Acute and Short-Term Administration of a Sulfonylurea (Gliclazide) Increases Pulsatile Insulin Secretion in Type 2 Diabetes

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The high-frequency oscillatory pattern of insulin release is disturbed in type 2 diabetes. Although sulfonylurea drugs are widely used for the treatment of this disease, their effect on insulin release patterns is not well established. The aim of the present study was to assess the impact of acute treatment and 5 weeks of sulfonylurea (gliclazide) treatment on insulin secretory dynamics in type 2 diabetic patients. To this end, 10 patients with type 2 diabetes (age 53 ± 2 years, BMI 27.5 ± 1.1 kg/m^2, fasting plasma glucose 9.8 ± 0.8 mmol/l, HbA$_{1c}$ 7.5 ± 0.3%) were studied in a double-blind placebo-controlled prospective crossover design. Patients received 40–80 mg gliclazide/placebo twice daily for 5 weeks with a 6-week washout period intervening. Insulin pulsatility was assessed by 1-min interval blood sampling for 75 min *1*) under baseline conditions (baseline), *2*) 3 h after the first dose (80 mg) of gliclazide (acute) with the plasma glucose concentration clamped at the baseline value, *3*) after 5 weeks of treatment (5 weeks), and *4*) after 5 weeks of treatment with the plasma glucose concentration clamped during the sampling at the value of the baseline assessment (5 weeks-elevated). Serum insulin concentration time series were analyzed by deconvolution, approximate entropy (ApEn), and spectral and autocorrelation methods to quantitate pulsatility and regularity. The *P* values given are gliclazide versus placebo; results are means ± SE. Fasting plasma glucose was reduced after gliclazide treatment (baseline vs. 5 weeks: gliclazide, 10.0 ± 0.9 vs. 7.8 ± 0.6 mmol/l; placebo, 10.0 ± 0.8 vs. 11.0 ± 0.9 mmol/l, P = 0.001). Insulin secretory burst mass was increased (baseline vs. acute: gliclazide, 43.0 ± 12.0 vs. 61.0 ± 17.0 pmol · l^{-1} · pulse^{-1}; placebo, 36.1 ± 8.4 vs. 30.3 ± 7.4 pmol · l^{-1} · pulse^{-1}, P = 0.047; 5 weeks-elevated: gliclazide vs. placebo, 49.7 ± 13.3 vs. 37.1 ± 9.5 pmol · l^{-1} · pulse^{-1}, $P < 0.05$) with a similar rise in burst amplitude. Basal (i.e., nonoscillatory) insulin secretion also increased (baseline vs. acute: gliclazide, 8.5 ± 2.2 vs. 16.7 ± 4.3 pmol · l^{-1} · pulse^{-1}; placebo, 5.9 ± 0.9 vs. 7.2 ± 0.9 pmol · l^{-1} · pulse^{-1}, P = 0.03; 5 weeks-elevated: gliclazide vs. placebo, 12.2 ± 2.5 vs. 9.4 ± 2.1 pmol · l^{-1} · pulse^{-1}, P = 0.016). The frequency and regularity of insulin pulses were not modified significantly by the antidiabetic therapy. There was, however, a correlation between individual values for the acute improvement of regularity, as measured by ApEn, and the decrease in fasting plasma glucose during short-term (5-week) gliclazide treatment (r = 0.74, P = 0.014, and r = 0.77, P = 0.009, for fine and coarse ApEn, respectively). In conclusion, the sulfonylurea agent gliclazide augments insulin secretion by concurrently increasing pulse mass and basal insulin secretion without changing secretory burst frequency or regularity. The data suggest a possible relationship between the improvement in short-term glycemic control and the acute improvement of regularity of the in vivo insulin release process. ***Diabetes*** **50:1778–1784, 2001**

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ApEn, approximate entropy; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GLP, glucagon-like peptide; OHA, oral hypoglycemic agent.

In healthy individuals, insulin release is characterized by a coordinated pattern of pulsatile secretion with an interval of 5–15 min, leading to oscillations in peripheral plasma insulin concentrations (1,2). The possible importance of the pulsatile mode of insulin delivery on hypoglycemic action, hepatic glucose production, and lipid metabolism has been verified by both in vivo and in vitro analyses (3–6). Abnormalities in pulsatile insulin secretion have been demonstrated not only in patients with established type 2 diabetes but also in their glucose-tolerant relatives and may be a part of the pathophysiological mechanisms leading to the development of overt type 2 diabetes (7–10).

Substrates (glucose), hormones (glucagon-like peptide [GLP]-1, somatostatin, and IGF-1), and antidiabetic compounds that regulate insulin secretion seem to exert their actions primarily via modulation of insulin secretory burst mass rather than frequency (11–19). Despite the widespread clinical use of insulin secretagogues in the treatment of type 2 diabetes, the influence of these antidiabetic drugs on the oscillatory pattern of insulin release has been elucidated only sparingly in diabetic humans (12).

It seems reasonable to assume that restoration of the physiological release pattern of insulin, in addition to attainment of relative insulin sufficiency, is of importance for the insulin actions in peripheral tissues as well as for acute and long-term β-cell performance. Thus, our hypothesis is that treatment with insulin secretagogues is likely to

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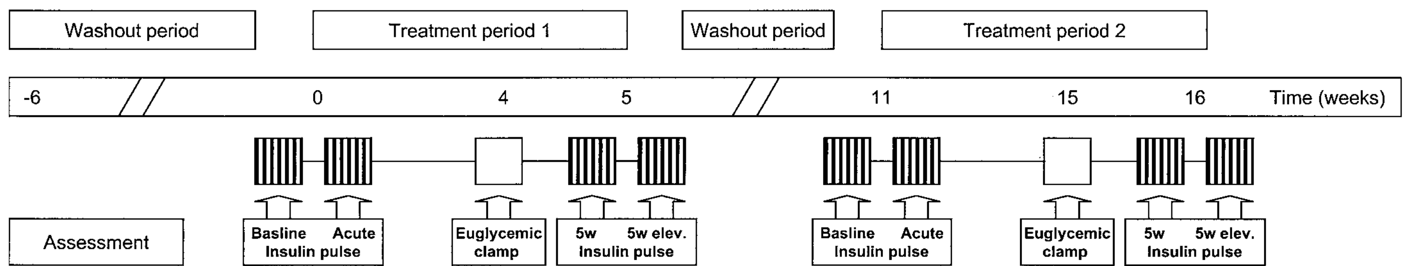

FIG. 1. Flow sheet. The effects of gliclazide on pulsatile insulin secretion were assessed in a double-blind placebo-controlled crossover design including washout periods before and between the intervention periods. Insulin pulsatility was assessed in the beginning and at the end of each intervention period. A hyperinsulinemic-euglycemic clamp was performed 1 week before the end of each intervention period. 5w, 5 weeks; 5w elev., 5 weeks-elevated.

influence the insulin delivery pattern qualitatively as well as quantitatively.

The present study is the first to explore the acute and short-term actions of an insulin secretagogue on insulin pulsatility in type 2 diabetic patients. To this end, we assessed the effect of gliclazide, a highly selective sulfonylurea compound for the β-cell–type K_{ATP} channel (20), on the high-frequency pulsatile insulin release patterns, as quantified by time series analysis of frequently sampled plasma insulin concentration time series. Therefore, we hoped to shed light on the potential restorative action of this therapeutic intervention on the mechanisms that drive physiologically oscillatory insulin production in humans.

RESEARCH DESIGN AND METHODS

The protocol was performed in accordance with the Helsinki Declaration and was approved by the local ethical committees of Aarhus and Vejle Counties.

Study subjects. Ten patients with type 2 diabetes according to the criteria of the World Health Organization were studied (age 53 ± 2 years, BMI 27.5 ± 1.1 kg/m^2, fasting plasma glucose 9.8 ± 0.8 mmol/l, HbA_{1c} 7.5 ± 0.3%). The median duration of diabetes was 1.5 years (ranging from 2 months to 8 years). None exhibited albuminuria, and one patient had early diabetic retinopathy. Before screening, patients were treated with an oral hypoglycemic agent (OHA) at less than one-half the maximal dose (sulfonylurea, n = 5; metformin, n = 2) or with diet alone (n = 3). In addition, six of the patients were treated for essential hypertension (ACE inhibitors, n = 5; thiazide diuretics, n = 1), and four patients were treated for hypercholesterolemia with 3-hydroxy-3-methylglutaryl–CoA reductase inhibitors.

Protocol. The design of the study is outlined in Fig. 1. The study was performed in a double-blind placebo-controlled randomized crossover design. Before inclusion, OHA-treated patients were withdrawn from antidiabetic treatment for a 6-week period. During the intervention, patients previously treated with diet alone received 40 mg gliclazide or placebo b.i.d. for the first week and thereafter received 80 mg b.i.d. Patients previously treated with OHA received 80 mg gliclazide or placebo b.i.d. throughout the intervention period. The last dose of gliclazide/placebo was taken 12–14 h before the assessment of insulin pulsatility or insulin sensitivity.

For each study day, the patients arrived at the research unit at 8:00 A.M., and intravenous catheters were placed in antecubital veins for infusion and sampling purposes. Patients were kept fasting during the assessment of insulin pulsatility. At the start of each treatment period, the impact of acute treatment with gliclazide was studied. After 30 min of rest (t = 0), blood was collected every minute for 75 min for later insulin measurements (baseline period). At 75 min, a single dose of gliclazide (80 mg) or placebo was administrated orally with a glass of water. Over the next 180 min, plasma glucose was measured every 5–15 min and clamped at the initial level by variable glucose infusion. The glucose infusion rate was modestly increased when necessary (and never decreased) throughout the study period. From 255 to 330 min, blood was sampled again every minute for insulin measurements (acute period). Details on the sampling procedure have been described previously (17).

After 5 weeks of treatment, pulsatile insulin secretion was again assessed. Similar to the day of acute assessment, patients were initially assessed, with no intervention at their fasting plasma glucose level, with blood sampling for 75 min (5 weeks). Thereafter, plasma glucose was clamped at the level of the first day of treatment by glucose infusion, and after an equilibrium period of 60–180 min, blood was collected again for 75 min (5 weeks-elevated). During all sampling periods, serum C-peptide, free fatty acids (FFAs), and plasma glucagon were measured in blood withdrawn every 15 min.

At week 4 in each treatment period, insulin sensitivity was assessed by a 180-min hyperinsulinemic-euglycemic clamp using an insulin infusion rate (Insulin Actrapid; Novo Nordisk, Bagsvaerd, Denmark) of 1.2 mU · kg^{-1} · min^{-1}. Plasma glucose was allowed to decline slowly to 5.5 mmol/l and was then clamped at this level. The mean glucose infusion rate was calculated during the so-called steady state period of 150–180 min.

Assays. All biochemical analyses were performed in duplicate. Plasma glucose was measured on a Beckman glucose analyzer (Beckman, Palo Alto, CA) using the glucose oxidation technique. All other blood samples were stored at −20°C and analyzed within a month. Serum insulin concentration was measured using a two-site immunospecific insulin enzyme-linked immunosorbent assay (ELISA). The intra- and interassay coefficients of variation were 3 and 5%, respectively. The antibodies cross-react at 30 and 63% with split-65,66 and des-64,65 proinsulin, respectively, whereas there is no cross-reactivity with pro-insulin, split-32,33 proinsulin, des-31,32 proinsulin, C-peptide, IGF-1, IGF-2, and glucagon. Samples for glucagon measurement were collected in tubes with aprotinin/EDTA solution in an ice bath and frozen immediately. Measurements were performed by radioimmunoassay (21). FFA was measured by a calorimetric method (Wako, Neuss, Germany). C-peptide was measured by a two-site monoclonal-based ELISA (K6218; Dako Diagnostics, Cambridgeshire, U.K.). This assay has an intra- and interassay coefficient of variation (in triplicate) of 2 and 3%, respectively.

Data analysis

Deconvolution analysis. Serum insulin concentration time series were analyzed in a blinded manner by deconvolution analysis to quantitate basal secretion, interpulse interval, secretory burst mass, and burst amplitude (22). Deconvolution was performed with a previously validated iterative multiparameter technique using the following assumptions: *1*) the hormone is secreted in a finite number of bursts with *2*) an individual amplitude, *3*) a common half-duration superimposed on a basal time-invariant secretory rate, and *4*) a bi-exponential disappearance rate, as previously described (23). The half-lives of insulin were set to be 2.8 and 5 min, with a fractional slow compartment of 28%. The fraction of insulin released in a pulsatile fashion was calculated as follows:

$$\frac{\text{Secretory burst mass/interpulse interval}}{\text{Secretory burst mass/interpulse interval} + \text{basal secretion}}$$

Detrending. To eliminate effects of non-stationarity in the data, approximate entropy (ApEn), spectral analysis, and autocorrelation analysis were performed on the residuals after subtraction of a seven-point moving average process (18,19,24).

ApEn. Regularity of insulin concentration time series was assessed by the model-independent and scale-invariant statistic ApEn (25). ApEn measures the logarithmic likelihood that runs in patterns that are close (within r) for m contiguous observations and remain close (within the tolerance width r) on subsequent incremental comparisons. A precise mathematical definition is given by Pincus (25). ApEn is a family of parameters that depends on the choice of the input parameters m and r and should be compared only when applied to time series of equal length, as done here. By application of a small r value (e.g., r = 0.2 × SD), ApEn evaluates fine (sub)patterns in the time series, whereas a larger r value (e.g., r = 1.0 × SD) is applied to evaluate more coarse patterns (26). A larger absolute value of ApEn indicates a higher degree of process randomness. ApEn is rather stable to noise that lies within the tolerance width r. To evaluate both the fine and the more coarse patterns in the time series, ApEn was calculated using r = 0.2 × SD and r = 1.0 × SD.

Spectral analysis. Spectral analysis was performed using noncommercial

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software. A Tukey window of 25 data points was used, and spectra were normalized assuming that the total variance in each time series was 100%. This enables comparison of spectral estimates, despite the different absolute values of insulin. The amplitudes of the dominant peak of the spectrum during placebo and gliclazide treatment were compared statistically.

Autocorrelation analysis. Autocorrelation analysis was performed without prior smoothing to analyze high frequency oscillations. The correlation coefficients of the first nonnegative peak of the autocorrelogram were compared statistically (24). Autocorrelation analysis was performed using SPSS version 9.0.

Autocorrelation analysis and spectral analysis were also performed on the derived secretion time series data as obtained by deconvolution analysis. The method used was similar as for the analyses of concentration data, but a truncation length of 150 data points was used for the spectral analyses because of the higher amount of estimated data points (300 data points).

Regression analysis. To assess the correlation between the induced changes in regularity and the changes in metabolic parameters, we assessed Pearson's parametric correlations between, on the one hand, *1*) the quantitative effect on the β-cells as measured by delta mean insulin concentrations, *2*) the change in insulin sensitivity as measured by Δ glucose infusion rate per mean insulin, and *3*) the effect on glucose control as measured by Δ fasting plasma glucose and, on the other hand, the change in regularity as measured by *1*) Δ ApEn ($m = 1, r = 0.2 \times$ SD, and $m = 1, r = 1.0 \times$ SD), *2*) Δ normalized spectral power, and *3*) Δ autocorrelation coefficient. Correlation analyses were performed on the data obtained in the acute and 5 weeks periods.

Statistical analysis. Statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC). Before statistical analysis, the validity of the data were verified by analyzing differences in baseline values, carryover effect, period effect, and sequence effect. None of these were found to be statistically significant. The efficacy of gliclazide treatment was assessed by analysis of variance, taking into account patient, period, sequence, and treatment factors. For the analysis of the acute effect, the baseline value is taken into account. *P* values refer to differences in gliclazide versus placebo treatment. The statistical significance level was 5%.

RESULTS

Circulating concentrations. Fasting plasma glucose concentrations decreased by 20% after 5 weeks of gliclazide treatment (gliclazide, 10.0 ± 0.9 vs. 7.8 ± 0.6 mmol/l; placebo, 10.0 ± 0.8 vs. 11.0 ± 0.9 mmol/l, $P = 0.001$) with a concomitant decrease in glycosylated hemoglobin (gliclazide, 7.8 ± 0.3 vs. 7.1 ± 0.3%; placebo, 7.5 ± 0.2 vs. 7.9 ± 0.3%, $P < 0.001$). Mean concentrations of glucose, insulin, C-peptide, glucagon, and FFA obtained during the pulsatility study are shown in Fig. 2. In the acute and 5 weeks-elevated sessions, plasma glucose levels were comparable in the gliclazide and placebo treatment period because of titrated glucose infusion. Serum insulin was significantly elevated after a single dose of gliclazide (baseline vs. acute: gliclazide, 84 ± 24 vs. 142 ± 40 pmol/l; placebo, 60 ± 11 vs. 64 ± 12 pmol/l, $P = 0.038$) and during the 5 weeks-elevated study session (gliclazide vs. placebo: 110 ± 25 vs. 83 ± 20 pmol/l, $P = 0.006$). The circulating insulin concentration at the 5 weeks period was comparable to baseline, probably reflecting the glucose-dependent action of gliclazide. Circulating C-peptide values followed those of serum insulin. Plasma glucagon was significantly decreased during the 5 weeks-elevated session (gliclazide vs. placebo: 57 ± 8 vs. 79 ± 10 pmol/l, $P = 0.011$). Serum FFA was suppressed at the acute assessment (44% reduction compared with placebo, $P = 0.006$) and at the 5 weeks-elevated session (29% reduction, $P = 0.006$) (Fig. 2).

Insulin secretion. High-frequency oscillations of insulin secretion were quantified by deconvolution analysis of insulin concentration time series. Primary data are listed in Table 1. Pulsatile insulin secretion, as measured by insulin secretory burst mass, increased by ~40% after acute stimulation with gliclazide (baseline vs. acute: gliclazide, 43.0 ±

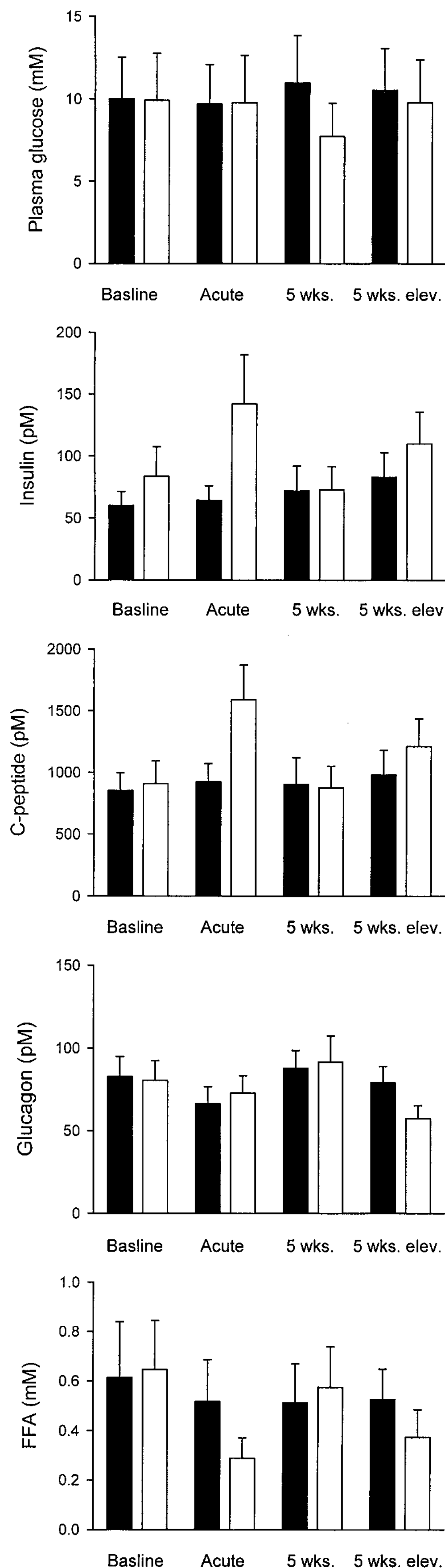

FIG. 2. Mean ± SE values of plasma glucose, serum insulin, C-peptide, plasma glucagon, and FFA in each of the four time series sessions (□, gliclazide; ■, placebo). 5 wks. elev., 5 weeks-elevated.

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TABLE 1
Insulin secretion characteristics based on deconvolution analysis and regularity analysis during basal conditions, acute stimulation, and 5-week treatment with placebo and gliclazide

	Placebo				Gliclazide			
	Basal	Acute	5 Weeks	5 Weeks-elevated	Basal	Acute	5 Weeks	5 Weeks-elevated
Deconvolution analysis								
Secretory burst mass (pmol · l^{-1} · $pulse^{-1}$)	36.1 ± 8.4	30.3 ± 7.4	42.4 ± 12.8	37.1 ± 9.5	43.0 ± 12.0	61.0 ± 17.0*	34.8 ± 9.7	49.7 ± 13.3*
Secretory burst amplitude (pmol · l^{-1} · min^{-1})	14.4 ± 3.3	12.1 ± 2.9	16.8 ± 5.0	14.8 ± 3.8	17.1 ± 4.8	24.3 ± 6.8*	13.9 ± 3.9	19.8 ± 5.3*
Basal secretion (pmol · l^{-1} · min^{-1})	5.9 ± 0.9	7.2 ± 1.1	6.8 ± 1.5	9.4 ± 2.1	8.5 ± 2.2	16.7 ± 4.3*	8.8 ± 2.1*	12.2 ± 2.5*
Interpulse interval (min/pulse)	6.7 ± 0.5	6.9 ± 0.7	7.1 ± 1.2	6.4 ± 0.5	5.9 ± 0.5	5.9 ± 0.4	6.5 ± 0.4	5.2 ± 0.4*
Regularity analysis								
Autocorrelation coefficient	0.16 ± 0.02	0.15 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.25 ± 0.04	0.22 ± 0.03	0.18 ± 0.03	0.21 ± 0.03
Spectral power	8.5 ± 0.7	7.5 ± 0.9	10.2 ± 0.9	9.4 ± 0.9	9.2 ± 1.0	9.6 ± 1.1	7.6 ± 0.8*	9.0 ± 0.9
Frequency (min/pulse)	6.9 ± 0.5	5.7 ± 0.4	6.7 ± 0.4	6.7 ± 0.4	7.0 ± 0.5	6.7 ± 0.3	7.1 ± 0.4	6.1 ± 0.5
ApEn (r = 0.2 × SD, m = 1)	1.496 ± 0.033	1.464 ± 0.016	1.505 ± 0.028	1.506 ± 0.023	1.482 ± 0.029	1.514 ± 0.027	1.483 ± 0.018	1.524 ± 0.019
ApEn (r = 1.0 × SD, m = 1)	0.642 ± 0.026	0.638 ± 0.027	0.662 ± 0.023	0.664 ± 0.019	0.639 ± 0.043	0.662 ± 0.025	0.631 ± 0.026	0.663 ± 0.027

Data are means ± SE. *$P < 0.05$, testing for treatment effect (gliclazide vs. placebo) in the crossover model.

12.0 vs. 61.0 ± 17.0 pmol · l^{-1} · $pulse^{-1}$; placebo, 36.1 ± 8.4 vs. 30.3 ± 7.4 pmol · l^{-1} · $pulse^{-1}$, $P < 0.047$), with a similar increase in burst amplitude. Likewise, basal insulin secretion increased significantly (baseline vs. acute: gliclazide, 8.5 ± 2.2 vs. 16.7 ± 4.3 pmol · l^{-1} · min^{-1}; placebo, 5.9 ± 0.9 vs. 7.2 ± 0.9 pmol · l^{-1} · min^{-1}, $P = 0.03$), whereas the interpulse interval was unaltered. After 5 weeks of treatment, the secretory burst mass was not different from baseline. Basal insulin secretion was increased by ~40% after gliclazide treatment ($P < 0.047$). When plasma glucose was elevated to the baseline level (5 weeks-elevated), both pulsatile and basal insulin secretion were significantly elevated during gliclazide treatment. The interpulse interval, as assessed by deconvolution analysis, was decreased after 5 weeks of treatment at elevated glucose ($P = 0.024$), but this could not be confirmed by spectral analysis. A representative example of insulin concentration time series and estimated insulin secretory rates during gliclazide treatment is shown in Fig. 3. Increased pulse amplitude and basal insulin secretion are evident in the acute (Fig. 3*F*) and 5 weeks-elevated (Fig. 3*H*) sessions. The fraction of insulin delivered in pulses was ~50% at baseline and was unaffected by acute and short-term gliclazide treatment.

Insulin sensitivity. Insulin sensitivity was assessed by the hyperinsulinemic-euglycemic clamp technique after 4 weeks of treatment. The glucose infusion rate tended to be higher during gliclazide treatment, though barely statistically significant (5.1 ± 0.8 vs. 4.2 ± 1.0 mg · kg^{-1} · min^{-1}, $P = 0.14$).

Regularity analysis. Regularity of insulin concentration time series was evaluated by complementary approaches. Regularity was unaltered by acute gliclazide administration. After 5 weeks of treatment, spectral power fell after gliclazide treatment ($P < 0.047$). However, this change was not verified by the two other regularity analyses. When glucose was subsequently elevated to the baseline level, there was still no difference in the regularity parameters between gliclazide and placebo treatment. Regularity analyses applied on the time series of derived insulin secretion data confirmed that the regularity of the release process was unaltered by gliclazide treatment (all $P > 0.05$) (Table 1).

Linear regression analysis showed that acute improvement in regularity on gliclazide treatment (i.e., decreased ApEn) correlated positively ($r = 0.74$ and 0.77 and $P = 0.014$ and 0.009 for fine and coarse ApEn, respectively) with the delayed (5 weeks) improvement in fasting plasma glucose (Fig. 4). Autocorrelation function and spectral power also tended to intimate correlations between changes in regularity and glycemic control, though the results were insignificant ($r = -0.466$ and -0.478 and $P = 0.18$ and 0.16, respectively). These differences likely reflect the high sensitivity of ApEn to relatively short time series. Changes in insulin sensitivity and in serum insulin concentration did not correlate to changes in any of the regularity measurements.

DISCUSSION

Enhancement of insulin secretion achieved by means of insulin secretagogues is a widely used approach in the treatment of type 2 diabetes. In the earlier stages of type 2 diabetes, sulfonylurea drugs (e.g., gliclazide) have been demonstrated to reestablish, at least in part, first-phase

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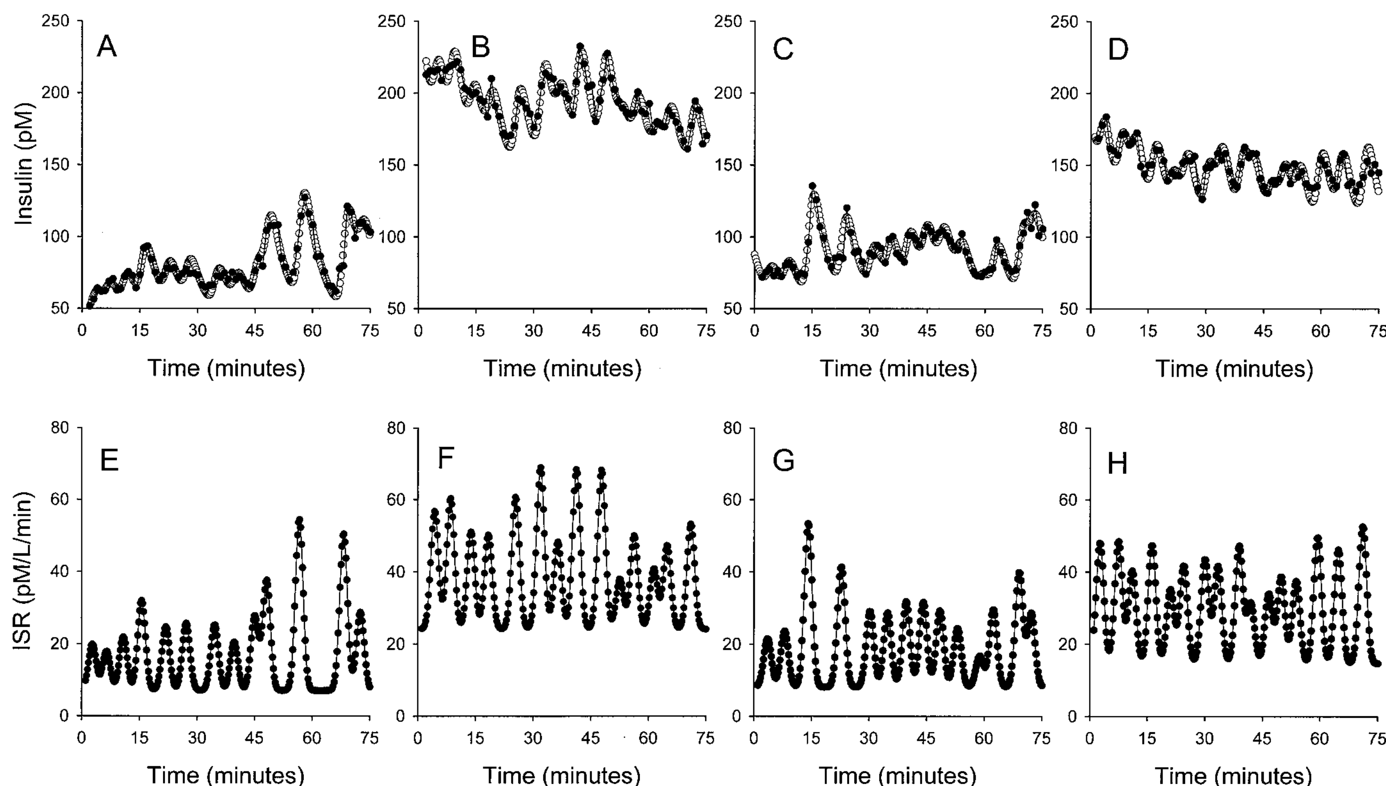

FIG. 3. Representative illustration of deconvolution analysis of serum insulin concentration time series. *A* and *D*: Measured serum insulin concentrations (●—●) together with a best-fit line estimated by bi-exponential deconvolution analysis (○○○). *E* and *H*: Estimated insulin secretory rates. *A* and *E*, baseline periods; *B* and *F*, acute periods; *C* and *G*, 5-week periods; and *D* and *H*, 5-week elevated periods. Increased insulin secretory burst amplitude and elevated basal insulin secretion rates are also shown (*F* and *H*).

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insulin secretion (27,28). This facet of insulin secretion involves a coordinate response to an exogenous glucose stimulus. Sulfonylureas appear to stimulate insulin release in a glucose-dependent manner (29), thus adjusting insulin release to the relative metabolic needs. Finally, gliclazide has been shown to increase the sensitivity of the β-cells, as assessed by the insulin response to glucose stimuli in a hyperglycemic clamp study (27). Because oscillatory insulin release may be maintained in part via a metabolic feedback loop involving oscillations in circulating glucose concentrations, these properties of sulfonylureas may be beneficial in maintaining or improving oscillations in insulin release. Hypothetically, increased regularity of high-frequency insulin release patterns might thus be obtained either by mechanisms that improve glucose control (i.e., reduced glucose and lipid toxicity) or via direct actions of gliclazide on the β-cells.

The present study is the first to explore acute and short-term (5 weeks) actions of an insulin secretagogue on high-frequency insulin oscillations in diabetic patients. The stimulatory actions of gliclazide could arise either via an increase in the pulsatile mode of secretion (by an increase of secretory burst mass or burst frequency) or via elevation of the basal insulin release rate. We found that the enhanced insulin secretion after acute and short-term gliclazide treatment is achieved by joint amplification of the pulsatile and the basal modes of insulin release, with an unchanged fraction of insulin delivered in pulses. There was no consistent effect on the frequency of insulin oscillations. These specific mechanistic findings are in accordance with the previous observation that acute tolbutamide infusion in healthy humans can increase the amplitude of insulin concentration profiles without modifying the high-frequency oscillatory rate (11). Similarly, glyburide enhanced insulin secretion in diabetic patients by augmenting oscillatory amplitude in a 24-h study of ultradian

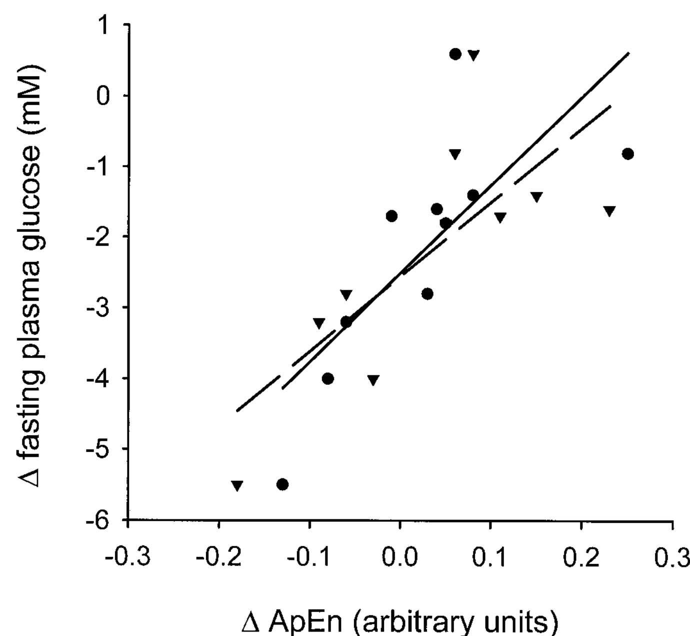

FIG. 4. Relation between the acute change in ApEn and short-term (5-week) change in fasting plasma glucose. ●, Coarse ApEn ($m = 1$, $r = 1.0 \times$ SD), $r = 0.77$, $P = 0.009$; – – –, fine ApEn ($m = 1$, $r = 0.2 \times$ SD), $r = 0.74$, $P = 0.014$.

oscillations (12). In nondiabetic dogs, tolbutamide infusion and ingestion likewise stimulated insulin secretion by dual effects on pulsatile secretion and basal secretion. No significant change in the fraction of pulsatile secretion and no change in pulse frequency were observed (13). In a recent study (19), the natural peptidyl insulin secretagogue GLP-1 promoted insulin secretion in diabetic patients by accentuating both basal and pulsatile insulin secretion after a short-term infusion. Another nonsulfonylurea insulin secretagogue, repaglinide, also exerted its action by amplifying pulse mass and amplitude without affecting pulse frequency in a single-dose study (18). Furthermore, repaglinide was found to decrease orderliness of the insulin release process, as measured by the regularity statistic ApEn, but because this study was carried out on healthy subjects (with normal insulin secretory patterns), results obviously cannot be compared with the current findings in type 2 diabetic patients with preexisting defects in the insulin release pattern.

In the present study, the regularity of the insulin release pattern remained unaffected by gliclazide treatment, as quantified by three complementary mathematical approaches, both in the acute state and after short-term treatment. Short-term actions were evaluated during the fasting glucose level and during clamped hyperglycemia to ensure comparability at the gliclazide and placebo period. However, the 5 weeks-elevated assessment might be influenced by the glucose infusion given, although steady state was achieved before the insulin release regularity was evaluated. Also, changes in glucagon and FFA might influence the results.

As a new approach to the analysis of insulin release pattern, regularity analyses were also performed on the secretion data derived by deconvolution of concentration time series. Results from these analyses confirmed the findings obtained by the analyses of the concentration data. GLP-1 infusion has been found to enhance β-cell sensitivity to glucose entrainment in subjects with impaired glucose tolerance but not in patients with overt diabetes (30). This could indicate that early intervention is mandatory to obtain significant improvement in the insulin pulsatility pattern. Alternatively, longer-term intervention might be required. In fact, in studies using perfused islets, early intervention with a thiazolidinedione compound partially antagonized the deterioration in insulin pulsatility, as assessed by entrainment in prediabetic but not in diabetic ZDF rats (31). However, precisely how entrainment data relate to spontaneous insulin pulsatility, as explored in the present study, is not yet clarified. On the other hand, pulse entrainment studies may be a sensitive tool for assessing that part of the pulse-generating mechanism driven by glucose oscillations (32,33).

Data from previous studies suggest that gliclazide acts primarily on the β-cell and that effects on peripheral insulin sensitivity are either slight or secondary to the improvement of the insulin secretory capacity (34). In vivo studies have shown increased insulin sensitivity during pulsatile insulin infusion compared with constant infusion (5). Likewise, we found only a trend toward increased insulin sensitivity after gliclazide treatment, despite augmented insulin pulsatility. However, the measure of insulin sensitivity was obtained during nonpulsatile hyperinsulinemia, and our data do not contradict these previous observations.

A potentially significant observation in the present study is the correlation between the improvement in ApEn in the acute state (3 h after dosing, at peak gliclazide concentration) and the decline in glycemia after 5 weeks of intervention. This might indicate that the hypoglycemic action of sulfonylureas depends in part on the ability of these drugs to improve physiological pulsatile insulin release. Thus, patients less capable of achieving such an acute response may also show lesser benefit to therapy. This notion is consistent with previous findings that a defect in β-cell coordination precedes the development of overt diabetes (9,10) and might imply that early intervention is preferential to maintain optimal β-cell function. Reduced glucotoxicity after 5 weeks of gliclazide treatment is unlikely to explain the aforementioned correlations because regularity changes were observed only in the acute state under glucose-clamped conditions. It will, however, be important to corroborate this correlation in further studies. The size of the study group is relatively small, and the correlation was only statistically significant for one of the regularity measurements applied (ApEn), although a trend was also seen for the complementary approaches.

In summary, gliclazide treatment amplifies insulin secretory burst mass with no effect on burst frequency or β-cell coordination. A correlation between the acute improvement of pattern regularity and the improvement of short-term (5-week) glycemic control could indicate a link between secretory orderliness and efficacy of gliclazide treatment. The fact that no sustained improvement in coordinate insulin release can be achieved in these diabetic patients, together with previous findings, might imply that early intervention is necessary to prevent the development of this facet of β-cell defect in type 2 diabetes.

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