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High Plasma Prorenin in Diabetes Mellitus and Its Correlation with Some Complications

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ABSTRACT. Plasma prorenin is abnormally high, whereas renin is normal or even low, in many patients with long-standing diabetes mellitus complicated by microvascular disease. Nephropathy or autonomic neuropathy has been put forward as a cause. We found that in 223 consecutive diabetics prorenin correlated positively with serum creatinine, the presence of macroalbuminuria (>250 mg/L), and the presence of diabetic retinopathy, particularly the proliferative type. This correlation did not depend on the presence of neuropathy or whether the patient was receiving insulin. It was also independent of sex, age, duration of diabetes, blood pressure, and blood levels of glucose and hemoglobin-A1c. The association between elevated prorenin and retinopathy remained significant after adjustment for creatinine and the presence of macroalbuminuria. Of the whole group of diabetics 94 consecutive patients were assessed for the presence of microalbuminuria (30–300 mg/24 h). Independently of the presence of micro- or macroalbuminuria, the mean level of prorenin was not above normal in the patients without retinopathy and was 2–3 times normal in those with

proliferative retinopathy. Thus, retinopathy appears to be a more important determinant of abnormally high prorenin than nephropathy. In addition, the renal vein to artery ratio of prorenin in 7 diabetics with both advanced nephropathy and proliferative retinopathy was not elevated, despite the high peripheral venous prorenin level and the impaired renal perfusion. Thus, the abnormally high prorenin level in these patients could not be explained by abnormal secretion by the kidneys. Finally, prorenin was not high in 16 nondiabetics with loss of sympathetic activity due to chronic autonomic neuropathy, which indicates that in the absence of diabetes, this type of autonomic failure is not sufficient to cause the high prorenin levels seen in diabetics. Our findings are evidence that abnormally high plasma prorenin levels in diabetics are not an immediate consequence of altered glucose metabolism. This abnormality is related to the development of microvascular disease in the eye and kidney and is at least in part due to decreased clearance of prorenin from the circulation, increased production from extrarenal sources, or both. (*J Clin Endocrinol Metab* 71: 1008–1015, 1990)

PRORENIN is an inactive precursor of renin, which in plasma is the rate-limiting enzyme responsible for the formation of angiotensin-II, a peptide with important vascular actions. In long-standing diabetes mellitus complicated by microvascular disease, the plasma level of prorenin is often 2–3 times normal (1, 2), whereas renin is within or below the normal range (3, 4). The cause of the elevated plasma prorenin in diabetes is unknown. Prorenin is synthesized in the renal juxtaglomerular cells, where it is converted to renin. The kidney releases both renin and prorenin into the circulation (5, 6), and it is possible that in diabetic nephropathy the quantity of prorenin released by the kidneys is disproportionately high because of abnormal enzyme processing or secretion (7).

Diabetic autonomic neuropathy has also been put forward as a cause. Loss of normal adrenergic stimulation of the renal juxtaglomerular cells might lead to a decrease in the secretion of renin stored in these cells and to a compensatory increase in the synthesis and secretion of prorenin. High plasma levels of prorenin have indeed been documented in some diabetics with autonomic neuropathy (8, 9). However, autonomic neuropathy was not the only diabetic complication in these patients.

In the present study we examined in a group of diabetics the statistical correlations between the plasma level of prorenin and the presence of nephropathy, neuropathy, and retinopathy. We also studied a group of nondiabetic patients with loss of sympathetic function due to autonomic neuropathy in order to examine whether loss of adrenergic stimulation, in the absence of diabetes and its complications, is associated with an elevated plasma prorenin level. We finally measured plasma prorenin in the renal veins of patients with diabetic nephropathy in an attempt to demonstrate increased secretion of prorenin by the kidney.

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Subjects and Methods

Out-patients with diabetes mellitus

This group ($n = 223$) consisted of all diabetics attending our out-patient facilities in 3 consecutive months, with the exception of patients with overt cardiac failure, hepatic disease, or renal failure (serum creatinine, $>200 \mu\text{mol/L}$). Patients on drugs likely to affect the plasma level of renin were also not included. Some clinical characteristics of these patients are given in Table 1. In the study group, 136 were receiving insulin, 82 were receiving oral antidiabetic drugs, and 5 were treated by diet alone. The patients were seen by an internist and an ophthalmologist, who were unaware of the results of renin measurements. Nontimed overnight urine collections obtained on 2 different days were tested for the presence of protein. Macroalbuminuria was defined as an albumin level above 250 mg/L in each of the 2 samples. An assessment of microalbuminuria was made in the patients visiting the out-patient clinic in the last 2 months, excluding those with macroalbuminuria and those with a history of kidney or urinary tract disease. Clinical characteristics of these patients are given in Table 2. Microalbuminuria was defined as albumin levels between $30\text{--}300 \text{ mg/24 h}$ in at least 2 of 3 consecutive 24-h urine collections (10).

Diabetic retinopathy was assessed by direct and indirect ophthalmoscopy after pupillary dilatation and in most cases also by fluorescein angiography. Grading of the severity of

TABLE 1. Clinical characteristics of diabetic patients with and without macroalbuminuria

	Macroalbuminuria		P value
	Absent	Present	
Sex (no. of female/male)	89/72	26/36	NS ^a
Age (yr)			
Median	56	54	NS
Range	21–87	20–92	
Duration of diabetes (yr)			
Median	9	15	<0.05
Range	1–55	1–47	
Insulin treatment (no. on insulin/not on insulin)	95/66	41/21	NS
Neuropathy (no. of subjects; absent/present)	127/34	44/18	NS
Diabetic retinopathy (no.)			
Absent/present	84/77	15/47	<0.001
Background/proliferative	49/28	11/36	<0.001
HbA1c [%; mean (SD)]	9.8 (1.2)	10.1 (0.9)	NS
Serum creatinine			
Mean (SD), $\mu\text{mol/L}$	82 (18)	101 (20)	<0.001
No. $>110 \mu\text{mol/L}$	10	19	<0.001
Blood pressure (mm Hg)			
Systolic [mean (SD)]	149 (24)	148 (21)	NS
Diastolic [mean (SD)]	81 (12)	86 (12)	NS
Renin (mU/L)			
Geometric mean	12.5	15.7	NS
95% confidence interval	2.4–65.0	3.3–76.4	
Prorenin			
Geometric mean	196	373	<0.001
95% confidence interval	44.1–873	88.0–1579	

^a $P > 0.05$.

TABLE 2. Clinical characteristics of diabetic patients with and without microalbuminuria

	Microalbuminuria		P value
	Absent	Present	
Sex (no. of female/male)	36/38	10/10	NS ^a
Age (yr)			
Median	49	55	<0.05
Range	21–76	38–86	
Duration of diabetes (yr)			
Median	11	13	NS
Range	1–39	1–45	
Insulin treatment (no. on insulin/not on insulin)	43/31	12/8	NS
Neuropathy (no. of subjects; absent/present)	62/7	17/3	NS
Diabetic retinopathy (no.)			
Absent/present	39/35	6/14	NS
Background/proliferative	17/18	9/5	NS
HbA1c [%; mean (SD)]	9.7 (1.2)	10.3 (0.9)	NS
Serum creatinine			
Mean (SD)	79 (19)	80 (20)	NS
No. $>110 \mu\text{mol/L}$	4	2	NS
Blood pressure (mm Hg)			
Systolic [mean (SD)]	144 (24)	160 (21)	<0.05
Diastolic [mean (SD)]	79 (19)	86 (12)	NS
Renin (mU/L)			
Geometric mean	11.4	7.6	NS
95% confidence interval	2.1–61.8	1.6–35.1	
Prorenin (mU/L)			
Geometric mean	166	163	NS
95% confidence interval	43.0–632	40.1–653	

^a $P > 0.05$.

diabetic retinopathy was based on the findings at ophthalmoscopy and was performed by the ophthalmologist. For the purpose of the present analysis, diabetic retinopathy was categorized as either background retinopathy or proliferative retinopathy. Background retinopathy was characterized as the presence of microaneurysms, whether or not they were combined with hemorrhages and hard exudates (11). Eyes with preproliferative retinopathy, characterized by multiple cotton wool spots, venous abnormalities, beading and duplications, intraretinal microvascular abnormalities, and diffuse large blot hemorrhages, were also categorized as background diabetic retinopathy. Patients with new vessels anywhere in the retina in either eye were categorized as having proliferative retinopathy. Of the 65 patients with proliferative diabetic retinopathy, 55 had been treated by laser photocoagulation of 1 or both eyes. Sensory or motor fiber neuropathy was defined as diminished sense of vibration at the lower or upper extremities and loss of Achilles or patellar tendon reflexes (12), which is generally associated with other signs of neuropathy or with symptoms such as sensory loss, distal paresthesia, or pain. No attempt was made to exclude or diagnose autonomic neuropathy, but none of the patients had symptomatic orthostatic hypotension.

Patients with diabetic nephropathy

Serum creatinine in these patients ($n = 7$) was above $300 \mu\text{mol/L}$, and total effective renal plasma flow measured by [^{131}I] hippurate clearance (13) was less than 130 mL/min . They also

TABLE 3. Plasma levels of prorenin in various categories of diabetics

Factor	Category	n	Prorenin (mU/L)	P value
Sex	Female	117	194 (43.0–844)	<0.001
	Male	106	291 (63.6–1330)	
Insulin	On insulin	87	203 (49.5–827)	<0.01
	Not on insulin	136	262 (51.5–1330)	
Proteinuria (>250 mg/L)	Absent	161	193 (43.2–848)	<0.0001
	Present	62	368 (85.7–1580)	
Neuropathy	Absent	172	219 (46.0–1040)	NS
	Present	51	276 (54.6–1400)	
Retinopathy	Absent	99	165 (46.5–589)	<0.01 ^a
	Background	60	228 (51.6–1010)	
	Proliferative	64	416 (101–1710)	

Prorenin is given as the geometric mean and 95% confidence interval. NS, $P > 0.05$. The difference between patients with and without neuropathy was of borderline significance ($P = 0.06$).

^a vs. Background.

FIG. 1. Plasma levels of prorenin in patients without diabetic retinopathy (NODR), background diabetic retinopathy (BGDR), or proliferative diabetic retinopathy (PDR). The patients are subdivided into those with and without macroalbuminuria. Horizontal lines indicate geometric means. Note the logarithmic scale. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (difference from the group without diabetic retinopathy). †, $P < 0.05$; ‡, $P < 0.01$ (difference from the group with background diabetic retinopathy). Hatched bar, normal range.

had proliferative diabetic retinopathy. There were no symptoms or signs of autonomic neuropathy. Orthostatic hypotension was absent, and the Valsalva response was normal. Peripheral venous plasma prorenin was elevated (>360 mU/L) in each patient. The patients were studied at the time they were undergoing aortic angiography in preparation of receiving a kidney transplant. Blood was sampled from the aorta and renal veins before the contrast injection was given.

Nondiabetic patients with chronic autonomic neuropathy

These patients ($n = 16$; aged 34–80 yr) have been described in detail previously (14). All had severe incapacitating orthostatic hypotension. Disruption of the integrity of the baroreflex

arc with loss of normal sympathetic function was confirmed by the absence of a systolic blood pressure overshoot in phase 4 of the Valsalva response. The resting plasma level of norepinephrine was low in the patients with pre- or postganglionic lesions. In all patients plasma norepinephrine did not respond to head up tilt on a tilting table for 30 min, causing a blood pressure drop of 20–25 mm Hg systolic and 10–15 mm Hg diastolic.

Blood sampling

In the group of out-patients with diabetes blood samples for measuring renin and prorenin were drawn from an antecubital vein after the patients had rested for 30 min in the sitting position. Blood samples for measuring creatinine, glucose, and

MICROALBUMINURIA

FIG. 2. Plasma levels of prorenin in patients without diabetic retinopathy (NODR), background diabetic retinopathy (BGDR), or proliferative diabetic retinopathy (PDR). The patients are subdivided into those with and without microalbuminuria. Horizontal lines indicate geometric means. Note the logarithmic scale. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (difference from the group without diabetic retinopathy). †, $P < 0.05$; ‡, $P < 0.01$ (difference from the group with background diabetic retinopathy). Hatched bar, normal range.

TABLE 4. Correlations of (log) prorenin with various factors in diabetes

	r	P value
Age	0.07	NS ^a
Duration of diabetes	0.24	<0.001
Systolic blood pressure	-0.07	NS
Diastolic blood pressure	0.02	NS
Serum creatinine	0.37	<0.001
Blood glucose	0.11	NS
HbA1c	0.09	NS

r is the Pearson correlation coefficient.

^a $P > 0.05$.

glycosylated hemoglobin (HbA1c; normal range, 5.5–7.3%) were also taken. In the nondiabetics with autonomic neuropathy peripheral venous blood was collected in the supine position. During the renal-vein catheterization studies blood was sampled from the renal vein on each side and at the same time from the abdominal aorta.

Measurements of renin, prorenin, and norepinephrine

The plasma levels of renin and prorenin were measured with the enzyme kinetic assay, in which the angiotensin-I generated by plasma *in vitro* was quantitated by RIA (15). For measuring prorenin, the proenzyme was quantitatively converted to renin at 4 C by immobilized trypsin (5, 16). That we were measuring true prorenin and not a renin-like enzyme, was supported by comparing the results of the enzyme kinetic assay with those of a direct RIA (16, 17). The latter was solid phase sandwich assay in which two monoclonal human renin antibodies, which reacted equally well with kidney renin and chorionic cell culture prorenin, were used. Measurements in plasma samples from diabetics with prorenin levels ranging from 100–1000 mU/L, as determined by the indirect enzyme kinetic assay, showed excellent agreement between the direct and indirect assays. The specific enzymatic activity of *in vitro* activated prorenin in plasma of diabetics was 0.8 ± 0.1 mU/ng (mean $\pm$ SD; $n = 8$), which is not different from the specific activity of purified

TABLE 5. Effects of diabetic retinopathy on mean (log) prorenin and SEs estimated by multiple regression analysis

Factor	Diabetic retinopathy grouping not included			Diabetic retinopathy grouping included		
	Effect	SE	P value	Effect	SE	P value
Constant (intercept)	0.94	0.26		1.09	0.24	
Creatinine	0.024	0.005	<0.0001	0.023	0.05	<0.001
Creatinine square	-0.000097	0.000024	<0.0001	-0.000092	0.000022	0.0001
Insulin therapy	0.08	0.04	0.06	0.02	0.04	0.7
Proteinuria (>250 mg/L)	0.19	0.05	<0.001	0.10	0.05	<0.05
No retinopathy	Not tested			-0.10	0.05	<0.05
Proliferative diabetic retinopathy	Not tested			0.19	0.06	0.001

Effects denote mean increase of (log) prorenin by: 1 U creatinine or creatinine square, insulin therapy *vs.* no insulin therapy (1 *vs.* 0), proteinuria present *vs.* absent (1 *vs.* 0), no diabetic retinopathy *vs.* background diabetic retinopathy (1 *vs.* 0), and proliferative diabetic retinopathy *vs.* background diabetic retinopathy (1 *vs.* 0).

TABLE 6. Plasma levels of prorenin, renin, and norepinephrine in nondiabetics with chronic autonomic neuropathy

Patient no.	Site of lesion	Etiology	Prorenin (mU/L)	Renin (mU/L)	Norepinephrine (nmol/L)
1-6	Preganglionic	Parkinson's disease	141 59-380	5.1 1.9-15.3	0.62 0.19-2.6
7-11	Postganglionic	Amyloidosis	189 121-327	10.2 5.0-30.4	0.33 0.09-1.2
12	Postganglionic	Progressive AF	103	8.1	0.26
13	Postganglionic	Progressive AF	271	0.8	0.75
14	Postganglionic	Progressive AF	176	0.9	0.14
15	Afferent	Iatrogenic	176	4.2	2.0
16	Afferent	Iatrogenic	236	14.6	3.2

Data in Parkinson's disease and amyloidosis are given as geometric means and range. AF, Autonomic failure; Iatrogenic, after radical neck dissection with preoperative irradiation.

TABLE 7. Renin and prorenin across the kidneys of patients with proliferative diabetic retinopathy and end-stage nephropathy

Sex	Female/male	n	2/5
Age	Median (range)	yr	51 (41-60)
Duration of diabetes	Median (range)	yr	21 (7-38)
Insulin treatment	On insulin/not on insulin	n	5/2
Albuminuria	Mean (SD)	g/L	3.8 (0.8)
Serum creatinine	Median (range)	μ mol/L	650 (311-730)
Blood pressure			
Systolic	Mean (SD)	mm Hg	179 (34)
Diastolic	Mean (SD)	mm Hg	94 (10)
[¹³¹ I]Hippurate clearance	Mean (SD)	mL/min	61 (38)
[¹³¹ I]Hippurate extraction			
Right kidney	Mean (SD)		0.36 (0.09)
Left kidney	Mean (SD)		0.30 (0.07) ^a
Renin			
Right kidney			
Artery	Geometric mean (range)	mU/L	9.4 (4.7-22.8)
Vein	Geometric mean (range)	mU/L	14.7 (8.2-27.7)
Vein/artery ratio	Mean (SD)		1.58 (0.28) ^b
Left kidney			
Artery	Geometric mean (range)	mU/L	9.4 (4.8-21.7)
Vein	Geometric mean (range)	mU/L	15.5 (10.0-29.7)
Vein/artery ratio	Mean (SD)		1.69 (0.35) ^b
Prorenin			
Right kidney			
Artery	Geometric mean (range)	mU/L	464 (316-914)
Vein	Geometric mean (range)	mU/L	471 (330-982)
Vein/artery ratio	Mean (SD)		1.02 (0.06) ^c
Left kidney			
Artery	Geometric mean (range)	mU/L	505 (308-896)
Vein	Geometric mean (range)	mU/L	526 (321-963)
Vein/artery ratio	Mean (SD)		1.04 (0.03) ^c

^a No significant difference between right and left kidney.

^b Significantly different from 1.00 ($P < 0.001$).

^c Not significantly different from 1.00 (no net secretion of prorenin).

human kidney renin (16).

Normal levels (geometric mean) of renin and prorenin in males ($n = 50$; aged 21-54 yr) were 18.2 (range, 7.5-52) and 166 mU/L (65-300), respectively. In normal women in the first 2-12 days of the menstrual cycle ($n = 54$; aged 20-37 yr) renin was 15.4 (4.7-59) mU/L, and prorenin was 151 (62-357) mU/L (16).

Plasma norepinephrine was measured by high performance liquid chromatography (14). In normal subjects ($n = 57$; aged 28-78 yr) plasma norepinephrine (geometric mean) was 1.5 (0.6-3.6) nmol/L.

Assessment of macro- and microalbuminuria

Macroalbuminuria was assessed in nontimed overnight urine samples with dipstick (Albustix, Miles, Naperville, IL), which has a detection limit of 250 mg/L. In case of a trace reaction, the urine sample was tested by the quantitative biuret reaction. Macroalbuminuria was defined as dipstick positive or biuret reaction of protein above 250 mg/L in two of two overnight urine samples. Microalbuminuria was assessed in three consecutive 24-h urine collections with radial immunodiffusion in agarose gel (18). The gel contained 30 μ L undiluted antiserum

against human serum albumin (Dakopatts, Glostrup, Denmark)/10 mL gel. The lower limit of detection was 2 mg/L, and the interassay variability was 8%. Microalbuminuria was defined as albuminuria of 30–300 mg/24 h in at least two of the 24-h urine collections, while all three 24-h urine collections were dipstick negative.

Statistical analysis

Differences between groups for discrete data were analyzed by χ^2 test or the Fisher exact test when appropriate. Differences for continuous data were analyzed with the unpaired *t* test or the Student-Newman-Keuls test for multiple comparisons, with the exception of the age of the patient and the duration of diabetes, which were analyzed with the median test (19). Renin and prorenin were not normally distributed. Logarithmic transformation yielded apparently normal distributions with similar SD in all groups. These transformed values were used for statistical analysis. Multiple regression analysis was performed with the use of the SPSS/PC+ program 1986 (SPSS, Inc., Chicago, IL).

Results

Correlations of prorenin with diabetic complications

In Table 1 the patients are grouped according to the presence or absence of macroalbuminuria. The groups did not differ significantly in female/male ratio, age, HbA1c, and systolic and diastolic pressure, or in the prevalence of insulin treatment and neuropathy. The prevalence of diabetic retinopathy was higher in the group with macroalbuminuria, and retinopathy in this group was more often of the proliferative type. Diabetes was of longer duration, and serum creatinine was higher in these patients. The plasma prorenin level was nearly 2 times higher, but renin levels were not significantly different.

In Table 2 the patients are grouped according to the presence or absence of microalbuminuria. Again, the groups did not differ significantly in female/male ratio, HbA1c, or the prevalence of insulin treatment and neuropathy. There was also no difference in serum creatinine. Systolic blood pressure was higher in the patients with microalbuminuria than in those without albuminuria. In contrast with the data obtained when the diabetics were grouped according to whether they had macroalbuminuria, no differences in plasma levels or prorenin or renin were found between the patients with and without microalbuminuria.

Prorenin was higher in the patients with diabetic retinopathy than in those without retinopathy, and the levels were highest in those with proliferative diabetic retinopathy, regardless of whether macro- or microalbuminuria was present (Figs. 1 and 2). The mean level of prorenin was 2–3 times normal in the patients with proliferative retinopathy, but was not above normal in the patients without retinopathy, and this was the case

in both the presence and absence of macro- or microalbuminuria.

Renin in the patients with either proliferative or background retinopathy was not elevated, and the renin levels were not significantly different from the levels in the patients without retinopathy.

The influence of various factors on the plasma level of prorenin was examined by multiple regression analysis (Tables 3–5). The factors studied were sex, age, duration of diabetes, insulin treatment, blood glucose, HbA1c, systolic and diastolic blood pressure, neuropathy, serum creatinine, macroalbuminuria, and finally, retinopathy. Statistically significant correlations were found with sex, duration of diabetes, insulin treatment, serum creatinine, proteinuria, and retinopathy. In the case of neuropathy, prorenin was also generally increased, but this finding was not statistically significant.

The factors most strongly correlating with prorenin were insulin treatment, serum creatinine, macroalbuminuria, and retinopathy. The correlation with creatinine was not linear, and the term creatinine square was introduced in the regression equation (Table 6). Once allowance was made for the presence of retinopathy, categorized as background diabetic retinopathy or proliferative diabetic retinopathy, the relations of prorenin with sex, duration of diabetes, and insulin treatment were no longer significant. This can be explained by interrelations between the various factors. In 34% of males proteinuria was present compared to 22% of females. Also, males had higher ($P < 0.001$) creatinine levels than females. Finally, with regard to the duration of diabetes, it appeared that longer duration generally implied insulin treatment and macroalbuminuria.

The multiple regression analysis, therefore, shows that high prorenin was associated with nephropathy and retinopathy and that other factors did not significantly contribute to this association. The analysis further indicated that the association with retinopathy was at least in part independent of the association with nephropathy. In contrast with the findings on prorenin, no significant correlations were found between the plasma level of renin and the various factors mentioned above.

Plasma prorenin in nondiabetics with autonomic neuropathy

Supine plasma renin was below the normal range in two patients with a preganglionic lesion and in two with a postganglionic lesion. In none of the patients with autonomic neuropathy did plasma renin rise after head up tilt for 30 min, despite a blood pressure drop of 20–25 mm Hg systolic and 10–15 mm Hg diastolic. Plasma prorenin was within the normal range in all patients (Table 6).

Renal venous plasma prorenin in diabetics with advanced nephropathy

Arterial plasma prorenin was markedly elevated in these patients (Table 7). The renal vein to artery prorenin ratio was 1.02 ± 0.03 on the right side and 1.04 ± 0.03 on the left (mean $\pm$ SD). In subjects with untreated essential hypertension ($n = 18$) the ratio was 1.03 ± 0.05 on the right side and 1.04 ± 0.06 on the left (20). Thus, the ratio in the diabetes was not higher than that in subjects with essential hypertension, despite the markedly reduced renal plasma flow in the former.

Peripheral venous renin in the diabetics was at the lower end of the normal range. The renal vein to artery ratio of renin was 1.58 ± 0.28 on the right side and 1.69 ± 0.35 on the left. In subjects with untreated essential hypertension the ratio was 1.28 ± 0.13 on the right side and 1.30 ± 0.14 on the left (20). The higher ratio in the diabetics is a consequence of the lower renal perfusion.

Discussion

Luetscher *et al.* were the first to report that the plasma level of prorenin is increased in patients with long-standing diabetes, and they suggested that prorenin might serve as a marker of microvascular disease. Our study offers further evidence in support of this. We found that in our patient population the elevated prorenin was associated with the presence of diabetic retinopathy, particularly the proliferative type, and that a number of confounding factors, *i.e.* sex, duration of diabetes, blood pressure, blood levels of glucose and HbA1c, or prevalence of neuropathy or insulin treatment, did not significantly contribute to this association.

In contrast with prorenin, plasma renin is normal or low in most diabetics (3, 4). In the patients in whom proliferative diabetic retinopathy was associated with macroalbuminuria we found plasma renin to be somewhat higher than in the groups without these complications. Higher renin levels in diabetics with albuminuria have also been reported by others (21). This finding might be an artifact caused by inadvertent activation of prorenin during blood sampling and processing. Samples from such patients contain relatively large quantities of prorenin, and inadvertent cryoactivation of prorenin as little as 1% sufficient to raise the measured level of renin by 50% (16).

Autonomic neuropathy is not uncommon in diabetes. Loss of adrenergic stimulation may lead to decreased secretion of renin by the juxtaglomerular cells, and it has been postulated that a compensatory increase in the synthesis and secretion of prorenin by these cells is the cause of the increase in plasma prorenin in diabetes (8, 9). However, our observations in nondiabetics with autonomic neuropathy do not support this explanation. Our data demonstrate that autonomic neuropathy in the ab-

sence of diabetes and its complications does not cause plasma prorenin to rise to the levels seen in diabetics, even when there is serious loss of adrenergic function.

It is possible that independently of the presence of autonomic neuropathy, the processing of prorenin to renin in the juxtaglomerular cells is somehow impaired and that the secretion of prorenin from these cells is increased when the kidney is affected by diabetic microvascular disease (7).

The mean level of prorenin was not above normal in the patients without retinopathy in either the presence or absence of macro- or microalbuminuria, whereas the mean level of prorenin was markedly elevated in the patients with proliferative retinopathy, not only when retinopathy was associated with macro- or microalbuminuria, but also when albuminuria was absent. These findings suggest that retinopathy is a more important determinant of abnormally high prorenin than nephropathy.

We measured the renal vein to artery ratio of prorenin in diabetics who had end-stage nephropathy and in whom peripheral venous plasma prorenin was elevated. The ratio in these patients was not significantly different from unity (no net secretion) and was not higher than that in patients with essential hypertension, despite the fact that renal plasma flow was much lower. Thus, the high plasma level of prorenin in the diabetics could not be fully explained by abnormal secretion by the kidneys.

This leaves us with two possibilities. One is reduced extrarenal clearance of prorenin and the other is increased extrarenal production. Nonenzymatic glycosylation of prorenin might lead to reduced clearance from the circulation. We found, however, no significant correlation between the levels of prorenin and HbA1c. It has been postulated that a cellular receptor exists, which binds the prosegment of prorenin (22). Such binding might initiate internalization of prorenin, for instance in the vascular endothelium. A number of endothelial functions are known to be impaired in diabetes (23–25), and this may also be true for prorenin internalization, with reduced clearance of prorenin from the circulation as a result.

With regard to the possibility of increased production of prorenin at extrarenal sites, it is of interest that large amounts of (pro)renin have been found in highly vascularized extrarenal tumors (26, 27). Neovascularization is an important feature of diabetic microvascular disease. In this context it might be relevant that the vitreous of the eye contains prorenin in concentrations that are, relative to levels of albumin and other plasma proteins, much higher than those in plasma (17). Prorenin is also present in extracts of the retina in concentrations too high to be explained by trapped plasma (28). It is also interesting that for a given level of albumin, the level of prorenin in the vitreous from eyes with retinal detach-

ment due to proliferative diabetic retinopathy was higher than that in eyes with spontaneous retinal detachment (17). It seems unlikely that the eye is an important source of circulating prorenin because of the small contribution of the eye to total body blood flow, but extensive areas of microvascular disease, possibly associated with new vessel formation, elsewhere in the body might produce enough prorenin to increase its level in plasma.

At any rate, the present study appears to indicate that the high plasma levels of prorenin found in diabetics are not an immediate consequence of altered glucose metabolism. These high levels are related to the development of microvascular disease in the eye and kidney, and they are at least in part due to decreased clearance of prorenin from the circulation, increased production at extrarenal sites, or both.

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