Comparison of the Inhibitory Effect of Insulin and Hypoglycemia on Insulin Secretion in Humans

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Although both insulin and hypoglycemia are known to inhibit endogenous insulin secretion, their potency to suppress insulin secretion has not been directly compared thus far. The serum C-peptide concentration was measured during 28 euglycemic and 28 stepwise hypoglycemic (4.1, 3.6, 3.1, and 2.6 mmol/L) clamp experiments using either a low-rate (1.5 mU · min^{-1} · kg^{-1}) or high-rate (15.0 mU · min^{-1} · kg^{-1}) insulin infusion. The experiments lasted 6 hours and were performed in 28 lean healthy men. During both the euglycemic and hypoglycemic clamps, serum insulin was approximately 40-fold higher during the high-rates versus low-rate insulin infusion (euglycemia, 24,029 ± 1,595 *v* 543 ± 34 pmol/L; hypoglycemia, 23,624 ± 1,587 *v* 622 ± 32 pmol/L). Under euglycemic conditions, serum C-peptide decreased from 0.54 ± 0.04 to 0.41 ± 0.05 nmol/L during the low-rate insulin infusion ($P < .05$) and from 0.55 ± 0.07 to 0.27 ± 0.09 nmol/L during the high-rate insulin infusion ($P < .001$). Under hypoglycemic conditions, serum C-peptide decreased from 0.50 ± 0.03 to 0.02 ± 0.01 nmol/L during the low-rate insulin infusion ($P < .001$) and from 0.46 ± 0.07 to 0.02 ± 0.01 nmol/L during the high-rate insulin infusion ($P < .001$). In the euglycemic clamp condition, the high-rate insulin infusion reduced the C-peptide concentration more than the low-rate insulin infusion ($P < .05$). Independent of the rate of insulin infusion, the decrease in C-peptide was distinctly more pronounced during hypoglycemia versus euglycemia ($P < .001$). These data indicate that insulin inhibits insulin/C-peptide secretion in a dose-dependent manner. Hypoglycemia is a much stronger inhibitor of insulin secretion than insulin itself.

SUPPRESSION OF ENDOGENOUS insulin secretion during mild hypoglycemia[1-5] likely represents a physiological mechanism protecting against the development of severe hypoglycemia. Previous studies indicated that this suppression of insulin secretion during hypoglycemia is mediated by both a direct effect on pancreatic β cells and an activation of the autonomic nervous system.[6] Hyperinsulinemia has also been found to decrease insulin secretion in most[7-13] but not all[1,14] studies. The contribution of hyperinsulinemia, often accompanying hypoglycemia, to the suppression of insulin release during hypoglycemia has been rarely assessed. In a hypoglycemic clamp experiment, Fanelli et al[2] found an enhanced suppression of insulin secretion by high serum insulin as compared with lower levels of serum insulin. However, Mellman et al[4] failed to find similar differences in a comparable experiment.

The present study aimed to compare the inhibitory effects of insulin and hypoglycemia on insulin secretion. Specifically, the question was whether and to what extent insulin suppresses its own secretion and, if so, to what extent this insulin effect contributes to the inhibition of insulin secretion induced by hypoglycemia. The measurement of serum C-peptide, which is released in equimolar amounts together with insulin, was used to estimate insulin secretion during 56 euglycemic and hypoglycemic clamp experiments using either a low-rate or high-rate insulin infusion.

SUBJECTS AND METHODS

Twenty-eight lean healthy men participated in the experiments (mean ± SEM age, 25.9 ± 0.5 years; range, 22 to 32; body mass index, 22.8 ± 0.3 kg/m^2; range, 18.6 to 26.0). Exclusion criteria were chronic or acute illness, current medication of any kind, smoking, alcohol or drug abuse, adiposity, and diabetes or hypertension in first-degree relatives. The study was approved by the local ethics committee of the Medical University of Luebeck. The purpose and potential risks of the study were carefully explained to all subjects, and their voluntary written informed consent was obtained before participation.

Each subject underwent a hypoglycemic clamp and a euglycemic clamp experiment, separated by an interval of at least 4 weeks. The order of conditions was balanced across subjects, and the experiments were performed in a single-blind fashion. The 28 subjects were randomly assigned to 2 different groups of 14 subjects each. In one group, insulin was infused at a rate of 1.5 mU · min^{-1} · kg^{-1} (low insulin) during the euglycemic and hypoglycemic clamp. In the other group, insulin was infused at a rate of 15.0 mU · min^{-1} · kg^{-1} (high insulin) during both clamp conditions.

The subjects reported to the medical research unit at 8 AM after an overnight fast of at least 10 hours. A cannula was inserted into a vein on the back of the hand, which was placed in a heated box (50° to 55°C) to obtain arterialized venous blood. A second cannula was inserted into an antecubital vein of the contralateral arm. Both cannulas were connected to long thin tubes that enabled blood sampling and adjustment of the rate of glucose infusion from an adjacent room without notice by the subject. After a 1-hour baseline period, insulin (H-insulin; Hoechst Marion Roussel, Frankfurt, Germany) was infused at a continuous rate of either 1.5 or 15.0 mU · min^{-1} · kg^{-1}, respectively. A 20% glucose solution was simultaneously infused at a variable rate to control the plasma glucose concentration. Arterialized blood was drawn every 5 minutes to measure the plasma glucose concentration (Beckman Glucose Analyser; Beckman, Munich, Germany). During the euglycemic clamps, plasma glucose between 5.0 and 5.5 mmol/L was maintained. During the hypoglycemic clamps, plasma glucose was reduced in a stepwise manner to achieve 4 plateaus at a concentration of 4.1, 3.6, 3.1, and 2.6 mmol/L, respectively. Each plateau was maintained for a 45-minute period, and the next-lower plateau was induced gradually within the next 45 minutes. During high insulin infusion, the potassium concentration was monitored at 30-minute intervals and substitution was given whenever the level decreased below 4.0 mmol/L. Blood samples for determination of serum insulin and C-peptide were obtained every 30 minutes.

All blood samples were immediately centrifuged, and the supernatants were stored at −24°C until assay. Serum insulin was determined by radioimmunoassay (Pharmacia Insulin RIA 100; Pharmacia Diagnos-

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tics, Uppsala, Sweden) with an interassay coefficient of variation (CV) less than 5.8% and an intraassay CV less than 5.4%. Serum C-peptide was determined by radioimmunoassay (C-Peptide-RIA; Hermann Biermann Diagnostica, Bad Nauheim, Germany) with an interassay CV less than 5.6%, an intraassay CV less than 3.2%, and a sensitivity of 0.017 nmol/L (0.05 ng/mL).

All values are presented as the mean ± SEM. Statistical analysis was based on the paired and unpaired Student's *t* test as appropriate and on ANOVA for repeated measurements including the factors insulin (high *v* low rate of insulin infusion) and plasma glucose (euglycemia *v* hypoglycemia). A *P* value less than .05 was considered statistically significant.

RESULTS

The plasma glucose concentration did not differ between the high- and low-insulin condition during either the euglycemic clamp or the hypoglycemic clamp (Fig 1A). The mean serum insulin was approximately 40-fold higher during the high-rate versus low-rate insulin infusion in both the euglycemic (24,029 ± 1,595 *v* 543 ± 34 pmol/L) and hypoglycemic (23,624 ± 1,587 *v* 622 ± 32 pmol/L) clamp condition.

During the euglycemic clamp condition, the low-rate insulin infusion decreased serum C-peptide to a steady-state level of 0.38 ± 0.06 nmol/L within the first 90 minutes of the clamp. During the high-rate insulin infusion, a distinctly lower steady-state level of 0.26 ± 0.03 nmol/L was reached already after 60 minutes of the clamp ($P < .05$ for the comparison of steady-state levels; Fig 1B). In contrast, during the hypoglycemic clamp condition, serum C-peptide progressively decreased to about 0.03 ± 0.01 nmol/L within the first 240 minutes of both the low-rate and high-rate insulin infusion. Thereafter, C-peptide remained essentially unchanged at this level (Fig 1B). Accordingly, the temporal dynamics of the decrease in C-peptide during hypoglycemia did not differ between the 2 insulin conditions. The decrease in C-peptide was significantly more marked during the hypoglycemic clamp as compared with the insulin infusion in the euglycemic condition independently of the rate of insulin infusion ($P < .001$). ANOVA showed a significant effect of plasma glucose ($P < .001$) and insulin ($P < .05$) on the serum C-peptide concentration and also a significant plasma glucose-insulin interaction ($P < .01$).

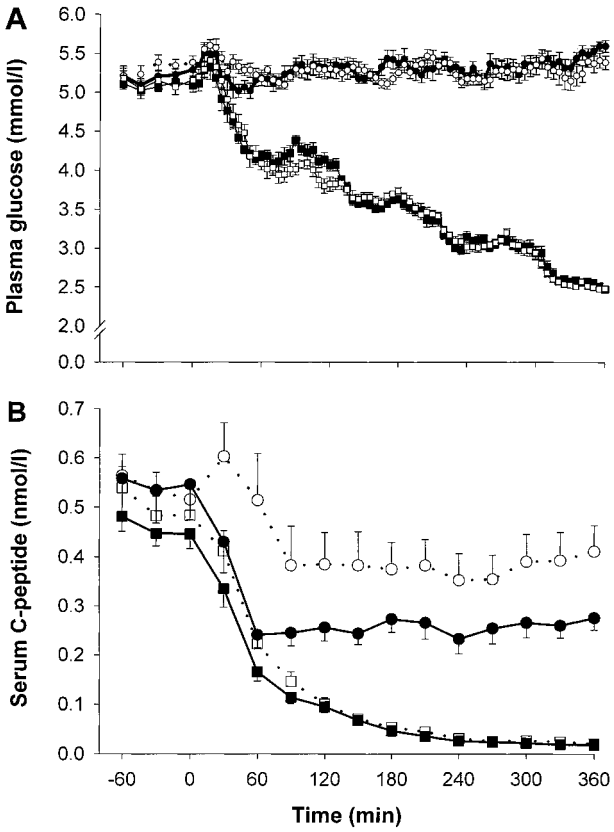

Fig 1. (A) Plasma glucose and (B) serum C-peptide during the euglycemic and hypoglycemic clamps. (○) Low-insulin euglycemic clamp, (●) high-insulin euglycemic clamp, (□) low-insulin hypoglycemic clamp, (■) high-insulin hypoglycemic clamp.

DISCUSSION

The present data indicate a dose-dependent inhibitory effect of insulin on endogenous C-peptide secretion as an estimate of insulin secretion. This result confirms the findings of previous studies.[15-18] Considering that serum insulin levels were clearly supraphysiological during the high-rate insulin infusion, the decrease in C-peptide during this experimental condition likely reflects a maximum suppressive effect of insulin on its own secretion, which may be quantified as approximately a 50% suppression. However, the magnitude of this suppression was much less than that of even mild hypoglycemia, as indicated by the present data.

During the hypoglycemic clamp, serum C-peptide decreased to extremely low levels within the first 240 minutes of insulin infusion, suggesting that endogenous insulin secretion was nearly abolished after this time interval. Considering the serum C-peptide half-life of approximately 30 minutes,[19] the maximum suppressive effect of hypoglycemia on insulin secretion was presumably already achieved 30 minutes earlier, ie, after about 210 minutes, when plasma glucose was about 3.3 mmol/L. The inhibition of insulin release by even mild hypoglycemia was so profound that an additional suppressive action of insulin was not detectable. Thus, the present data indicate that low plasma glucose is the major inhibitor of insulin secretion, while insulin itself is only a minor inhibitor. These results agree with previous findings by Turner et al[20] showing that ethanol-induced hypoglycemia nearly abolishes insulin secretion even in the absence of exogeneous insulin.

The finding that the maximum suppressive effect of insulin on its own secretion is about 50%, not 100%, is not surprising, considering that the half-life of a pulse of insulin is 5 to 7 minutes. If there were 100% inhibition of endogenous insulin release by insulin itself, it would be amazing for insulin to increase for prolonged stimulatory periods. Therefore, the present data, by quantifying a 50% suppression as maximal, may explain why insulin levels increase during prolonged stimulation periods despite the inhibitory influence of insulin on its own secretion.

The mechanism by which insulin inhibits its own secretion cannot be determined by the present study, since our study design did not allow us to distinguish between a potential peripheral and central nervous effect of insulin. However, previous findings favor a central nervous mediation rather than

a direct inhibition of pancreatic β cells.[21-23] For instance, Luzi et al[23] showed that in patients with denervated pancreas, ie, after well-functioning pancreas transplantation, euglycemic hyperinsulinemia fails to suppress endogenous C-peptide secretion, indicating that the feedback inhibition of insulin secretion is neurally mediated. Further evidence for this view derives from the finding that insulin increases sympathetic nervous system activity, as well as plasma catecholamine levels, during euglycemia,[24-26] which in turn can decrease pancreatic insulin release.[6]

The downregulation of endogenous insulin secretion with a low plasma glucose concentration appears to be a physiological defense mechanism against the development of severe hypoglycemia. Patients with type 1 diabetes[27] have a much higher risk for severe hypoglycemia than those with type 2 diabetes.[28] Type 1 diabetes is characterized by an absolute dependency on exogenous insulin, whereas in type 2 diabetes, the insulin demand is, at least in part, covered by endogenous insulin. Thus, patients with type 1 diabetes are unable to regulate plasma glucose by modulating endogenous insulin secretion. Although previous data by Turner et al[29] suggested that the suppression of insulin secretion by decreasing plasma glucose is impaired in type 2 diabetes, a more recent study by Shamoon et al[30] showed a similar decrease in C-peptide during hypoglycemia in type 2 diabetic patients compared with healthy control subjects. Thus, patients with type 2 diabetes presumably will have a downregulation of endogenous insulin secretion during developing hypoglycemia to prevent a further decrease of plasma glucose. This in addition to the preserved counterregulatory hormone response to hypoglycemia in type 2 diabetic patients[31,32] may contribute to the lower risk for severe hypoglycemia in type 2 diabetics in comparison to type 1 diabetics. Support for this view derives from the finding that type 1 diabetic patients with residual insulin/C-peptide secretion are also at lower risk for severe hypoglycemia than those with a complete loss of insulin secretion.[27]

In summary, the present study demonstrates a dose-dependent inhibition of insulin/C-peptide secretion by insulin. However, the inhibitor effect of hypoglycemia on endogenous insulin secretion is much stronger than that of insulin itself.

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