blood glucose profiles in response to meal ingestion were also found in kidney transplant patients, a role of the immunosuppressive treatment rather than liver

Fig 1. Plasma somatostatin, blood glucose, plasma insulin, C-peptide, and glucagon concentrations (mean ± SEM) in 10 liver transplant patients (●), 10 normal subjects (○), 10 cirrhotic patients (△), and 7 kidney transplant patients (□) before and after test meal ingestion.

Table 2. Fasting Values and Incremental Postprandial Response Areas Over 180 Minutes for Blood Glucose and Hormonal Parameters in Controls and Patients

Group	Somatostatin (pmol/L)*	Glucose (mmol/L)†	Insulin (nmol/L)‡	C-Peptide (nmol/L)‡	Glucagon (pmol/L)*
Fasting values					
Controls	7.6 ± 0.6‖	4.3 ± 0.1	0.08 ± 0.006‖	0.59 ± 0.05	13.8 ± 0.7
Liver transplant patients	7.3 ± 1.3‖	5.0 ± 0.4	0.08 ± 0.019‖	0.70 ± 0.09	41.7 ± 5.5§
Cirrhotics	19.5 ± 1.1	4.7 ± 0.2	0.22 ± 0.023	0.84 ± 0.10	36.2 ± 5.3§
Kidney transplant patients	7.5 ± 0.8‖	4.9 ± 0.2	0.06 ± 0.009‖	0.82 ± 0.08	37.0 ± 8.4§
AUC					
Controls	1811 ± 270	130 ± 22	15.1 ± 1.3	90 ± 7¶	159 ± 103
Liver transplant patients	2547 ± 95§‖	339 ± 36§	20.3 ± 1.7‖	153 ± 29	1129 ± 366§
Cirrhotics	987 ± 164§	333 ± 40§	34.8 ± 4.0§	101 ± 9¶	311 ± 124
Kidney transplant patients	2578 ± 164§‖	366 ± 42§	17.4 ± 5.0	223 ± 20	1268 ± 325§

For AUC, units are as follows: *pmol/L · min, †mmol/L · min, ‡nmol/L · min.
§$P < .05$ *v* controls.
‖$P < .05$ *v* cirrhotics.
¶$P < .05$ *v* kidney transplant patients.

transplantation or preexisting liver failure seems likely. However, the various effects of the two main immunosuppressants (cyclosporine and prednisone) on islets and peripheral tissues cannot be readily disentangled. It has been demonstrated that therapeutic doses of cyclosporine inhibit insulin and increase somatostatin insular immunoreactivity in rats.[26] Furthermore, cyclosporine has also been shown to decrease insulin secretory reserve in nondiabetic kidney transplant and psoriasis patients.[7] Our findings are in agreement with these results and suggest that cyclosporine may play a role in somatostatin secretion, either by acting directly on somatostatin secretory cells or by affecting insulin secretion. Finally, the hyperglucagonemia in OLT patients suggests the hypothesis that pancreatic α-cell hyperplasia and/or hypersensitivity to aminogenic stimulation[27] persists after liver transplantation despite normalization of liver function. However, the occurrence of hyperglucagonemia in kidney transplant patients also indicates that this increase in glucagon secretion, which prevails over the inhibitory effect of somatostatin released after the meal, may be due to immunosuppressive treatment and in particular to corticosteroids. Indeed, it is well established that glucocorticoid administration enhances glucagon secretion in normal man.[28]

The physiological and/or pathological significance of this pattern of somatostatin secretion is at present unclear. Since somatostatin inhibits a number of gastric, intestinal, and pancreatic exocrine secretions and affects splanchnic blood flow and the rate of intestinal glucose and amino acid absorption,[29] it is possible that the meal-induced hypersomatostatinemia observed in OLT patients reduces the rate of these gastrointestinal processes, consequently influencing the absorption of some nutrients and, in turn, nutritional status.

Table 3. Somatostatin Chromatographic Profiles and S-28/S-14 Ratio Before and After Meal Ingestion in Controls and Patients

	Controls		Liver Transplant Patients		Cirrhotics		Kidney Transplant Patients	
	Basal	Peak	Basal	Peak	Basal	Peak	Basal	Peak
S-28	35.0	122.5	33.4	147.1	99.3	142.8	24.6	105.1
S-14	19.9	62.2	18.8	72.5	46.2	67.2	13.2	51.7
S-28/S-14 atio	1.8	2.0	1.8	2.0	2.0	2.1	1.9	2.0

Data are expressed as total pmol recovered in the corresponding elution volume.

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