Minimal Increases in Glucagon Levels Enhance Glucose Production in Man with Partial Hypoinsulinemia

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SUMMARY

In man a small dose of somatostatin (50 μg/h) suppressed moderately basal insulin (5 μU/ml) and glucagon (40 pg/ml) levels. This resulted in a short-lasting hypoglycemia, which was then followed by marginal hyperglycemia throughout the experiment. The addition of a minimal dose of glucagon (0.50 ng/kg/min) to somatostatin normalized basal glucagon levels and resulted in a significant and sustained hyperglycemia. During the first 2 h, hyperglycemia was mainly due to increased glucose production, whereas later on it was maintained by decreased glucose uptake. We conclude that, in man moderately deprived of insulin, even a marginal change in glucagon level induces a long-lasting hyperglycemia. DIABETES *32*:633–636, July 1983.

It is well known that glucagon in addition to insulin plays an important role in the acute regulation of glucose metabolism in normal man and animals. The role of glucagon could be clarified when the discovery of somatostatin offered the first model to study glucagon deficiency. A number of animal experiments suggests that glucagon is necessary for the marked hepatic overproduction of glucose and ketones that characterizes the insulin-deficient state.[1–5] Presently and in variance with the results of another group,[6] we demonstrate that only a marginal change in glucagon level provokes sustained hyperglycemia and enhances glucose production even in the moderately insulin-deficient man. Glucagon replacement was given for 6 h via a peripheral vein during a partial suppression of insulin release by concomitantly infusing a low dose of somatostatin.

MATERIAL AND METHODS

Ten healthy volunteers, one woman and nine men aged 29–42 yr, participated in this study. Informed consent was obtained from all subjects. The Ethical and Isotope Committees of Karolinska Hospital had given their approval to the study. All subjects had normal i.v. glucose tolerance[7] and a normal insulin response to glucose infusion.[8] Their body weight ranged from 81% to 105% of ideal (Metropolitan Insurance Company). The subjects were on a free carbohydrate diet (approximately 200 g/day) and reported to the laboratory on the morning after an overnight fast. Experiments started at 8 a.m. with the subjects in a recumbent position. Teflon catheters were inserted into a superficial vein of each arm and kept open with a slow drip of saline.

In the study designed to elucidate the effect of increasing doses of glucagon on prevailing blood glucose levels, somatostatin was administered alone or in combination with glucagon. Somatostatin was given as an i.v. bolus of 200 μg followed by a continuous infusion of 50 μg/h during 6 h. This dose is known not to interfere with splanchnic circulation.[9] Glucagon was administered as a continuous intravenous infusion (0.25, 0.50, 1.50 ng/kg/min) for 6 h. Blood samples were collected in chilled heparinized test tubes with Trasylol at −30, 0, 10, 30, 60, 120, 180, 240, 300, and 360 min.

In the second series of experiments we investigated the effect of a combined 6-h infusion of somatostatin and glucagon on glycemia and glucose turnover. Somatostatin (see above) and glucagon (0.50 ng/kg/min) were administered after an equilibration period of 2 h. Glucose turnover was measured using a primed infusion of [3-^{3}H]glucose. The ratio of the priming and the continuous infusion dose was 1:120. The rate of tracer infusion was 0.167 μCi/min. Blood samples were taken at 10-min intervals during the last 30 min of the equilibration period and thereafter at 20-min intervals. The rates of endogenous glucose production (Ra), glucose utilization (Rd), and metabolic clearance of glucose (MCR) were determined by the tracer infusion technique.[10–12] A sliding-fit technique that employed three consecutive val-

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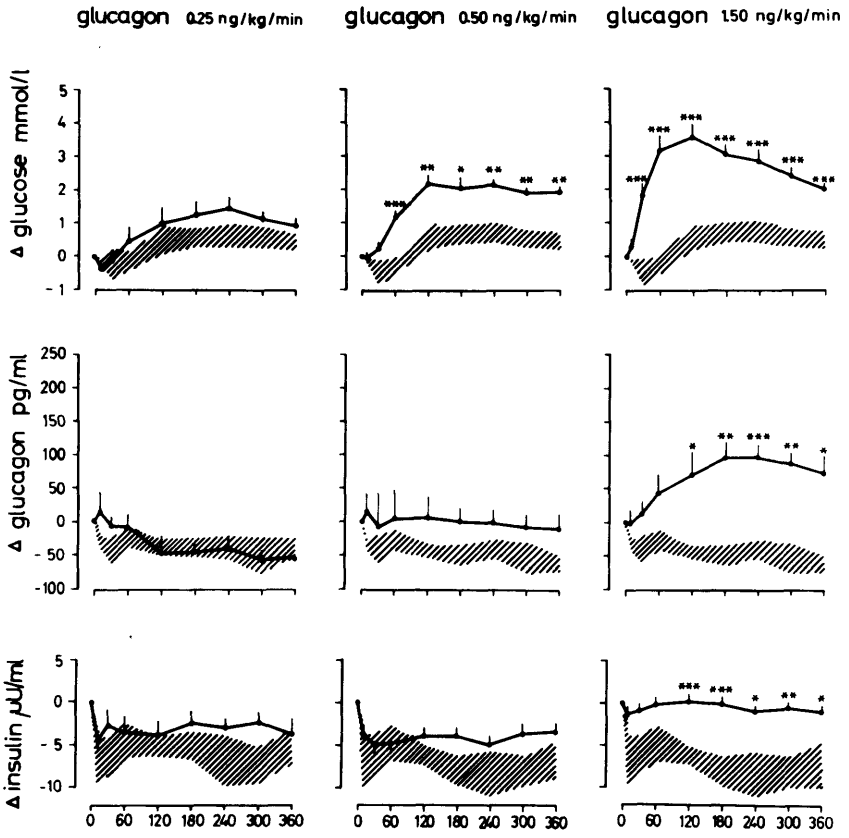

FIGURE 1. Effect of combined infusions of somatostatin (200 μg i.v. + 50 μg/h) and graded doses of glucagon (0.25, 0.5, 1.5 ng/kg/min) over 6 h on glucose, insulin, and glucagon concentrations in five healthy subjects (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.005. Shaded area indicates control experiment with somatostatin alone (mean ± SEM).

ues of glucose concentration and specific activity was used in the calculations.[13]

Glucose concentration was measured by a glucose-oxidase method.[14] Insulin was measured by a double-antibody radioimmunoassay technique using antibodies raised in guinea pigs against porcine insulin. Porcine insulin also served as standard. Plasma glucagon was determined by a charcoal separation radioimmunoassay technique[15] using antibody 30K.

Cyclic somatostatin was provided by the Research Department of the Kabi Group, Stockholm. Glucagon was purchased from Eli Lilly and Company (Indianapolis, Indiana) and [3-^{3}H]glucose from New England Nuclear (Boston, Massachusetts). Student's *t* test for paired observations was used in the statistical analyses.

RESULTS

The infusion of somatostatin reduced peripheral glucagon by approximately 40 pg/ml (Figure 1). When somatostatin and glucagon were given together, plasma glucagon levels varied depending on the dose of the exogenously administered hormone. With 0.50 ng/kg/min, somatostatin-induced suppression of glucagon was fully compensated whereas 0.25 ng/kg/min did not appreciably affect measurable glucagon concentrations. In the presence of 1.5 ng/kg/min of glucagon, plasma concentrations of the hormone increased over the basal level by approximately 100 pg/ml.

Basal insulin was decreased by 5 μU/ml with somatostatin alone (Figure 1). The very low dose of glucagon (0.25–0.50 ng/kg/min) did not significantly interfere with this suppressive effect of somatostatin. When the higher glucagon dose (1.5 ng/kg/min) was given, basal insulin levels were maintained because the inhibitory effect of somatostatin was counteracted by hyperglycemia and glucagon.

With somatostatin there was a transient decrease of plasma glucose during the first hour, which was then followed by marginal hyperglycemia. This pattern was not significantly altered by addition of 0.25 ng/kg/min of glucagon, but 0.5 ng/kg/min prevented the initial hypoglycemia and induced a marked and sustained hyperglycemia. In the presence of 1.5 ng/kg/min the maximal increment of blood glucose was 3 mmol/L at 2 h. Thereafter glucose concentration gradually declined, and after 6 h it was still elevated but identical to the level obtained with 0.5 ng/kg/min of glucagon.

In the tracer experiments (Figure 2) somatostatin infusion was combined with a small amount of glucagon (0.50 ng/kg/min). Again, a significant sustained hyperglycemia and hypoinsulinemia were found, although glucagon was kept at basal concentrations. The basal hepatic glucose production (Ra) was 1.97 ± 0.05 mg/kg/min. During the combined infusion of glucagon and somatostatin Ra was elevated for about 2 h with a maximum of 2.80 ± 0.24 mg/kg/min at 40 min, but after 3 h Ra was lower than basal. Glucose utilization (Rd) was decreased initially and during the last 3 h of the experiment. The metabolic clearance rate (MCR) was decreased during the whole experimental period.

DISCUSSION

We demonstrate that in man a dose of glucagon as low as 0.50 ng/kg/min exerts significant and sustained hyperglycemia even when basal insulin is only partially suppressed by somatostatin. During the first 2 h hyperglycemia is mainly

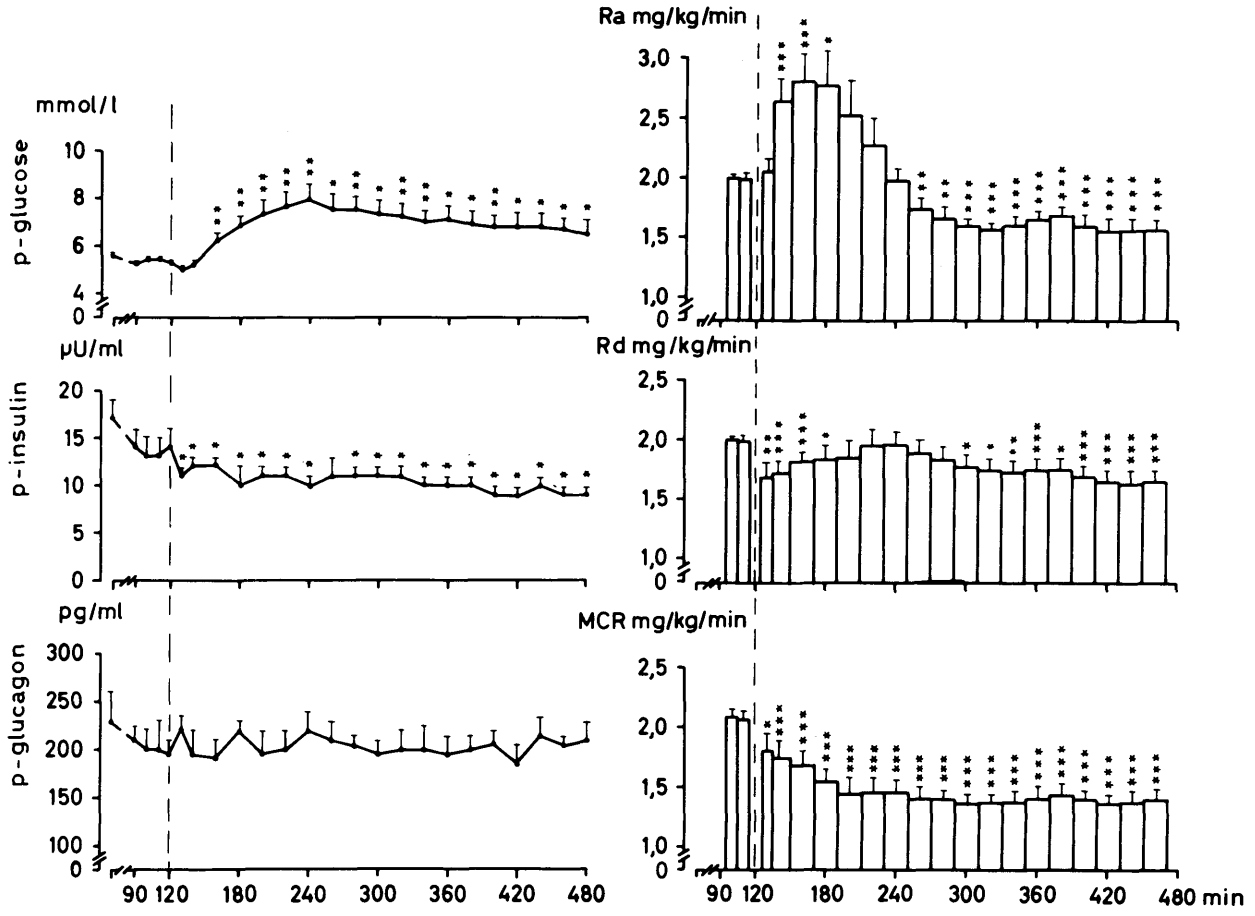

FIGURE 2. Effect of a 6-h combined infusion of somatostatin (200 μg i.v. + 50 μg/h) and glucagon (0.5 ng/kg/min) on glucose, insulin, and glucagon levels and on hepatic glucose production (Ra), glucose utilization (Rd), and metabolic clearance rate (MCR) in five healthy subjects (mean ± SEM). *P < 0.05, **P < 0.01, ***P < 0.005.

due to increased glucose production, whereas later on it is maintained by decreased glucose uptake. In a similar study in a normal man,[6] in which glucagon replacement was also given via a peripheral vein during somatostatin infusion, plasma glucose initially increased twofold but subsequently declined, and at 2–3 h the levels were comparable to those observed with somatostatin alone. They concluded that glucagon lack was not a modifying factor after the initial hyperglycemic response to prolonged infusions of somatostatin. This apparent controversy between these and our data could be due to a small but critical difference in the experimental design. They administered 10 times more somatostatin which, when given together with glucagon, was already 1 h after the start of infusion, accompanied by increasing insulin levels presumably due to marked hyperglycemia. In contrast, in our experiments the initial suppression of insulin was less marked but was sustained throughout the 6-h experimental period. We agree with Unger's opinion[16] that the effect of glucagon can be appropriately evaluated only if suppression of insulin secretion is sustained.

The important role of glucagon in induction of hyperglycemia has also been demonstrated in dogs.[4] When plasma glucagon was maintained at basal levels by infusing glucagon intraportally, the somatostatin-mediated insulin suppression was associated with a prompt rise in hepatic glucose production and overt hyperglycemia. When glucagon was not replaced (combined insulin and glucagon deficiency), hepatic glucose production did not increase and glucose concentration increased much less.

It has been shown that glucagon suppression by somatostatin can lower plasma glucose in diabetic patients, and the question was raised whether or not glucagon suppression could be useful as an adjunct to insulin therapy.[16] In alloxan-diabetic dogs hyperglycemia was improved by somatostatin-induced glucagon suppression[1] and this was due solely to suppression of glucose production.[17] Also, in depancreatized dogs, the administration of somatostatin immediately following either pancreatectomy[2,3] or the withdrawal of insulin treatment[5] prevented the expected rise in plasma glucose.

The critical importance of partial insulin deprivation in sensitizing the liver to the effect of endogenously released glucagon was demonstrated recently.[18] Depancreatized dogs were maintained either on basal or 30% subbasal intraportal insulin infusion. A marginal epinephrine-induced increase in extrapancreatic glucagon had a major impact on increasing glucose production and glycemia in such partially insulin-deprived dogs. The degree of insulin deprivation of these dogs was equivalent to the insulin deprivation induced in our present study with the low dose of somatostatin.

In conclusion, our study in man as well as the previously published animal studies suggest that glucagon suppres-

sion can be effective in lowering glycemia in subjects with partial suppression of insulin release by somatostatin or in animals with decreased insulin release (alloxan diabetic) or with partial insulin deprivation (depancreatized dogs shortly after surgery or withdrawal of insulin treatment). In moderately insulin-deprived man even marginal replacement of glucagon results in sustained hyperglycemia.

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