PLASMA RENIN ACTIVITY AND URINARY KALLIKREIN EXCRETION IN RESPONSE TO INTRAVENOUS FUROSEMIDE IN DIABETIC PATIENTS

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The cause for the increased incidence of associated hypertension and nephropathy in diabetes mellitus is not as yet fully understood and the role of renal vasoactive substances has been suggested. A number of studies have appeared in literature during the past few years indicating changes in plasma renin activity4,5, urinary prostaglandins26 and kallikrein excretion2,19, and plasma inactive renin concentrations17 in diabetics. Variable plasma renin activity (PRA) ranging from low5,23, normal11 to high values6, have been described. Renin release in response to orthostatic tilt21 and isoproterenol infusion24 is reported to be blunted in these patients. Apart from the renin-angiotensin system, there is evidence of abnormalities in renal kallikrein excretion in diabetics. Decreased kallikrein in patients with hypertension and nephropathy2, and high kallikrein in poorly controlled diabetics have been recently reported19.

Furosemide, a loop diuretic when administered intravenously, is known to affect these hemodynamic mechanisms9,16,26. The purpose of the present study was to investigate whether PRA and kallikrein excretion in response to furosemide is altered in diabetics and, if so, if it could be correlated to the initiation and progression of hypertension and/or nephropathy.

MATERIALS AND METHODS

Forty-six diabetic patients (27 males and 19 females, aged 45.8 ± 8.8 years) and 10 age-matched controls (7 males and 3 females, aged 49.1 ± 14.2 years) without any family history of diabetes mellitus or hypertension and not taking any medication were studied. Diabetes had been diagnosed 6 months to 20 years before, on the basis of increased fasting and post-prandial plasma glucose levels. Patients were prospectively selected for the study according to age, duration of disease, type of diabetes (34 NIDDM and 12 IDDM) and presence or absence of complications. Fifteen (Group I) had neither hypertension nor clinical evidence of nephropathy; 11 (Group II) had hypertension without evidence of nephropathy; 8 (Group III) had nephropathy.

Key-words: Diabetic nephropathy; Furosemide; Plasma renin activity; Urinary kallikrein.

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Tab. 1 - Clinical characteristics of study subjects. (FBS = fasting blood sugar; SBP = systolic blood pressure; DBP = diastolic blood pressure. Data are expressed as means ± SE).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>age (years)</th>
<th>NIDDM/IDDM</th>
<th>duration of disease (years)</th>
<th>complications (years)</th>
<th>FBS (mg/dl)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>serum creatinine (mg/dl)</th>
<th>blood urea (mg/dl)</th>
<th>urinary albumin (mg/day)</th>
<th>baseline PRA (ng/ml·h)</th>
<th>kallikrein excretion (ng·ml·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>10</td>
<td>45.8 ± 8.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>88.4 ± 10.6</td>
<td>150.0 ± 9.0</td>
<td>80.0 ± 6.6</td>
<td>0.92 ± 0.18</td>
<td>86.0 ± 2.8</td>
<td>28.8 ± 0.2</td>
<td>7.8 ± 0.5</td>
<td>1.82 ± 0.17</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>42.2 ± 12.3</td>
<td>9/6</td>
<td>&lt;1-2</td>
<td>-</td>
<td>138.0 ± 8.3</td>
<td>131.6 ± 4.0</td>
<td>83.0 ± 4.0</td>
<td>0.94 ± 0.21</td>
<td>28.8 ± 1.2</td>
<td>25.6 ± 0.3</td>
<td>2.50 ± 0.11</td>
<td>99.5 ± 0.44</td>
</tr>
<tr>
<td>Group II</td>
<td>11</td>
<td>48.3 ± 6.8</td>
<td>9/2</td>
<td>2-14</td>
<td>1-2</td>
<td>126.8 ± 10.0</td>
<td>180.0 ± 8.6</td>
<td>100.0 ± 5.4</td>
<td>0.98 ± 0.15</td>
<td>28.4 ± 1.3</td>
<td>29.3 ± 0.3</td>
<td>2.97 ± 0.29</td>
<td>71.27 ± 0.75</td>
</tr>
<tr>
<td>Group III</td>
<td>8</td>
<td>54.0 ± 9.4</td>
<td>6/2</td>
<td>1-17</td>
<td>&lt;1</td>
<td>140.2 ± 6.5</td>
<td>140.0 ± 4.0</td>
<td>85.0 ± 4.8</td>
<td>1.68 ± 0.44</td>
<td>28.2 ± 2.0</td>
<td>128.0 ± 9.6</td>
<td>1.12 ± 0.18</td>
<td>79.12 ± 0.74</td>
</tr>
<tr>
<td>Group IV</td>
<td>12</td>
<td>52.5 ± 10.2</td>
<td>10/2</td>
<td>3-20</td>
<td>1-3</td>
<td>138.5 ± 9.4</td>
<td>191.3 ± 10.0</td>
<td>105.0 ± 5.0</td>
<td>1.80 ± 0.34</td>
<td>62.4 ± 3.8</td>
<td>180.0 ± 9.0</td>
<td>2.02 ± 0.32</td>
<td>56.75 ± 0.56</td>
</tr>
</tbody>
</table>

with proteinuria of 1 g or more daily; and 12 (Group IV) had hypertension with diabetic nephropathy. The clinical and laboratory characteristics of the groups as well as the data for blood pressure, fasting blood sugar, serum creatinine, blood urea and 24 h urinary albumin are reported in tab. 1.

The protocol was approved by the Ethics Committee of All-India Institute of Medical Sciences and informed consent from all the patients and volunteers was obtained before the study.

For the control of diabetes mellitus, with the exception of 2 patients in Group I treated with diet alone and 3 patients each in Groups III and IV treated with insulin plus diet, the remainder received glibenclamide (Daonil®, Hoechst) for NIDDM and insulin for IDDM. Anti-hypertensive medication (beta-blockers, calcium channel blockers and captopril), with the exception of diuretics, was withdrawn 24 h prior to investigation and a low salt diet (<2 g/day) was recommended to both patients and controls 5 days before the study.

All patients and controls were instructed to collect 4 h urine after discarding the night sample and to come to SRB Centre of Clinical Pharmacology of AIIMS. A peripheral sample was obtained 30-60 min after, with the patient in a supine position, and collected into chilled tube containing EDTA for estimation of PRA. Furosemide 0.5 mg/kg was administered intravenously immediately following the withdrawal of blood and 10 min later another blood sample was withdrawn. A 4 h urine sample for urinary kallikrein and electrolytes (Na⁺ and K⁺) was collected before and after furosemide.

PRA was measured by the radioimmunoassay of Ang-I and kallikrein was estimated by a bioassay procedure using the micromethod of Marin Grecz and Carretero, which employs the enzymatic property of the urinary kallikrein to generate kinin after incubation with a substrate derived from dog plasma. Matching assay was carried out by the superfusion method on an estrogenized rat uterus preparation using Tyrode's solution. Urinary sodium and potassium were measured by flame photometer. Albumin excretion rate was measured by the method of Henry et al.

The sensitivity of the radioimmunoassay procedure for Ang-I was 40-52 pg/ml/h. The mean interassay and intrainassay variations were 11.44% and 2.24%, respectively, and the mean recovery of standard Ang-I was 112.7%.

The sensitivity of estrogenized uterine preparation for standard bradykinin varied between 50-100 pg. The reliability criteria of the assay were checked and fell within acceptable limits.

The statistical analysis of the data before and after furosemide was carried out by the paired t-test, and between groups by the one-way analysis of variance (ANOVA).