Plasma Metanephrine Levels Are Decreased in Type 1 Diabetic Patients with a Severely Impaired Epinephrine Response to Hypoglycemia, Indicating Reduced Adrenomedullary Stores of Epinephrine

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A defective epinephrine response to hypoglycemia is a common disorder in type 1 diabetes. We assessed the role of the adrenomedullary capacity to secrete epinephrine in this disorder by measuring plasma metanephrine levels in affected type 1 diabetic patients compared with those in matched nondiabetic controls. Metanephrine is formed from epinephrine that leaks from adrenomedullary storage vesicles by catechol-*O*-methyl transferase (COMT) and is continuously released into the circulation. Thus, plasma metanephrine levels reflect adrenomedullary epinephrine content and, provided there is normal COMT activity, the adrenomedullary capacity to secrete epinephrine. Diabetic patients had approximately 25% lower plasma metanephrine levels than controls (0.18 ± 0.09 *vs.* 0.24 ± 0.02 nmol/liter; $P = 0.012$), whereas plasma epinephrine, norepinephrine, and normetanephrine levels were comparable between patients and controls. In response to hypoglycemia, the increments in plasma epinephrine and plasma metanephrine levels were both significantly lower in diabetic patients than in controls ($P < 0.001$), but the increase in plasma metanephrine as a percentage of the increase in plasma epinephrine was identical, indicating similar COMT activity. We conclude that type 1 diabetic patients with an impaired epinephrine response to hypoglycemia have lower plasma metanephrine levels than matched controls, reflecting decreased adrenomedullary stores of epinephrine and indicating reduced adrenomedullary capacity to secrete epinephrine. (*J Clin Endocrinol Metab* **89:** 2057–2061, 2004)

HYPOGLYCEMIA IS A very common phenomenon for virtually all patients with type 1 diabetes. The risk for iatrogenic hypoglycemia sharply increases when an already absent glucagon response is accompanied by failure to sufficiently secrete epinephrine in response to hypoglycemia (1). In addition, because of epinephrine's role in the appearance of hypoglycemic (warning) symptoms, impaired epinephrine responses may adversely affect the perception of hypoglycemia and, as such, contribute to unawareness of hypoglycemia. In the absence of autonomic neuropathy, it has been proposed that the impaired epinephrine response to hypoglycemia is caused by resetting the threshold value for epinephrine release to a lower glucose level (2). This hypothesis is based on observations that glycemic thresholds for epinephrine release shift to lower glucose levels after antecedent hypoglycemia (3) and after improvement of metabolic control (4–9), whereas they shift to higher glucose levels when hypoglycemia is avoided (10–12) and metabolic control is relaxed (13).

Recent observations, however, suggest that a reduced adrenomedullary secretory capacity contributes to the impaired epinephrine response. For instance, antecedent hypoglycemia has been found to suppress adrenergic responses

to subsequent exercise (14), and reduced adrenergic responses to exercise have been reported in well controlled diabetic patients without autonomic neuropathy (15, 16). Others, however, found no effect of antecedent hypoglycemia on adrenergic responses to exercise (17) or reported that the capacity to secrete epinephrine during exercise remained unaltered in diabetic patients despite recurrent hypoglycemia (18). Differences in the intensity and duration of, and the compliance with the exercise protocols used in the various studies may explain some of these conflicting data. In addition, exercise tests are not the most profound stimulus for the adrenal medulla, so normal epinephrine responses can occur despite reduced adrenomedullary secretory capacity.

The introduction of a sensitive method for measurements of free plasma metanephrines has provided a reliable means to estimate adrenomedullary capacity (19–21). Within the adrenal medulla, epinephrine leaking from storage vesicles into the cytoplasm is converted to metanephrine by catechol-*O*-methyl transferase (COMT). In contrast to epinephrine, metanephrine is continuously released into the bloodstream, almost independently of adrenergic stimulation (22). As over 90% of circulating metanephrine has an adrenomedullary source (23), plasma metanephrine levels reflect adrenomedullary stores of epinephrine and, provided that COMT activity is normal, the adrenomedullary capacity to secrete epinephrine. In the present study we measured plasma metanephrine levels to determine whether the adrenomedullary secretory capacity is reduced in type 1 diabetic patients with

impaired epinephrine responses to hypoglycemia. We used a hyperinsulinemic normoglycemic-hypoglycemic glucose clamp to correct for an effect of hyperglycemia (24) and to investigate the effect of hypoglycemia on metanephrine release in relation to epinephrine release.

Subjects and Methods

From a previous study we selected 10 patients who had an epinephrine response to hypoglycemia that was below the lower limit of the normal response and 10 matched nondiabetic controls (25). All patients used regular insulin or insulin analogs in a basal-bolus regimen or in an sc pump. No patients had signs of autonomic neuropathy, as measured by standard cardiovascular reflex tests (heart rate responses during deep breathing, heart rate response to Valsalva maneuver, heart rate and blood pressure responses to standing, and blood pressure response to sustained handgrip) (26), and no one used medication other than insulin or oral contraceptives. The study was approved by the local hospital ethics committee, and all participants gave written informed consent.

Hyperinsulinemic clamp

Patients were instructed to reduce the evening insulin dose or nocturnal insulin infusions before the studies to avoid nocturnal hypoglycemia. Both patients and controls were admitted to the research unit at 0800 h after an overnight fast and having abstained from caffeine-containing beverages for at least 48 h. The brachial artery of the nondominant arm was cannulated under local anesthesia for blood sampling and blood pressure recording. The antecubital vein of the contralateral arm was cannulated for infusion of insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) and glucose. Baseline variables were obtained after a stabilization period of 30–45 min. Thereafter, a hyperinsulinemic (360 pmol/m^{-2}·min) hypoglycemic glucose clamp procedure was performed, as described previously (25). Using a variable infusion of 20% glucose, the arterial plasma glucose concentration was sequentially clamped at 5.0, 3.5, and 2.5 mmol/liter at hourly intervals, guided by plasma glucose levels measured in duplicate every 5 min by the glucose oxidation method (Glucose Analyzer II, Beckman, Fullerton, CA). Arterial blood was sampled for measurements of plasma catecholamines and metanephrines at baseline, during the final 30 min of clamped normoglycemia, and during the final 30 min of the second hypoglycemic phase (2.5 mmol/liter).

Analytical methods

Plasma epinephrine and norepinephrine were analyzed by HPLC with fluorometric detection, as described previously (27). Plasma metanephrine and normetanephrine levels were determined by HPLC and electrochemical detection (interassay coefficient of variation of 6.4% for metanephrine and of 6.8% for normetanephrine) (28). Plasma insulin and C peptide were measured by RIAs (25).

Calculations and statistics

COMT activity was assessed by the following methods. Firstly, based on earlier reports in rodents, metanephrine/epinephrine (M/E) ratios were calculated for each individual participant at baseline and during normoglycemia (24). Secondly, we calculated the absolute increase in plasma metanephrine level above baseline as a percentage of the absolute increase in plasma epinephrine level in response to hypoglycemia. Serial data were compared by two-way ANOVA, and means were compared by *t* test. For data that had no normal distribution, we used the Wilcoxon signed rank test and Mann-Whitney U test to compare paired and unpaired data, respectively. For calculations and statistical analyses, the SPSS personal computer software package version 9.0 (SPSS, Inc., Chicago, IL) was used, and $P < 0.05$ was considered statistically significant. Results are presented as the mean ± SEM unless otherwise specified.

Results

The characteristics of the participants are given in Table 1. As expected, type 1 diabetic patients had higher hemoglobin

TABLE 1. Baseline characteristics

	Type 1 DM patients	Healthy controls
No. (M/F)	10 (5/5)	10 (5/5)
Age (yr)	32.7 ± 10.9	32.2 ± 8.7
BMI (kg/m^2)	23.4 ± 2.6	22.9 ± 2.1
Duration of diabetes (yr)	15.0 ± 6.4	
HbA$_{1c}$ (%)	7.3 ± 0.5[a]	5.1 ± 0.2
Fasting plasma glucose (mmol/liter)	12.2 ± 3.2[a]	5.2 ± 0.5
C peptide (mmol/liter)	<0.01[a]	0.40 ± 0.14
Insulin (pmol/liter)	337 ± 215[b]	65 ± 18

Data are the mean ± SD. To convert plasma glucose values to mg/dl, multiply by 18; to convert plasma insulin values to μU/ml, divide by 6.0. BMI, Body mass index; DM, diabetes mellitus; HbA$_{1c}$, hemoglobin A.

[a] $P < 0.005$ *vs.* controls.
[b] $P < 0.05$ *vs.* controls.

A$_{1C}$, fasting plasma glucose and insulin levels, and lower C peptide levels than controls (all $P < 0.05$). At baseline, there were no differences in arterial plasma epinephrine, norepinephrine, or normetanephrine levels between patients and controls, but plasma metanephrine levels were 25% lower in diabetic patients than in controls ($P = 0.012$; Table 2). As a consequence, diabetic patients had a lower M/E ratio than controls (0.93 ± 0.11 *vs.* 1.72 ± 0.30; $P = 0.034$).

Normoglycemia

Plasma glucose levels obtained during the normoglycemic phase of the clamp were similar in patients and controls (5.0 ± 0.0 *vs.* 4.9 ± 0.1 mmol/liter; $P = 0.16$). Plasma insulin levels increased by similar amounts in both groups, so that insulin levels remained somewhat higher in patients than in controls (836 ± 92 *vs.* 559 ± 24 pmol/liter; $P = 0.013$). During normoglycemia, plasma epinephrine levels did not change significantly in control or diabetic subjects. Plasma metanephrine levels decreased in controls ($P = 0.008$) and tended to decrease in patients ($P = 0.10$), but remained about 25% lower in patients than in controls ($P = 0.019$). As a consequence, the M/E ratio decreased in both patients and controls (both $P < 0.05$) during normoglycemia, but remained lower in the patients (0.58 ± 0.07 *vs.* 1.08 ± 0.12; $P = 0.005$). Plasma norepinephrine levels increased in both patients ($P = 0.007$) and controls ($P = 0.005$), whereas plasma normetanephrine levels did not change in either group.

Hypoglycemia

Plasma glucose levels obtained during the hypoglycemic phase of the clamp were 2.4 ± 0.0 mmol/liter in the patients and 2.5 ± 0.0 mmol/liter in the controls ($P = 0.24$). Hypoglycemia increased plasma epinephrine and metanephrine levels in both patients and controls (all $P < 0.01$; Table 2). Plasma epinephrine levels increased by 52-fold in the control and by 12-fold in the diabetic subjects ($P < 0.001$). Plasma metanephrine levels increased by 2.9-fold in the control subjects and by 2.0-fold in the diabetic patients ($P < 0.001$). The absolute increase above baseline in plasma concentration of metanephrine as a percentage of the absolute increase in plasma concentration of epinephrine was 6.7 ± 0.9% in control subjects and 6.7 ± 1.5% in diabetic patients ($P = 0.97$; Fig.

TABLE 2. Plasma levels of catecholamines and metanephrines at baseline and during the normoglycemic and hypoglycemic phases

	Type 1 DM patients	Healthy controls	*P* value
Epinephrine (nmol/liter)			
Baseline	0.22 ± 0.03	0.19 ± 0.03	NS
Normoglycemia (5.0 mmol/liter)	0.31 ± 0.05	0.22 ± 0.03	NS
Hypoglycemia (2.5 mmol/liter)	2.49 ± 0.29[a]	7.60 ± 0.87[a]	<0.0001
Metanephrine (pmol/liter)			
Baseline	0.18 ± 0.01	0.24 ± 0.02	0.012
Normoglycemia (5.0 mmol/liter)	0.16 ± 0.01	0.20 ± 0.01[b]	0.019
Hypoglycemia (2.5 mmol/liter)	0.34 ± 0.03[a]	0.69 ± 0.05[a]	<0.0001
Norepinephrine (nmol/liter)			
Baseline	0.76 ± 0.11	0.68 ± 0.09	NS
Normoglycemia (5.0 mmol/liter)	1.15 ± 0.12[b]	0.90 ± 0.07[b]	NS
Hypoglycemia (2.5 mmol/liter)	1.80 ± 0.21[a]	2.92 ± 0.55[a]	0.005
Normetanephrine (pmol/liter)			
Baseline	0.16 ± 0.01	0.20 ± 0.02	NS
Normoglycemia (5.0 mmol/liter)	0.17 ± 0.01	0.16 ± 0.01	NS
Hypoglycemia (2.5 mmol/liter)	0.25 ± 0.02[a]	0.26 ± 0.03[a]	NS

Data are the mean ± SEM. *P* values given denote differences between patients and controls. To convert plasma values for epinephrine and norepinephrine to pg/ml, multiply by 183.2 and 169.2, respectively; to convert plasma values for metanephrine and normetanephrine to pg/ml, divide by 5.08 and 5.46, respectively.

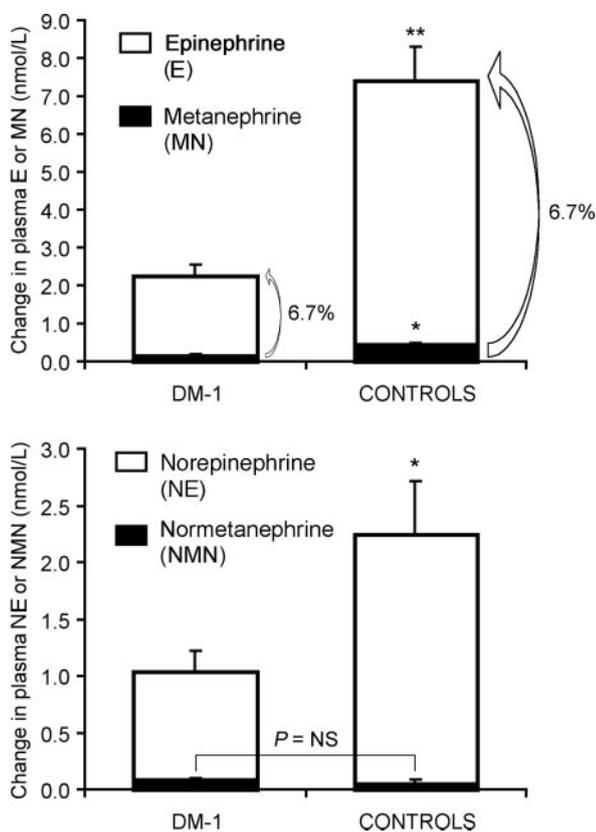[a] $P < 0.01$ for trend from baseline (by ANOVA).
[b] $P < 0.01$ *vs.* baseline.

FIG. 1. Mean (±SEM) increases in plasma epinephrine and metanephrine (*top*) and in plasma norepinephrine and normetanephrine (*bottom*) in response to hypoglycemia. In both patients and controls, the increase in plasma metanephrine was 6.7% that of plasma epinephrine (P = NS). *, $P < 0.05$; **, $P < 0.001$ (higher plasma concentration *vs.* that in diabetic patients).

1). In response to hypoglycemia, plasma norepinephrine levels increased by 2.7-fold in diabetic patients and by 4.3-fold in controls (P = 0.015), whereas plasma normetanephrine levels increased slightly in diabetic patients and controls, but to a similar extent (Fig. 1).

Discussion

The results of the present study indicate that type 1 diabetic patients with an impaired epinephrine response to hypoglycemia have a reduced adrenomedullary capacity to secrete epinephrine. This conclusion is based on the finding of lower plasma metanephrine levels in patients compared with matched controls both at baseline and after obtaining normoglycemia in either group. In contrast, plasma levels of epinephrine, norepinephrine, or normetanephrine were comparable between patients and controls at baseline and throughout normoglycemia. A finding that has not been documented previously is that in addition to the reduced responses of plasma epinephrine and norepinephrine to hypoglycemia, plasma metanephrine levels increased significantly less during hypoglycemia in diabetic patients than in controls. However, the hypoglycemia-induced increase in plasma metanephrine calculated as a percentage of the increase in plasma epinephrine was identical in patients and controls, suggesting similar peripheral (extraadrenal) conversion of epinephrine to metanephrine.

In humans, over 90% of plasma metanephrine is produced from epinephrine leaking from storage vesicles into the cytoplasm of chromaffin cells (23). This conversion is catalyzed by intracytoplasmatic COMT. Because metanephrine is continuously released into the bloodstream, reduced plasma metanephrine levels reflect reduced adrenomedullary storage of epinephrine (21), which, in turn, indicates a reduced capacity to secrete epinephrine. Our findings of a reduced adrenomedullary secretory capacity may help to explain previous observations of reduced adrenergic responses to exercise tests in well controlled diabetic patients, *i.e.* patients with probable impaired counterregulatory function (15, 16). Because hypoglycemia is one of the most profound stimuli of epinephrine secretion, a reduced capacity to secrete epinephrine is likely to be clinically relevant. However, the

finding of 25% lower plasma metanephrine levels in the diabetic patients, which is quantitatively less than the impaired epinephrine response to hypoglycemia, suggests that it is an additive component to the previously described resetting of the glycemic threshold value (2).

Under hypoglycemic conditions, plasma levels of metanephrine increased in both patients and controls. This increase in plasma metanephrine levels above baseline results from the metabolism of elevated epinephrine levels either after reuptake by the adrenals or by extraadrenal conversion, both of which are catalyzed by COMT (22, 29). Because hypoglycemia induced a lower epinephrine response in the diabetic patients, our observation of a blunted increase in plasma metanephrine during hypoglycemia was not unexpected. To assess whether an impairment in the metabolism of epinephrine contributed to the blunted increase in plasma metanephrine, we calculated the absolute change in plasma metanephrine as a percentage of the absolute change in plasma epinephrine induced by hypoglycemia. The increase in plasma metanephrine relative to the increase in plasma epinephrine levels was identical in patients and controls, which matches values reported previously (22) and indicates similar rates of metabolism and similar COMT activity in patients and controls, at least under hypoglycemic conditions. This is important because it makes it highly unlikely that the lower plasma metanephrine levels in diabetic patients at baseline were the result of reduced COMT activity within the adrenal medulla, as has been suggested by studies in liver homogenates from hyperglycemic streptozotocin-induced diabetic rats (24). It also challenges the usefulness of the M/E ratio as a surrogate marker for COMT activity (24).

It seems unlikely that our findings are explained by diabetic autonomic neuropathy. First of all, all patients had normal responses to cardiovascular reflex tests (26). Secondly, the normal basal plasma epinephrine levels in the diabetic patients argue against clinically relevant autonomic neuropathy. As adrenomedullary cells secrete their content directly into the circulation upon stimulation, basal plasma epinephrine levels generally reflect basal neuronal outflow to the adrenal medulla (21), which explains findings of reduced plasma epinephrine levels in patients with diabetic autonomic neuropathy (30–32). Finally, the normal plasma norepinephrine levels at baseline and the normal response to hyperinsulinemia (during clamped normoglycemia) further support the idea that autonomic nervous function was not substantially impaired.

Limitations of our study concern the small sample size and the indirect nature of our method to assess adrenomedullary epinephrine content and secretory capacity. Comparing adrenomedullary secretory capacity between diabetic patients and controls directly would require a stimulus of adrenomedullary epinephrine secretion other than hypoglycemia that is at least as profound. However, such a stimulus is unavailable for *in vivo* studies in humans. Another limitation is that it cannot be determined from our data whether the reduced plasma metanephrine levels in diabetic patients reflect a temporal depletion of adrenal stores or a structural loss of adrenal mass. Because hypoglycemia has been reported to suppress the adrenergic response to subsequent exercise (14), temporal exhaustion of adrenal stores seems a plausible phenomenon in patients with type 1 diabetes. In rats, recurrent hypoglycemia was found to both suppress the epinephrine response to subsequent hypoglycemia and to chronically enhance adrenal sympathetic tone. This observation indicates that the defect in epinephrine release occurred as a result of or at least persisted despite maximal stimulation, both of which are compatible with depletion of adrenal stores (33). It could be hypothesized that repeated hypoglycemia-induced exhaustion of adrenal stores may eventually become a structural defect. A structural loss of adrenal mass could explain why epinephrine responses to exercise tests remain lower in diabetic patients despite scrupulous avoidance of hypoglycemia (15, 16), and why hypoglycemia prevention programs do not completely restore the impaired epinephrine response to hypoglycemia (10).

In conclusion, our study shows that diabetic patients with established (hypoglycemia-induced) counterregulatory failure have low plasma metanephrine levels compared with nondiabetic controls. Assuming similar metabolic rates within the adrenal medulla, the lower plasma metanephrine levels reflect reduced epinephrine storage and reduced adrenomedullary secretory capacity. To what extent a reduced adrenomedullary capacity contributes to the failure to secrete sufficient amounts of epinephrine under hypoglycemic conditions cannot be derived from the present study, but assuming that hypoglycemia requires the maximum adrenal reserve, it is likely to be clinically relevant.

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