Abnormal Glucose Handling by the Kidney in Response to Hypoglycemia in Type 1 Diabetes

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The frequent occurrence of hypoglycemia in people with type 1 diabetes is attributed to abnormalities in the blood glucose counterregulatory response. In view of recent findings indicating that the kidney contributes to prevent and correct hypoglycemia in healthy subjects, we decided to investigate the role of renal glucose handling in hypoglycemia in type 1 diabetes. Twelve type 1 diabetic patients and 14 age-matched normal individuals were randomized to hyperinsulinemic-euglycemic (n = 6 diabetic subjects and n = 8 control subjects) or hypoglycemic (n = 6 each) clamps with blood glucose maintained either stable near 100 mg/dl (5.6 mmol/l) or reduced to 54 mg/dl (3.0 mmol/l). All study subjects had their renal vein catheterized under fluoroscopy, and net renal glucose balance and renal glucose production and utilization rates were measured using a combination of arteriovenous concentration difference with stable isotope dilution technique. Blood glucose and insulin were comparable in both groups in all studies. In patients with diabetes, elevations in plasma glucagon, epinephrine, and norepinephrine were blunted, and both the compensatory rise in endogenous glucose production and in the net glucose output by the kidney seen in normal subjects with equivalent hypoglycemia were absent. Renal glucose balance switched from a mean ± SE baseline net uptake of 0.6 ± 0.4 to a net output of 4.5 ± 1.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in normal subjects, but in patients with diabetes there was no net renal contribution to blood glucose during similar hypoglycemia (mean ± SE net glucose uptake [baseline 0.7 ± 0.4] remained at 0.4 ± 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the final 40 min of hypoglycemia; $P < 0.01$ between groups). We conclude that adrenergic stimulation of glucose output by the kidney, which represents an additional defense mechanism against hypoglycemia in normal subjects, is impaired in patients with type 1 diabetes and contributes to defective glucose counterregulation.
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Intensive therapy that effectively lowers mean blood glucose concentrations toward the nondiabetic range increases the risk of severe iatrogenic hypoglycemia in people with type 1 diabetes (1). This has been largely attributed to compromised counterregulation, which includes defective hormonal responses and hypoglycemia unawareness. It is known that in patients with C-peptide–negative diabetes of long duration, lower blood glucose concentrations are required to elicit autonomic nervous system and symptomatic responses to hypoglycemia. As a consequence, compensatory adjustments in the rates of endogenous glucose production and utilization are delayed, and result in severe and prolonged hypoglycemia (2). The fact that patients with renal insufficiency are also at increased risk for developing hypoglycemia (3) has raised the question of whether glucose handling by the kidney plays a role in the body's defense against hypoglycemia. Although the mechanisms behind the episodes of hypoglycemia in patients with renal insufficiency are not as clearly defined as in diabetes, it is conceivable that the kidney plays a more critical role in the maintenance of blood glucose concentration than previously appreciated.

The biochemical capacity and physiological reserve of the kidney to produce and release glucose into the circulation have been characterized and were emphasized several years ago by a series of studies performed in fasting humans. Using arteriovenous glucose concentration differences, Cahill et al. (4–6) demonstrated that the net contribution of the kidney to blood glucose, which was negligible in the initial 24 h, increased significantly with prolonged fasting, such that by the fourth week of fasting, the kidney was responsible for one-half of the total glucose appearing in the circulation. Our understanding of the role of the kidney in glucose homeostasis has improved substantially with the recent development of an isotopic method capable of partitioning individual rates of renal glucose production and utilization (7). There is now evidence that in healthy individuals renal glucose production is stimulated by epinephrine (8) and suppressed by insulin (9), and that increased glucose production by the kidney contributes to maintenance of blood glucose even in early fasting (10). Additionally, and perhaps of greater clinical significance, mild-to-moderate hypoglycemia sustained by continuous insulin infusion is associated with net glucose output by the kidney. This is primarily due to enhanced rates of renal glucose production combined with minimal or no changes in renal glucose utilization (11,12). The reversal to net glucose output by the kidney during sustained hypoglycemia appears to complement the simul-

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No abbreviations used.

TABLE 1
Characteristics of patients with type 1 diabetes and normal control subjects studied during either euglycemic or hypoglycemic hyperinsulinemic clamp

	Normal subjects		Type 1 diabetes	
	Euglycemia	Hypoglycemia	Euglycemia	Hypoglycemia
n	8	6	6	6
Age (years)	33.0 ± 3.8 (23–42)	32.2 ± 6.4 (20–47)	29.0 ± 3.9 (20–38)	32.0 ± 6.8 (22–40)
Sex				
Male	5	3	3	3
Female	3	3	3	3
Weight (kg)	73 ± 8	67 ± 9	70 ± 8	71 ± 11
BMI (kg/m^2)	23.8 ± 1.8	22.8 ± 2.4	24.6 ± 3.2	24.3 ± 2.6
Ethnicity				
Caucasian	4	4	4	3
African American	2	1	2	2
Hispanic	2	1	0	1

*Data are means ± SD (range) or *n*.

taneous rise in glucose production by the liver, thus contributing to the attenuation of the fall and restoration of normal blood glucose concentration (13). These adaptive changes in glucose kinetics are brought about by the counterregulatory response, which includes immediate release of glucagon and epinephrine into the circulation and neural sympathetic activation. Since glucagon secretion in response to hypoglycemia, which affects primarily the liver, is lost early in patients with type 1 diabetes (14), prevention of hypoglycemia relies entirely on adrenergic mechanisms (15). Thus, we have postulated that adjustments in renal glucose handling in these patients must play an important role in defense against hypoglycemia. This study was therefore undertaken to examine the hypothesis that inadequate renal response to hypoglycemia contributes to the defective blood glucose counterregulation in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study subjects. We studied 12 patients with type 1 diabetes and 14 normal subjects. Among the patients with diabetes, six were women and six were men, with a mean (± SD) age of 31 ± 5 years. The duration of diabetes was 15 ± 4 years. All patients were taking insulin through either multiple daily injections ($n = 9$) or continuous subcutaneous delivery with the assistance of a pump ($n = 3$). The average (mean ± SD) total dose of insulin was 1.2 ± 0.5 units/kg daily, with a mean HbA_{1c} value of 8.0 ± 0.5% (normal value, <6.5). No patients had clinical evidence of autonomic neuropathy; severe hypoglycemia within the preceding 4 weeks; or a blood glucose concentration (ascertained by self-monitoring four to six times daily) <68 mg/dl (3.8 mmol/l) 3 days before the experiment. Patients were not receiving medication other than insulin, and none had acute illness or evidence of hepatic or renal dysfunction, hypertension, or vascular diseases. Among the normal subjects, eight were men and six were women, with a mean age of 33 ± 5 years. None had a history of diabetes in first-degree relatives or conditions that might affect glucose regulation or hormonal responses to hypoglycemia. Characteristics of all 24 individuals are summarized in Table 1. The study was approved by the University Hospital review board and the State University of New York–Stony Brook Human Investigation Committee, and all subjects gave written informed consent.

Protocol. All subjects were admitted to the University Hospital General Clinical Research Center at 6:00 P.M. the evening before the study, and they consumed a standard meal; no food was allowed after dinner. For 3 days before the study, all subjects had abstained from alcohol and had been on a weight-maintaining diet containing at least 200 g of carbohydrate per day. Patients with diabetes had the intermediate- and long-acting insulin discontinued 24 h before the experiment and received only subcutaneous injections of regular insulin at breakfast, lunch, and dinner. At 12 A.M. (midnight), a continuous intravenous insulin infusion was started and adjusted to maintain blood glucose concentration between 80 and 120 mg/dl (4.4 and 6.6 mmol/l) according to a scale using hourly capillary blood glucose measurements. The insulin infusion rate was fixed 2 h before the commencement of the study each morning. All experiments were carried out between 7:00 A.M. and 3:00 P.M. after a 10- to 12-h overnight fast, and each subject was randomly assigned to a single experimental protocol.

Procedures. To ensure a standardized hypoglycemic stimulus and to factor out the independent effects of hyperinsulinemia, one-step hypoglycemic- and euglycemic-clamp procedures analogous to those described by Simonson et al. (16) were performed in two separate experiments in normal subjects and diabetic patients alike. Two cannulas were inserted, one in each antecubital vein, for infusion of insulin, glucose, para-aminohippurate, and stable isotopes, and a third cannula was inserted in a dorsal hand vein for blood sampling. The hand was placed in a heated (65°C) box to arterialize the venous blood. Primed continuous infusions of [6,6-$^2H_2$]glucose and [2-^{13}C]glycerol (Cambridge Isotope Laboratories, Andover, MA), together with a continuous infusion of para-aminohippurate (Merck & Co, West Point, PA), were started in all subjects at the beginning of each experiment. During the 180-min equilibration period, the subjects were transported to the radiology suite and had the left renal vein catheterized under fluoroscopy through the right femoral vein using local anesthesia (lidocaine 2%). The position of the catheter tip was ascertained by injecting iodinated contrast material, and the catheter was then infused continuously with heparinized saline solution (4.0 units/min).

After baseline determinations, all subjects were given continuous intravenous infusions of insulin (0.5 mU · kg^{-1} · min^{-1}), and target plasma glucose values were achieved by varying the rate of an infusion of 10% glucose in water with added [6,6-$^2H_2$]glucose to keep plasma glucose isotope enrichments constant, as described by Butler et al. (17). Plasma glucose was measured at the bedside at 10-min intervals with a glucose analyzer (Beckman, Fullerton, CA). Plasma glucose concentrations were stabilized between 100 and 140 mg/dl (5.5 and 7.7 mmol/l) in patients with diabetes, and between 90 and 105 mg/dl (5.0 and 5.8 mmol per liter) in normal subjects during 40 min (baseline) before the induction of either hypoglycemia or euglycemia with hyperinsulinemia. The plasma glucose concentration was reduced to ~55 mg/dl (~3.0 mmol/l) over a period of 180 min by combining insulin infusion with a reduction in the rate of exogenous glucose infusion. Plasma glucose concentrations were then maintained at that level for a further 40 min (hypoglycemia phase). In the euglycemic clamp studies, plasma glucose concentrations between 90 and 120 mg/dl (5.0 and 6.6 mmol/l) were achieved over a period of 180 min by combining insulin infusion with exogenous glucose infusion. Plasma glucose concentrations were then maintained at that level for a further 40 min (euglycemia phase). During all studies, vital signs, cognitive functions, and peripheral pulses were monitored frequently; all subjects were resting quietly in bed with free access to water but not to food. Normal saline (0.9% sodium chloride) was given at the rate of 75 ml/h to all subjects for the duration of the experiment.

Measurements. Blood samples were taken simultaneously from the arterialized hand vein and the left renal vein at 10-min intervals during the 40-min baseline euglycemic period and again during the final 40 min of the hyperinsulinemic clamp (either hypoglycemia or euglycemia phase of the study period) for measurements of blood glucose and glycerol concentrations and isotope enrichments; plasma para-aminohippurate, free fatty acids, and insulin; and C-peptide, glucagon, and catecholamines. Renal plasma flow was estimated by para-aminohippurate clearance rates and corrected to renal blood flow by the (1-hematocrit) factor. Net renal glucose balance was determined using arterial-renal vein blood glucose concentration differences. Cal-

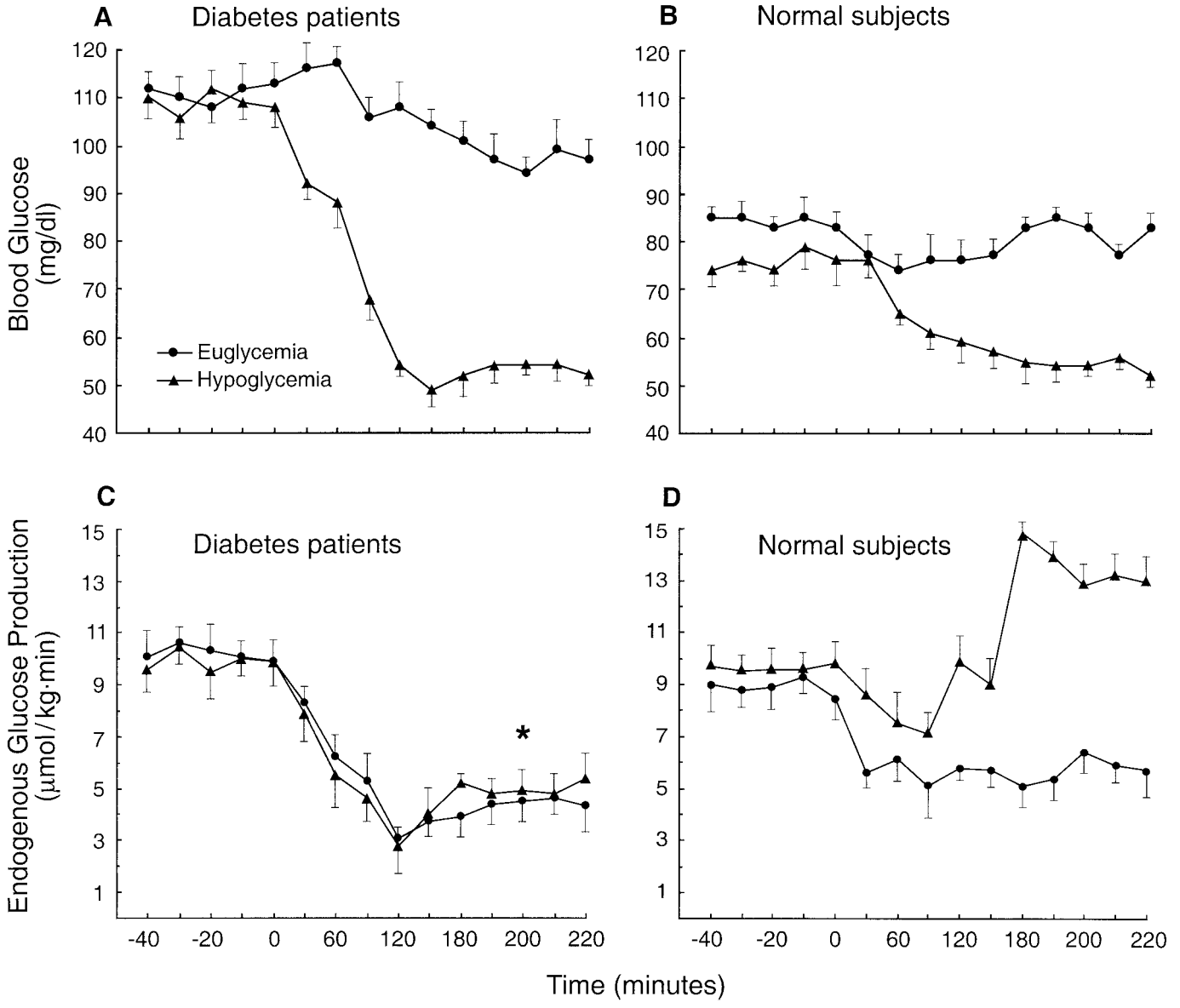

FIG. 1. Mean ± SE blood glucose concentrations (*A* and *B*) and endogenous glucose production rates (*C* and *D*) in 12 patients with type 1 diabetes and 14 normal subjects during 220-min periods of hyperinsulinemia with either euglycemia (●) or hypoglycemia (▲). To convert blood glucose values to millimoles per liter, multiply by 0.05551. The zero on the *x*-axis indicates the beginning of the hyperinsulinemic clamp periods. **P* < 0.001 for differences between euglycemia and hypoglycemia in diabetic patients versus normal subjects.

culations of the rates of systemic renal glucose production and utilization and of glycerol conversion to glucose were based on systemic and renal dilution and fractional extraction of isotopes according to previously described methods (7).

In all subjects, symptoms of hypoglycemia were assessed at 15-min intervals, and the subjects were asked to rate if these were mild, moderate, or severe. The symptoms were pounding heart, shakiness, sweating, headache, difficulty in thinking, or slowed thinking. Heart rates were measured continuously and averaged over 30-s intervals; blood pressure and peripheral pulses in lower extremities were examined at 30-min intervals. Urine samples for measurements of glucose were obtained at the beginning and at the end of each study in all patients with diabetes.

Analytical techniques. Blood glucose and glycerol concentrations were measured by enzymatic assays (18), and isotope enrichments were measured by gas chromatography–mass spectrometry, as previously described (9). Plasma para-aminohippurate was measured by a colorimetric assay (19), and plasma insulin (20), C-peptide (21), and glucagon (22) were measured by double-antibody radioimmunoassays. Plasma free fatty acids (after alcohol-heptane extraction [23]) and catecholamines (24) were measured by high-performance liquid chromatography. All of the samples from each subject were analyzed in a single assay. The intra-assay variations for blood glucose and glycerol assays were <5%; for isotope enrichment, determinations were <3%. The intra-assay variations for para-aminohippurate assay and for insulin, C-peptide, and glucagon assays were <10%, and for free fatty acids, epinephrine, and norepinephrine, they were 15, 17, and 12%, respectively.

Statistical analysis. The blood glucose concentrations, renal glucose balance (net uptake or net output), systemic and renal glucose and glycerol kinetics, and hormone responses in all four groups of individuals studied were compared by analysis of variance with repeated-measures design when appropriate. Baseline values for each parameter were defined as the means of all five values obtained during the 40-min interval preceding the hyperinsulinemic clamp period. Hypoglycemia- and euglycemia-phase values for the same parameters were defined as the means of all five values obtained during the final 40-min interval of the hypoglycemic or euglycemic period, respectively. All statistical tests were two-sided.

RESULTS

Systemic and renal glucose kinetics. The mean blood glucose concentrations during the hypoglycemic- and euglycemic-clamp procedures were comparable in the patients with diabetes and the normal subjects, and during each study the target blood glucose of ~54 mg/dl (~3.0

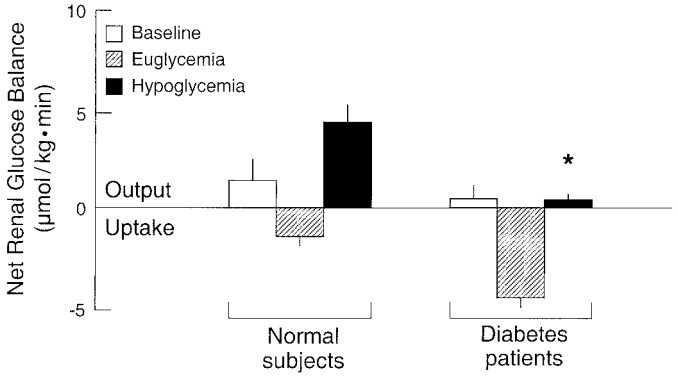

FIG. 2. Mean ± SE net renal glucose balance in 14 normal subjects and in 12 patients with type 1 diabetes in the baseline (□) and during the last 40 min of 220-min periods of hyperinsulinemia with either euglycemia (▨) or hypoglycemia (■). *$P < 0.01$ for differences between euglycemia and hypoglycemia in diabetic patients versus normal subjects.

mmol/l) was reached (Fig. 1*A* and *B*). The rates of endogenous (whole-body) glucose production were equally decreased during the euglycemic clamp procedures, though the compensatory rise in endogenous glucose production during the hypoglycemic clamp procedures in normal subjects (Fig. 1*D*) was not observed in patients with diabetes (Fig. 1*C*).

The mean (± SE) renal glucose balance changed from output to net uptake during the euglycemic clamp procedures and to a higher net output during the hypoglycemic clamp procedures (from −0.8 ± 0.4 to 1.4 ± 0.5 and to −4.5 ± 1.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively). However, in patients with diabetes, the small renal glucose output changed to an uptake during the euglycemic clamp procedures (from −0.3 ± 0.5 to 4.5 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), which was greater than in control subjects ($P < 0.05$). Also, the renal glucose output during comparable hypoglycemic clamp procedures was small and not statistically significant (−0.4 ± 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (Fig. 2). After insulin infusion, changes in net renal glucose balance in the euglycemic periods were due to concomitant increases in renal glucose utilization and decreases in renal glucose production documented both in normal subjects and in patients with diabetes. During hypoglycemia, however, the twofold increase in renal glucose production rates, which contributed to net renal glucose output in normal subjects, was not seen in patients with diabetes (Table 2).

Plasma free fatty acids and glycerol kinetics. The mean plasma free fatty acid, blood glycerol concentration, and whole-body glycerol turnover rates—all indexes of adipose tissue lipolysis—decreased significantly in all euglycemic clamp procedures and increased significantly during all hypoglycemic clamp procedures. Although the increments were consistently greater in normal subjects than in patients with diabetes, differences between groups were not statistically significant (Table 3).

In the baseline, rates of whole-body glycerol conversion to glucose, an index of systemic gluconeogenesis, and rates of renal glycerol conversion to glucose, an index of renal gluconeogenesis, were twofold higher in patients with diabetes ($P = 0.02$), though these showed equal percent decreases during the euglycemic clamp. The increments in systemic and renal glycerol gluconeogenesis during the hypoglycemic clamp procedures were attenuated in patients with diabetes when compared with normal subjects, but these differences did not reach statistical significance.

Plasma hormone responses to hypoglycemia. Elevations in the plasma insulin concentrations were comparable during all clamp procedures. The plasma C-peptide levels were much lower in the patients with diabetes than in normal subjects in the baseline, consistent with the diagnosis of type 1 diabetes. As expected, there was a substantial decline in the plasma C-peptide levels in the normal subjects after insulin administration in all clamp procedures (Table 4). Also, in the patients with diabetes, the plasma glucagon concentrations were lower than in the normal subjects and remained low during the hypoglycemic clamp procedures, whereas in the normal subjects, the plasma glucagon concentrations increased by ~50% (Table 4).

Plasma catecholamine responses to hypoglycemia. The plasma epinephrine and norepinephrine concentrations did not change significantly during euglycemic hyperinsulinemia in either group during any of the studies (Table 4). In the normal subjects, the plasma epinephrine concentrations increased approximately eightfold during hypoglycemia, and the plasma norepinephrine concentrations nearly doubled, whereas during comparable hypoglycemia in the patients with diabetes, the plasma epinephrine and norepinephrine concentrations increased by only threefold and 50%, respectively (Table 4). In the patients with diabetes, markedly blunted catecholamine responses to hypoglycemia were also documented across the kidney by measuring renal norepinephrine spillover, an index of renal autonomic sympathetic activity (Fig. 3). In the normal subjects, the mean renal plasma norepinephrine balance increased during hypoglycemia, from an output of 0.23 ± 0.07 to 1.37 ± 0.29 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (from 1.36 ± 0.42 to 8.10 ± 1.71 $pmol \cdot kg^{-1} \cdot min^{-1}$), but during

TABLE 2
Renal glucose production and utilization rates in normal subjects and patients with diabetes in the baseline and the last 40 min of a 220-min hyperinsulinemic clamp period of either euglycemia or hypoglycemia

	Baseline	Euglycemia	Baseline	Hypoglycemia
Glucose production				
Normal subjects	3.39 ± 1.25	1.37 ± 1.36*	2.44 ± 1.28	5.50 ± 1.20*
Diabetic patients	2.96 ± 1.31	0.67 ± 1.36*	2.05 ± 1.36	0.65 ± 1.24*
Glucose utilization				
Normal subjects	1.24 ± 1.32	2.76 ± 1.65*	3.08 ± 1.52	1.01 ± 1.46*
Diabetic patients	1.75 ± 1.62	5.20 ± 1.93*	2.71 ± 1.52	0.25 ± 1.36*

Data are means ± SE in μmol/kg · min. *$P < 0.05$ for differences between euglycemia and hypoglycemia in diabetes patients versus normal subjects.

TABLE 3
Plasma free fatty acid concentration and glycerol kinetics in normal subjects and patients with diabetes in the baseline and the last 40 min of a 220-min hyperinsulinemic clamp period with either euglycemia or hypoglycemia

	Baseline	Euglycemia	Baseline	Hypoglycemia	*P* values*
Normal subjects					
Free fatty acids	565 ± 71	282 ± 37	683 ± 56	962 ± 103	0.001
Glycerol	72 ± 4	32 ± 2	74 ± 14	85 ± 8	0.001
Glycerol turnover	2.75 ± 0.08	1.35 ± 0.10	1.87 ± 0.06	4.00 ± 0.05	0.001
WB Glyc-to-Glc	0.71 ± 0.02	0.51 ± 0.05	0.44 ± 0.18	0.60 ± 0.15	0.020
Renal Glyc-to-Glc	0.26 ± 0.04	0.15 ± 0.02	0.28 ± 0.09	0.48 ± 0.11	0.040
Diabetic patients					
Free fatty acids	694 ± 42	62 ± 5	629 ± 67	764 ± 91	0.001
Glycerol	68 ± 6	45 ± 5	78 ± 8	96 ± 6	0.001
Glycerol turnover	2.39 ± 0.34	1.40 ± 0.11	2.42 ± 0.40	3.47 ± 0.80	0.001
WB Glyc-to-Glc	1.39 ± 0.17	1.18 ± 0.08	1.47 ± 0.21	1.76 ± 0.16	0.040
Renal Glyc-to-Glc	0.53 ± 0.24	0.35 ± 0.27	0.66 ± 0.30	0.79 ± 0.45	0.050

Data are means ± SE in micromoles per liter for free fatty acid and glycerol concentrations, and in micromoles per kilograms times minute for rates of glycerol turnover and glycerol conversion to glucose (Glyc-to-Glc) in whole body (WB) or in the kidney (renal). **P* values for comparisons of the differences between baseline and clamp periods in each group. Differences between groups did not reach statistical significance.

equivalent hypoglycemia in the patients with diabetes, the renal norepinephrine balance only increased from 0.16 ± 0.08 to 0.65 ± 0.31 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (from 0.95 ± 0.47 to 3.84 ± 1.83 $pmol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.02$, for the comparison of the changes from baseline between groups). The net renal norepinephrine balance did not change during euglycemic hyperinsulinemia in either group during any of the studies.

Symptoms. Both the patients with diabetes and the normal subjects developed mild symptoms of hypoglycemia during the hypoglycemic clamp procedures (data not shown). The mean heart rates did not change significantly during the periods of hypoglycemia in either the patients with diabetes (at baseline, 82 ± 6 beats/min; during the final 40 min of hypoglycemia, 92 ± 7 beats/min) or the normal subjects (at baseline, 80 ± 5 beats/min; during the final 40 min of hypoglycemia, 88 ± 6 beats/min).

DISCUSSION

We found that adrenergic stimulation of renal glucose output in response to hypoglycemia is impaired and contributes to defective counterregulation in patients with long-standing type 1 diabetes and no clinical evidence of classical autonomic neuropathy. This impairment would be expected to make them more susceptible to severe and prolonged episodes of hypoglycemia. Because patients with type 1 diabetes cannot dissipate exogenously administered insulin and lack the glucagon secretion necessary to rapidly reverse the insulin-suppressed hepatic glucose production, activation of the autonomic nervous system becomes their main defense against hypoglycemia (25). As a result, the attenuation and correction of the fall in blood glucose concentration depend heavily on adrenergic stimulation of gluconeogenesis, a process shared by the liver and kidney (8,25). Although epinephrine also stimulates hepatic glycogen degradation, gluconeogenesis is the predominant source of endogenous glucose production during prolonged hypoglycemia (25). Recurrent episodes of hypoglycemia have been shown to reduce autonomic and symptomatic responses to subsequent hypoglycemia (15,16), thus increasing the risk of iatrogenic and asymptomatic hypoglycemia. Considering that our patients did not report any episodes of hypoglycemia requiring either hospitalization or the assistance of others during the 4-week period preceding the study, and that there were no capillary blood glucose measurements <3.8 mmol/l (68 mg/dl) 3 days before the experiment, the subnormal adrenergic responses observed in our studies are consistent with the concept of "elevated" glycemic threshold for epinephrine secretion, i.e., lower blood glucose concentrations are required to elicit autonomic and symptomatic responses to hypoglycemia (15,16,25). This threshold abnormality is characteristically seen in patients with type 1 diabetes with >10 years' duration, and it is particularly common in those who are C-peptide–negative and have adequate HbA_{1c} levels. The susceptibility to recurrent hy-

TABLE 4
Plasma concentrations of insulin, C-peptide, glucagon, and catecholamines in normal subjects and patients with diabetes in the baseline and the last 40 min of a 220-min hyperinsulinemic clamp period with either euglycemia or hypoglycemia

	Baseline	Euglycemia	Baseline	Hypoglycemia
Insulin				
Normal subjects	49 ± 7	206 ± 15	43 ± 12	167 ± 22
Diabetic patients	56 ± 12	262 ± 5	45 ± 7	240 ± 21
C-peptide				
Normal subjects	581 ± 28	122 ± 18	692 ± 25	188 ± 14
Diabetic patients	188 ± 25	112 ± 14	136 ± 26	122 ± 13
Glucagon				
Normal subjects	106 ± 4	81 ± 8*	95 ± 14	148 ± 21*
Diabetic patients	52 ± 8	47 ± 5*	58 ± 12	63 ± 16*
Epinephrine				
Normal subjects	74 ± 14	83 ± 21*	63 ± 12	457 ± 41*
Diabetic patients	99 ± 15	115 ± 14*	79 ± 25	227 ± 31*
Norepinephrine				
Normal subjects	164 ± 14	155 ± 34*	151 ± 14	235 ± 19*
Diabetic patients	92 ± 17	142 ± 21*	123 ± 10	182 ± 31*

Data are means ± SE in picomoles per liter for plasma insulin and C-peptide, and in picograms per milliliter for plasma glucagon, epinephrine and norepinephrine. To convert plasma epinephrine and norepinephrine values to picomoles per liter, multiply by 5.458 and 5.911, respectively. **P* < 0.05 for differences between euglycemia and hypoglycemia in diabetic patients versus normal subjects.

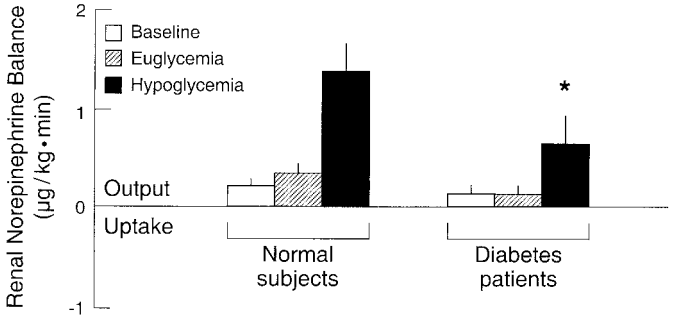

FIG. 3. Mean ± SE net renal norepinephrine balance in 14 normal subjects and in 12 patients with type 1 diabetes in the baseline (□) and during the last 40 min of 220-min periods of hyperinsulinemia with either euglycemia (▨) or hypoglycemia (■). *P = 0.02 for differences between euglycemia and hypoglycemia in diabetic patients versus normal subjects.

poglycemia is believed to be secondary to undetected episodes of nocturnal hypoglycemia, which occur frequently during intensive insulin therapy (26–28).

Interest in the potential role of the kidney in the regulation of glucose metabolism has been revived since our original report (7). In humans, postabsorptive renal glucose production and utilization rates have been estimated in the range of 5–28% of whole-body glucose turnover by various groups of investigators (8–10). A more recent study has concluded that this variability is partially due to methodological noise and that the renal contribution to endogenous glucose production lies between 4 and 18% (29). In our experience, with studies performed in a heterogeneous group of healthy male and female volunteers (n = 42) combining left renal vein catheterization and arterialized hand vein sampling with infusion of [6,6-$^2H_2$] glucose, postabsorptive renal glucose production averages 1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (~14% of endogenous glucose production), with a standard deviation of 4.16 and a coefficient of variation of 2.44. These results are in close agreement with those obtained by others (8–10,29) and further support the notion that because quantitation of renal glucose flux is technically difficult and variable, highly sensitive methods and a large number of subjects must be used in studies designed to evaluate renal glucose production and utilization rates. These data also confirm that the contribution of the kidney to glucose appearance rates in postabsorptive humans is negligible compared with that of the liver, although observations that renal glucose production increases substantially with prolonged fasting (6), in type 2 diabetes (30), in insulin-induced hypoglycemia (11,12), and during the anhepatic phase of liver transplantation (31) strongly suggest that the kidney plays a more important role in glucose homeostasis than previously appreciated.

There is little or no controversy regarding the fact that the liver is the primary site of glucose production in most circumstances. In this study, for example, the relative contribution of the kidney to endogenous glucose production during hypoglycemia in normal subjects did not exceed 35% (i.e., 5.5 of 15.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and net glucose output increased to a maximum of 4.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Hence, the inadequate compensatory rise in renal glucose production in diabetic patients represents a small abnormality in comparison with that of the liver. Hepatic glucose production, which accounted for ~65% (i.e., 9.5 of 15.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of endogenous glucose production during hypoglycemia in normal subjects, was responsible for nearly 100% (~6.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of glucose appearing in the circulation in diabetic patients under a comparable degree of hypoglycemia. Therefore, our studies uncover an abnormality in glucose handling by the kidney, in addition to that of the liver, in response to hypoglycemia and further reaffirm that the liver represents the predominant organ responsible for the compensatory rise in endogenous glucose production during hypoglycemia in both normal subjects and patients with diabetes. In theory, however, the body's ability to prevent and correct hypoglycemia without the full contribution of the kidney is incomplete and sufficient to impair normal blood glucose recovery.

The findings that plasma glucagon remained essentially unchanged, and that elevations in both plasma catecholamines and renal norepinephrine overflow were markedly attenuated during sustained hypoglycemia, indicate that the lack of compensatory rise in endogenous (hepatic and renal) glucose production observed in patients with diabetes was secondary to insufficient hormonal stimulation of glucose production. Specifically, our data demonstrate that both adrenomedullary (circulating epinephrine) and sympathetic neural (renal norepinephrine overflow) responses to a given level of hypoglycemia are reduced early in these patients. These results are in agreement with those reported by others (32,33) and further suggest that insufficient stimulation of renal, in addition to hepatic, gluconeogenesis due to reduced autonomic responses provides incomplete protection against hypoglycemia in patients with type 1 diabetes. The extent to which the lack of glucose release into the circulation by the kidney in these experimental conditions of mild-to-moderate hypoglycemia is the result of limited substrate supply remains unclear. Nonetheless, these abnormalities are analogous to other defects in the glucose counterregulatory responses (34), and with no evidence of intrinsic renal damage, they may be reversed by scrupulous avoidance of hypoglycemia.

Demonstration that net glucose output by the kidney, which represents an additional physiological mechanism in the defense against hypoglycemia, is impaired in patients with diabetes is of considerable significance. Hypoglycemia is the limiting factor and the most feared complication in the management of diabetes (2), and it is associated with substantial morbidity, particularly in patients on intensive insulin therapy (1). To significantly reduce the occurrence of hypoglycemia in these patients, we must learn how to avoid and compensate for the compromised glucose counterregulatory responses. Our findings indicate that protection against the fall in blood glucose concentration in patients with diabetes is largely accomplished by the activation of the autonomic nervous system and relies partly on stimulation of renal gluconeogenesis. This may represent an adaptation to the loss of glucagon secretion during hypoglycemia. Therefore, besides improving our ability to deliver more physiological insulin replacement and implementing strategies that aim to reduce the conventional risk factors (34,35), preservation of renal blood flow and function may be critical in

restoring normal glucose counterregulation in patients with type 1 diabetes.

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