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Glucose homeostasis during prolonged suppression of glucagon and insulin secretion by somatostatin

(glucose turnover/diabetes mellitus)

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ABSTRACT **Somatostatin was infused for 5–8 hr into five normal men and eleven normal, conscious dogs. This infusion resulted in a persistent decline in plasma glucagon (40–60%) and insulin (30–45%). Plasma glucose fell 15–25% during the initial 1–2 hr, but subsequently rose to hyperglycemic levels (130–155 mg/100 ml) by 3–6 hr, despite persistent hypoglucagonemia. Glucose production initially declined by 40–50%, but later rose to levels 15–20% above basal rates while peripheral glucose utilization fell to levels 20–30% below basal, thereby accounting for hyperglycemia. Infusion of exogenous insulin so as to restore plasma insulin to preinfusion values or cessation of the somatostatin infusion with restoration of endogenous insulin secretion resulted in a prompt reduction of plasma glucose to baseline values. Prevention of the initial somatostatin-induced hypoglycemic response by intravenous infusion of glucose failed to prevent the delayed hyperglycemia. We conclude that somatostatin causes only transient hypoglycemia in normal subjects and that hyperglycemia eventually develops as a consequence of insulin deficiency. These data indicate that basal glucagon secretion is not essential for the development of fasting hyperglycemia and support the conclusion that insulin deficiency rather than glucagon excess is the primary factor responsible for abnormal glucose homeostasis in the diabetic.**

A bihormonal disturbance involving glucagon excess as well as insulin deficiency has been postulated as the pathogenetic defect in diabetes mellitus (1, 2). The hypothesis that glucagon is essential for the development of hyperglycemia in diabetes has been supported by studies using somatostatin (growth hormone-release inhibiting factor), an agent that inhibits insulin, glucagon, and growth hormone secretion (3–5), while at the same time lowering blood glucose concentration (6, 7). However, such studies have generally involved short (1 to 2-hr) infusions of somatostatin. Whether more prolonged periods of hypoglucagonemia would be equally effective in suppressing blood glucose levels has not been established. Furthermore, studies involving the response to physiologic doses of exogenous glucagon in normal and diabetic subjects (8) and observations in pancreatectomized patients (9) have raised serious doubts as to the essentiality of glucagon in the pathogenesis of diabetes. The present study was consequently undertaken to determine whether prolonged (5–6 hr) suppression of glucagon and insulin secretion by somatostatin results in persistent hypoglycemia. Our findings indicate that after an initial, transient decline, the plasma glucose concentration increases to hyperglycemic levels despite ongoing hypoglucagonemia. These data suggest that insulin deficiency rather than glucagon availability is the essential factor in the development of diabetes.

METHODS

Five healthy, male volunteer subjects, 20–28 years of age, were studied. All were within 20% of ideal body weight (Metropolitan Life Insurance Tables, 1959). They consumed weight-maintaining diets containing a minimum of 200 g of carbohydrate. The subjects were informed of the nature, purpose, and possible risks of the study before their written, voluntary consent to participate was obtained.

The subjects were studied in the postabsorptive state after a 12 to 15-hr overnight fast. An indwelling catheter was inserted in an antecubital vein for blood sampling and in the contralateral vein for administration of somatostatin. In order to study net splanchnic glucose balance, in two of the subjects blood samples were also obtained from catheters placed in a brachial artery and an hepatic vein, by techniques described previously (10). In each of the subjects three or four simultaneous samples of arterial and hepatic venous blood were obtained at 10-min intervals in the basal state and during the final 30 min of the somatostatin infusion. Linear somatostatin (kindly provided by Drs. Roger Guillemin and Jean Rivier of the Salk Institute, San Diego, Calif.) was prepared as a 0.1% (wt/vol) solution in pyrogen-free saline, passed through a 22-μm filter apparatus (Millipore Corp., Bedford, Mass.), and stored at 4° until used. The somatostatin infusate was administered as a continuous infusion via a peristaltic pump (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.) in a dose of 9–10 μg/min for 5 hr.

Studies were also conducted in six normal, conscious dogs, weighing 17–31 kg. The dogs were studied after an 18-hr overnight fast. Cyclic somatostatin (Bachem Inc., Marina Del Rey, Calif.) was infused in a dose of 0.15–0.20 μg/kg per min for 6–8 hr. A total of 11 somatostatin infusions were performed (five dogs were studied on two occasions). In five of the studies, [3-^{3}H]glucose (New England Nuclear, Boston, Mass.) was administered as a primed-continuous infusion before and during the somatostatin infusion (6 hr) to evaluate glucose production and utilization (11). In each of these studies the specific activity of the [3-^{3}H]glucose in blood had reached a plateau before administration of somatostatin. Four dogs received a continuous infusion of porcine crystalline insulin (Eli Lilly and Co., Indianapolis, Ind.) at a rate of 0.15 milliunit/kg per min during the final 40 min of the somatostatin infusion. One dog received a variable rate (0.14–0.87 mg/kg per min) intravenous glucose infusion for the initial 175 min of the somatostatin infusion in order to prevent the early hypoglycemic response to somatostatin.

Three of the dogs also received infusions of saline for 6 hr under conditions identical with those used for the somatostatin infusions.

Plasma glucose was determined by the glucose oxidase method (12) on a Beckman Glucose Analyzer. Methods used for the determination of plasma immunoreactive glucagon (with Unger antibody, 30K), plasma immunoreactive insulin, and specific activity of plasma glucose have been described (11, 13). Rates of endogenous glucose production and total glucose utilization were calculated in the steady state before somatos-

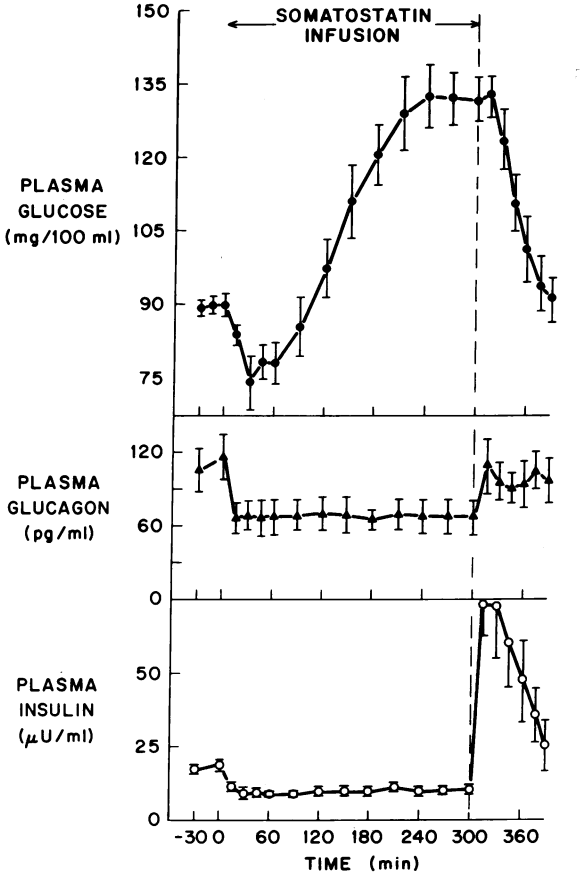

FIG. 1. Effect of prolonged somatostatin infusion (5 hr) on plasma glucose, glucagon, and insulin concentrations (mean ±SE) in normal humans.

tatin infusion (14) and during nonsteady state conditions after somatostatin administration using 0.65 of the initial glucose pool size as the rapidly mixing compartment of the glucose pool (15, 16). Serum growth hormone was determined by radioimmunoassay (17), and plasma cortisol by a fluorometric method (18). Hepatic blood flow was estimated by the continuous infusion technique (19), with indocyanine green dye (20).

Statistical analyses were performed with the Student's *t* test (the paired *t* test was used when applicable) and linear regression analysis (21). Data in the *text* are presented as the mean ±SE.

RESULTS

Response to prolonged somatostatin infusion in normal humans

In Fig. 1 the effect of prolonged (5 hr) somatostatin administration on plasma glucagon, insulin, and glucose concentrations in normal subjects is shown. As expected (3, 4), somatostatin infusion resulted in a prompt decline in plasma glucagon and insulin concentrations. Glucagon levels fell by 56 ± 10 pg/ml ($P < 0.005$), while insulin declined by 9 ± 2 microunits/ml ($P < 0.01$). Suppression of both hormones persisted throughout the infusion of somatostatin.

The pattern of response of blood glucose was distinctly different from that of the pancreatic hormones. As anticipated (6, 7), plasma glucose levels initially declined by 15 ± 3% ($P < 0.01$) during the first 60–90 min. This hypoglycemic effect did not, however, persist, as indicated by the return of plasma glucose levels to basal values by 120 min and the subsequent development of fasting hyperglycemia in all subjects ($P < 0.001$). Plasma glucose rose within 4–5 hr to 120–150 mg/100 ml (mean 136 ± 5), an increase of 30–60 mg/100 ml (mean 47 ± 4) above baseline levels. When the somatostatin infusion was discontinued, concentrations of plasma glucagon and insulin promptly rose and that of plasma glucose declined to preinfusion, basal levels (Fig. 1). Serum growth hormone (2.0 ± 0.3 ng/ml in the basal state) and plasma cortisol (15 ± 3 μg/100 ml in the basal state) were unchanged throughout the study.

In the two subjects studied by the hepatic venous catheter technique, splanchnic glucose production rose by 15–20% after 4–5 hr of the somatostatin infusion. In these subjects, splanchnic glucose production was 1.13 ± 0.03 and 0.83 ± 0.03 mmol/min in the basal state and rose to 1.38 ± 0.05 and 0.99 ± 0.02 mmol/min, respectively, during the final 30 min of the infusion ($P < 0.01$).

Studies of somatostatin infusion in normal, conscious dogs

The response of plasma glucagon, insulin, and glucose to somatostatin infusion in normal, conscious dogs was virtually identical to that observed in humans. Concentration of plasma glucagon (42 ± 7 pg/ml in the control period) fell by 50–60% immediately after somatostatin infusion and remained suppressed throughout the infusion. Similarly, plasma insulin concentration (19 ± 2 microunits/ml in the control period) decreased by 30–35% and remained reduced throughout the infusion period. Plasma glucose concentration (95 ± 1 mg/100

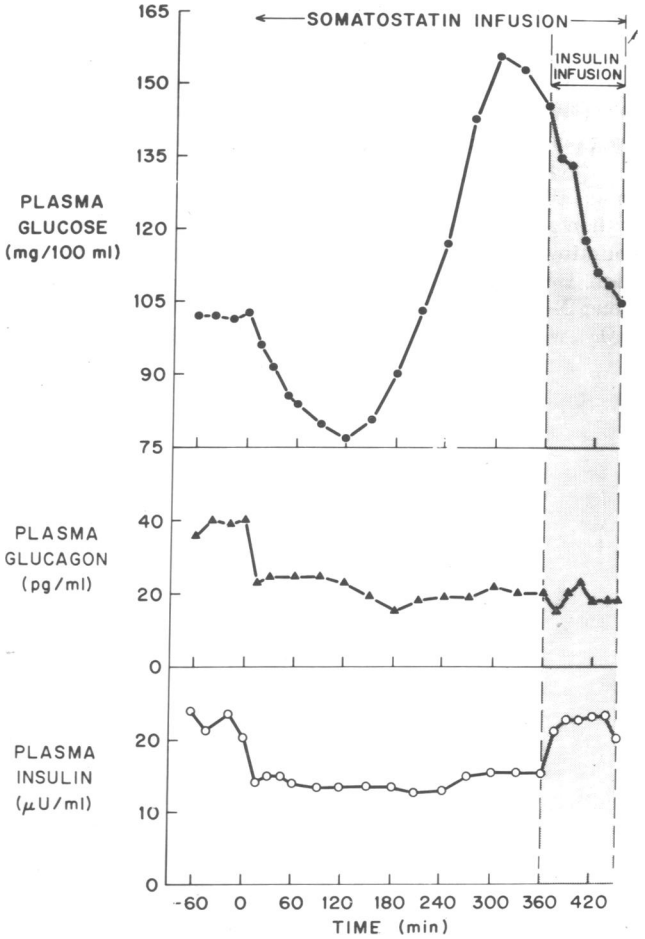

FIG. 2. Effect of intravenous infusion of insulin (0.15 milliunit/kg per min) on somatostatin-induced hyperglycemia in a normal, conscious dog. Restoration of basal plasma insulin levels resulted in normalization of plasma glucose concentration.

ml in the basal state) initially declined by 22 ± 4%. However, by 2 hr the hypoglycemic effect waned and hyperglycemia invariably occurred. Plasma glucose concentration increased to peak levls of 138 ± 4 mg/100 ml ($P < 0.001$) at 3–7 hr, a rise of 30–65 mg/100 ml above preinfusion, control levels. A direct linear correlation was observed between the rise in blood glucose and the fall in plasma insulin concentrations ($r = 0.61$, $P < 0.05$). In contrast, dogs receiving saline demonstrated a small (5–10 mg/100 ml) decline in glucose concentration over 6 hr.

In dogs rendered hyperglycemic by prolonged infusion of somatostatin, intravenous administration of exogenous insulin in amounts calculated to restore plasma insulin levels to normal (0.15 milliunit/kg per min) resulted in a prompt decline in plasma glucose to basal, preinfusion values after 60–90 min (Fig. 2). In four dogs infused with insulin during the final 60–90 min of the somatostatin infusion, plasma glucose fell from peak levels of 138 ± 6 mg/100 ml to 99 ± 10.

To evaluate whether the delayed hyperglycemia induced by somatostatin was dependent on the prior induction of hypoglycemia, we infused glucose at a variable rate for the initial 175 min of the somatostatin infusion so as to maintain plasma glucose at basal levels. Despite prevention of early hypoglycemia, plasma glucose levels increased to 145–155 mg/100 ml in association with prolonged infusion of somatostatin (Fig. 3).

The effects of the somatostatin infusion on endogenous glucose production and glucose utilization are shown in Fig. 4. Glucose production decreased by 45–50% ($P < 0.01$) during the first 90 min of somatostatin administration and then returned to baseline values after 180 min. At the conclusion of the somatostatin infusion, endogenous glucose production (2.5 ± 0.4 mg/kg per min) was slightly, but not significantly, increased above preinfusion values (2.2 ± 0.4 mg/kg per min). Total glucose utilization, on the other hand, decreased to a lesser extent than glucose production during the first 90 min, thereby accounting for the initial decline in plasma glucose concentration. However, as the somatostatin infusion was continued beyond 2–3 hr, total glucose utilization remained depressed ($P < 0.05$), which, in association with the rise in glucose production, resulted in the development of fasting hyperglycemia (Fig. 4). During the final 30 min of the infusion both glucose production and glucose utilization had returned to basal, preinfusion levels, accounting for stabilization of plasma glucose at hyperglycemic concentrations (Fig. 4).

DISCUSSION

The present findings confirm previous observations demonstrating a hypoglycemic response to a 60- to 120-min infusion of somatostatin in normal subjects (6, 7). Particularly noteworthy in the current study, however, was the observation that hyperglycemia developed as the infusion of somatostatin was extended beyond 2 hr. In normal humans as well as healthy conscious dogs, blood glucose concentration increased to 30–65 mg/100 ml above pre-infusion fasting levels as somatostatin infusion was continued for 4–6 hr. Interestingly, in a recent report, Altszuler *et al.* noted that in normal dogs rendered hypoglucagonemic for 2 hr by continuous infusion of somatostatin, after an initial decline, glucose production increased by 90 min, despite ongoing suppression of glucagon (22).

With respect to the mechanism of the delayed, somatostatin-induced hyperglycemia, insulin deficiency would appear to be the primary etiologic factor. Restoration of endogenous insulin secretion by cessation of the infusion (Fig. 1), or administration of exogenous insulin in physiologic replacement doses (Fig. 2), resulted in a prompt decline in blood glucose concentrations to baseline values. Furthermore, a direct correlation was observed between the rise in plasma glucose concentration after somatostatin infusion and the extent of decline in serum insulin. In contrast, it is unlikely that increased secretion of counter-regulatory hormones (e.g., epinephrine, cortisol, and growth hormone) during the initial, hypoglycemic phase of the infusion is responsible for the hyperglycemic response. First, concentrations of plasma cortisol and growth hormone were not increased during the infusion. Second, prevention of somatostatin-induced hypoglycemia by infusion of intravenous glucose failed to prevent the delayed hyperglycemia (Fig. 3).

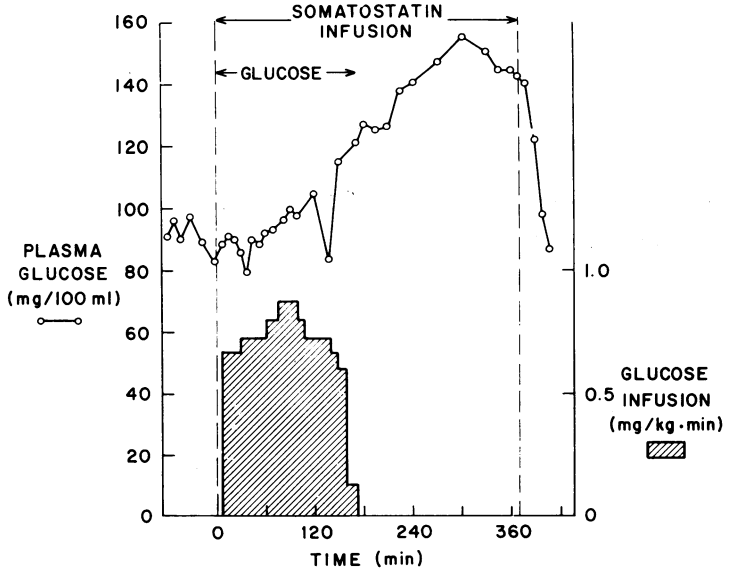

FIG. 3. Effect of prevention of the initial hypoglycemic response to somatostatin on somatostatin-induced hyperglycemia in a normal, conscious dog. Fasting hyperglycemia developed upon cessation of a variable rate glucose infusion (indicated by the hatched area), which maintained plasma glucose at basal levels for the initial 175 min. Plasma glucagon and insulin concentrations were reduced by 40–45% throughout the period of somatostatin administration.

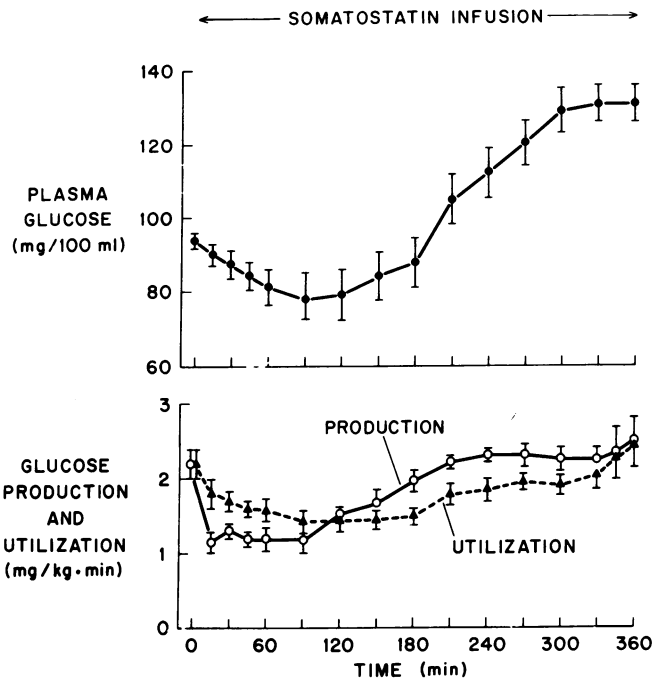

FIG. 4. Effect of prolonged somatostatin infusion (6 hr) on plasma glucose concentration, endogenous glucose production, and total glucose utilization in five normal conscious dogs. Glucose production fell ($P < 0.01$) during the first 90 min, accounting for the initial hypoglycemic response. After 180 min, glucose production returned to normal levels whereas total glucose utilization remained depressed ($P < 0.05$), resulting in fasting hyperglycemia. Plasma insulin and glucagon concentrations were significantly ($P < 0.05$) reduced throughout the study.

As for the basis of the biphasic blood glucose response to somatostatin (hypoglycemia followed by hyperglycemia), time-dependent effects of insulin and/or glucagon may be responsible. The initial hypoglycemia may reflect the persistence of insulin's action unopposed by the presence of glucagon. Previous studies have shown that in contrast to glucagon, insulin's effect on the liver as well as on peripheral tissues may persist for 40 min or more after its disappearance from the bloodstream (23, 24). Alternatively, the initial hypoglycemia may reflect an evanescent response to lack of glucagon, independent of insulin action. Favoring the latter hypothesis is the demonstration that persistent hyperglucagonemia has only a transient stimulatory effect on hepatic glucose production, which lasts for less than 30 min (25). Thus, decreased as well as increased secretion of glucagon may be characterized by evanescent effects on the liver.

The current findings are of particular interest with respect to the pathogenesis and treatment of diabetes mellitus. An essential role for glucagon in all forms of diabetes has been suggested (1, 2), primarily on the basis of the fall in blood glucose concentration observed during 120 to 180-min infusions of somatostatin in alloxan-diabetic dogs (26) and in diabetic humans (27). The present study demonstrates that despite ongoing suppression of glucagon secretion, fasting hyperglycemia develops in normal subjects infused with somatostatin for more than 2–3 hr. Both splanchnic balance studies (in humans) and radioactive tracer data (in dogs) indicate that the development of hyperglycemia during prolonged infusion of somatostatin is associated with an increase in hepatic glucose output. Glucose production, initially reduced by 40–50%, rises to values 15–20% above basal, preinfusion levels. The rise in glucose production coupled with a reduction in glucose utilization results in a progressive elevation in plasma glucose. Hyperglycemia is thus the result of relative or absolute glucose overproduction (reflecting the important contribution of the liver) in conjunction with reduced glucose utilization rates (Fig. 4). The condition produced with somatostatin is thus analogous to the situation in spontaneous diabetes. In spontaneously diabetic patients, as in the subjects given somatostatin, glucose production (determined either by radioactive tracer techniques or splanchnic balance studies) is slightly increased or normal, but is inappropriate for the accompanying hyperglycemia (28–30). These findings thus support the conclusion that insulin deficiency rather than glucagon excess is the primary hormonal disturbance in the pathogenesis of diabetes mellitus. A similar conclusion has been reached on the basis of studies in which infusions of exogenous glucagon failed to induce fasting or postprandial hyperglycemia in normal subjects or insulin-treated diabetic patients (8). Furthermore, in pancreatectomized patients, clinical diabetes with fasting hyperglycemia develops in the absence of detectable, circulating pancreatic glucagon (9).

It should be noted that the fasting hyperglycemia produced by ongoing infusion of somatostatin was relatively mild. This may be a consequence of residual insulin secretion, as indicated by the fact that plasma insulin levels fell by only 30–45% (Figs. 1 and 2). On the other hand, the lack of the contributory action of glucagon in exaggerating the hyperglycemic consequences of insulin deficiency may be an additional or alternative explanation. A hyperglycemic effect of glucagon in the insulin-deficient diabetic has been observed (8). Thus, while the current findings indicate that glucagon is not essential for the development of fasting hyperglycemia, they do not exclude the possibility that glucagon contributes to the diabetic state by increasing blood glucose levels in circumstances of insulin deficiency.

The response to short-term (2–3 hr) infusions of somatostatin has led to the suggestion that this agent may be potentially useful as an adjunct to insulin administration in the management of diabetes (27). It should, however, be noted that many diabetics treated for 20 years or more with exogenous insulin retain some degree of endogenous insulin secretion (31). This

residual insulin secretion has in fact been implicated as a major factor that distinguishes the more readily manageable patient from the "brittle" diabetic (32). To the extent that somatostatin induced fasting hyperglycemia in normal subjects, intensification rather than amelioration of diabetes may occur in patients with residual insulin secretion. These findings, coupled with recent observations indicating that somatostatin interferes with glucose absorption from the gastrointestinal tract (33), thus raise serious questions concerning the potential usefulness of this agent in the treatment of patients with diabetes.

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