Renin Regulation in Type II Diabetes Mellitus: Influence of Dietary Sodium

Angelina Trujillo, Peter Eggena, Jack Barrett, and Michael Tuck

Numerous abnormalities in the renin-angiotensin system have been described in diabetes mellitus. Plasma renin activity (PRA) has been noted to be low, normal, and high in diabetic patients; these variable results may be explained by differences in patient selection and standardization of study conditions. We evaluated PRA and inactive renin responses in Type II normotensive (n=7) and hypertensive (n=12) diabetic patients specifically selected for no or minimal evidence (background retinopathy) for microvascular complications. Patients were studied in a metabolic ward after 7 days on a constant low sodium (20 meq/day) and 7 days on a high sodium (250 meq/day) diet. Nondiabetic control subjects (n=7) were evaluated under similar conditions. On low sodium intake, mean PRA levels were significantly reduced in the hypertensive diabetic group, but were not different between the control and normotensive diabetic groups. Hypertensive diabetic patients on high sodium intake also had greater reductions in PRA responses compared with the other study groups. In general, diabetic subjects on high sodium intake excreted less sodium and had more cumulative sodium retention than control subjects. Levels of inactive renin were not significantly different between the normotensive and hypertensive diabetic patients and were comparable with the levels in control subjects. Inactive renin levels changed in a similar direction and magnitude as PRA in response to sodium intake and posture in the three study groups. Infusion of angiotensin II led to comparable reductions in PRA in both diabetic groups and in the control group, suggesting an intact short feedback loop control. We conclude that Type II hypertensive diabetic patients with no or minimal complications have abnormalities of renin secretion that are best brought out under conditions of dietary sodium balance. The reduced PRA responses in these patients cannot be explained by defective activation of renin or by abnormalities of the angiotensin II short feedback loop of PRA control. Advanced age and abnormalities in renal sodium excretion in diabetic patients in combination with elevated blood pressure may account for the abnormal PRA responses in Type II diabetic hypertensive subjects. (Hypertension 1989; 13:200–205)

Several alterations in the renin-angiotensin system have been described in diabetes mellitus. In patients with established autonomic neuropathy, decreased plasma renin activity (PRA) responses have been found, which suggests that neural control of renin release is altered in this disorder.¹⁻⁴ Patients with diabetic nephropathy and retinopathy also have decreased PRA levels.⁵⁻⁶ However, one study actually noted high levels of PRA in diabetics with retinopathy.⁷ In contrast, normal levels of PRA have been found in most studies of diabetic patients who had no clinical evidence of microvascular complications.⁸⁻¹¹ These findings suggest that changes in the renin-angiotensin system may be linked to the onset of microvascular complications in diabetes mellitus.

Changes in the renin-angiotensin system in diabetes mellitus could also be secondary to abnormalities in sodium and volume control since increases in total exchangeable sodium have been consistently found in diabetic patients.⁸⁻⁹ Such observations indicate that knowledge of the state of sodium balance is very important in the evaluation of the renin-angiotensin system in diabetic patients. However, many investigations of the status of the renin-angiotensin system in diabetes mellitus have not rigorously controlled for sodium balance, which makes comparison of results difficult. Other potential reasons for the variable PRA results reported in diabetic patients include selection of mixed study populations without distinguishing between patients with Type I or II diabetes mellitus or between normotensive and hypertensive subjects.

Abnormalities in renin processing could also contribute to the changes in PRA levels described in
diabetes mellitus. Increased levels of inactive renin have been noted in several studies of diabetic patients, which suggests an inability in patients with this disorder to normally activate renin.12–18 High levels of inactive renin have been noted most consistently in diabetics with microvascular complications.17

The present study was designed to evaluate in Type II diabetic patients the responses of PRA and inactive renin under conditions of controlled high and low dietary sodium intake. We selected normotensive and hypertensive diabetic patients who demonstrated no clinical evidence of renal disease or neuropathy and no or very minimal evidence for retinopathy.

Subjects and Methods

We studied 19 subjects with diabetes mellitus: 12 hypertensive and 7 normotensive patients who were admitted under informed consent to the Metabolic Study Ward of the Veterans Administration Medical Center, Sepulveda, California. All patients had Type II diabetes mellitus treated with either oral hypoglycemic agents (n=5), insulin (n=13), or diet alone (n=1). Diabetes control was maintained relatively constant during the study with fasting blood glucose levels ranging from 150 to 240 mg/dl. Duration of diabetes for the hypertensive patients was from 9 to 25 years (mean, 13.5 years) and for the normotensive diabetic patients from 1 to 30 years (mean, 18.3 years). The ages of the hypertensive study group ranged from 53 to 71 years and the normotensive group from 54 to 69 years. Hypertension was defined as diastolic blood pressure level of 90 mm Hg or above as determined on three separate occasions. Secondary forms of hypertension were excluded by appropriate testing. All antihypertensive medications were withdrawn 2–3 weeks before the study. Presence of retinopathy was evaluated by ophthalmoscopic examination in the Ophthalmology Clinic and renal function by creatinine clearance and quantitative urine protein measurement. The presence or absence of neuropathy was excluded by complete neurological examination. Control subjects (n=7) were healthy, nondiabetic individuals ranging in age from 32 to 54 years.

Patients were admitted to a study ward and, on the second day, were placed on a 20 meq sodium/100 meq potassium constant diet for 7 days. They were then changed to a 250 meq sodium/100 meq potassium constant diet for another 7 days. Dietary sodium balance was checked by daily collection of 24-hour urine samples for measurement of sodium, potassium, and creatinine. In general, urinary sodium excretion was stable after the 5th day of each diet and studies were started the following day. Body weight was measured at 8:00 AM daily, and supine and upright blood pressure recorded twice daily at 8:00 AM and 4:00 PM by a member of the investigative team.

On the sixth day of each dietary protocol, a posture study was performed with blood samples taken to determine PRA and inactive renin responses.

Patients remained supine from 10:00 PM the evening before the study, and at 7:00 AM the next morning a scalp vein needle was inserted into an arm vein for sampling. After supine PRA and inactive renin samples were obtained, patients remained in a standing position with mild exercise for 3 hours at which time the second sample was taken.

On the seventh day of each diet, a graded-dose angiotensin II infusion was performed to evaluate the effect of angiotensin II on renin release. At 7:00 AM a 19-gauge needle was inserted into an antecubital vein for infusion of angiotensin II and a second needle inserted in the opposite arm for withdrawal of blood samples. Blood pressure was monitored every 5 minutes with an automatic blood pressure–monitoring device (Arteriosonde, Roche Laboratories, Santa Ana, California) placed over the right brachial artery. At 8:00 AM the infusion of angiotensin II (Hypertensin, Ciba Pharmaceutical Co., Summit, New Jersey) in 5% dextrose and water (60 ng/ml) was begun, using rates of 0.5, 1.0, 2.0, and 3.0 ng/kg/hr, via a Harvard infusion pump (Harvard Apparatus, Millis, Massachusetts) with each dosage infused for 30 minutes. Samples for determining PRA were obtained at 0, 30, 60, 90, and 120 minutes.

Serum and urinary electrolytes were determined by flame photometry, and lithium was used as an internal standard. PRA was measured as previously described19 by incubation of plasma with 0.1 vol 1 M sodium phosphate buffer and angiotensinase inhibitors (pH 7.4) for 1 or 3 hours. Generated angiotensin I was determined by radioimmunoassay. To derive inactive renin, total renin was determined by a 1-minute preincubation of a plasma sample with 1 mg/ml final concentration of trypsin (Sigma Chemical Co., St. Louis, Missouri). The trypsin activity was 12,000 BAEE units/mg protein as analyzed by Sigma Chemical Co. The reaction was terminated by the addition of twice the concentration of soybean trypsin inhibitor and the sample was then incubated as described for PRA.20 Inactive renin was the difference between total renin and PRA.

Group mean±SEM is presented as the index of dispersion. Student’s t test was used to evaluate statistical probability and Dunnett’s test used for multiple comparisons from baseline. The null hypothesis was rejected when p<0.05.

Results

Table 1 demonstrates the clinical characteristics of each study group. None of the patients had proliferative diabetic retinopathy, as documented by ophthalmoscopic examination performed within 6 months before the study. However, six hypertensive diabetic patients demonstrated mild background retinopathy, whereas the remaining six had normal examinations. Of the normotensive diabetics, one had background retinopathy and six had normal examinations. None of the patients demonstrated clinical evidence of diabetic nephropathy; they all had normal creatinine clearance and normal
TABLE 1. Clinical Characteristics and Sodium Balance in Non-diabetic Control Subjects and in Normotensive and Hypertensive Diabetic Patients

<table>
<thead>
<tr>
<th>Data</th>
<th>Controls (n=7)</th>
<th>Normotensive diabetics (n=7)</th>
<th>Hypertensive diabetics (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48±4*</td>
<td>60±5</td>
<td>62±6</td>
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<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.8±0.1</td>
<td>1.1±0.1</td>
<td>1.2±0.2</td>
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<tr>
<td>Creatinine clearance (ml/min)</td>
<td>105±11</td>
<td>96±11</td>
<td>93±8</td>
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</table>

Dietary sodium balance

Low sodium diet (20 meq)

<table>
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<th>Normotensive diabetics (n=7)</th>
<th>Hypertensive diabetics (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>68±5*</td>
<td>82±9</td>
<td>87±5</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>86±4</td>
<td>88±2</td>
<td>97±3*</td>
</tr>
<tr>
<td>Urinary Na (meq/24 hr)</td>
<td>23±3</td>
<td>20±3</td>
<td>19±4</td>
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</table>

High sodium diet (250 meq)

<table>
<thead>
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<th>Hypertensive diabetics (n=12)</th>
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</thead>
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<tr>
<td>Weight (kg)</td>
<td>71±5*</td>
<td>84±9</td>
<td>90±4</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>87±4</td>
<td>90±3</td>
<td>113±4*</td>
</tr>
<tr>
<td>Urinary Na (meq/24 hr)</td>
<td>218±14</td>
<td>186±18</td>
<td>190±13</td>
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</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure.

* p<0.01 control group vs. diabetic groups; † p<0.01 hypertensive diabetic group vs. normotensive diabetic and control groups; ‡ p<0.05 control group vs. diabetic groups.

Urinary protein excretion. Diabetic neuropathy was excluded by demonstrating normal neurological examinations in all patients.

Urinary sodium excretion recorded on the seventh day of each diet (Table 1) showed that subjects were in balance on the low sodium diet and sodium excretion was comparable between study groups. On the seventh day of the high sodium diet, there were no significant differences in urinary sodium excretion between the diabetic study groups; however, mean sodium excretion was significantly less in both the normotensive and hypertensive diabetic groups compared with the control group (Table 1). Cumulative sodium balance data indicated that the diabetic subjects retained more sodium than the control subjects during the high sodium intake period. Although the hypertensive diabetic group gained approximately 3 kg compared with 2 kg in the normotensive diabetic group when changing from low to high sodium intake, the increase in mean body weight was not significantly different between study groups.

Plasma Renin Activity

Figure 1 shows the supine and upright mean values for PRA for the control and the two diabetic study groups. The hypertensive diabetic group on the high sodium diet had significantly (* p<0.05) lower upright PRA values compared with the control and normotensive diabetic groups. With low sodium intake, supine and upright PRA values were not significantly different between the control and normotensive diabetic groups whereas the hypertensive diabetic group had significantly lower mean PRA levels. Evaluation of the incremental PRA response from supine to upright posture showed that, during the low sodium diet period responses were similar in the three groups. During the high salt diet period, PRA incremental posture responses were significantly lower in the hypertensive diabetic group.

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Figure 1. Bar graph showing mean supine and upright values of plasma renin activity (PRA) in the control group and in normotensive (NTDM) and hypertensive (HTDM) patients with Type II diabetes mellitus after 6 days on a low (20 meq) and after 6 days on a high (250 meq) sodium (Na) diet.

Figure 2. Bar graph showing mean supine inactive renin and plasma renin activity values in the control group and in normotensive (NTDM) and hypertensive (HTDM) patients with Type II diabetes mellitus after 6 days on a low (20 meq) sodium diet and after 6 days on a high (250 meq) sodium (Na) diet.
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Inactive Renin Levels

Mean values for supine PRA and inactive renin are shown in Figure 2 for the normotensive and hypertensive diabetic and control groups. In general, changes in mean inactive renin levels were similar in direction and magnitude to those of PRA in that the levels were lower on the high sodium diet. There were no differences between inactive renin levels in the three study groups. Only one hypertensive patient had an inactive renin level greater than 45 ng/ml/hr, which is the upper limit of normal for our laboratory. Mean levels of inactive renin were not different between diabetic patients with background retinopathy and those without retinopathy.

Plasma Renin Activity Responses to Angiotensin II Infusion

Infusion of angiotensin II in graded doses produced a stepwise reduction in PRA values from baseline in normotensive (Figure 4) and hypertensive (Figure 5) diabetic subjects of similar magnitude to the control subjects. PRA reductions with angiotensin II were less pronounced during the period of high sodium intake in all study groups but the decrements from baseline were not different among the groups. Peak incremental mean arterial pressure (MAP) responses to angiotensin II were not significantly different between the normotensive and hypertensive diabetic groups on either diet but were higher in the diabetic than in the nondiabetic control group.

Discussion

The present study differs from most previous studies of the renin-angiotensin system in diabetes mellitus, as it was designed to control for sodium intake. We also specifically selected Type II diabetic patients who had no or very minimal (background retinopathy) clinical evidence of microangiopathic complications. The results show impairment of PRA responses in Type II diabetic patients that can best be brought out under conditions of dietary sodium balance. Thus with high sodium intake, posture PRA responses were subnormal in diabetic patients with hypertension compared with the normotensive diabetic patients and control subjects. With low sodium intake, supine and upright levels of PRA in the diabetic hypertensive patients were lower than in the control subjects, while the normotensive diabetic patients did not differ. These findings indicate that, under stimulatory conditions for renin release (sodium restriction and upright posture), the hypertensive Type II diabetic patient is not able to mount normal PRA responses. Thus, the presence of hypertension in diabetes mellitus may contribute to some compromise in the activity of the renin-angiotensin system. However, as depicted in Figure 1, there is a general trend toward reduced levels of PRA when comparing the control group with both the normotensive and hypertensive diabetic groups, which suggests that other processes in diabetes may affect the renin-angiotensin system.

Several confounding factors that influence the renin-angiotensin system must also be considered in
evaluating our results. There is a well established decline in the activity of the renin-angiotensin system with advancing age. As the mean age of the diabetic patients was approximately 12 years greater than the control subjects, we cannot exclude an effect of age to explain the lower PRA values in the diabetic patients. However, the diabetic study groups did not differ in age, and yet the diabetic hypertensive group still had further suppression of PRA. There also was no correlation between PRA levels and duration of diabetes. Differences in renal sodium excretion in response to high salt intake could account for the lower PRA levels in the diabetic groups as both age and diabetes can influence sodium excretion. On the seventh day of the high sodium diet, urinary sodium excretion in the diabetic patients was significantly less than in the control subjects despite careful monitoring of dietary intake. These observations suggest that diabetic patients do not come into sodium balance on a high sodium diet as readily as nondiabetic individuals and are in agreement with the literature describing abnormalities in sodium homeostasis in diabetes mellitus. Alterations in sodium handling, which lead to net sodium accumulation in the diabetic patients, could contribute to the reduced PRA responses. The level of glycemic control has also been shown to influence blood pressure and PRA levels in diabetic patients, with better control leading to mild reductions in PRA and blood pressure. However, there was no correlation between blood glucose and PRA in our patients and glycemic control was held relatively constant during the study.

Reports of PRA levels in diabetes mellitus have been highly variable with some investigators finding low values, many reporting normal, and a few noting high PRA levels. These inconsistent findings for PRA in diabetes could be attributed to different study conditions, selection of different diabetic populations, or failure to correct for age, blood pressure, and other factors that affect PRA levels. Sodium balance is one of the major determinants in the control of the renin-angiotensin system, yet many studies of PRA in diabetes mellitus have not rigorously controlled for this factor. Proper selection and definition of the diabetic study population is also an important consideration. Thus, the presence or absence of microvascular complications and the type of complication may have bearing on the activity of the renin-angiotensin system. Christlieb has consistently reported low levels of PRA in Type I diabetic patients with renal disease. Likewise, patients with established diabetic autonomic neuropathy also have decreased PRA levels. However, Drury and Bodansky, while examining Type I diabetic patients with microangiopathy (proliferative retinopathy), actually found high levels of PRA. Diabetic patients without evidence of microvascular complications or with mild neuropathy generally have been found to have normal PRA values. Our results partially confirm these reports, showing that during sodium restriction PRA responses in normotensive Type II diabetic patients are comparable with control subjects. However, the hypertensive diabetic patients had submaximal PRA levels during both high and low sodium intake. In Type II diabetic patients without nephropathy who were evaluated while on a controlled regular sodium diet, O'Hare et al found low PRA levels; an observation that would agree in part with the results found in our diabetic hypertensive patients during the high sodium diet period.

Plasma inactive renin is elevated in some patients with diabetes mellitus and is most consistently seen in those with microvascular complications. Leut scher et al have suggested that increased plasma inactive renin may be a marker for microvascular complications in diabetes mellitus. Increases in plasma inactive renin in diabetes may also be a consequence of coexistent autonomic nervous system dysfunction. Misbin et al reported that diabetic subjects with microangiopathy but no neuropathy had normal inactive renin, whereas patients with neuropathy had elevated levels. The present study demonstrates that, in Type II diabetic patients with no or minimal microvascular changes (background retinopathy), inactive renin values are similar between control and diabetic subjects with and without hypertension. Our results also show that inactive renin levels vary with sodium balance in a fashion similar to PRA values. Other studies have found reciprocal changes in active and inactive renin in normal subjects during acute stimulation of the renin-angiotensin system, which suggests that active renin is derived from inactive renin.

Ash et al have recently reported that in some diabetic patients renal conversion of inactive renin to active renin may be impaired, which results in elevated inactive renin levels with reduced PRA levels. We actually found a direct relation between inactive renin and PRA levels in both the control and diabetic groups in response to dietary sodium and posture. This direct relation between PRA and inactive renin would appear not to support the proposal that active renin is derived from inactive renin. However, the low PRA levels in the face of normal inactive renin levels could be explained by a partial defect in renin activation. While our diabetic patients graphically appeared to have higher levels of inactive renin in response to low sodium intake (Figure 2), the levels were not statistically different from the normal control group.

Infusion of angiotensin II in normal subjects produces a dose-dependent decline in circulating renin levels as part of the short feedback loop mechanism of angiotensin II on renin release. Patients with hypertension show decreased effectiveness of the short feedback loop for angiotensin with incomplete suppression of PRA during infusion of this peptide. Because of the multiple abnormalities in renin control in diabetes mellitus, we examined the angiotensin II short feedback loop during angiotensin II infusion in our patients. Compared with essential
hypertensive subjects, diabetic normotensive and hypertensive patients had normal reductions in PRA during angiotensin II infusion. Thus, in contrast to abnormalities in volume, sodium, and neural control of renin release in diabetes mellitus, the angiotensin II short feedback loop for control of renin release appears to function normally.

References


KEY WORDS • renin • diabetes • sodium • microcirculation
Renin regulation in type II diabetes mellitus: influence of dietary sodium.
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Hypertension. 1989;13:200-205
doi: 10.1161/01.HYP.13.3.200

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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