Glucagon Supports Postabsorptive Plasma Glucose Concentrations in Humans With Biologically Optimal Insulin Levels

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OBJECTIVE—Based on the premise that postabsorptive patients with type 1 diabetes receiving intravenous insulin in a dose that maintains stable euglycemia are receiving biologically optimal insulin replacement, we tested the hypothesis that glucagon supports postabsorptive plasma glucose concentrations in humans.

RESEARCH DESIGN AND METHODS—Fourteen patients with type 1 diabetes were studied after an overnight fast on up to five occasions. Insulin was infused intravenously to hold plasma glucose concentrations at ~100 mg/dl (5.6 mmol/l) overnight and fixed from −60 to 240 min the following morning. From 0 through 180 min the patients also received *1*) saline, *2*) octreotide 30 ng · kg^{-1} · min^{-1} with growth hormone replacement or octreotide with growth hormone, plus *3*) glucagon in doses of 0.5 ng · kg^{-1} · min^{-1}, *4*) 1.0 ng · kg^{-1} · min^{-1}, and *5*) 2.0 ng · kg^{-1} · min^{-1}.

RESULTS—Compared with a mean ± SE of 98 ± 5 mg/dl (5.4 ± 0.3 mmol/l) at 180 min during saline, mean plasma glucose concentrations declined to 58 ± 1 mg/dl (3.2 ± 0.1 mmol/l) ($P <$ 0.001) at 180 min during octreotide plus saline and were 104 ± 16 mg/dl (5.8 ± 0.9 mmol/l) (NS), 143 ± 13 mg/dl (7.9 ± 0.7 mmol/l) ($P = 0.004$), and 160 ± 15 mg/dl (8.9 ± 0.8 mmol/l) ($P < 0.001$) at 180 min during octreotide plus glucagon in doses of 0.5, 1.0, and 2.0 ng · kg^{-1} · min^{-1}, respectively.

CONCLUSIONS—In the setting of biologically optimal insulin replacement, suppression of glucagon secretion with octreotide caused a progressive fall in plasma glucose concentrations that was prevented by glucagon replacement. These data document that glucagon supports postabsorptive glucose concentrations in humans. ***Diabetes*** **59:2941–2944, 2010**

The current interest in the development of drugs that block the action or secretion of glucagon for the treatment of diabetes rests on the premise that glucagon supports the plasma glucose concentration and, therefore, that glucagon, in concert with insulin deficiency, may play a role in the pathogenesis of hyperglycemia in diabetes (1–4). That premise is based largely on studies with somatostatin including those with the pancreatic clamp technique (4,5). That technique involves infusion of somatostatin (or of the somatostatin analog octreotide) (6), which suppresses insulin and glucagon secretion among other actions, alone and with insulin replacement, glucagon replacement, and both insulin and glucagon replacement to document the roles of suppression of those hormones in the changes in glycemia (or in glucose kinetics when glucose concentrations are clamped) that result from administration of somatostatin (7–10).

Obviously, the biological appropriateness of the putative replacement doses of insulin and glucagon are critical to the interpretation of pancreatic clamp data (11,12). Excessive insulin replacement (or insufficient glucagon replacement) would confound the data. Insulin has been infused peripherally in doses of 0.14 (13), 0.15 (7), 0.20 (14), and 0.24 (15) mU · kg^{-1} · min^{-1} in humans to replace basal insulin levels during infusion of somatostatin. However, we found that intravenous insulin doses as low as 0.15 mU · kg^{-1} · min^{-1} are excessive; when infused alone in healthy humans they drove plasma glucose concentrations down to subnormal levels and thus activated glucose counterregulatory systems (nearly complete suppression of insulin secretion and stimulation of glucagon and epinephrine secretion) (11). Although it also lowered plasma glucose concentrations, insulin infused in a dose of 0.10 mU · kg^{-1} · min^{-1} did not drive glucose down to subnormal levels and therefore did not activate glucose counterregulatory systems, at least over 2 h (11). Accordingly, we used the latter lower insulin replacement dose in additional pancreatic clamp studies in healthy adults (12). Octreotide infusion caused plasma glucose concentrations to decrease and then increase as expected (6–10); octreotide plus insulin in a dose of 0.10 mU · kg^{-1} · min^{-1} caused a sustained decrease in plasma glucose consistent with the interpretation that glucagon, in concert with insulin, supports the postabsorptive plasma glucose concentration. However, the addition of glucagon in a dose of 1.0 ng · kg^{-1} · min^{-1}, a putative replacement dose (16), to octreotide and insulin did not raise glucose levels to those observed during infusion of octreotide alone. Therefore, the insulin dose was still too high, the glucagon dose was too low, or both.

To clarify this issue, we tested the hypothesis that glucagon supports the postabsorptive plasma glucose concentration in insulin-sufficient patients with type 1 diabetes. Our premise is that demonstrably endogenous insulin-deficient patients with type 1 diabetes infused intravenously with insulin in a dose that maintains stable euglycemia are receiving a biologically optimal insulin replacement dose.

RESEARCH DESIGN AND METHODS

Fourteen patients with type 1 diabetes—ten women and four men with a mean ± SD age of 33 ± 9 years, weight of 78 ± 17 kg, BMI of 26.7 ± 4.3 kg/m^2, duration of diabetes of 16 ± 11 years, and A1C of 7.8 ± 0.8%—gave their

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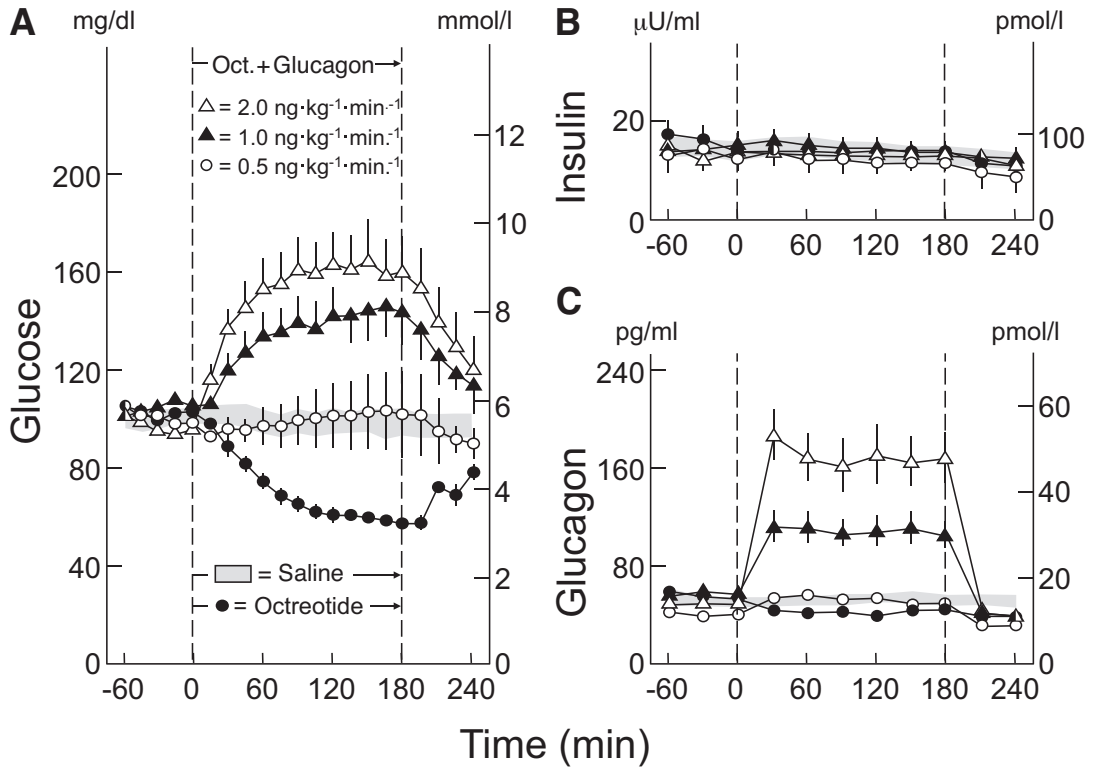

FIG. 1. Mean ± SE plasma glucose concentrations (*A*) and plasma insulin (*B*) and glucagon (*C*) concentrations in patients with type 1 diabetes infused with insulin in doses that maintain euglycemia from −60 to 240 min with infusions of *1*) saline (shaded area) (n = 14), *2*) octreotide (with growth hormone replacement) (●) (n = 14) (glucose was infused if necessary to prevent glucose levels <55 mg/dl [3.0 mmol/l]), *3*) octreotide (with growth hormone) plus glucagon 0.5 ng · kg^{-1} · min^{-1} (○) (n = 6), *4*) plus glucagon 1.0 ng · kg^{-1} · min^{-1} (▲) (n = 14), and *5*) plus glucagon 2.0 ng · kg^{-1} · min^{-1} (△) (n = 10). Oct., octreotide.

written consent to participate in this study, which was approved by the Washington University Human Research Protection Office and conducted at the Washington University Clinical Research Unit (CRU). Six patients were using a multiple daily injection insulin regimen with a long-acting insulin analog as the basal insulin and a rapid-acting insulin analog as the prandial insulin. Eight patients were using a continuous subcutaneous insulin infusion regimen with a rapid acting insulin analog. With values less than the detection unit of 0.1 ng/ml assigned that value, their mean ± SD fasting plasma C-peptide concentrations were 0.2 ± 0.2 ng/dl (<0.1 ± 0.1 nmol/l).

Subjects were studied, after an overnight fast, on up to five occasions. They took their last dose of long-acting insulin, if used, the morning of the day prior to study and managed their diabetes with a rapid-acting insulin that day. They reported to the CRU the evening prior to the study. An intravenous line was inserted, and the plasma glucose concentration was held at ~100 mg/dl (5.6 mmol/l) overnight with intravenous infusion of variable doses of regular insulin (Novolin R; Novo Nordisk, Princeton, NJ). The following morning, that insulin dose was adjusted, if necessary, and infused from −60 to 240 min. Along with insulin, infused on all study occasions, initially the subjects received intravenous *1*) saline (n = 14), *2*) octreotide 30 ng · kg^{-1} · min^{-1} (n = 14), *3*) octreotide plus glucagon 1.0 ng · kg^{-1} · min^{-1} (n = 14), or *4*) octreotide plus glucagon 2.0 ng · kg^{-1} · min^{-1} (n = 10), in random sequence, from 0–180 min. After 10 studies, the glucagon dose of 2.0 ng · kg^{-1} · min^{-1} was discontinued and six patients received octreotide plus the glucagon dose of 0.5 ng · kg^{-1} · min^{-1} from 0–180 min. Growth hormone (Genotropin; Pharmacia and Upjohn, Kalamazoo, MI) was infused, in a dose of 4.7 ng · kg^{-1} · min^{-1} (15), along with octreotide. Glucose was infused intravenously, if necessary to prevent plasma glucose concentrations <55 mg/dl (3.0 mmol/l). Blood samples were drawn through an indwelling line in a hand, with that hand kept in a ~55°C plexiglas box to provide arterialized venous samples, every 15 min for plasma glucose determinations and every 30 min for plasma insulin, glucagon, growth hormone, and epinephrine and norepinephrine measurements from −60 to 240 min. Blood pressure and heart rate were recorded (Dash 3000; GE Healthcare, Fairfield, CT) at 30-min intervals, and the electrocardiogram was monitored throughout.

Analytical methods. Plasma glucose concentrations were measured with a glucose oxidase method (YSI Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin, C-peptide, and growth hormone were measured with two-site chemiluminescent assays (Immulite 1000; Siemens, Los Angles, CA), plasma glucagon with the Linco radioimmunoassay (Millipore, Temecula, CA), and plasma epinephrine and norepinephrine with a single isotope derivative (radioenzymatic) method (17).

Statistical methods. Except where the SD is specified, data are reported as the mean ± SE. The time- and condition-related data were analyzed by repeated-measures mixed model ANOVA. P values <0.05 were considered to indicate statistically significant differences.

RESULTS

Plasma glucose concentrations. Plasma glucose concentrations (Fig. 1*A*) were stable during saline infusion; they were 101 ± 3 mg/dl (5.6 ± 0.2 mmol/l) at 0 min and 98 ± 3 mg/dl (5.4 ± 0.2 mmol/l) at 180 min. Glucose levels declined, from 102 ± 3 mg/dl (5.7 ± 0.2 mmol/l) to 58 ± 1 mg/dl (3.2 ± 0.1 mmol/l) (P vs. saline <0.001) during octreotide infusion. Indeed, to prevent glucose levels <55 mg/dl (3.0 mmol/l), glucose was infused (0.9 ± 0.2 mg · kg^{-1} · min^{-1}) in the 3rd h in 10 of the 14 patients. Glucose levels were stable during octreotide plus glucagon 0.5 ng · kg^{-1} · min^{-1} (98 ± 2 mg/dl [5.4 ± 0.1 mmol/l] to 104 ± 15 mg/dl [5.8 ± 0.9 mmol/l]), rose from 104 ± 3 mg/dl (5.8 ± 0.2 mmol/l) to 143 ± 13 mg/dl (7.9 ± 0.7 mmol/l) (P vs. saline = 0.004) during octreotide plus glucagon 1.0 ng · kg^{-1} · min^{-1} and rose from 97 ± 3 mg/dl (5.4 ± 0.2 mmol/l) to 160 ± 15 mg/dl (8.9 ± 0.8 mmol/l) (P vs. saline < 0.001) during octreotide plus glucagon 2.0 ng · kg^{-1} · min^{-1}. The insulin infusion rates were 0.12 ± 0.02, 0.14 ± 0.02, 0.13 ± 0.02, 0.16 ± 0.02, and 0.13 ± 0.02 mU · kg^{-1} · min^{-1}, respectively, in the five studies.

Plasma insulin and glucagon concentrations. Plasma insulin concentrations (Fig. 1*B*) were stable (13 ± 2 μU/ml [78 ± 12 pmol/l] at 0 min and 12 ± 2 μU/ml [72 ± 12 pmol/l] at 180 min) during saline infusion and the other

study conditions. Plasma glucagon concentrations (Fig. 1*C*) were also stable (50 ± 3 pg/ml [14.4 ± 0.9 pmol/l] at 0 min and 52 ± 4 pg/ml [14.9 ± 1.1 pmol/l[rsqb[at 180 min) during saline infusion. They decreased to 43 ± 3 pg/ml (12.3 ± 0.9 pmol/l) during infusion of octreotide alone and increased to 51 ± 5 pg/ml (14.6 ± 1.4 pmol/l), 105 ± 10 pg/ml (30.1 ± 2.9 pmol/l), and 166 ± 19 pg/ml (47.7 ± 5.5 pmol/l) during infusion of octreotide plus glucagon in doses of 0.5, 1.0, and 2.0 ng · kg^{-1} · min^{-1}, respectively.

Other data. Because growth hormone was infused, plasma growth hormone concentrations were stable during all octreotide infusions (e.g., 2.2 ± 0.6 ng/ml [97 ± 26 pmol/l] at 0 min and 1.7 ± 0.2 ng/ml [75 ± 9 pmol/l] at 180 min during octreotide with growth hormone without glucagon replacement). Plasma epinephrine concentrations tended to increase when plasma glucose levels fell during infusion of octreotide with growth hormone without glucagon replacement (data not shown). Heart rate, systolic blood pressure, and diastolic blood pressure were similar under the five study conditions (data not shown).

DISCUSSION

The optimal insulin replacement dose during pancreatic clamps in humans remains to be determined (11,12). There is evidence that insulin doses of 0.14–0.24 mU · kg^{-1} · min^{-1}, used earlier (7,13–15), are excessive; indeed, an insulin dose of 0.10 mU · kg^{-1} · min^{-1} lowered plasma glucose concentration in healthy humans (11). When that dose was infused with octreotide, the addition of a putative replacement dose (16) of glucagon did not recreate the glycemic pattern observed during infusion of octreotide alone (12). The latter finding suggests that the insulin dose was still too high, the glucagon dose was too low, or both. The biologically optimal insulin replacement dose would be one that maintains euglycemia in the absence of endogenous insulin secretion. That condition is met in patients with type 1 diabetes, who have virtually no endogenous insulin secretion, receiving infusions of insulin in doses that maintain stable euglycemia over time.

In such patients receiving biologically optimal insulin replacement in fixed doses, we tested the hypothesis that glucagon supports the postabsorptive plasma glucose concentration by comparing the plasma glucose concentrations during saline infusion with those during infusion of the somatostatin analog octreotide (with growth hormone replacement) alone and with three doses of glucagon. During infusion of saline, plasma glucose levels were stable. During infusion of octreotide without glucagon, plasma glucose levels declined progressively to subnormal levels. Indeed, glucose infusions were required to prevent glucose levels <55 mg/dl (3.0 mol/l) in 10 of the 14 patients. Remarkably, that decline in plasma glucose was prevented, and euglycemia was maintained, by coinfusion of only 0.5 ng · kg^{-1} · min^{-1} of glucagon with octreotide. The latter finding provides additional evidence that such low glucagon doses are biologically active (4,16,18,19). Thus, these data indicate that even the lower insulin dose used in our previous pancreatic clamp study (12) was still too high and that the glucagon dose was not too low. They convincingly confirm that glucagon supports the postabsorptive plasma glucose concentration in humans. Indeed, the glycemic response to the lowest dose of glucagon and the hyperglycemic responses to the higher doses suggest a substantial impact of glucagon.

Limitations of the present study include the fact that although four limbs—saline, octreotide without glucagon, octreotide plus glucagon 1.0 ng · kg^{-1} · min^{-1}, and octreotide plus glucagon 2.0 ng · kg^{-1} · min^{-1}—were conducted in random sequence, the octreotide plus glucagon 0.5 ng · kg^{-1} · min^{-1} limb was performed toward the end of the study in a subset of the patients. In addition, the glucagon radioimmunoassay that was used cross-reacts with molecular species in addition to 3,500 Da glucagon (4,5). Thus, despite clear effects on plasma glucose concentrations, the decrement in measured plasma glucagon during infusion of octreotide and the increment during infusion of octreotide with the lowest dose of glucagon were small. Finally, other neuroendocrine and cardiovascular effects of octreotide were not studied. However, complete reversal of the glucose lowering effect of octreotide by coinfusion of a relatively low dose of glucagon suggests that these other effects play little, if any, role in the changes in plasma glucose levels.

Interestingly, the insulin infusion doses that maintained euglycemia in these patients were slightly greater than 0.10 mU · kg^{-1} · min^{-1}, a dose we used earlier (12) and now judge excessive in nondiabetic individuals. However, patients with type 1 diabetes are known to be somewhat resistant to the actions of insulin (20–22).

These data document that glucagon, in concert with insulin, supports the postabsorptive plasma glucose concentrations in humans. Thus, they support the notion that glucagon, in concert with insulin deficiency, plays a role in the pathogenesis of hyperglycemia in diabetes (1–4) and the interest in the development of drugs that block the action or secretion of glucagon for the treatment of diabetes (4).

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B.A.C. and P.E.C. planned the study. B.A.C. performed the study. B.A.C. and P.E.C. wrote the manuscript.

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