Low Plasma Renin Activity in Normotensive Patients with Diabetes Mellitus: Relationship to Neuropathy

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SUMMARY To determine the effect of diabetes mellitus on the renin-aldosterone system, independent of age, nephropathy, or hypertension, 16 nonnotensive diabetics with long-term disease (mean duration, 15 years) and no (14) or minimal (2) proteinuria, were compared to nine age-matched, normotensive controls. Plasma renin activity (PRA) measured supine and after 4 hours of quiet ambulation, both on an ad libitum diet and on Day 4 of a 10 mEq low sodium diet, was always lower in the diabetics (31%-56% of control values). After the combined stimulus of sodium depletion and ambulation, PRA was 2.2 ± 0.4 in the diabetics compared to 3.4 ± 0.2 ng/ml/hr in controls (p < 0.025).

On the low sodium diet, PRA and the postural response of PRA correlated directly with the degree of autonomic dysfunction as quantitated by the velocity of esophageal peristalsis (r = 0.60, p < 0.05; r = 0.75, p < 0.005 respectively), suggesting that autonomic neuropathy was an important factor contributing to low PRA in these patients. No other parameters correlated with PRA. Plasma renin substrate (PRS) tended to be lower in diabetics (1053 ± 95 vs 1358 ± 132 ng AI/ml; p < 0.07) but not sufficiently so to account for the substantial difference in PRA. Furthermore, PRS did not correlate with PRA. Fasting blood sugar, while higher in diabetics (209 vs 96 mg/dl), and creatinine clearance, which was lower (112 ± 13 vs 78 ± 4 ml/min; p < 0.01), also did not correlate with PRA. Other factors, including serum creatinine, serum potassium, urinary aldosterone, blood pressure, and body weight, and the responses of these parameters to sodium depletion, were similar in diabetics and controls. These data Implicate visceral neuropathy as a major factor in the hyporeninemia of these diabetics. (Hypertension 3: 87-92, 1981)

KEY WORDS • diabetes mellitus • plasma renin activity • plasma renin substrate • aldosterone • peripheral neuropathy • autonomic neuropathy • esophageal peristalsis

HYPERTENSION occurs in over 50% of the diabetics who have developed retinopathy and nephropathy. Plasma renin activity (PRA) and aldosterone excretion are low in these hypertensive diabetics, but not in normotensive diabetics who have no complications from their disease. Hyporeninemia also occurs in normotensive diabetics with advanced diabetic complications, including orthostatic hypotension, retinopathy, and nephropathy. Hypertensive diabetics with nephropathy have lower PRA than those without it. On the basis of studies such as the above, a major role for diabetic nephropathy in the production of hyporeninemia has been postulated. The role of neuropathy has never been assessed independently, however. In one of the studies above, 21 of 28 diabetic patients with nephropathy also had neuropathy, and in another, at least four of seven patients with nephropathy (creatinine clearance, 47 cc/min), hyperkalemia, and hyporeninemia had neuropathy. To evaluate the role of neuropathy independently, the present study utilizes a group of normotensive diabetics with mild-to-moderate neuropathy, but with minimal nephropathy (creatinine clearance 78 cc/min, proteinuria less than 0.4 mg/24 hr). Visceral autonomic function is quantitated by measurement of the velocity of esophageal peristalsis. Because PRA decreases with age, an age-matched, nondiabetic control group has been selected.
Materials and Methods

All patients and control volunteers gave informed consent and were admitted to the research center in the Philadelphia Naval Hospital for the duration of the study. These individuals were selected from a larger population who had participated in previously reported studies of esophageal motility. Selection was based on willingness to participate in further studies, excluding only those with established clinical renal disease. Their clinical characteristics are summarized in Table 1. The normotensive nondiabetic subjects had no evidence of renal or cardiovascular disease, and were not taking medication at the time of the study or chronically.

All subjects were given an ad libitum hospital diet for 4 days, followed by a diet of 10 mEq sodium and 75 mEq potassium for 4 days. All received 40 mg of furosemide orally at the start of the low salt diet. The PRA was determined supine and after 4 hours of ambulation on the last day of each diet. To construct the PRA-sodium nomogram, additional values obtained from Day 3 of each diet were included when available. In addition, fasting blood sugar, serum electrolytes, BUN, and creatinine were monitored daily. Peripheral neuropathy was evaluated by a neuro- logical examination testing the biceps, patellar and Achilles reflexes, 2-point discrimination, vibratory sense, light touch, and proprioception, as previously reported. Motor nerve conduction studies of the median, ulnar, peroneal and tibial nerves, and sensory nerve conduction studies of the median, ulnar, and sural nerves, were also performed as previously described. Motor latency, nerve conduction, velocity and sensory latency of the appropriate nerves were determined. Responses for each test were rated either as normal or abnormal, and total ratings were compiled for the neurological examination (maximum score 12). Visceral autonomic function was evaluated by measurement of the velocity of esophageal peristalsis in 13 of the 16 volunteers. Retinopathy was evaluated by fundoscopic examination. Diabetic patients, all treated with insulin and diet, continued their usual therapy during the study. Neither ketosis or clinical hypoglycemia developed in any of the patients; FBs was monitored daily.

Serum and urine samples for creatinine, sodium, potassium, and glucose were obtained by routine clinical procedures and analyzed by an automated analyzer using a picric acid method (creatinine), a direct potentiometric method (sodium and potassium), and a glucose oxidase method (glucose) (Technicon Instruments Corp., Tarrytown, New York). Blood samples for PRA were centrifuged immediately after collection and frozen at -20° until assay. PRA was measured by radioimmunoassay of angiotensin I after incubation of plasma for 3 hours at 37°C. The incubation mixture of 1.115 ml contained 1.0 ml of plasma (with 1.5 mg disodium EDTA), 3.4 μmoles of 8-hydroxyquinoline sulfate, 40.1 μmoles of dimercaprol, and 400 μmoles of Tris buffer (pH 7.2) to adjust the final pH to 7.4. Inter- and intra-assay variance averaged 8% during the period of study for plasma pools with both high and low PRA. Plasma renin substrate was determined by a modification of the method of Katz and Smith. Partially purified angiotensinase-free human renin was prepared by Method A of Haas et al. Standard human renin was kindly provided by the World Health Organization (WHO) International Laboratories, Holly Hill, England. Each 0.02 ml plasma sample was incubated at 37° for 24 hours with excess human renin (0.05 G.U.), 0.01 ml of 0.34 M 8-hydroxyquinoline, 0.002 ml of 0.806 M dimercaprol, 0.01 ml of neomycin sulfate (0.25 mg/ml) in a final volume of 1.0 ml made up by adding 0.01 M Tris chloride buffer, pH 7.4 with 0.5% human serum albumin. The angiotensin I was measured by radioimmunoassay. Aldosterone excretion was determined by radioimmunoassay. Inter-assay variance averaged 15%. Statistical analyses were performed using Student's t test. The mean and standard error are indicated.

Results

In the diabetics PRA was lower under all conditions than in age-matched controls (figs. 1, 2). After maximum stimulation (4 hours of ambulation and 4 days of sodium restriction), PRA was 2.2 ± 0.4 in the diabetics compared to 3.4 ± 0.2 ng/ml/hr in the controls (p < 0.025). The importance of age-matching is evident from a comparison of these PRA values to the mean PRA in 15 young normotensive controls (age 20 to 30 years) previously studied under the same conditions in our laboratory; their mean PRA was 9.1 ± 1.3 ng/ml/hr. When expressed as a percent increase from baseline, PRA in the diabetics responded normally to both sodium depletion and posture. On the low salt diet, five of 16 diabetics failed to achieve sodium balance, and as a result the mean sodium excretion for all diabetics was 29.7 ± 8.8 compared to

| TABLE 1. Clinical Characteristics of Normotensive Diabetics With Neuropathy and Age-Matched Controls |
|---------------------------------------------------------------|---------|---------|
| Clinical characteristic | Controls (n = 9) | Diabetics (n = 10) |
| Age (yrs) | 52 ± 2 | 50 ± 1 |
| Weight (lbs) | 173 ± 10 | 165 ± 10 |
| Sex (M/F) | 9/0 | 14/2 |
| Race (Caucasian/Black) | 8/1 | 12/4 |
| Duration of diabetes (yrs) | — | 15 ± 2 |
| Fasting blood sugar (mg/dl) | 96 ± 5 | 209 ± 18* |
| Serum creatinine (mg/dl) | 1.1 | 1.1 |
| Urinary protein < 0.1 g/24 hrs | 9 | 14* |
| Retinopathy | — | 1 |

*Computed from the average of daily fasting blood sugar determinations for each subject; p < 0.001.

†Urinary proteins for the remaining two diabetics were 0.2 and 0.4 g/24 hrs.
only 7.5 ± 1.6 mEq/24 hrs ($p < 0.02$) in controls. The mean PRA for these five, however, was no different from the group mean. Urinary sodium excretion in diabetics did not correlate with the concurrent quantitative 24-hour urine glucose, PRA, the degree of neuropathy, or urinary aldosterone.

By using either a PRA-urinary sodium excretion nomogram or the criterion of a PRA value less than 2.1 ng/ml/hr at noon on Day 4 of the low sodium diet, eight of the 16 diabetic patients had a maximal PRA less than the minimum control PRA under the same conditions. The mean PRA of this "low renin" group was 1.0 ± 0.2 vs 3.3 ± 0.4 ng/ml/hr in the "normal renin" group. The mean age, weight, change in weight with sodium restriction, duration of diabetes, fasting blood sugar, urinary sodium, aldosterone, and glucose excretion, creatinine clearance, plasma renin substrate, and indices of peripheral neuropathy were all similar in low and normal PRA groups. One of the two women and three of the four blacks were in the low renin group. The velocity of esophageal peristalsis in the low renin group was markedly lower: 2.5 ± 0.3 vs 3.5 ± 0.4 cm/sec ($p < 0.001$). Fourteen normal volunteers, mean age 54 years, studied by one of us (D.O.C.) had a mean velocity of esophageal peristalsis of 3.3 ± 0.2 cm/sec.11

The severity of the peripheral neuropathy was quite variable, ranging from practically no abnormalities, to abnormality of every function tested. However, only one patient had a significant orthostatic drop in blood pressure (40 mm Hg) indicative of severe autonomic neuropathy. Despite this range in severity, no correlation could be made between PRA values on either of the diets, posturally stimulated or not, and the ratings for peripheral neuropathy, either by neurological exam (data not shown) or by nerve conduction studies.
values ranged from 0.06 to 0.34). Neurological exam scores for peripheral neuropathy did correlate with those from nerve conduction studies ($r = 0.68; p < 0.05$), but neither correlated with esophageal peristaltic velocity ($r = 0.14$ for both). On the other hand, there was a good correlation between esophageal peristaltic velocity and PRA under conditions of maximal stimulation (ambulation on Day 4 of the low sodium diet) (fig. 3). This relationship was closer when the increment of PRA related to ambulation was considered. No significant correlation was found under conditions of lesser stimulation of PRA.

Urinary aldosterone (table 2) was not significantly different in diabetics and controls despite the differences in PRA. Plasma renin substrate was $1053 \pm 95 \text{ng AI/ml}$ in diabetics compared to $1358 \pm 132$ in controls ($p < 0.07$).

The effect of the low sodium diet on serum sodium, potassium, creatinine, blood pressure, and weight was similar in the diabetics and controls (table 2). Mean fasting blood sugar did not correlate with PRA on linear regression analysis. Quantitative urine glucose ranged from zero to 90 g/24 hrs, with a mean of 15 g in the diabetics on the last day of sodium depletion, but showed no correlation with concurrent PRA. Despite selection of a group of diabetics with minimal or no proteinuria by conventional quantitative urinary protein analysis, the creatinine clearance, at least on the ad libitum diet, was less in the diabetics (table 2). However, no significant correlation between creatinine clearance and PRA could be found.

**Discussion**

These data document hyporeninemia in patients with long-standing, insulin-dependent diabetes mellitus complicated by neuropathy, but not by hypertension or substantial compromise of renal function. Although this group of selected diabetics represents a narrow spectrum of disease, it has allowed separation of neuropathy from the other complications often present in long-standing diabetes. The association between neuropathy and low PRA is further strengthened and further defined by the observation of a correlation between the degree of hyporeninemia and the severity of autonomic neuropathy as quantitated by velocity of esophageal peristalsis. The validity of this parameter as a measure of autonomic function is based on the demonstration of normal esophageal smooth muscle function coupled with slowed velocity of peristalsis in diabetics with peripheral neuropathy as compared to those without. Although autonomic neuropathy is more likely to be present in diabetics with peripheral neuropathy than in those without, the
lack of a close correlation between these parameters in diabetics has been well documented. Therefore our failure in our small group of diabetics, all with neuropathy, to demonstrate a close correlation between our approximate clinical index of peripheral neuropathy on the one hand, and direct measurements of esophageal peristaltic velocity or PRA on the other, while disappointing in terms of clinical utility, is not unexpected. Use of heart rate variability as a sensitive index of early autonomic dysfunction in diabetics would be helpful in further establishing the validity of this test.

The observed association between diabetic autonomic neuropathy and renin secretion is compatible with current understanding of these processes. Section of the renal nerve bearing autonomic fibers to the juxtaglomerular apparatus (JGA) lowers basal PRA and of the renal nerve bearing autonomic fibers to the juxtaglomerular apparatus (JGA) lowers basal PRA and of the renal nerve bearing autonomic fibers to the juxtaglomerular apparatus (JGA). Other factors may complicate an evaluation of diabetic autonomic neuropathy on renin secretion. Rapid changes in glucose concentration may stimulate an adrenergic response. Markedly elevated blood sugar (450 mg/dl) in rats is associated with low PRA, possibly on the basis of expansion of extra cellular fluid volume. Inactive PRA may be increased in some patients with diabetes, failure to guard against cryoactivation of inactive renin, or use of any assay that activates inactive renin, may result in falsely normal or elevated PRA in diabetics.

A role for autonomic neuropathy does not exclude other factors that might lower renin in our patients, or certainly in other patients with long-term diabetes. However, none was significantly associated with hyporeninemia in our patients, including mildly decreased renal function, elevated blood sugar, or slightly lower plasma renin substrate. The observed disassociation between PRA and aldosterone in diabetics has been well-described recently by de Chatel and coworkers. In some of their patients, as in ours, aldosterone (measured in plasma) was the same in diabetics with or without low PRA. Although sodium space was expanded in their diabetics, it was equally so in those with low or normal PRA. They found no differences in potassium or cortisol either, and concluded that an unidentified factor was influencing aldosterone secretion. Alternative explanations include an increase in adrenal sensitivity to angiotensin II, known to occur in patients with low renin hypertension and diabetics with retinopathy, or an alteration in aldosterone metabolism. Since a primary defect in renin secretion caused by a destructive lesion in the autonomic system should lower aldosterone secretion, some additional process must be involved in our patients.

The clinical implication of our observation that autonomic neuropathy is associated with low PRA is that some patients with long-standing diabetes mellitus have a primary defect in renin secretion. This implies that these patients have a potential impairment of homeostatic mechanisms involved in maintenance of vascular tone, plasma volume, and potassium balance. The extent to which this defect is compensated, or overcompensated, for by other processes such as nephropathy or changes in sensitivity to angiotensin II, remains a subject for further investigation.
References

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