Prorenin and Angiotensin-Dependent Renal Vasoconstriction in Type 1 and Type 2 Diabetes

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Prorenin is a powerful marker for risk of nephropathy and retinopathy in diabetes, but the responsible mechanism remains unclear. Studied were 35 patients with diabetes (18 with type 1 and 17 with type 2) and 69 age-matched healthy subjects with para-aminohippurate and inulin clearances and their response to captopril. All patients with diabetes had normal renal function and no microalbuminuria. Prorenin was calculated as the difference between total renin and active renin. Active renin level in patients with diabetes (11.6 ± 0.9 μU/ml) was significantly lower than in normal subjects (14.5 ± 1.3 μU/ml; $P < 0.05$); despite this, the renal vascular response to captopril was much larger (82.9 ± 11.5 *versus* 13.6 ± 5.8 ml/min per 1.73 m^2; $P < 0.01$). Prorenin in both patients with type 1 and type 2 diabetes (175.7 ± 15.1 μU/ml) also was significantly higher than in normal subjects (128 ± 5.8 μU/ml; $P < 0.01$). Active renin correlated with prorenin in normal subjects ($r = 0.44$, $P = 0.0002$), and this correlation was much more striking in patients with diabetes ($r = 0.72$, $P = 0.0001$). The active renin and prorenin correlation was identical in type 1 and type 2 diabetes. There was a clear correlation between plasma prorenin and the renovascular response to captopril in patients with diabetes ($P < 0.01$) but not in normal subjects ($P > 0.13$). The strong correlation between plasma prorenin concentration and the renovascular response to captopril in diabetes supports the hypothesis of a direct effect of prorenin, but the unanticipated high degree of correlation between plasma prorenin and active renin limits the conclusions that can be drawn.

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We have presented evidence that the intrarenal renin system often is activated in the patient with diabetes, reflected in an increased renal vasodilator response to angiotensin-converting enzyme inhibition with captopril or an angiotensin receptor blocker (1). The increase in plasma prorenin concentration in diabetes and the remarkable increase in risk for microvascular disease that is associated with that increase makes prorenin an attractive candidate as the source of increased intrarenal angiotensin in diabetes (2–15). In the kidney, prorenin serves as the precursor to renin but until recently had no known role once it reaches the circulation.

Recent studies have identified tissue-binding sites for prorenin that could account for prorenin bioactivity at the tissue level, which does not occur in plasma (8–12). Nguyen *et al.* (10) described a mesangial receptor that has affinity for renin and increases the catalytic activity of renin approximately five-fold. Of specific interest for the prorenin story is that this receptor binds prorenin as effectively as it binds renin; thereby, prorenin is activated. Observations in animal models suggest that this pathway may be important under some circumstances (15).

We have been unable to show a correlation between plasma renin activity or active renin and renovascular responses to captopril in diabetes (1). Our hypothesis in this study was that if prorenin is involved in the enhanced angiotensin-dependent control of the renal circulation and diabetes, then a correlation would be found between plasma prorenin levels and the renovascular response.

Materials and Methods

We studied 104 individuals, including 35 men and women with diabetes (18 with type 1 and 17 with type 2), who ranged in age from 20 to 52 yr (mean ± SEM 37 ± 2.0) and 69 healthy age-matched men and women who ranged in age from 21 to 57 yr (mean ± SEM 36 ± 1.8). The duration of diabetes ranged from 10 to 45 yr (mean 16 ± 4.0). Diabetes was diagnosed according to accepted guidelines (16). All were otherwise healthy, normotensive, and free of sustained microalbuminuria and other complications of diabetes. The participants were studied during admission to a metabolic ward, the General Clinical Research Center of the Brigham and Women's Hospital, where balance was achieved on a controlled diet.

All participant were placed on a high-salt isocaloric diet starting 2 d before admission and continuing throughout the hospitalization, with a daily sodium intake of 200 mmol. Daily dietary potassium (100 mmol) and fluid intake (2500 ml) were constant. Twenty-four-hour urine samples were collected daily and analyzed for sodium, potassium, creatinine, and protein. The protocol was approved by the Partners Human Subjects Committee Internal Review Board, and written informed consent was obtained from each participant.

Renal Hemodynamic and Hormonal Responses to Captopril

On the morning of the renal hemodynamic study, an intravenous catheter was placed in each arm of each participant, one for infusion of p-aminohippurate (PAH), inulin, and dextrose 5% in water and the

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other for blood sampling. In the patients with diabetes, a third intravenous line was placed for continuous infusion of insulin that was started at 0.015 μ/kg per h. Blood glucose was measured every 30 min (Precision PCX; Abbott Laboratories, Chicago, IL). The insulin infusion was adjusted to maintain blood glucose below the renal threshold but without inducing hypoglycemia, at levels of 100 to 140 mg/dl. The participants were supine and had been fasting for at least 8 h.

The study day began with a 60-min baseline infusion of PAH and inulin before captopril administration (25 mg orally) to determine baseline renal plasma flow (RPF) and GFR, respectively. In a subset, we compared the renal hemodynamic response to captopril and to candesartan: On the first morning, the patients received captopril (25 mg orally); on the next morning, the patients received candesartan (16 mg orally). These dosages were chosen because both represent the top of the relationship between dosage and RPF response. The responses were highly correlated ($r = 0.77$, $P < 0.01$). PAH and inulin infusions continued for 3 h in the case of captopril and 4 h in the case of candesartan, and blood samples were drawn at 45-min intervals. BP was recorded during each infusion by an automatic recording device (Dinamap; Critikon, Tampa, FL) at 5-min intervals.

Renal Clearance Studies

PAH (Merck, Sharp & Dohme, Rahway, NJ) and Inutest (Fresenius Pharma Austria, Linz, Austria) clearances were assessed after metabolic balance was achieved. A control blood sample was drawn, and then loading doses of PAH (8 mg/kg) and inulin (50 mg/kg) were given intravenously. A constant infusion of PAH and inulin was initiated immediately at a rate of 12 and 30 mg/min, respectively, with an IMED pump (Alaris Medical System, San Diego, CA). This achieved a plasma PAH concentration in the middle of the range in which tubular secretion dominates excretion. At this plasma level of PAH, clearance is independent of plasma concentration and represents approximately 90% of RPF when corrected for individual body surface area. Likewise, at the level of plasma inulin achieved, inulin clearance reflects GFR. RPF and GFR determinations were made at baseline and at 45-min intervals thereafter for 4 h while the participants remained supine.

Laboratory Procedures

Blood samples were collected on ice and spun immediately, and the plasma was frozen until assay. Urinary and serum sodium and potassium levels were measured using the ion-selective electrode. PAH and inulin were measured using an autoanalyzer technique. Plasma renin activity (PRA) and aldosterone were determined by RIA (17). Measurements of total renin and active renin were made by RIA as described by Hurwitz *et al.* (18). Prorenin was calculated as the difference between total renin and active renin. Hemoglobin A_{1c} was measured by HPLC. The normal range is 4.4 to 6.3%.

Immunoradiometric Assay. The direct assay of active renin and total renin was performed using the active renin immunoradiometric assay (IRMA) kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) as described for this laboratory (18). Two mAb were used: R3.36.16 (Ciba Geigy, Basel, Switzerland), specific for active and inactive renin, and (R1.20.5, Ciba Geigy), specific only for active renin. The analytic sensitivity of the method is 2.7 μU/ml. Prorenin was calculated by subtracting the amount of active renin from total renin.

PRA. PRA was measured by using a commercial antibody-coated tube RIA kit (Incstar, Stillwater, MN) as previously described (18). The sensitivity of the assay is 0.018 ng/ml per h (0.01 nmol/L per h).

Other Analyses. Urinary sodium and potassium levels were measured using an ISE analyzer (NOVA Biomedical, Waltham, MA). The urinary creatinine level was measured with a discrete analyzer (Beckman Instruments, Brea, CA) using the Jaffe reaction.

Statistical Analyses

Group means were calculated with the SEM as the index of dispersion. For renal hemodynamic data, the baseline value taken was the average of three predrug determinations, and the peak response was the average of the two highest consecutive values. Statistical comparisons were made by the *t* test or ANOVA where appropriate. Linear correlation and regression was used to examine relationships. $P < 0.05$ was considered to be statistically significant.

Results

The patients with diabetes and the normal subjects were similar in age, gender distribution, serum creatinine, and serum sodium and potassium (Tables 1 and 2). There was a significant difference in body mass index (30 ± 1.0; 26.7 ± 0.6; $P < 0.05$). Systolic and diastolic BP, hemoglobin A_{1c}, and fasting blood sugars, as anticipated, all were higher in the patients with diabetes (Tables 1 and 2). Our goal of similar suppression of the renin-angiotensin system with a high-salt diet was achieved; 24-h sodium in the patients with diabetes was 242 ± 30 mEq *versus* 236 ± 32 mol; PRA was 0.63 ± 0.25 *versus* 0.40 ± 0.1; and plasma aldosterone concentration was 2.3 ± 0.8 *versus* 2.4 ± 0.8 ng/ml (Tables 1 and 2).

Total renin did not differ significantly between normal subjects (143 ± 10 μU/ml) and patients with diabetes (187 ± 20 μU/ml). However, the contribution of prorenin and active renin to total renin did differ (Tables 2 and 3). Active renin was significantly lower in the patients with diabetes ($P < 0.01$), whereas prorenin was significantly higher ($P < 0.01$). PRA did not differ between the two groups.

In the patients with diabetes, the relation between active renin and prorenin (Figure 1A) was striking and highly statistically significant ($r = 0.72$, $P = 0.0001$). More than 50% of the variation of active renin could be accounted for on the basis of prorenin. The strong relation between active renin and prorenin was identical in type 1 and type 2 diabetes. Active renin and prorenin were similarly distributed in both diabetes groups (Table 3). Prorenin and active renin in plasma also correlated in normal subjects but much less strikingly (Figure 1B).

The renal vasodilator response to captopril, again as anticipated, was much larger in the patients with diabetes (82.9 ± 11.5) than in the normal subjects (13.6 ± 5.8 ml/min per 1.73 m^2; $P < 0.01$; Tables 2 and 4) despite the lower active renin level in the patients with diabetes. Change in systolic and diastolic BP before and after captopril in relation to the prorenin and active renin levels was NS for both normal subjects and patients with diabetes ($0.004 < r < 0.14$, $0.28 < P < 0.99$).

The relationships between prorenin, active renin, and the renovascular response to captopril differed strikingly in healthy subjects and in patients with diabetes. In the normal subjects, the renovascular response to captopril did not show a significant correlation either with active renin ($r = 0.17$, $P = 0.15$; Figure 2A) or with prorenin ($r = 0.18$, $P = 0.13$; Figure). In the patients with diabetes, prorenin showed a statistically significant relationship to the renovascular response to captopril

Table 1. Baseline characteristics[a]

Parameter	Diabetes	Healthy
n	35	69
Age (yr)	37.0 ± 2.0	36.0 ± 1.8
Gender distribution (male/female)	12/23	30/39
BMI (kg/m^2)	30.0 ± 1.0[b]	26.7 ± 0.6
Baseline SBP (mmHg)	127.0 ± 2.0	118.0 ± 2.0
Baseline DBP (mmHg)	72.0 ± 1.0	68.0 ± 1.0
SBP at peak RPF (mmHg)	119.0 ± 4.0	115.0 ± 2.0
DBP at peak RPF (mmHg)	67.0 ± 1.0	66.0 ± 1.0
Duration of diabetes (yr)	16.0 ± 4.0	—
Diabetes type (1/2)	18/17	—
Hemoglobin A_{1C} (%)	7.3 ± 0.7	—
Fasting blood sugar (mg/dl)	119.0 ± 8.0[c]	67.0 ± 8.0
Serum creatinine (mg/dl)	0.84 ± 0.06	0.75 ± 0.08
Serum sodium (mEq/L)	139.0 ± 0.8	137.0 ± 0.9
24-H urine sodium (mEq)	242.0 ± 30.0	236.0 ± 32.0

[a]Data are means ± SEM. BMI, body mass index; DBP, diastolic BP; RPF, renal plasma flow; SBP, systolic BP.
[b]$P < 0.05$.
[c]$P < 0.01$.

Table 2. Normal subjects and patients with diabetes[a]

Parameter	Normal	Diabetes
Baseline PRA (ng/AngI per ml/h)	0.40 ± 0.1	0.63 ± 0.25
Peak PRA after captopril (ng AngI per ml/h)	1.4 ± 0.3[b]	3.0 ± 0.4
Prorenin (μU/ml)	128.7 ± 5.8[b]	175.7 ± 15.1
Active renin (μU/ml)	14.5 ± 1.3[b]	11.6 ± 0.9
Delta RPF (ml/min per 1.73 m^2)	13.6 ± 5.8[c]	82.9 ± 11.5
Plasma aldosterone (ng/ml)	2.3 ± 0.8	2.4 ± 0.8

[a]Data are means ± SEM. AngI, angiotensin I; PRA, plasma renin activity.
[b]$P < 0.05$.
[c]$P < 0.01$.

Table 3. Patients with type 1 and type 2 diabetes and normal subjects[a]

Parameter	Diabetes		Normal
	Type 1	Type 2	
Total renin (μU/ml)	189.0 ± 23.0	186.0 ± 20.0	143.0 ± 10.0
Prorenin (μU/ml)	176.7 ± 22.4	175.0 ± 21.0	128.7 ± 5.8
Active Renin (μU/ml)	12.0 ± 1.3	10.8 ± 1.0	14.5 ± 1.3
Δ RPF (ml/min per 1.73 m^2)	79.7 ± 15.5	86.0 ± 17.0	13.6 ± 5.8
Δ GFR	4.0 ± 2.5	9.4 ± 6.0	−1.0 ± 4.7

[a]Data are means ± SEM.

($r = 0.42$, $P = 0.01$; Figure 2A) and a relation of marginal statistical significance with active renin ($r = 0.31$, $P = 0.06$; Figure 3A).

Discussion

Plasma prorenin concentration in the patient with diabetes has represented a major puzzle. On the one hand, it has provided a very powerful predictor of risk for microvascular disease (2–7). On the other hand, prorenin has been widely considered to be inactive, neither producing angiotensin peptides nor converting to renin (1). The recent identification of a prorenin receptor, which could lead to angiotensin formation *via* prorenin without conversion to renin, led to this analysis (10). Our hypothesis was that in some patients with diabetes, the

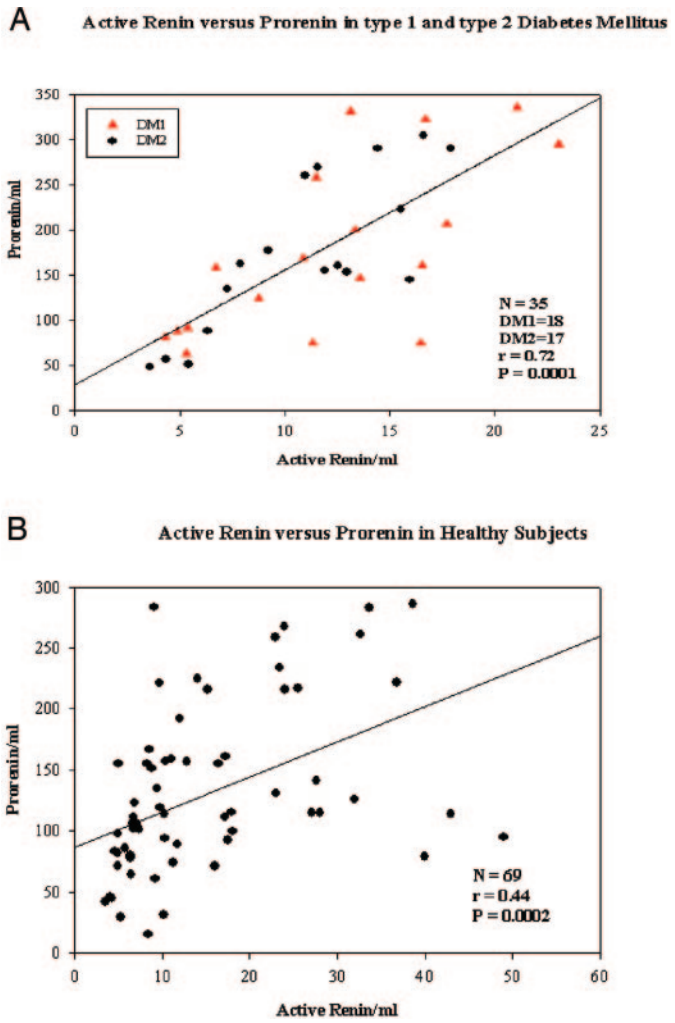

Figure 1. (A) Relationship between active renin and prorenin is shown in patients with diabetes. The correlation was strong ($r = 0.72$) and highly significant ($P = 0.0001$). The relationships essentially were identical in 18 patients with type 1 diabetes and 17 with type 2 diabetes. (B) Relation between active renin and prorenin in healthy subjects. A statistically significant ($P = 0.0002$) relationship was found in normal subjects as well but less striking than in that in patients with diabetes ($r = 0.44$).

pathway would lead to an angiotensin-dependent influence on the renal circulation. We and others have documented activation of the intrarenal renin-angiotensin system in the majority of patients with diabetes (1,19) but without a clear explanation. A special action of prorenin in diabetes could provide such an explanation.

Our studies confirm earlier reports that active renin is reduced and prorenin is increased in the patient with diabetes (2–7). Previous studies focused on measuring prorenin levels primarily in patients with type 1 diabetes. We report significantly higher levels of prorenin in both type 1 and type 2 diabetes when compared with prorenin measured in healthy subjects. Despite the lower active renin level, the renovascular response to captopril was much larger in the patients with diabetes than in normal subjects. There was a substantial difference in the relation between prorenin levels and the renovascular response to captopril, significant in the patients with diabetes ($P < 0.01$), whereas in the normal subjects, the same relationship was NS ($P > 0.13$). The pattern of the relationship with active renin was similar to that seen for prorenin in patients with diabetes in that the relationship is more statistically significant in patients with diabetes ($P < 0.06$) than in normal subjects ($P > 0.15$).

Interpretation of these data is complicated by the fact that active renin and prorenin were strongly correlated in both the normal subjects and the patients with diabetes. The correlation between active renin and prorenin in normal subjects confirms the observation of Hurwitz *et al.* (18) and extends it to diabetes. The strong relation between active renin and prorenin essentially was identical in type 1 and type 2 diabetes. The higher the active renin, the higher the prorenin. To our knowledge, the strong correlation between active renin and prorenin in type 1 and type 2 diabetes has not been discussed previously. There is no reason *a priori* to suspect that active renin and prorenin levels in plasma would be highly correlated. The release of active renin from the kidney is under tight control and varies with a number of conditions, including salt intake, BP, and sympathetic nervous system activity: The release of prorenin, however, is thought to be constitutive (20). Moreover, all of the circulating active renin comes from the kidney, but prorenin has multiple sites of origin, including especially the ovary. For all of these reasons, a correlation was not anticipated.

The early studies on the predictive power of prorenin in identifying risk for nephropathy and retinopathy largely focused on the patients with type 1 diabetes and microvascular complications (2–7). In this study, we examined patients with both type 1 and type 2 diabetes and discerned no difference in the pattern of relationships. One possible mechanism, *via* the renin-prorenin receptor, was reviewed. Nothing is known of the influence of diabetes on the state of this receptor. As an alternative, recent studies identified in a transgenic murine model an influence of diabetes on the pathway for prorenin processing (21). In brief, they found that in diabetic mice but not normal mice, a cardiac prorenin-converting enzyme was present. Such enzyme activation in humans with diabetes would account nicely for our findings.

A murine model has provided what probably is the most direct and persuasive evidence for a role for prorenin in the pathogenesis of diabetic nephropathy (15). Prorenin is rendered inactive by the prosegment, which folds into the active site cleft of mature renin to prevent interaction with substrate. A prorenin-binding protein, such as the renin-prorenin receptor (8–10), leads to prorenin activation by interacting with a special region of prorenin, the "handle" region, leading to a conformational change in the enzyme. Ichihara *et al.* (15) tested the hypothesis that a peptide with the structure of this handle region might bind to the crucial site as a decoy and thereby inhibit the nonproteolytic activation of prorenin. In streptozotocin-induced diabetes in rats, they showed that treatment with the handle-region peptide inhibited almost completely the development of diabetic nephropathy without an influence on glycemic control. Although we need to know more about the

Table 4. Renal hemodynamics after captopril in normal subjects and patients with diabetes[a]

Parameter	Normal	Diabetes
Baseline RPF (ml/min per 1.73 m^2)	567.0 ± 9.8	555.0 ± 28.0
Peak RPF (ml/min per 1.73 m^2)	580.0 ± 10.8	638.0 ± 42.0

[a]Data are means ± SEM.

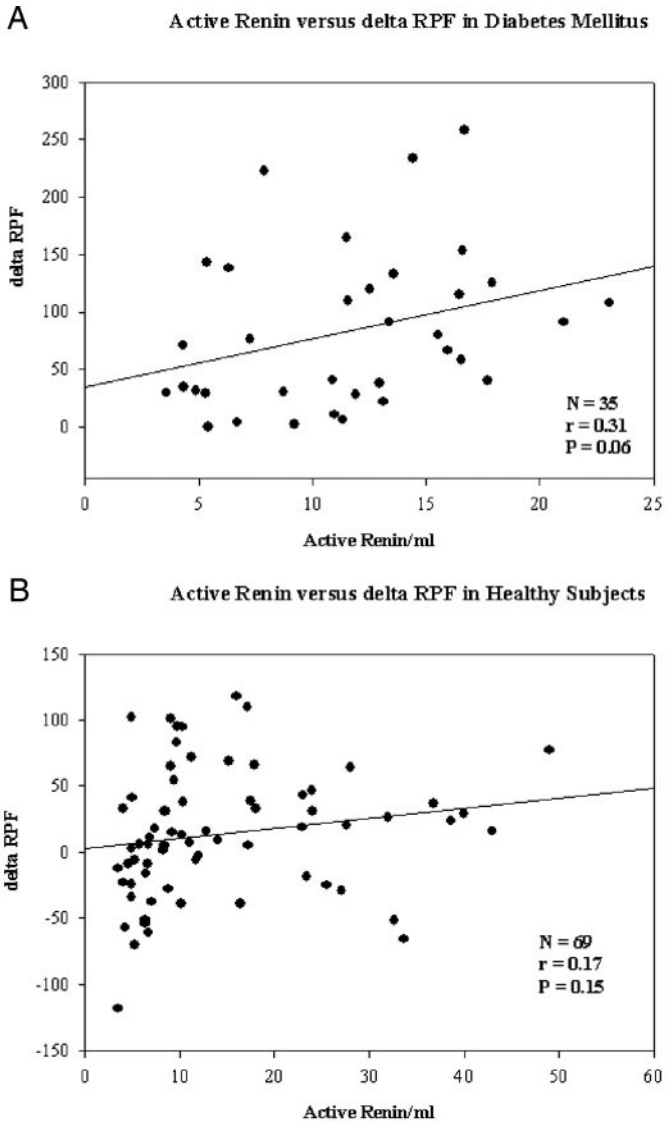

Figure 2. (A) In patients with diabetes, there was a borderline relation between active renin level and the renovascular response to captopril ($r = 0.31$, $P = 0.06$). (B) In normal subjects, there was no relation between active renin and the renovascular response to captopril ($r = 0.17$, $P = 0.15$).

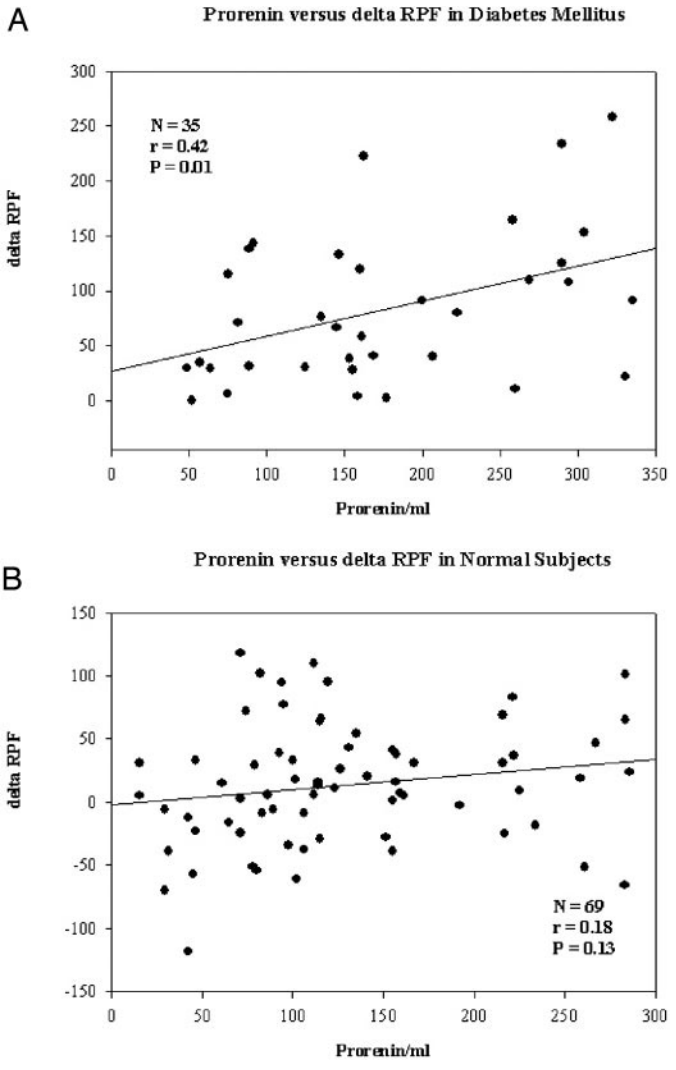

Figure 3. (A) Relation between prorenin and the renovascular response to captopril in patients with diabetes. The correlation was strong ($r = 0.42$) and statistically significant ($P = 0.01$). (B) In normal subjects, the relation between prorenin level and the renovascular response to captopril was minimal and did not achieve statistical significance ($r = 0.18$, $P = 0.13$).

pharmacology of the decoy peptide, it seems very likely that its action is *via* an interference with prorenin activation.

The most widely cited evidence for the biologic inactivity of prorenin came from a study by Lenz *et al.* (22), who examined the relatively acute hemodynamic and hormonal effects of prorenin in cynomolgus monkeys. The absence of a response to prorenin in this setting well may reflect primarily that conversion of prorenin and its activation and biologic effect may occur over a longer time span than the duration of such experiments.

Approximately twice as many women as men were enrolled in the diabetes group, and there was a substantial excess of

women in the control group. We have been interested in the role of gender in the responses studied in this investigation (23) and have found that only the use of oral contraceptives influences the renin system contribution to renovascular tone in our model.

The generalizability of any study is an important issue. In this study, the patients with diabetes were remarkably healthy, generally free of hypertension and proteinuria. This did not reflect policy, as the patients were enrolled consecutively. It very well may reflect a bias that was created by our referral physicians, who probably are happier to refer for study patients who are free of hypertension or proteinuria than those who require renin-system blockade because they had these problems. We have shown similar patterns of renovascular response to pharmacologic interruption of the renin system in patients with type 2 diabetes and advanced nephropathy (24).

Because of the strong correlation between active renin and prorenin in all subject groups, especially in those without diabetes, an identification of a specific role for an action of prorenin was impossible. One attractive possibility is that one or more of the renin inhibitors that currently are being assessed will interfere with this pathway (17). Alternative approaches will be necessary to identify the specific role of prorenin in angiotensin-dependent responses. However, this study was robust, involving >100 participants, and all of the assays used represent substantial experience in our laboratory. The hypotheses and the findings reflect important issues in the risk for microvascular consequences in patients with type 1 and type 2 diabetes.

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