Increasing the Decrement in Insulin Secretion Improves Glucagon Responses to Hypoglycemia in Advanced Type 2 Diabetes

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OBJECTIVE — In advanced β-cell failure, counterregulatory glucagon responses may be impaired due to a reduced decrement in insulin secretion during the development of hypoglycemia. The present studies were therefore undertaken to test the hypothesis that these may be improved by increasing this decrement in insulin secretion.

RESEARCH DESIGN AND METHODS — Twelve subjects with type 2 diabetes who have been insulin required were studied as a model of advanced β-cell failure. Glucagon responses were examined during a 90-min hypoglycemic clamp (≅2.8 mmol/l) on two separate occasions. On one occasion, tolbutamide was infused for 2 h before the clamp so that the decrement in insulin secretion during the induction of hypoglycemia would be increased. On the other occasion, normal saline was infused as a control.

RESULTS — Before the hypoglycemic clamp, infusion of tolbutamide increased insulin secretion ~1.9-fold (P < 0.001). However, during hypoglycemia, insulin secretion decreased to similar rates on both occasions (P = 0.31) so that its decrement was approximately twofold greater following the tolbutamide infusion (1.63 ± 0.20 vs. 0.81 ± 0.17 pmol·kg⁻¹·min⁻¹, P < 0.001). This was associated with more than twofold greater glucagon responses (42 ± 11 vs. 19 ± 8 ng/l, P < 0.002) during the hypoglycemic clamp but unaltered glucagon responses to intravenous arginine immediately thereafter (449 ± 50 vs. 453 ± 50 ng/l, P = 0.78).

CONCLUSIONS — Increasing the decrement in insulin secretion during the development of hypoglycemia improves counterregulatory glucagon responses in advanced β-cell failure. These findings further support the concept that the impaired counterregulatory glucagon responses in advanced β-cell failure may at least partially be due to a reduced decrement in insulin secretion.

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Increased secretion of glucagon is considered most important for counterregulation of hypoglycemia. Epinephrine responses, although normally not critical, become critical when glucagon secretion is deficient (1). Counterregulation of hypoglycemia is markedly impaired when both glucagon and epinephrine responses are compromised (1). Defective glucagon responses to hypoglycemia, as they occur in type 1 diabetes and markedly insulin-deficient type 2 diabetes, therefore play an important role in the pathophysiology of glucose counterregulation.

Growing evidence suggests that a decrease in insulin secretion may be an important signal for the increased glucagon secretion during hypoglycemia as originally proposed by Samols et al. (2). Absence or a marked reduction in this signal may therefore be a plausible explanation for the impaired glucagon responses in type 1 diabetes and advanced type 2 diabetes. For example, Robertson’s group (3,4) showed that both normal isolated human and rat islets and islets from streptozotocin-administered rats (which characteristically do not release glucagon when exposed to a very low glucose concentration) can respond to glucose deprivation by releasing glucagon if they are first provided with increased endogenous or exogenous insulin so that the decrement in intraislet insulin during hypoglycemia was increased. This group of investigators also demonstrated that in streptozotocin-induced diabetic rats with near complete β-cell failure, glucagon responses to hypoglycemia, which had been absent, were restored when the decrement in intraislet insulin was reestablished by an infusion of insulin into the superior pancreaticoduodenal artery that was switched off when blood glucose fell into the hypoglycemic range (4).

Whether a decrement in insulin secretion and the absence thereof play a similarly important role in the physiology and pathophysiology of glucagon responses to hypoglycemia in humans is unclear. Banerer et al. (5) found that in nondiabetic subjects, counterregulatory glucagon responses were blunted when the fall in insulin secretion during hypoglycemia was prevented by an infusion of an insulin secretagogue tolbutamide. In subsequent human studies, counterregulatory glucagon responses were found to be ~50 and ~30% reduced when insulin secretion was inhibited before hypoglycemia by oral diazoxide (6) or an infusion of somatostatin (7), respectively, so that the decrement in insulin secretion during hypoglycemia, during which diazoxide or somatostatin were no longer being given, was ~50% diminished or prevented. However, interpretation of these studies is limited for the following reasons. First, in the tolbutamide experiments, insulin secretion increased above baseline during hypoglycemia by the tolbutamide infu-
Insulin and glucagon responses to hypoglycemia

sion, and since insulin suppresses glucagon release (8), the blunted glucagon responses might have been merely the result of intrascllet hyperinsulinemia. Second, in the diazoxide experiments, insulin secretion was incompletely inhibited before hypoglycemia so that its decrement during the development of hypoglycemia was reduced but not prevented. And third, in the somatostatin experiments the reduced glucagon responses might have been caused by ongoing suppression of glucagon secretion; conversely, a rebound in glucagon secretion following the somatostatin infusion (9) might have underestimated the effect of the absent decrement in insulin secretion. Aside from the limitations of these studies, and perhaps most importantly, proof of concept usually requires its confirmation by complimentary approaches, which has yet to be provided for the hypothesis that a decrease in insulin secretion is an important signal for the increased glucagon secretion during hypoglycemia.

The present studies were therefore undertaken to test the hypothesis that restoring the decrement in insulin secretion during hypoglycemia improves glucagon responses in humans in whom this decrement is diminished. For these purposes, we studied counterregulatory glucagon responses in insulin-requiring type 2 diabetic subjects, who were expected to have a reduced decrement in insulin secretion during hypoglycemia as a result of advanced β-cell failure, on two separate occasions. On one occasion, the diabetic subjects’ insulin secretion was increased before inducing hypoglycemia by an infusion of tolbutamide so that its decrement during hypoglycemia would be artificially increased. On the other occasion, subjects received an infusion of normal saline instead of tolbutamide.

**RESEARCH DESIGN AND METHODS** — Informed written consent was obtained from 12 subjects with type 2 diabetes after the protocol had been approved by the Institutional Review Board of the Carl T. Hayden Veterans Affairs Medical Center and the University of Rochester. All subjects had failed a combination of at least two oral hypoglycemic agents to maintain an HbA1c (A1C) of <8.0% and were being treated with insulin at the time of the study as an index of advanced β-cell failure. Subjects (10 men and 2 women) were 54.3 ± 1.4 years of age and had a BMI of 32.8 ± 1.4 kg/m², a known diabetes duration of 10 ± 2 years, and an A1C of 7.3 ± 0.4%. Exclusion criteria included the presence of circulating GAD antibodies, renal insufficiency, autonomic neuropathy, untreated proliferative retinopathy, coronary artery disease, anemia, episodes of severe hypoglycemia, or hypoglycemia unawareness. In addition to insulin, one subject was being treated with metformin, eight subjects were being treated with a sulfonylurea and metformin, and one subject was being treated with a sulfonylurea, metformin, and rosiglitazone. All oral hypoglycemic agents were withdrawn 4 days before each experiment. The last injection of long- or intermediate-acting insulin was at bedtime 2 days before each study. If needed, preprandial injections of short-acting insulin were given the day before the studies.

All subjects were studied on two separate occasions 13.4 ± 3.6 days apart (range 10–19). For each study, subjects were admitted to the Clinical Research Center of the Carl T. Hayden Veterans Affairs Medical Center (n = 10) or the University of Rochester General Clinical Research Center (n = 2) between 5:00 and 6:00 p.m. the evening before experiments. Subjects received a standard dinner (10 kcal/kg; 50% carbohydrate, 35% fat and 15% protein) between 6:30 and 7:00 p.m. and fasted thereafter except for water ad lib until the experiments were completed. At ~10:00 p.m., an antecubital vein was cannulated and an overnight intravenous insulin infusion was started to restore near normoglycemia. During this period, blood glucose concentrations were measured at ~30 min intervals and levels <90 mg/dl (5.0 mmol/l) were avoided. The insulin infusion, which had rendered the diabetic subjects near normoglycemic, was maintained during the blood sampling period.

At ~7:00 a.m. the following morning, a dorsal hand vein was cannulated in a retrograde fashion and kept in a thermoregulated Plexiglass box at 65°C for sampling arterialized venous blood (10). Approximately 1 h later, two blood samples were collected at 30 min intervals (~150 and ~120 min) for measurement of baseline concentrations of plasma glucose, insulin, C-peptide, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol. At ~120 min, a 2-h intravenous infusion of tolbutamide (1 g/h) was begun on one occasion to artificially increase the decrement in insulin secretion during the subsequent hypoglycemic clamp; on the other occasion, an infusion of normal saline was given instead. Infusions were given in a single-blinded randomized fashion, and an equal number of subjects were studied in both orders. During the 2-h infusions, plasma glucose was maintained at baseline levels by an intravenous infusion of glucose if needed. At 0 min, a continuous infusion of insulin (1.5 mU · kg⁻¹ · min⁻¹) was begun, and plasma glucose was allowed to decrease to 45–50 mg/dl (2.5–2.8 mmol/l) and subsequently maintained at this level until 120 min with glucose concentrations being measured every 5 min. Blood samples were collected as described above at ~90, ~60, ~30, 0, 30, 60, 75, 90, 105, and 120 min.

Blood samples were collected for plasma insulin, C-peptide, glucagon, cortisol, and growth hormone in EDTA tubes containing a protease inhibitor, and for plasma catecholamines in EDTA tubes. Plasma glucose was immediately determined with a glucose analyzer (Yellow Springs Instruments). For other determinations, samples were placed immediately in a 4°C ice bath, and plasma was subsequently separated by centrifugation at 4°C. For consistency, all samples of a given subject were analyzed together in the same assay. Plasma insulin, C-peptide, glucagon, growth hormone, and cortisol concentrations were determined by standard radioimmunoassays at the University of Rochester, and plasma epinephrine and norepinephrine concentrations were measured by a radioenzymatic method at the Washington University School of Medicine as previously described (11).

**Calculations** — B-Cell function was assessed by using homeostasis model assessment of β-cell function (HOMA-%B), which was calculated as C-peptide (pmol/l) × 3.33/ (glucose [mmol/l] − 3.5) using the average fasting plasma concentrations. C-peptide was used in place of the originally proposed plasma insulin because of the exogenous infusion of insulin (12). Rates of insulin secretion were calculated by deconvolution analyses of plasma C-peptide using an open two-compartmental model and population-based transition coefficients as described by Hovorka and Jones (13). The software (ISEC, version 2) was kindly provided by Dr. R. Hovorka (Center for Measurement and Information in Medicine, City University, London, U.K.).
Statistical analyses

Unless stated otherwise, data are expressed as means ± SD. Paired two-tailed Student’s t tests were used to compare corresponding data of both sets of experiments. Means of plasma hormone concentrations at 105 and 120 min and the increments of these means above baseline (mean of −150 and −120 min) were used for comparisons of hormonal responses to hypoglycemia. Pearson correlation coefficients were used to determine the relationship between decrements in insulin secretion and glucagon responses.

RESULTS

Plasma glucose, insulin, and C-peptide and rates of insulin secretion

At baseline, arterialized venous plasma insulin concentrations were similar on both occasions. During the infusion of tolbutamide, plasma insulin increased slightly, but this did not result in significantly greater levels than those during the infusion of saline ($P = 0.15$). Subsequently, during the infusion of insulin, plasma insulin increased to comparable levels in both sets of experiments ($991 ± 83$ vs. $998 ± 114$ pmol/l, $P = 0.91$). Since glycemia was controlled, plasma glucose levels were virtually identical before the insulin infusion and fell similarly during the insulin infusion to $2.8 ± 0.1$ mmol/l on both occasions (Fig. 1).

Arterialized venous concentrations of plasma C-peptide and rates of insulin secretion were comparable at baseline in both sets of experiments; HOMA-%B averaged $575 ± 107$ (pmol/l)/(mmol/l). After the baseline period, plasma C-peptide increased significantly during the infusion of tolbutamide but decreased, albeit not significantly, during the infusion of saline. This resulted in $\sim 75\%$ greater plasma C-peptide concentrations during the last 30 min before the insulin infusion in the tolbutamide experiments than in the saline experiments ($715 ± 98$ vs. $414 ± 74$ pmol/l, $P < 0.001$). Subsequently, during the insulin infusion, during which tolbutamide or saline were no longer being infused, plasma C-peptide declined to $294 ± 46$ pmol/l in the tolbutamide experiments and to $161 ± 30$ pmol/l in the saline experiments. Accordingly, the decrement in plasma C-peptide during hypoglycemia was increased $\sim 65\%$ by the antecedent tolbutamide infusion ($421 ± 53$ vs. $253 ± 49$ pmol/l, $P < 0.001$). Similarly, rates of insulin secretion increased during the infusion of tolbutamide ($P < 0.001$) but tended to decrease during the infusion of saline ($P = 0.07$) so that rates were $\sim 90\%$ greater before the insulin infusion in the tolbutamide experiments ($1.90 ± 0.24$ vs. $1.02 ± 0.19$ pmol · kg$^{-1}$ · min$^{-1}$, $P < 0.001$). Subsequently, during the infusion of insulin, insulin secretion decreased to similar rates in the tolbutamide and saline experiments ($0.27 ± 0.05$ vs. $0.21 ± 0.04$ pmol · kg$^{-1}$ · min$^{-1}$, $P = 0.31$). Since insulin secretion had been stimulated in the tolbutamide experiments before the insulin infusion, the decrement in insulin secretion during the insulin infusion was approximately two-
fold increased (1.63 ± 0.20 vs. 0.81 ± 0.17 pmol · kg⁻¹ · min⁻¹, P < 0.001) (Fig. 2).

Plasma counterregulatory hormones

At baseline, plasma glucagon was comparable on both occasions. Subsequently, plasma glucagon remained unaltered during the infusion of saline (P = 0.20) but tended to decrease during the infusion of saline (P < 0.07) (Fig. 3). This resulted in a trend toward slightly greater plasma glucagon levels during the last 30 min before the insulin infusion in the tolbutamide experiments (93 ± 6 vs. 86 ± 5 ng/l, P < 0.06). However, during the initial 30 min of the insulin infusion, during which tolbutamide was no longer being infused, plasma glucagon decreased to comparable levels in both experiments (86 ± 6 vs. 81 ± 7 ng/l, P = 0.14). Plasma glucagon increased similarly thereafter with the onset of hypoglycemia until 75 min but subsequently to a greater extent in the tolbutamide experiments than in the saline experiments. Accordingly, during the last 15 min of the hypoglycemic clamp, during which plasma glucagon levels plateaued, glucagon responses were significantly increased by the antecedent tolbutamide infusion; both the absolute plasma glucagon concentrations (131 ± 17 vs. 112 ± 14 ng/l, P < 0.04) and the increments of plasma glucagon above baseline (42 ± 11 vs. 19 ± 8 ng/l, P < 0.002) were significantly improved. Plasma glucagon and its increments during hypoglycemia correlated with the decrements in insulin secretion in the tolbutamide (r = 0.625, P < 0.03) and tolbutamide (P = 0.05, respectively) and saline experiments (r = 0.658 and r = 0.652, respectively, both P < 0.02) and when both experiments were analyzed together (r = 0.623 and r = 0.659, respectively, both P < 0.001). In contrast to the findings during hypoglycemia, plasma glucagon increased to virtually identical levels during the infusion of arginine on both occasions (449 ± 50 vs. 453 ± 50 ng/l, P = 0.78).

Counterregulatory responses of epinephrine (1.789 ± 27.6 vs. 1.888 ± 302 pmol/l, P = 0.67), norepinephrine (1.026 ± 147 vs. 955 ± 138 pmol/l, P = 0.76), cortisol (312 ± 61 vs. 306 ± 68 nmol/l, P = 0.90), and growth hormone (2.4 ± 1.6 vs. 3.0 ± 1.0 μg/l, P = 0.65) were comparable in both sets of experiments.

CONCLUSIONS — The present study was undertaken to further assess the concept that a decrement in insulin secretion is an important factor for glucagon responses during hypoglycemia. For these purposes, we took the opposite approach of previous studies and tested the hypothesis whether restoring the decrement in insulin secretion improves glucagon responses in humans in whom this decrement is diminished because of advanced β-cell failure. As a model, we selected type 2 diabetic subjects who were insulin requiring, a clinical index of insulin deficiency, which was subsequently confirmed by our finding that fasting HOMA-%B was ~70% reduced compared with previously studied nondiabetic subjects with similar demographic characteristics (14).

Consistent with previous reports (15), we found that plasma glucagon concentrations increased very little in response to hypoglycemia in type 2 diabetic subjects with poor β-cell function. However, when the decrement in insulin secretion during hypoglycemia was increased approximately twofold by an antecedent infusion of the insulin secretagogue tolbutamide, glucagon responses approximately doubled, resembling those in nondiabetic subjects during similar experimental conditions (7). Because arterial concentrations of glucose, insulin, and catecholamines were virtually identical in both sets of experiments of the present study, these findings cannot be ascribed to differences in the hypoglycemic stimulus, peripheral hyperinsulinemia, or activation of the sympathoadrenal system.

Tolbutamide has been found to increase glucagon secretion in isolated rat α-cells (16) and in insulin-deficient type 1 diabetes in some (17) but not all (18) studies, and in the present studies in patients with advanced type 2 diabetes, plasma glucagon tended to be greater during the infusion of tolbutamide than during the infusion of saline. It may therefore be argued that the increased glucagon responses following tolbutamide may be explained by ongoing stimulation of glucagon secretion or simply greater plasma glucagon levels immediately before inducing hypoglycemia. This explanation appears, however, unlikely given...
the following considerations. First, insulin secretion decreased to similar rates during hypoglycemia, and plasma glucagon increased to similar levels in response to arginine. Second, tolbutamide had no residual effects at the end of the hypoglycemic clamp when counterregulatory glucagon responses were analyzed. Second, infusion of tolbutamide has been shown to decrease, not to increase, glucagon responses to hypoglycemia in humans (5,19), which is probably indirectly mediated via its effects on β-cells. Third, after the infusions of tolbutamide or saline were stopped, plasma glucagon levels were similar on both occasions from ~30 to 75 min of the insulin infusion as well as during the infusion of arginine after hypoglycemia. If the increased glucagon responses during the last 30 min of hypoglycemia in the tolbutamide experiments were the result of ongoing stimulation of glucagon secretion or slightly greater glucagon levels immediately before the insulin infusion, one would however expect increased glucagon levels during the entire period following the tolbutamide infusion. And finally, due to its short t/2, plasma glucagon achieves a steady state within ~30 min of a constant delivery into the systemic circulation in humans (20,21). Plasma glucagon would therefore be expected to reach equivalent concentrations at the end of the hypoglycemic clamp in both experiments if glucagon secretion were comparable regardless of prior levels.

The time course of events during the hypoglycemic clamp may be of interest. In the tolbutamide experiments, glucagon levels started to increase to a greater extent at ~90 min. At this time point, insulin secretion was still greater than in the saline experiments despite an increased decrement in insulin secretion during the antecedent 90 min. These observations suggest that in the present studies, the rate of fall of insulin secretion might have been more important than the actual rate of insulin secretion for stimulation of glucagon secretion in response to hypoglycemia.

We would like to point out that the present studies do not exclude a role of defective autonomic stimulation (22), or other mechanisms, in the impaired counterregulatory glucagon responses in type 1 diabetes and advanced type 2 diabetes. Furthermore, they do not permit insight as to the exact mechanism(s) responsible for the increased glucagon responses to hypoglycemia when the decrement in insulin secretion was increased. First of all, for this mechanism to be operative, at least a large number of α-cells would have to be perfused after β-cells. Although there are at least three models of the microcirculation of islets of Langerhans (23), evidence indicates that this is probably the case in humans (23). Second, there are several factors cosecreted with insulin that may be involved. These include, but are not limited to, amylin and zinc, which have been shown to suppress glucagon secretion (24,25). An increased decrement of these and potentially other factors in addition to insulin might have thus been a signal for the increased counterregulatory glucagon secretion. Moreover, we cannot exclude the possibility that the increased glucagon responses in the tolbutamide experiments were the result of a direct cell-to-cell interaction, where by the β-cell signal to α-cells would be mediated by processes within the β-cells that are associated with intracellular insulin dynamics.

Regardless of the mechanism(s), our studies do demonstrate that increasing the decrement in insulin secretion during the development of hypoglycemia improves counterregulatory glucagon responses in humans in whom this decrement is diminished as a result of advanced β-cell failure. Accordingly, together with the results of previous studies, the hypothesis that a decrease in insulin secretion is an important signal for the increased glucagon secretion during hypoglycemia has now been confirmed by complimentary approaches in humans.

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References
амид стимулирует эксклизию глюкагона
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ATP-чувствительного K⁺-канала (KATP) в гиппагоне