Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes

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Received 20 December 1999; accepted in final form 24 March 2000

Laedtke, Thomas, Lise Kjems, Niels Pørksen, Ole Schmitz, Johannes Veldhuis, Pai C. Kao, and Peter C. Butler. Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. Am J Physiol Endocrinol Metab 279: E520–E528, 2000.—Impaired insulin secretion in type 2 diabetes is characterized by decreased first-phase insulin secretion, an increased proinsulin-to-insulin molar ratio in plasma, abnormal pulsatile insulin release, and heightened disorderliness of insulin concentration profiles. In the present study, we tested the hypothesis that these abnormalities are at least partly reversed by a period of overnight suspension of β-cell secretory activity achieved by somatostatin infusion. Eleven patients with type 2 diabetes were studied twice after a randomly ordered overnight infusion of either somatostatin or saline with the plasma glucose concentration clamped at ~8 mmol/l. Controls were studied twice after overnight saline infusions and then at a plasma glucose concentration of either 4 or 8 mmol/l. We report that in patients with type 2 diabetes, 1) as in nondiabetic humans, insulin is secreted in discrete insulin secretory bursts; 2) the frequency of pulsatile insulin secretion is normal; 3) the insulin pulse mass is diminished, leading to decreased insulin secretion, but this defect can be overcome acutely by β-cell rest with somatostatin; 4) the reported loss of orderliness of insulin secretion, attenuated first-phase insulin secretion, and elevated proinsulin-to-insulin molar ratio also respond favorably to overnight inhibition by somatostatin. The results of these clinical experiments suggest the conclusion that multiple parameters of abnormal insulin secretion in patients with type 2 diabetes mechanistically reflect cellular depletion of immediately secretable insulin that can be overcome by β-cell rest.

pulsatile insulin secretion; hyperglycemia; orderliness; C-peptide; somatostatin; proinsulin

HUMANS WITH TYPE 2 DIABETES exhibit defective insulin secretion, which is most obviously manifest under conditions of insulin resistance (12, 15, 23, 24, 41, 42). Impaired insulin secretion in type 2 diabetes is often characterized by a decreased first-phase insulin response (41), abnormal pulsatile insulin release (14, 21, 23), an increased ratio of proinsulin to insulin in the systemic circulation (30, 36, 43), and reduced orderliness of plasma insulin patterns (31). Many, but not all, of these parameters of insulin secretion were measured in patients with type 2 diabetes during hyperglycemia and compared with control subjects during normoglycemia. Under these conditions, the overnight fasting plasma insulin concentration (12) and measured insulin secretion rate (23) in patients with type 2 diabetes and nondiabetic controls are comparable, which is presumably due to the offsetting effects of hyperglycemia and impaired insulin secretion.

Chronic hyperglycemia per se may have deleterious effects on insulin secretion (5, 10, 17, 18, 29), which can be partly ameliorated by decreasing β-cell demand through either weight loss (10, 34), insulin treatment (5, 6, 37, 38), or inhibition of insulin secretion by diazoxide (9, 19). Based on these observations, one proposition is that chronic β-cell stimulation is important in the pathogenesis of impaired insulin secretion in type 2 diabetes (15, 24, 37, 42). In humans with type 2 diabetes, there appears to be a deficiency of β-cell mass compared with appropriately matched (comparatively obese) nondiabetic controls (13). In patients with type 2 diabetes, therefore, a decreased number of β-cells are exposed to an environment of chronic hyperglycemia and insulin resistance, which could establish the pathophysiological basis for chronically increased β-cell stimulation.

The absent and/or defective insulin pulses previously reported in patients with type 2 diabetes could be caused either by a primary defect in the pacemaker and/or islet coordination system or by a defect at the level of the islet. We hypothesized that the latter is true, such that a normally generated impulse (healthy pacemaker) is delivered in an appropriately synchronized manner (healthy coordination) to islets but that these fail to respond appropriately to this stimulus. We

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further hypothesized that such a deficient response may be due to deficient immediately releasable insulin stores. A primary purpose of the present study was to determine whether a period of β-cell rest imposed by overnight somatostatin at physiological hyperglycemia (a strategy designed to enhance immediately secretable insulin stores) restores pulsatile insulin release. If a defect in pacemaker or islet coordination is responsible for the abnormal pattern of pulsatile insulin release in patients with diabetes, we would not anticipate restoration of pulsatile insulin release by a period of somatostatin-imposed β-cell rest. To meet this primary objective, therefore, the crucial comparison in the present studies is between the pattern of insulin release in patients with type 2 diabetes after either somatostatin or saline treatment.

Thus, in the present study, we sought to address the hypothesis that, in patients with type 2 diabetes, the pattern of impaired insulin secretion is at least partly reversible after a period of β-cell rest imposed via somatostatin (which inhibits insulin secretion but not synthesis). This circumstance would argue against the presence of specific β-cell defects leading to a loss of first-phase insulin secretion, pulsatile insulin secretion, disordered insulin secretion, or decreased conversion of proinsulin to insulin, and rather imply that these defects are at least in part a consequence of chronically increased β-cell stimulation in the setting of an inadequate number of β-cells.

METHODS

Study Subjects

Eleven patients with type 2 diabetes (yr since diagnosis 2.4 ± 0.6, range 0.5–6 yr), and six nondiabetic control subjects were studied in the General Clinical Research Center (GCRC) of the Mayo Clinic. The studies were approved by the Mayo Clinic Institutional Review Board (IRB), and all volunteers provided written informed consent. Inclusion criteria for patients with type 2 diabetes were documented fasting hyperglycemia (>7.8 mmol/l) on at least two occasions, a positive family history of type 2 diabetes, onset of diabetes after age 35, and the absence of diabetic ketoacidosis. The inclusion criterion for controls was a normal fasting blood glucose value of <6.1 mmol/l. Exclusion criteria for both type 2 diabetes and control subjects were pregnancy, abnormal liver or renal function tests or a history of hepatic or renal disease, a history of pancreatitis, anemia, symptomatic ischemic heart disease or cardiac failure, and treatment with glucocorticoid or anticonvulsants. Patients with type 2 diabetes and controls were matched for age, gender, and body mass index (Table 1). The diabetic patients were managed with diet (n = 4), sulfonylurea (n = 3), or insulin (n = 4). Sulfonylurea treatment was discontinued 48 h before each study, and NPH or Lente insulin was discontinued 24 h before each study. During those 48 h, patients were monitored frequently to avoid fasting hyperglycemia (>16 mmol/l) and were treated with soluble insulin if required.

Study Design

Patients with type 2 diabetes were studied twice in the Mayo Clinic GCRC, once after overnight hyperglycemia (SAL) to reproduce the typical metabolic environment observed by the β-cell in type 2 diabetes (chronically increased demand for insulin secretion), and once after comparable overnight inhibition of insulin secretion with an intravenous infusion of somatostatin (SMS). Control subjects were also studied twice after an overnight stay in the GCRC, either at normal fasting blood glucose concentrations (4-SAL) or after the plasma glucose concentration was raised to 8 mmol/l (8-SAL).

Study Protocol

Diabetic patients. All 11 patients with type 2 diabetes were studied twice, after either an overnight SAL or an overnight SMS. On each occasion, the study protocol can be conveniently divided into the overnight period (2000–0700), when subjects were prepared for study (SAL vs. SMS), and the study period per se (0700–0832), when the dynamics of insulin secretion were measured. On both occasions, volunteers were admitted to the GCRC at 1500, fed a standard meal at 1600, and had two forearm peripheral intravenous catheters placed before the initiation of the protocol at 2000. During the overnight period of the study (2000 to 0700), blood was sampled for measurement of the plasma glucose concentration at 30-min intervals, and the plasma glucose concentration was clamped at 8 mmol/l on both occasions. During SAL, the plasma glucose concentration was maintained via glucose infusion (50% dextrose, 0.9% normal saline at 30 ml/h). During the overnight SMS, somatostatin was infused at 60 ng·kg⁻¹·min⁻¹ from 2000 to 0700 along with replacement infusions of growth hormone (3 ng·kg⁻¹·min⁻¹), glucagon (0.65 ng·kg⁻¹·min⁻¹), and insulin given at a variable rate (mean 0.19 mU·kg⁻¹·min⁻¹, range 0.11 to 0.38 mU·kg⁻¹·min⁻¹) to maintain the plasma glucose concentration at 8 mmol/l. At 0700, the saline or somatostatin (and hormone replacement) infusions begun at 8 mmol/l by variable rate were discontinued, and the study period (0700–0832) was begun. On both occasions, blood was sampled from an arterialized dorsal hand vein at 5-min intervals from 0700 to 0832 for measurement of the plasma glucose concentration. The plasma glucose concentration was clamped at 8 mmol/l by variable exogenous glucose infusion (50% dextrose). To assess the pulsatility and orderliness of insulin secretion, blood was sampled at 1-min intervals from 0700 to 0800 from a forearm vein into chilled EDTA tubes that were immediately centrifuged, and the plasma was stored at –20°C for subsequent assay for insulin, C-peptide, and proinsulin. At 0820, a 1-mg bolus of glucagon was given intravenously to elicit first-phase insulin secretion. After the intravenous glucagon bolus, blood was sampled at 1-min intervals for 12 min to allow measurement of first-phase insulin secretion. The studies were concluded at 0832.

Control subjects. Normal volunteers were also studied twice after overnight admission to the GCRC. For control
the intensive sampling period (parameters for obese controls and subjects with type 2 diabetes as modified by Van Cauter et al. (39), which validated these index, and sex, according to the method of Eaton et al. (4) and calculations with standard parameters adjusted for age, body mass calculated by deconvolution of plasma C-peptide concentrations. The plasma proinsulin concentration was measured by a two-site immunospecific ELISA, as previously described (Dako Diagnostics, Cambridgeshire, UK) (27). Proinsulin was measured for a commercial available kit, as previously described (27, 38). Asymmetry highly correlated joint variance spaces were calculated for each parameter by the Monte Carlo support plane procedure (40). Secretory rates are expressed as mass units of insulin (pmol) released per unit of distribution volume (liter) per unit of time (min). The mass of hormone secreted per burst (time integral of the calculated secretory burst) is thus computed as picomoles of insulin released per liter of volume of distribution. The percentage of insulin delivered into the circulation in discrete insulin bursts was calculated as previously described (27, 38).

Orderliness of insulin secretion. The orderliness of insulin secretion was calculated by a scale- and model-independent statistic termed approximate entropy (ApEn; Ref. 22). We have previously described in detail the use of this tool to determine the orderliness of insulin secretion (32). ApEn assigns a single nonnegative number to a time series in which larger values correspond to greater apparent process randomness, and smaller values, conversely, correspond to more instances of recognizable patterns or consistent features in the data. Briefly, ApEn measures the logarithmic likelihood that runs of patterns that are similar (within a certain threshold, which is here taken as 20% of each series SD, r) for m consecutive observations (plasma insulin concentrations) will remain similar on the next incremental comparisons.

First-phase insulin secretion. First-phase insulin secretion in response to glucagon injection was quantified by calculating the area under the curve of the plasma insulin concentration profile during the 12 min after glucagon injection.

Proinsulin-to-insulin ratio. The plasma proinsulin-to-insulin molar ratio was calculated by dividing the concentration of proinsulin (pmol/l) by insulin (pmol/l) obtained during the end of the 60-min intensive sampling period in each patient. To examine the trend of this with time, the corresponding values were also calculated at the beginning (t = 0) and middle (t = 30) of the intensive sampling period.

Statistical Analysis

Data are presented as means ± SE. Statistical comparison between groups was made with the Student’s two-tailed t-test (nonpaired between diabetic or control groups and paired for comparisons within groups). Linear regression analysis was used to determine correlation between parameters within a group. A probability of <5% due to occurrence by chance alone (P < 0.05) was taken as evidence of statistical significance.

RESULTS

Experimental Conditions

In patients with type 2 diabetes, the plasma glucose concentrations were successfully clamped at equiva-
lent hyperglycemia overnight during the SMS and SAL nights (Fig. 1), and this clamp was maintained after discontinuation of these infusions during the intensive sampling period. Suppression of insulin secretion by SMS was confirmed by suppression of the plasma C-peptide concentration during SMS vs. SAL. In control subjects, the plasma glucose concentration remained at \( \sim 5 \) mmol/l during both nights and was then increased to \( \sim 8 \) mmol/l during the intensive sampling period on the SAL visit (Fig. 1).

**Insulin Secretion**

The calculated insulin secretion rates by deconvolution of C-peptide kinetics are shown in Fig. 2. As previously reported, insulin secretion in patients with type 2 diabetes is defective compared with relevantly matched controls (3.03 \( \pm \) 0.35 vs. 5.10 \( \pm \) 0.78 pmol·kg\(^{-1}\)·min\(^{-1}\), SAL vs. 8-SAL, \( P < 0.001 \)), i.e., when measured at comparable glucose concentrations (8 mmol/l in the present study). However, after previous somatostatin treatment, the mean insulin secretion rate in type 2 diabetic patients increased to rates comparable with the rate observed in nondiabetic controls [5.00 \( \pm \) 0.83 vs. 5.10 \( \pm \) 0.78 pmol·kg\(^{-1}\)·min\(^{-1}\), SMS vs. 8-SAL, \( P = \) not significant (NS)].

**Pulsatile Insulin Secretion**

The calculated proportion of posthepatic insulin delivery derived from discrete insulin bursts was \( \sim 40\% \) in both diabetic and nondiabetic subjects. This lower percentage than the \( \sim 90\% \) observed with prehepatic sampling (32) presumably reflects hepatic extraction of the insulin pulses delivered into the portal circulation (31). Inspection of the 1-min plasma insulin concentration profile in control subjects and patients with type 2 diabetes revealed high-frequency oscillations (Figs. 3 and 4). These oscillations were markedly enhanced after discontinuation of overnight inhibition of insulin secretion with SMS. Formal pulse detection and quantification by deconvolution revealed that the frequency of pulses was comparable in patients with type 2 diabetes after either overnight SAL or SMS treatment (interburst interval 5.9 \( \pm \) 0.4 vs. 4.7 \( \pm \) 0.3 min, SAL vs. SMS, \( P = \) NS). This frequency was also comparable with that observed in control subjects studied at euglycemia (7.3 \( \pm \) 1.5 min, 4-SAL) or hyperglycemia (6.6 \( \pm \) 1.1 min, 8-SAL). In contrast, there were major differences in the pulse mass (and parallel changes in pulse amplitude) between groups. Thus the pulse mass (Fig. 5) was similar in controls studied during euglycemia to that in patients with type 2 diabetes after overnight inhibition of insulin secretion with SMS.
diabetes studied at hyperglycemia (27.4 ± 5.8 vs. 18.8 ± 3.0 pmol/l, SAL vs. 4-SAL, $P = \text{NS}$). In contrast, when controls were studied at comparable plasma glucose concentrations (8 mmol/l) to those observed in patients with type 2 diabetes, the defect in insulin pulse mass was evident (27.4 ± 5.8 vs. 67.6 ± 15.5 pmol/l, SAL vs. 8-SAL, $P < 0.02$). However, after overnight SMS treatment, this defect in pulse mass was overcome (77.8 ± 19.3 vs. 67.6 ± 15.5 pmol/l, SMS vs. 8-SAL). Both the increase in pulse mass (increment in pulse mass in each patient) ($r = 0.8, P < 0.01$) and the increase in total insulin secretion rate ($r = 0.9, P < 0.01$) after overnight SMS infusion (Fig. 6) was strongly related to the basal C-peptide concentration (Fig. 6). The increment in pulse mass and total insulin secretion was also positively correlated with the basal insulin secretion rate ($P < 0.01$). This observation suggests that the extent of the increment in insulin pulse mass after $\beta$-cell rest depends on the residual $\beta$-cell mass present in any particular patient. Inspection of Fig. 5 reveals that, within the group of patients studied, patients whose diabetes could be controlled with diet in general showed the greatest basal C-peptide concentration and had the greatest capacity to enhance insulin secretion as well as increase insulin pulse mass after SMS treatment, whereas patients who required insulin treatment had the lowest basal C-peptide concentrations and lowest increment in insulin secretion and pulse mass. Patients on sulfonylurea treatment had intermediate responses. First-phase insulin response after intravenous glucagon (Fig. 7) was also enhanced after SMS treatment (3.1 ± 0.9 vs. 4.6 ± 1.4 nmol·l$^{-1}$·min$^{-1}$, SMS vs. SAL, $P < 0.001$).
Orderliness of Insulin Secretion

Under the conditions studied, the orderliness of insulin secretion measured by ApEn was not significantly higher in patients with type 2 diabetes compared with control subjects (Fig. 8). However, the orderliness of insulin secretion increased markedly in patients with type 2 diabetes after SMS treatment ($1.34 \pm 0.05$ vs. $1.09 \pm 0.05$, SAL vs. SMS, $P < 0.05$). Acute hyperglycemia in control subjects did not cause an increase in ApEn.

Proinsulin-to-Insulin Ratio

The proinsulin-to-insulin molar ratio was increased in patients with type 2 diabetes, as previously reported ($0.61 \pm 0.2$ vs. $0.29 \pm 0.04$, SAL vs. 4-SAL, $P < 0.05$). However, after overnight inhibition of insulin secretion, the proinsulin-to-insulin ratio fell markedly to normal in patients with type 2 diabetes ($0.61 \pm 0.2$ vs. $0.14 \pm 0.04$, SAL vs. SMS, $P < 0.01$). Acute hyperglycemia did not affect the molar proinsulin-to-insulin ratio in nondiabetic controls ($0.29 \pm 0.04$ vs. $0.24 \pm 0.01$, 4-SAL vs. 8-SAL, $P = \text{NS}$). Inspection of Fig. 9 reveals that the proinsulin-to-insulin ratio was stable for 60 min of observation after discontinuation of somatostatin.

DISCUSSION

The present study affirms the hypothesis that the pattern of multiply dysregulated insulin secretion typical in patients with type 2 diabetes is, at least temporarily, reversible by short-term $\beta$-cell rest achieved via an overnight somatostatin infusion. The results of this clinical experiment thus imply that $\beta$-cell rest is a logical therapeutic strategy to restore the pattern of insulin secretion in patients with type 2 diabetes.

In the present studies, we applied validated deconvolution techniques (4, 25, 26, 39) to identify and quantify total and pulsatile insulin secretion and approximate entropy to estimate the orderliness of the insulin release process (32, 22) in patients with type 2 diabetes and in control subjects, who were matched for body mass index with patients with diabetes and were therefore obese. The insulin secretion dynamics in the
controls are therefore those of obese insulin-resistant subjects. In contrast to earlier comparisons of pulsatile insulin secretion in patients with type 2 diabetes and controls (14, 21), we studied patients with type 2 diabetes and control subjects at comparable plasma glucose concentrations. Also, we used a novel, highly sensitive insulin assay that has minimal cross-reactivity with insulin split products or proinsulin (2). Under these experimental conditions, in contrast to previous reports (14), we observe that the frequency of insulin secretory pulses in humans with type 2 diabetes is no different from that in control subjects (interpulse interval ~6 min). The frequency reported here is comparable with that observed by direct sampling of the portal vein in dogs (25, 26) and humans (35) by previously available insulin radioimmunoassays but greater than that observed in the systemic circulation with those assays (14, 21, 25). This pulse frequency is also comparable with that reported in the isolated perfused pancreas (7) and perifused islet (3, 20). The normal insulin pulse frequency in patients with type 2 diabetes reported here implies that the pacemaker intensity driving pulsatile insulin secretion and the system for coordination of insulin secretory bursts among islets are both intact in these diabetic patients. In contrast, we were able to record a markedly decreased amount of insulin secreted with each burst (burst mass) in patients with type 2 diabetes studied at blood glucose concentrations comparable with those in controls. These data allow the speculation that the mechanism underlying defective insulin secretion in patients with type 2 diabetes is insufficiently released insulin by β-cells in response to each ~6-min (synchronous) wave of depolarization through the endocrine pancreas. The observation that this defect can be resolved by a period of overnight somatostatin-induced inhibition of insulin secretion supports the concept that defective insulin secretion in type 2 diabetes may be partly caused by depletion of the immediately secretable insulin pool in β-cells of these patients.

We also report that orderliness of insulin secretion is enhanced in response to previous overnight inhibition of insulin secretion by somatostatin. However, in contrast to conditions where the plasma glucose concentration in patients with type 2 diabetes is allowed to fluctuate liberally during the night before study (31), under the conditions of the present study, where the glucose concentration was fixed overnight (and during intensive sampling) at 8 mmol/l, the calculated approximate entropy was no higher in patients with diabetes than it was in control subjects. Therefore, one explanation for the increased disorderliness of insulin concentration behavior in patients with type 2 diabetes could be the greater disorderliness of the plasma glucose concentration.

Although it is well established that the molar ratio of proinsulin to insulin concentrations in the systemic circulation is increased in patients with type 2 diabetes (30, 36, 43), the cause of this anomaly is controversial. On the one hand, this may represent a primary defect in the proinsulin-to-insulin conversion present in patients with type 2 diabetes (28); alternatively, it may reflect release of immature insulin granules as a consequence of chronically increased β-cell demand (1, 16). The current study supports the latter hypothesis, because the proinsulin-to-insulin molar ratio measured by two highly specific sandwich assays decreased to normal in patients with type 2 diabetes after overnight β-cell inhibition. Such data are also consistent with the observation that a decreased β-cell mass in humans with a partial pancreatectomy (33) results in increased circulating levels of proinsulin relative to insulin. Given the longer half-life of proinsulin compared with insulin, one possible theoretical explanation for this apparent restoration of the proinsulin-to-insulin ratio is a non-steady-state artifact. However, the proinsulin-to-insulin ratio remained quite stable during the 60 min of study.

Many of the characteristics of abnormal insulin secretion present in type 2 diabetes are observed in a variety of models of decreased β-cell mass. These features include decreased first-phase insulin secretion (8, 18, 19, 33), reduced amplitude of pulsatile insulin secretion (8), and an elevated molar ratio of the proinsulin/insulin concentration in the systemic circulation (33). In the present study, we report that overnight inhibition of insulin secretion in humans with type 2 diabetes results in restoration of these dynamic abnormalities of insulin secretion. Such experimental observations support the hypothesis that many characteristics of abnormal insulin secretion typical of type 2 diabetes are, at least in part, a consequence of chronically increased demand (hyperglycemia and insulin resistance) on a decreased β-cell mass.

In summary, we report that, in humans with type 2 diabetes, impaired pulsatile insulin secretion is marked by an alteration of insulin pulse mass, which
defect can be overcome by β-cell rest. Furthermore, we report that β-cell rest enhances first-phase insulin secretion, decreases the abnormally elevated proinsulin-to-insulin ratio in plasma, and restores a significantly more orderly time/mode of insulin release. Taken together, these data suggest that, in humans with type 2 diabetes, the immediately secretable insulin pool, consisting of docked vesicles, is depleted and replaced by relatively less mature vesicles with incompletely processed proinsulin. Somatostatin and/or β-cell rest constitutes effective islet cell secretory dynamics, at least in the short term, pointing to key role(s) of relatively deficient somatostatin restraint of β-cell secretory activity and/or attrition of total β-cell mass in this disease.

We gratefully acknowledge the excellent technical skills of Jane Kahl and Karen Laasko, the superb care given by the nursing staff of the Mayo Clinic GCRC, and especially the volunteers who participated in the research studies. The current study was funded by a grant from the US Public Health Service, National Institutes of Health Grants DK-4434 (P. C. Butler) and MO1 R0858 (GCRC Mayo Clinic), the Mayo Foundation (P. C. Butler), the National Science Foundation Science and Technology Center in Biological Timing (University of Virginia), National Institutes of Health Research Career Development Award IK04 HD-00634 (J. Veldhuis), The Wellcome Trust Grant 049853 (P. C. Butler), and the University of Edinburgh.

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