Effects of Sustained Insulin-Induced Hypoglycemia on Cardiovascular Autonomic Regulation in Type 1 Diabetes

Minna L. Koivikko,[1] Pasi I. Salmela,[1] K.E. Juhani Airaksinen,[2] Juha S. Tapanainen,[3] Aimo Ruokonen,[4] Timo H. Mäkikallio,[1] and Heikki V. Huikuri[1]

Effects of hypoglycemia on cardiac autonomic regulation may contribute to the occurrence of adverse cardiac events. This study assessed the effects of sustained hyperinsulinemic hypoglycemia on cardiovascular autonomic regulation in type 1 diabetic patients and their nondiabetic counterparts. The study consisted of 16 type 1 diabetic patients and 8 age-matched healthy control subjects who underwent euglycemic and hypoglycemic clamp procedures in a random order. Heart rate variability was measured from continuous electrocardiogram recordings by time and frequency domain methods, along with Poincaré plot analysis during both a hyperinsulinemic-euglycemic and hypoglycemic clamp at three different glucose levels (4.5–5.5, 3.0–3.5, and 2.0–2.5 mmol/l). Controlled hypoglycemia resulted in an increase of supine heart rate in both the diabetic patients (from 72 ± 9 to 80 ± 11 bpm, $P < 0.01$) and the control subjects (from 59 ± 5 to 65 ± 5 bpm, $P < 0.05$) and progressive reductions of the high-frequency spectral component and beat-to-beat heart rate variability (SD1; $P < 0.05$ in the diabetic patients and $P < 0.01$ in control subjects). No significant changes in heart rate variability occurred during the euglycemic clamp. We conclude that hypoglycemia results in a reduction of cardiac vagal outflow in both diabetic and nondiabetic subjects. Altered autonomic regulation may contribute to the occurrence of cardiac events during hypoglycemia. *Diabetes* 54:744–750, 2005

During hypoglycemia the release of counterregulatory hormones serves to maintain blood glucose homeostasis. The response of catecholamines is normally not important, but it becomes critical when the response of glucagon is deficient, as in type 1 diabetic patients, who are largely dependent on epinephrine to correct and prevent hypoglycemia (1,2). Many studies have documented impaired secretory responses of epinephrine to plasma glucose decrements in type 1 diabetic patients (1,3). This response impairs gradually over several years after the diagnosis of diabetes. In diabetic patients, the blunted response of counterregulatory hormones may result in a loss of the ability to recognize developing hypoglycemia (1,3,4). Recurrent iatrogenic hypoglycemia also elevates the glycemic thresholds for symptoms and for the activation of the glucose counterregulation system (3–9). It has also been hypothesized that hypoglycemia may play a role in the "dead in bed" syndrome, i.e., the sudden nocturnal death of young individuals with type 1 diabetes (10,11).

Heart rate variability has been increasingly used to assess autonomic function in various physiological and pathological settings, including the assessment of autonomic dysfunction in diabetic patients (12–18). There are some reports on changes in heart rate variability during hypoglycemia among healthy subjects (19–20), but less is known about hypoglycemia-induced changes in cardiac autonomic regulation in diabetic patients. The present study was designed to assess and compare changes in heart rate and heart rate variability during a hyperinsulinemic-hypoglycemic and euglycemic clamp among type 1 diabetic patients and age-matched nondiabetic subjects.

RESEARCH DESIGN AND METHODS

A total of 16 patients (7 men and 9 women), mean age of 32 ± 8 years (range 18–46 years), with type 1 diabetes and 8 healthy subjects (3 men and 5 women), mean age 34 ± 10 years (22–48 years), participated in the study (Table 1). The known duration of diabetes was 13 years (2–29 years), and glycemic control was fairly strict (mean HbA_{1c} 7.0%, 5.8–8.7%). The diabetic patients and healthy subjects were all normal by weight (BMI 23.2 ± 1.7 vs. 24.1 ± 2.3 kg/m^2, respectively). None of the patients or the healthy control subjects had a previous history of cardiovascular disease or clinical evidence of heart disease, and all had normal electrocardiograms. There were no signs of diabetic complications, with the exception of nonproliferative and treated proliferative retinopathy. There was no clinical evidence of neuropathy, and all bedside autonomic tests (Valsalva, deep breathing, and orthostatic test) were within the reported normal range (21,22). The study protocol was approved by the local ethical committee at Oulu University Hospital, and all of the patients and healthy subjects signed informed consent forms approved by the ethical committee.

Protocol. Both euglycemic and hypoglycemic clamp procedures were performed at intervals of at least 1 week in a random order. The study subjects arrived in the laboratory in the morning after an overnight fast. The diabetic patients had taken their last subcutaneous insulin injection before bedtime and did not take their morning insulin dose on the day of the study. They were instructed to avoid hypoglycemia (symptoms or blood glucose <3.0 mmol/l) during the preceding 24 h and to eat a light snack if their bedtime glucose was <6.0 mmol/l. On the morning of the study day, a cannula was inserted into an antecubital vein of the left arm for infusion of glucose and insulin. A second

From the [1]Department of Internal Medicine, University of Oulu, Oulu, Finland; the [2]Department of Internal Medicine, University of Turku, Turku, Finland; the [3]Department of Gynecology and Obstetrics, University of Oulu, Oulu, Finland; and the [4]Department of Clinical Chemistry, University of Oulu, Oulu, Finland.

Address correspondence and reprint requests to Heikki Huikuri, MD, Department of Internal Medicine, University of Oulu, P.O. Box 5000 (Kajaanintie 50), FIN-90014 University of Oulu, Finland. E-mail: heikki.huikuri@oulu.fi.

Received for publication 9 September 2004 and accepted in revised form 15 November 2004.

BP, blood pressure; GH, growth hormone.

TABLE 1
Characteristics and the autonomic test results of the diabetic patients and nondiabetic subjects

	Diabetic patients	Healthy subjects
n	16	8
Age (years)	32 ± 8	34 ± 10
Sex (F/M)	9/7	5/3
Diabetes duration (years)	13 ± 9	—
HbA_{1c} (%)	7.0 ± 0.9	—
BMI (kg/m^2)	23.2 ± 1.7	24.1 ± 2.3
Systolic BP (mmHg)	126 ± 12	124 ± 22
Diastolic BP (mmHg)	77 ± 8	76 ± 15
BP response to standing (mmHg)		
Systolic BP	16 ± 18	28 ± 20
Diastolic BP	23 ± 15	32 ± 14
Valsalva ratio	2.01 ± 0.43	1.92 ± 0.25
Mean DBD (bpm)	26 ± 10	29 ± 12

Data are means ± SD. DBD, deep breathing difference.

cannula was placed into a large vein on the right forearm for blood sampling. The hand and forearm were placed in a heated box (45°C) to ensure arterialization of venous blood. The R-R interval was measured continuously with a real-time microprocessor-based QRS detection system (Polar Electro, Kempele, Finland) (23). The R-R interval recorder was connected to the patient via two dermal electrodes. At every glucose level, a 12-lead electrocardiogram was recorded. Blood pressure (BP) was measured from a finger of the left hand using a Finapress finger-cuff method. Blood samples were taken for analyses of blood glucose, serum potassium, and counterregulatory hormones. Hypoglycemic symptoms were registered using a symptom questionnaire, which listed 11 different symptoms: tremor, heart pounding, nervousness, sweating, tingling, hunger, tiredness, faintness, dizziness, difficulty in thinking, and blurry vision. The subjects rated the intensity of each symptom from 0 (none) to 10 (severe).

A primed continuous infusion of 80 $mU \cdot m^{-2} \cdot min^{-1}$ of regular human insulin was started, and the glucose clamp technique was used to adjust the blood glucose concentration (24). Blood glucose was measured every 2.5–5 min. The study subjects were blinded to the blood glucose concentration throughout the study. Glucose concentration was initially stabilized between 4.5 and 5.5 mmol/l. During a hypoglycemic clamp, blood glucose was permitted to slowly decrease to the first target level of 3.0–3.5 mmol/l (mild hypoglycemia), and then to the second target level of 2.0–2.5 mmol/l (moderate hypoglycemia). Thereafter, the glucose concentration was slowly increased, and the study was continued during euglycemia. During the euglycemic clamp, blood glucose was kept at the level of 4.5–5.5 mmol/l throughout the study, but the measurements were performed four times (as during the hypoglycemic clamp) at regular intervals. All of the measurements were performed only after clamping blood glucose at a desired range.

Measurements. Blood glucose was analyzed at the bedside, using the glucose dehydrogenase method (B-Glucose Analyzer; HemoCue, Ängelholm, Sweden). Blood HbA_{1c} (Pharmacia, Uppsala, Sweden), plasma epinephrine, and norepinephrine (Acclaim; Bio-Rad, Hercules, CA) were determined by high-performance liquid chromatography. The glucagon (Diagnostic Products, Los Angeles, CA), growth hormone (GH; Pharmacia), intact adrenocorticotropic hormone (Nichols Institute Diagnostics, San Juan Capistrano, CA), and cortisol (Orion Diagnostica, Oulunsalo, Finland) concentrations were determined by radioimmunoassay, as recommended by the manufacturers. Serum potassium was measured by an ion-selective electrode.

The analysis of heart rate variability was accomplished with a special software package (Hearts7; Heart Signal, Kempele, Finland). At the steady state of every glucose level, a 15-min period when no other tests were performed was selected for the analysis of heart rate variability. The spectral analysis was accomplished as described by the Task Force of European Society of Cardiology and the North American Society of Pacing and Electrophysiology (25). The high-frequency (0.15–0.40 Hz) and low-frequency (0.04–0.15 Hz) components and the ratio of low to high frequency were calculated. For dynamic measures of heart rate variability, a quantitative analysis of Poincaré plot was used as described in detail previously (26). Of the quantitative parameters, beat-to-beat R-R interval variability (SD1 of Poincaré plot analysis) and long-term heart rate variability (SD2) were calculated. The standard deviation of N-N intervals ($SDNN_{15}$ of the Poincaré plot) was used as a time domain measure of heart rate variability.

Statistical methods. Standard statistical methods were used for the calculation of means and SDs. Because of the skewed distributions, a logarithmic transformation to the natural base was performed on the measures of heart rate variability. Changes in the variables at the different glucose levels were studied using ANOVA for repeated measurements. The differences between groups at each glucose level were further analyzed by independent-samples *t* test. The nonparametric Friedman test was used on the absolute values of spectral analysis of heart rate variability. The results are presented as the means ± SD and Spearman's bivariate correlation coefficients (*r*).

RESULTS

Changes in heart rate, heart rate variability, and BP during the hyperinsulinemic clamp. At every glucose level, heart rate was higher and all other heart rate variability indexes, except the ratio of low to high frequency, were lower in the diabetic patients than the healthy control subjects (Table 2). During hypoglycemia, there was a similar increase of supine heart rate in both groups (Table 2). Mean BP showed a nonsignificant decreasing trend at hypoglycemia, and the change was similar in both groups.

Total heart rate variability ($SDNN_{15}$) did not change during hypoglycemia among the diabetic patients (Table 2). However, the high-frequency spectral component of heart rate variability decreased significantly, and a nonsignificant trend toward a decrease of the low-frequency component was also observed (Table 2, Fig. 1). There was also a nonsignificant trend toward an increasing ratio of low to high frequency. SD1 also decreased at hypoglycemic glucose levels, but long-term heart rate variability (SD2) remained unchanged (Fig. 1). The high-frequency spectral component and SD1 did not decrease significantly during mild hypoglycemia, but a pronounced and significant decrease of these measures was observed at moderate hypoglycemia (Fig. 2).

Among the nondiabetic subjects, the changes in the measures of heart rate variability at hypoglycemia were similar to those observed in the diabetic patients (Table 2). The high-frequency spectral component and SD1 decreased significantly, and the ratio of low to high frequency showed a nonsignificant increasing trend. No significant differences were observed in the heart rate or heart rate variability responses between the diabetic patients and control subjects during the entire clamp procedure.

At euglycemia, there were no significant changes in the heart rate, the high- and low-frequency spectral components, or SD1 among all the study subjects. Only a modest increase of the ratio of low to high frequency occurred at the last euglycemic step in the diabetic patients ($P < 0.05$). During the euglycemic clamp, the high- and low-frequency power of heart rate variability and SD1 were significantly lower among the diabetic patients compared with the healthy control subjects ($P < 0.01$ for all).

The response of counterregulation hormones during the hyperinsulinemic clamp. In both groups, the serum concentrations of potassium decreased during the hypoglycemic and euglycemic clamp (Table 3). As expected, the diabetic patients had an impaired response of glucagon to hypoglycemia compared with the healthy subjects (Table 3). The response of epinephrine was also blunted in the diabetic patients (from 0.2 ± 0.1 to 1.7 ± 1.6 nmol/l in the diabetic group and from 0.2 ± 0.1 to 3.1 ± 1.4 nmol/l in the control group) (Table 3). The response of norepinephrine tended to be attenuated in the diabetic group (from 1.3 ±

TABLE 2
Mean heart rate, BP, and heart rate variability in diabetic patients and healthy control subjects at each glycemic plateau during a hypoglycemic clamp

	Blood glucose (mmol/l)				
	4.5–5.5	3.0–3.5	2.0–2.5	4.5–5.5	*P*
Heart rate (bpm)					
Diabetic patients	72 ± 9*	76 ± 11*	80 ± 11*	73 ± 9†	<0.01
Control subjects	59 ± 5	63 ± 5	65 ± 5	58 ± 5	<0.05
Mean BP (mmHg)					
Diabetic patients	81 ± 7	79 ± 8	76 ± 10	82 ± 9	NS
Control subjects	85 ± 16	84 ± 8	76 ± 16	83 ± 14	NS
Standard deviation of R-R intervals (ms)					
Diabetic patients	65.2 ± 26.6†	63.6 ± 26.5*	61.1 ± 27.2*	72.7 ± 29.0†	NS
Control subjects	122.3 ± 35.1	111.8 ± 42.3	98.3 ± 35.3	129.6 ± 30.0	NS
High-frequency power of heart rate variability					
Diabetic patients Ln (ms^2)	5.53 ± 1.34*	5.22 ± 1.56‡	4.77 ± 1.32*	5.54 ± 1.26*	<0.05
Control subjects Ln (ms^2)	7.18 ± 0.96	6.52 ± 0.87	6.08 ± 0.85	7.00 ± 1.19	<0.01
Low-frequency power of heart rate variability					
Diabetic patients Ln (ms^2)	6.49 ± 0.82†	6.42 ± 1.05*	6.03 ± 1.07*	6.64 ± 0.88*	NS
Control subjects Ln (ms^2)	7.56 ± 0.54	7.37 ± 0.85	7.05 ± 0.69	7.51 ± 0.59	<0.01
Ratio of low to high frequency					
Diabetic patients	3.7 ± 2.9*	4.6 ± 3.7	4.8 ± 3.7	4.0 ± 2.8	NS
Control subjects	1.7 ± 0.8	2.5 ± 1.0	2.8 ± 1.1	2.1 ± 1.5	NS
SD1					
Diabetic patients Ln (ms)	3.01 ± 0.62†	2.90 ± 0.72*	2.70 ± 0.71*	3.05 ± 0.57*	<0.05
Control subjects Ln (ms)	3.76 ± 0.46	3.48 ± 0.44	3.30 ± 0.40	3.72 ± 0.54	<0.01
SD2 (ms)					
Diabetic patients	85.8 ± 33.7‡	85.9 ± 36.6†	82.6 ± 37.8*	100.9 ± 40.2‡	NS
Control subjects	165.7 ± 45.6	153.5 ± 57.9	135.2 ± 50.1	176.7 ± 38.5	NS

Data are means ± SD. Δ, changes in a parameter between the third and first measurements at hypoglycemia and between the fourth and first measurements at euglycemia; SD1, standard deviation of instantaneous beat-to-beat R-R interval variability; SD2, standard deviation of continuous beat-to-beat R-R interval variability. The symbols indicate the difference between the diabetic patients and healthy control subjects in independent samples *t* test: *$P < 0.01$; †$P < 0.001$; ‡$P < 0.05$.

0.3 to 1.8 ± 0.6 nmol/l) compared with the control group (from 1.3 ± 0.3 to 2.2 ± 0.4 nmol/l). The increases in the concentrations of cortisol, adrenocorticotropic hormone, and GH were significant among the healthy control subjects at hypoglycemia. The response of these counterregulation hormones was blunted in the diabetic patients (Table 3). During the euglycemic clamp, there were no significant increments in glucagon, epinephrine, norepinephrine, or other hormone concentrations in either group (Table 3).

None of the baseline variables (e.g., the age or the duration of diabetes) correlated with the changes in heart rate variability measures during controlled hypoglycemia. The rise of the plasma epinephrine or norepinephrine level, or other hormones, had no significant correlation with the changes in any of the heart rate variability

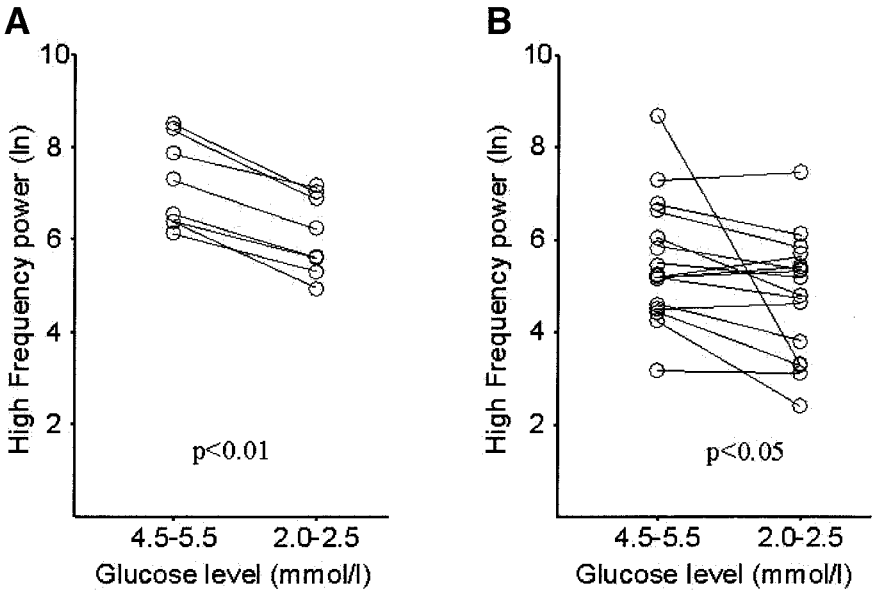

FIG. 1. Individual values of high-frequency spectral component at euglycemic and hypoglycemic glucose levels of nondiabetic control subjects (*A*) (*n* = 8) and diabetic patients (*B*) (*n* = 16), respectively.

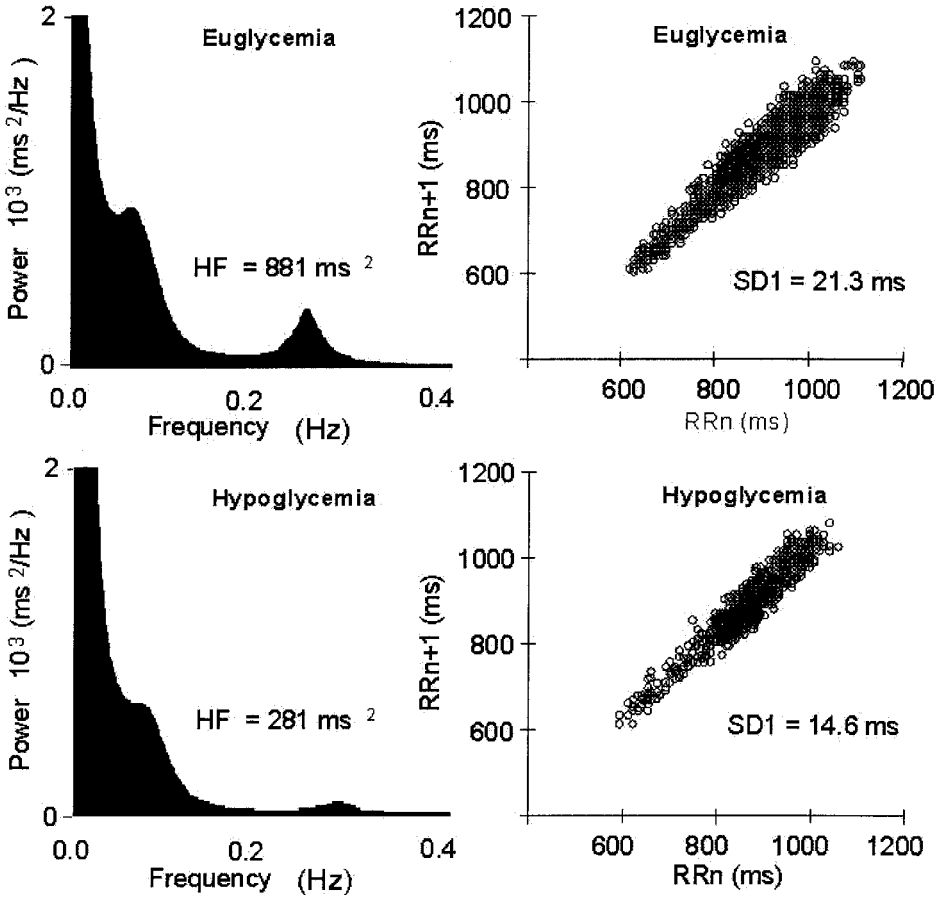

FIG. 2. Examples of power spectra and Poincaré plots from a diabetic patient during euglycemia and during hypoglycemia. Hypoglycemia typically shows reduced low and particularly reduced high-frequency (HF) spectral power and reduced beat-to-beat variability (SD1) in Poincaré plot.

measures in either the diabetic patients or the healthy control subjects. As expected, the healthy subjects had more hypoglycemic symptoms at the lowest glucose level compared with the diabetic patients (31 ± 25 vs. 18 ± 10 points, $P < 0.01$). The severity of symptoms did not correlate with changes in heart rate variability indexes.

TABLE 3
Serum potassium, cortisol, and GH concentrations and plasma glucagon, epinephrine, norepinephrine, and ACTH concentrations at each glycemic plateau during a hypoglycemic clamp and a euglycemic clamp

	Hypoglycemic clamp					Euglycemic clamp				
	BG 4.5–5.5 mmol/l	BG 3.0–3.5 mmol/l	BG 2.0–2.5 mmol/l	BG 4.5–5.5 mmol/l	*P*	BG 4.5–5.5 mmol/l	BG 4.5–5.5 mmol/l	BG 4.5–5.5 mmol/l	BG 4.5–5.5 mmol/l	*P*
Potassium (mmol/l)										
Diabetic patients	3.7 ± 0.2	3.7 ± 0.2	3.6 ± 0.3	3.5 ± 0.2	<0.001	3.9 ± 0.4	3.7 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	<0.01
Control subjects	3.7 ± 0.3	3.6 ± 0.3	3.4 ± 0.2	3.5 ± 0.4	<0.05	3.7 ± 0.2	3.6 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	<0.05
Glucagon (ng/l)										
Diabetic patients	15.6 ± 4.6	14.8 ± 3.9*	16.9 ± 4.7†	14.3 ± 3.9	<0.05	16.5 ± 4.4	15.3 ± 3.6	15.0 ± 3.8	14.6 ± 4.8	NS
Control subjects	19.5 ± 8.3	24.5 ± 8.0	42.4 ± 9.1	18.4 ± 8.8	<0.001	18.5 ± 9.7	17.4 ± 4.9	18.7 ± 9.8	18.3 ± 7.7	NS
Epinephrine (nmol/l)										
Diabetic patients	0.2 ± 0.1	0.4 ± 0.3	1.7 ± 1.6	0.4 ± 0.3	<0.01	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	NS
Control subjects	0.2 ± 0.1	0.3 ± 0.2	3.1 ± 1.4	0.4 ± 0.2	<0.001	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	NS
Norepinephrine (nmol/l)										
Diabetic patients	1.3 ± 0.3	1.5 ± 0.3	1.8 ± 0.6	1.5 ± 0.4‡	<0.01	1.4 ± 0.4	1.4 ± 0.4‡	1.4 ± 0.5	1.3 ± 0.3	NS
Control subjects	1.4 ± 0.3	1.4 ± 0.4	2.2 ± 0.4	2.0 ± 0.7	<0.01	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	NS
Cortisol (μmol/l)										
Diabetic patients	0.4 ± 0.2	0.4 ± 0.3‡	0.4 ± 0.3	0.4 ± 0.3	NS	0.4 ± 0.2*	0.4 ± 0.3‡	0.3 ± 0.2	0.3 ± 0.2	<0.01
Control subjects	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	<0.001	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	NS
ACTH (pmol/l)										
Diabetic patients	6.8 ± 11.9	4.8 ± 3.1	11.3 ± 9.6‡	4.6 ± 2.1	NS	4.1 ± 2.6	4.8 ± 3.8	3.6 ± 1.3	3.6 ± 1.4	NS
Control subjects	4.2 ± 1.7	4.1 ± 1.2	23.5 ± 11.4	5.7 ± 2.7	<0.01	3.4 ± 1.3	4.3 ± 1.6	4.1 ± 1.1	4.0 ± 1.1	NS
GH (μmol/l)										
Diabetic patients	3.4 ± 5.7	4.9 ± 9.1	9.0 ± 6.6	8.4 ± 6.9	<0.05	2.2 ± 3.5	3.2 ± 7.4	2.6 ± 4.9	2.6 ± 4.1‡	NS
Control subjects	0.5 ± 0.6	0.3 ± 0.3	11.6 ± 9.6	10.5 ± 9.5	<0.01	1.5 ± 2.4	0.3 ± 0.4	0.8 ± 1.7	0.2 ± 0.2	NS

Data are means ± SD. The symbols indicate the difference between the diabetic patients and healthy control subjects in independent samples *t* test. *$P < 0.01$; †$P < 0.001$; ‡$P < 0.05$. Δ, changes in a parameter between the third and first measurements at hypoglycemia and between the fourth and first measurements at euglycemia; ACTH, adrenocorticotropic hormone; BG, blood glucose.

DISCUSSION

The results of this study showed that cardiac vagal activity, as assessed by the high-frequency component and SD1, decreases progressively during controlled hypoglycemia. The changes in cardiac autonomic regulation were similar between the diabetic patients and the nondiabetic subjects during the hypoglycemic clamp. Thus, prolonged hypoglycemia seems to be associated with a decrease in cardiac vagal outflow.

Autonomic function in type 1 diabetic patients. The average heart rate was higher and all heart rate variability indexes lower at baseline and at all glucose levels during the euglycemic and hypoglycemic clamps among the diabetic patients compared with the control subjects, suggesting impaired cardiovascular autonomic function. These findings concur with previous studies showing impaired heart rate variability in diabetes (14,16,17). However, the routine tests of the autonomic nervous system were within normal limits in diabetic patients (12–18). It is obvious that these tests are not sensitive enough in detecting subtle abnormalities in cardiac autonomic function, which can be better uncovered by assessment of continuous heart rate variability (17) or by measurement of baroreflex sensitivity (27,28). Despite the impaired autonomic function, the responses of cardiac autonomic regulation to hypoglycemia were within the same range between the diabetic patients and the nondiabetic control subjects.

Effects of hypoglycemia on cardiovascular autonomic regulation. The high-frequency spectral component of heart rate variability and SD1 analyzed from Poincaré plots have been previously shown to reflect the cardiac vagal outflow (29–31). Both high-frequency oscillations of heart rate and short-term heart rate oscillations (SD1) measured from return plots are almost absent after vagal blockade (30,31). The latter index (SD1) was used in this study in addition to spectral analysis because it is less sensitive to trends in heart rate itself when compared with analysis of the high-frequency power spectral component (26). The increase of heart rate itself, regardless of its origin, may also result in a reduction of these heart rate variability indexes. However, in the present study, the increase of heart rate and the decrease of high-frequency power and SD1 were not related to an increase of epinephrine or norepinephrine, suggesting that adrenomedullary sympathoexcitation was not the primary cause of altered heart rate variability, but that these changes were most likely a result of reduced cardiac vagal outflow caused by hypoglycemia itself.

Previous studies have provided conflicting results regarding the effect of hypoglycemia on heart rate and heart rate variability. Premature ventricular beats, severe nodal bradycardia, and an increase in heart rate have been observed (32,33). Russel et al. (34) found an increase in heart rate in nondiabetic and intensively treated type 1 diabetic patients during both hypoglycemic and euglycemic hyperinsulinemia, without a statistically significant difference between the euglycemic and hypoglycemic conditions. To our knowledge, our study is the first reporting the effects of hypoglycemia on heart rate variability among diabetic patients.

Regarding the effect of hypoglycemia on heart rate and heart rate variability in healthy subjects, the present findings differ from the results of two recent studies (19,20). Laitinen et al. (19) did not observe any responses in cardiac parasympathetic regulation during a hyperinsulinemic-hypoglycemic clamp in healthy nondiabetic subjects. Schächinger et al. (20) did not find any significant changes in heart rate during a hyperinsulinemic-hypoglycemic clamp, but they did observe a small increase in the high-frequency spectral component (20). There are salient differences between the previous studies and the present one, which probably explain the divergent results. Laitinen et al. used a single-day study protocol in which hypoglycemia followed euglycemia, which differed from our protocol. They also targeted higher blood glucose values (3.0 mmol/l) with weaker stimulation of the autonomic nervous system. Schächinger et al. used a 2-day single-blinded crossover design similar to ours, but they also targeted a higher glucose level (2.7 mmol/l) than we did here. In both studies, a shorter period (5 min) was used for the analysis of changes in heart rate and heart rate variability compared with the present study (15 min). Short recording periods may distort the analysis of spectral components of heart rate variability because of trends in the average heart rate itself. Together, these observations support the view that mild short-term hypoglycemia does not result in significant changes in either the heart rate or heart rate variability, but more marked and prolonged hypoglycemia, as in the present study, clearly increases the heart rate and decreases the high-frequency oscillations of heart rate.

Effects of hyperinsulinemia. The responses to hypoglycemia were assessed here during a hyperinsulinemic clamp. There is some evidence that supraphysiological concentrations of insulin per se may have effects on neuroendocrine responses. At comparable levels of hypoglycemia, epinephrine, norepinephrine, cortisol, and GH responses appear to increase along with greater hyperinsulinemia (35). However, no significant changes occurred in any of the heart rate variability indexes during the hyperinsulinemic-euglycemic clamp in the present study, suggesting that the observed changes in vagal indexes during the hypoglycemic clamp were caused by hypoglycemia itself but not caused by hyperinsulinemia. Furthermore, the last measurements of heart rate variability were assessed after reaching euglycemia during the hypoglycemic clamp, and these measurements revealed the restoration of high- and low-frequency power to the level preceding hypoglycemia. Similarly, a previous study did not observe any changes in the average heart rate during a hyperinsulinemic-euglycemic clamp (36). A small increase of the ratio of low to high frequency was observed in the euglycemic hyperinsulinemic state in the diabetic subjects, suggesting that insulin itself may have some effects on the sympatho-vagal balance. Another study also documented an increase in the ratio of low to high frequency after high- and low-rate insulin infusions among healthy women at euglycemia (37).

Implications. The present study showed that moderate prolonged hypoglycemia results in reduced cardiac vagal outflow in both diabetic patients and nondiabetic subjects. Several clinical and experimental studies have documented the cardioprotective role of vagal activity during various clinical settings (38–41). Because diabetic pa-

tients already have reduced cardiac vagal outflow at the euglycemic state, and because hypoglycemia further attenuates this outflow, it is possible that this phenomenon may have some clinical importance in contributing to adverse clinical events (e.g., the occurrence of "dead-in-bed" syndrome), but further studies are obviously needed to confirm this association. Finally, the experimental conditions were artificial here and differ from real life, where the rate and depth of hypoglycemia are not controlled. Therefore, more studies during spontaneous hypoglycemia will be needed to assess more definitively the role of autonomic regulation during hypoglycemia.

ACKNOWLEDGMENTS

This study was supported by a research grant from the Finnish Diabetes Research Foundation, by Polar Electro, and the Medical Council of the Academy of Finland, Helsinki, Finland.

REFERENCES

1. Cryer PE, Fisher JN, Shamoon H: Hypoglycemia (Review). *Diabetes Care* 17:734–755, 1994
2. Bolli GB, Fanelli CG: Physiology of glucose counterregulation to hypoglycemia. *Endocrinol Metab Clin North Am* 28:467–493, 1999
3. Cryer PE: Hypoglycemia unawareness in IDDM (Review). *Diabetes Care* 16 (Suppl. 3):40–47, 1993
4. Cryer PE: Symptoms of hypoglycemia, thresholds for their occurrence, and hypoglycemia unawareness. *Endocrinol Metab Clin North Am* 28:495–500, 1999
5. Davis MR, Mellman M, Shamoon H: Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 41:1335–1340, 1992
6. Amiel SA, Pottinger RC, Archibald HR, Chusney G, Cunnah DT, Prior PF, Gale EA: Effect of antecedent glucose control on cerebral function during hypoglycemia. *Diabetes Care* 14:109–118, 1991
7. The Diabetes Control and Complications Trial Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 46:271–286, 1997
8. CG Fanelli, L Epifano, AM Rambotti, S Pampanelli, A Di Vincenzo, F Modarelli, M Lepore, B Annibale, M Ciofetta, P Bottini, et al.: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
9. Lingenfelser T, Buettner U, Martin J, Tobis M, Renn W, Kaschel R, Jakober B: Improvement of impaired counterregulatory hormone response and symptom perception by short-term avoidance of hypoglycemia in IDDM. *Diabetes Care* 18:321–325, 1995
10. Tattersall RB, Gill GV: Unexplained deaths of type 1 diabetic patients. *Diabet Med* 8:49–58, 1991
11. Weston PJ, Gill GV: Is undetected autonomic dysfunction responsible for sudden death in type 1 diabetes mellitus? The 'dead in bed' syndrome revisited. *Diabet Med* 16:626–631, 1999
12. Ewing DJ, Neilson JM, Travis P: New method for assessing cardiac parasympathetic activity using 24 hour electrocardiograms. *Br Heart J* 52:396–402, 1984
13. Pagani M, Malfatto G, Picrimi S, Casati R, Masu AM, Poli M, Guzzetti S, Lombardi F, Cerutti S, Malliani A: Spectral analysis of heart rate variability in the assessment of autonomic diabetic neuropathy. *J Autonom Nerv Syst* 23:143–153, 1988
14. Malpas SC, Maling TJ: Heart-rate variability and cardiac autonomic function in diabetes. *Diabetes* 39:1177–1181, 1990
15. Ewing DJ, Neilson JM, Shapiro CM, Stewart JA, Reid W: Twenty hour heart rate variability: effects of posture, sleep, and time of day in healthy controls and comparison with bedside tests of autonomic function in diabetic patients. *Br Heart J* 65:239–244, 1991
16. Freeman R, Saul JP, Roberts MS, Berger RD, Broadbridge C, Cohen RJ: Spectral analysis of heart rate in diabetic autonomic neuropathy: a comparison with standard tests of autonomic function. *Arch Neurol* 48:185–190, 1991
17. Bellavere F, Balzani I, De Masi G, Carraro M, Carenza P, Cobelli C, Thomaseth K: Power spectral analysis of heart-rate variations improves assessment of diabetic cardiac autonomic neuropathy. *Diabetes* 41:633–640, 1992
18. Ziegler D, Dannehl K, Mûhlen H, Spûler M, Gries FA: Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses at various stages of diabetic neuropathy. *Diabet Med* 9:806–814, 1992
19. Laitinen T, Huopio H, Vauhkonen I, Camaro C, Hartikainen J, Laakso M, Niskanen L: Effects of euglycaemic and hypoglycaemic hyperinsulinaemia on sympathetic and parasympathetic regulation of haemodynamics in healthy subjects. *Clin Sci* 105:315–322, 2003
20. Schächinger H, Port J, Brody S, Linder L, Wilhelm FH, Huber PR, Cox D, Keller U: Increased high-frequency heart rate variability during insulin-induced hypoglycemia in healthy humans. *Cli Sci (Lond)* 106:583–588, 2004
21. Piha SJ: Cardiovascular autonomic reflex tests: normal responses and age-related reference values. *Clin Physiol* 11:277–290, 1991
22. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 8:491–498, 1985
23. Tapanainen JM, Seppänen T, Laukkanen R, Loimaala A, Huikuri HV: Significance of the accuracy of RR interval detection for analysis of new dynamic measures of heart rate variability. *ANE* 4:10–18, 1999
24. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am Physiol* 237:E214–E223, 1979
25. Task force of European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Eur Heart J* 17:354–381, 1996
26. Tulppo MP, Mäkikallio TH, Takala TES, Seppänen T, Huikuri HV: Quantitative beta-to-beta analysis of heart rate dynamics during exercise. *Am J Physiol* 271:H244–H252, 1996
27. Weston PJ, James MA, Panerai R, McNally PG, Potter JF, Thurston H, Swales JD: Abnormal baroreceptor-cardiac reflex sensitivity is not detected by conventional tests of autonomic function in patients with insulin-dependent diabetes mellitus. *Clin Sci* 91:59–64, 1996
28. Weston PJ, Panerai RB, MsCullogh A, McNally PG, James MA, Potter JF, Thurston H, Swales JD: *Diabetologia* 39:1385–1391, 1996
29. Katone PG, Felix JIH: Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. *J Appl Physiol* 39:801–805, 1975
30. Tulppo MP, Mäkikallio TH, Takala TES, Seppänen T, Shoemaker K, Tutungi E, Hughson RL, Huikuri HV: Effects of pharmacological adrenergic and vagal modulation on fractal heart rate dynamics. *Clin Physiol* 21:515–523, 2001
31. Penttilä J, Helminen A, Jartti T, Kuusela T, Huikuri HV, Tulppo MP, Scheinin H: Effect of cardiac vagal outflow on complexity and fractal correlation properties of heart rate dynamics. *Auton Autacoid Pharmacol* 23:173–179, 2003
32. Shimada R, Nakashima T, Nunoi K, Kohno Y, Takeshita A, Omae T: Arrhythmia during insulin-induced hypoglycemia in a diabetic patient. *Arch Intern Med* 144:1068–1069
33. Lindström T, Jorfeldt L, Tegler L, Arnqvist HJ: Hypoglycaemia and cardiac arrhythmias in patients with type 2 diabetes mellitus. *Diabet Med* 9:536–541, 1992
34. Russell RR III, Chyun D, Song S, Sherwin RS, Tamborlane WV, Lee FA, Pfeifer MA, Rife F, Wackers FJT, Young LH: Cardiac responses to insulin-induced hypoglycemia in nondiabetic and intensively treated type 1 diabetic patients. *Am J Physiol Endocrinol Metab* 281:E1029–E1036, 2001
35. Galassetti P, Davis SN: Effects of insulin per se on neuroendocrine and metabolic counter-regulatory responses to hypoglycaemia. *Cli Sci* 99:351–362, 2000
36. Airaksinen J, Lahtela JT, Ikäheimo MJ, Sotaniemi EA, Takkunen JT: Intravenous insulin has no effect on myocardial contractility or heart rate in healthy subjects. *Diabetologia* 28:649–652, 1985
37. Bellavere F, Cacciatori V, Moghetti P, Gemma ML, Dellera A, Tosi F, Negri C, Thomaseth K, Muggo M: Acute effect of insulin on autonomic regulation of the cardiovascular system: a study by heart rate spectral analysis. *Diabet Med* 13:709–714, 1996
38. Schwartz PJ, Billman GE, Stone HL: Autonomic mechanisms in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with healed myocardial infarction: an experimental preparation for sudden cardiac death. *Circulation* 69:790–800, 1984
39. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ, the Multicenter Postinfarction

Research Group: Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 59:256–262, 1987
40. Hull SS Jr, Evans AR, Vanoli E, Adamson PB, Stramba-Badiale M, Albert DE, Foreman RD, Schwartz PJ: Heart rate variability before and after myocardial infarction in conscious dogs at high at low risk of sudden death. *J Am Coll Cardiol* 16:978–985, 1990
41. Bigger JT, Fleiss JL, Steinmann RC, Rohitzky LM, Kleiger RE, Rottman JN: Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 85:164–171, 1992