Original Article

Urinary albumin excretion rate during angiotensin II infusion in microalbuminuric patients with insulin and non-insulin-dependent diabetes mellitus

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Abstract As angiotensin-converting enzyme inhibition is accompanied by a marked decrease in glomerular protein loss, the hypothesis was tested that an increase of the glomerular transcapillary hydraulic pressure difference by exogenous angiotensin II would increase microalbuminuria in patients with insulin (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). Acute effects of increasing doses of angiotensin II (1, 3 and 6 ng/kg/min) were studied on mean arterial pressure (MAP), glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), total renal vascular resistance (TRVR), and urinary albumin excretion rate (UAER) in 11 IDDM and 11 NIDDM microalbuminuric patients. Angiotensin II infusion changed MAP from 100±3 mmHg at baseline to 105±3, 111±3, and 116±3 mmHg (P<0.001), ERPF from 542±29 to 478±24, 429±23, and 382±19 ml/min (P<0.001), FF from 20.2±0.06 to 23.1±0.7, 27.1±1.1, and 29.8±1.2% (P<0.001), and TRVR from 9454±809 to 11 158±930, 13 310±1206, and 15 538±1362 dyne s cm⁻⁵ (P<0.001). GFR and UAER, however, did not change significantly. Therefore, during angiotensin II infusion ERPF decreased, while FF and TRVR increased. As UAER and GFR remained unchanged, the presumed rise in intraglomerular capillary pressure by exogenous angiotensin II did not increase UAER. We suggest that during manipulation of the renin–angiotensin system, as in other renal diseases with proteinuria, factors other than glomerular transcapillary hydraulic pressure determine the degree of urinary albumin loss in microalbuminuric IDDM and NIDDM patients.

Key words: angiotensin II; diabetes mellitus; filtration fraction; microalbuminuria; urinary albumin excretion rate

Introduction

A urinary albumin excretion rate (UAER) between 30 and 300 mg 24 h, so-called microalbuminuria, forecasts the development of clinically manifest diabetic nephropathy in insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) [1]. Newly diagnosed patients with IDDM have an elevated glomerular filtration rate (GFR) of 20–50% above that of age-matched, non-diabetic subjects [2,3]. The issue that this so-called hyperfiltration may provoke proteinuria has been examined further in experimentally diabetic rats. Micropuncture studies in streptozotocin-induced diabetic rats have shown an increased single-nephron glomerular filtration rate (SNGFR), and an increased glomerular transcapillary hydraulic pressure difference too, and has been postulated to contribute to the occurrence of microalbuminuria in patients with IDDM as well as NIDDM [5].

Angiotensin-converting enzyme (ACE) inhibitors have been shown to normalize intraglomerular capillary pressure and to prevent the increase in the albumin excretion rate in experimentally diabetic rats [6] and to decrease microalbuminuria in normotensive adults with IDDM [7]. Although more than one mechanism is likely to be involved [8], the haemodynamic effect of ACE inhibition has been considered to be largely responsible for the reduction in urinary albumin excretion of microalbuminuric as well as proteinuric diabetic patients using these drugs. However, studies with angiotensin II infusion in patients with chronic renal diseases and proteinuria did not confirm the suggestion that an increase of the intraglomerular capillary pressure magnifies proteinuria [9,10].

Therefore, the aim of this study was to test the hypothesis that if microalbuminuria is largely governed by haemodynamic factors, an increase in filtration...
fraction (FF) by exogenous angiotensin II would increase microalbuminuria in diabetic patients.

Subjects and methods

Subjects

Patients were recruited from our out-patient diabetic clinic after written informed consent. The study protocol had been approved by the local ethical committee.

All patients fulfilled the following criteria before the start of the study: the presence of microalbuminuria (defined as an albumin excretion rate of 30–300 mg albumin/24 h shown in at least three out of four 24 h urine specimens), and diabetes mellitus (IDDM or NIDDM) treated with insulin, tolbutamide, glibenclamide or diet only. Exclusion criteria were glycosylated haemoglobin percentage (HbA1c) > 12.0%, age < 18 or > 65 years, serum creatinine > 120 µmol/l, diabetes duration more than 30 years, body mass index > 35.0 kg/m², untreated systolic blood pressure > 170 mmHg, untreated diastolic blood pressure > 95 mmHg, treatment with antihypertensive or non-steroidal anti-inflammatory drugs, a history of any renal disease, and females in the fertile period using no adequate contraceptive methods.

A full medical history was taken and a complete physical examination performed the day before the start of the study. Blood samples for haematological (haematocrit) and chemical measurements (glycosylated haemoglobin; serum creatinine) were drawn and determined the same day. A sample of urine was taken for examination of the centrifuged urinary sediment to exclude urinary tract infection. If any of these measurements was seriously aberrant, patients were not accepted for the study.

Study design

Patients were asked to refrain from strenuous exercise, smoking and alcoholic beverages for 3 days before the beginning of the experiments.

On the day of the experiments patients came to the out-patient clinic after an overnight fast. The morning dose of insulin or oral hypoglycaemic agents had been omitted. All subjects remained supine, but during the test it was sometimes necessary to permit sitting on the edge of the bed for voiding.

At the beginning of the test (t = 0) two intravenous canules were inserted, one for blood sampling and one for infusion. The blood glucose level (Refloflux®, Boehringer Mannheim, Mannheim, Germany) was maintained between 4 and 8 mmol/l by means of a variable glucose and insulin infusion with, respectively, an Infusomat® Secura and a Perfusor® Secura FT (both B. Braun Melsungen AG, Melsungen, Germany). The glucose infusion rate was kept constant (50 ml/h) and the insulin infusion varied, although sometimes it was necessary to administer a higher rate of glucose infusion. Blood glucose level was measured each 30 min but, if necessary, more often.

Blood pressure measurements were performed at 5–10 min intervals with an automatic device (Datascope®, Datex Instrumentarium Corporation, Helsinki, Finland). Electrocardiogram monitoring (Hewlett Packard) was performed during the whole test period for reasons of safety.

GFR and effective renal plasma flow (ERPF) were measured according to the method of Donker et al. [11] with 125I-hippuran and 51Cr-EDTA (a solution of 200 µCi/3.7 Mbq 125I-hippuran and 200 µCi/3.7 Mbq 51Cr-EDTA in 400 ml saline 0.9%). At the start of the study (t = 0) a priming dose of these radiopharmaceuticals was administered intravenously. Thereafter, a continuous infusion (Imed® type 928) with the dissolved radiopharmaceuticals was started. One and a half hours was allowed for stabilization of the blood glucose and radiopharmaceutical levels. The next 2 h (t = 1.5–3.5) were used for baseline clearance studies.

After the baseline clearance studies, increasing dosages of angiotensin II (AII; Hypertensin®, Ciba-Geigy AG, Basel, Switzerland), 1, 3 and 6 ng AII/kg body weight/min, were administered intravenously (Perfusor®), each for the period of 1 h. Stable AII and aldosterone levels are reached within 20 min in a normal human [12].

To ensure adequate diuresis patients drank 200 ml tap water each hour. At regular time intervals blood was drawn and urinary samples were taken for measurement of renin, AII, GFR, ERPF, and UAER.

Calculations

FF (%) was determined by the quotient 100 × GFR/ERPF. Mean arterial pressure (MAP, mmHg) was calculated as two times diastolic plus one time systolic blood pressure divided by three. Renal blood flow (RBF) was calculated as ERPF/(1 – haematocrit), and expressed in l/min. Total renal vascular resistance (TRVR) was calculated as (MAP/RBF) × 80, and expressed in dyne s cm⁻².

Analytical methods

Urinary albumin concentration was measured by RIA (albumin double antibody RIA kit, Diagnostic Products Corp., Los Angeles, California, USA).

Plasma AII concentration was measured by RIA (angiotensin II antibody RIA kit, Nichols Institute Diagnostics, Los Angeles, California, USA). Plasma AII concentrations greater than 200 pg/ml could not be determined exactly, and are reported as 200 pg/ml in the results.

Plasma renin concentration was measured by RIA (renin antibody RIA kit, E.R.I.A. Diagnostics Pasteur, Marnes-la-Coquette, France). Plasma renin concentrations smaller than 3.5 pg/ml could not be determined exactly and are reported as 3.5 pg/ml in the data.

Radioactivity of plasma and urine samples for measurement of GFR and ERPF, and of RIA samples for measurement of urinary albumin concentration, plasma AII and renin concentrations, was determined with a gamma scintillation counter (2 inch well-type crystal, ICN 3.33, ICN Tracer Lab., Belgium).

Glycosylated haemoglobin percentage (HbA1c) was measured by means of high-performance liquid chromatography (HPLC system, Pharmacia/LKB, Upsala, Sweden).

Creatinine concentration was measured by means of a Jaffé technique (creatinine kit for BM/Hitachi 717, Boehringer Mannheim, Germany).

Statistical analysis

Data were obtained at each time point of blood and urine sampling from all 22 subjects. The equilibrium period lasted 1.5 h from the start of the experiment. The means of the first two measurements of GFR, ERPF, MAP, and UAER were used as the baseline values for the AII infusion period.

For comparison with the AII infusion period results, the mean baseline value of the above variables was used to
correct for individual differences. Analysis of variance with polynomial contrasts was used to assess changes over time. A significance level of 0.05 was used for all statistical tests.

**Results**

Twenty-three patients participated in the study, but only 22 completed the experiments. The infusions were prematurely stopped in one patient because he became very upset during the experiment; his data were excluded from the analysis.

The clinical and physical characteristics of the patients are shown in Table 1. The IDDM patients with microalbuminuria were younger than the NIDDM patients (mean ± SD = 35 ± 11 and 51 ± 11 years, respectively), and had a longer history of diabetes (20 ± 5 and 12 ± 7 years, respectively). Three patients in the NIDDM group were not treated with insulin. Body mass index tended to be lower in IDDM than in NIDDM (24.5 ± 2.7 and 27.6 ± 4.1 kg/m², respectively; Mann–Whitney U-test, P = 0.06). No other differences could be found between the IDDM and the NIDDM patients for the remaining data.

During the whole experiment, apart from the equilibrium period, blood glucose levels were successfully maintained between 4 and 8 mmol/l in 19 patients. On one occasion, blood glucose level reached a hypoglycaemic value 2 h after the start of the experiment, but that level was immediately corrected. In two other cases, blood glucose levels were unstable for approximately 90 min after the equilibrium period, but remained between 2.5 and 8 mmol/l.

Results are shown in Table 2. GFR did not change during the infusion of increasing dosages of AII, but ERPF decreased significantly. As a consequence, FF increased markedly. MAP increased progressively. Therefore, TRVR increased significantly. However, no changes in UAER were observed (P = 0.49).

Compared to baseline values plasma renin concentration dropped and AII concentration increased during the AII infusion period (Table 3).

**Discussion**

At filtration pressure equilibrium, SNGFR can be expressed as:

\[
\text{SNGFR} = (1 - \frac{\pi_A}{P_{thp}}) \times Q_A
\]

where \(\pi_A\) is the afferent colloid osmotic pressure of plasma, \(P_{thp}\) the mean glomerular transcapillary hydraulic pressure difference, and \(Q_A\) the afferent arteriolar plasma flow rate [13].

In the present study GFR did not change, while ERPF decreased and FF increased markedly during AII administration. There was no obvious reason for a changed colloid osmotic pressure (\(\pi\)) during the study. Thus, looking at the above equation, it is reasonable to assume an increase in glomerular transcapillary hydraulic pressure difference during the AII infusion period. The increase in TRVR during exogenous AII is in agreement with these findings. However, the lack of an effect of AII infusion on UAER in our study does not support our postulated hypothesis, since the presumed rise in glomerular transcapillary hydraulic pressure did not affect UAER.

Comparable effects of exogenous AII on urinary albumin excretion have been described previously in patients with chronic renal disease and proteinuria [9,10]. Thus, our and other studies suggest that factors other than changes in renal haemodynamics are also involved in the antiproteinuric effect of ACE inhibitors. This suggestion is supported by observations made earlier in experimentally diabetic, spontaneously hypertensive rats. These rats have a higher intraglomerular pressure compared with normotensive, experimentally diabetic rats, but do not develop more proteinuria or histological damage [14]. Conversely, AII infusion magnifies proteinuria in normal rats [15,16] and in rats with experimentally glomerular diseases [17,18], but the doses of AII used in those rats exceeded markedly the doses of AII used in our and other human studies.

Debate exists whether filtration equilibrium, as described above, does exist in humans. If it does not, filtration dysequilibrium implies that the ultrafiltration coefficient (\(K_f\)) and the tone of the afferent and efferent arterioles have additional or opposing effects on SNGFR, SNFF and \(P_{thp}\) [8]. In the present study AII was administered in doses leading to a significant increase in MAP. This marked rise in systemic blood pressure will have resulted in an increased glomerular afferent arteriolar resistance [13]. Despite the decrease in ERPF, GFR remained essentially unchanged in our study.

The total rate of glomerular filtration of a single nephron independent of equilibrium can be expressed as:

\[
\text{SNGFR} = K_f P_{zf}
\]

where \(P_{zf}\) is the difference between the mean transcapillary hydraulic and colloid osmotic pressure difference [13]. Intravenous infusion of AII in pressor doses in rats resulted in a substantial decrease in glomerular plasma flow and a marked increase in afferent and efferent vasoconstriction [19]. However, efferent arteriolar vasoconstriction dominated the afferent arteriolar vasoconstriction [19]. Thus, the mean transcapillary hydraulic pressure difference and, therefore, \(P_{zf}\) had to increase and \(K_f\) to decrease for the maintenance of
Table 2. The effect of angiotensin II (1, 3 and 6 ng/kg/min) on GFR, ERPF, MAP, TRVR, and UAER in IDDM (n = 11) and NIDDM (n = 11) (mean ± SEM)

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>Renin (pg/ml)</th>
<th>Angiotensin II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16.0 ± 2.2</td>
<td>12.3 ± 1.6</td>
</tr>
<tr>
<td>All inf 1 ng/kg/min</td>
<td>13.1 ± 1.7</td>
<td>36.6 ± 4.7</td>
</tr>
<tr>
<td>All inf 3 ng/kg/min</td>
<td>11.2 ± 1.6</td>
<td>97 ± 12</td>
</tr>
<tr>
<td>All inf 6 ng/kg/min</td>
<td>10.2 ± 1.5</td>
<td>164 ± 11</td>
</tr>
</tbody>
</table>

All inf, angiotensin II infusion.

References

16. Eisenbach GM, Liew JB van, Boylan JW. Effect of angiotensin

Table 3. Plasma concentrations (mean ± SEM) of renin and angiotensin II in IDDM (n = 11) and NIDDM (n = 11)

<table>
<thead>
<tr>
<th>GFR (ml/min)</th>
<th>110 ± 7</th>
<th>110 ± 6</th>
<th>116 ± 7</th>
<th>114 ± 7</th>
<th>0.34</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPF (ml/min)</td>
<td>542 ± 29</td>
<td>478 ± 24</td>
<td>429 ± 23</td>
<td>382 ± 19</td>
<td>0.000</td>
</tr>
<tr>
<td>FF (%)</td>
<td>20.2 ± 0.6</td>
<td>23.1 ± 0.7</td>
<td>27.1 ± 1.1</td>
<td>29.8 ± 1.2</td>
<td>0.000</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>100 ± 3</td>
<td>105 ± 3</td>
<td>111 ± 3</td>
<td>116 ± 3</td>
<td>0.000</td>
</tr>
<tr>
<td>TRVR (dyne s cm–2)</td>
<td>9454 ± 809</td>
<td>11158 ± 930</td>
<td>13310 ± 1206</td>
<td>15538 ± 1362</td>
<td>0.000</td>
</tr>
<tr>
<td>UAER (mg/h)</td>
<td>6.4 ± 2.0</td>
<td>5.7 ± 1.4</td>
<td>4.7 ± 1.3</td>
<td>6.7 ± 2.2</td>
<td>0.49</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure; TRVR, total renal vascular resistance; UAER, urinary albumin excretion rate.

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