Evidence for the local occurrence of angiotensin II in rat kidney and its modulation by dietary sodium intake and converting enzyme blockade

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Summary
1. Angiotensin II (ANG II) was measured in acid-ethanol homogenates of rapidly frozen rat kidneys by a method involving ion-exchange and immunoabsorbent purification of peptides before radioimmunoassay.
2. Concentrations of ANG II found in kidney were 10–20 times that in plasma.
3. Perfusion of the kidneys via the renal artery with isotonic sodium chloride solution or with disodium EDTA solution did not alter the concentrations of intrarenal ANG II.
4. Animals fed on a sodium-deficient diet for 8 days had markedly higher concentrations of intrarenal ANG II, plasma renin activity and kidney renin concentration than sodium-replete animals.
5. After oral sodium loading for 3 weeks, rats had suppressed plasma renin activity and kidney renin concentration but unchanged intrarenal ANG II when compared with animals on a normal sodium intake.
6. One hour after the administration of a converting enzyme inhibitor (SQ 20881) plasma renin activity was elevated, kidney renin concentration unchanged and intrarenal ANG II was depressed.
7. These results demonstrate the presence of ANG II in the extravascular compartment of the kidney. They further suggest that its quantity is influenced by sodium intake and that angiotensin I converting enzyme is essential for its formation.

Key words: angiotensin II, converting enzyme, juxtaglomerular apparatus, macula densa, renin, feedback.

Abbreviations: ANG I, ANG II, angiotensin I and angiotensin II.

Introduction
It has been widely postulated that angiotensin II (ANG II) might serve a local regulatory role in the kidney (Thurau, 1963; Guyton, Langston & Navar, 1964; Thurau, 1974). One possible mechanism for this action might be as a transmitter substance in the tubulo-glomerular feedback system (Thurau & Schnerrmann, 1965; Schnerrmann, Wright, Davis, Stackelberg & Grill, 1970), which servo-regulates glomerular filtration rate (GFR) of each nephron according to the NaCl concentration reaching its macula densa. By this means intrarenal ANG II might be involved in the regulation of salt and water excretion (Thurau, 1974).

Despite this interest in local tissue ANG II, there have been no previous published reports of attempts to measure the concentrations of ANG II in kidney to test this hypothesis. This paper reports the use of a method developed to measure kidney ANG II (Mendelsohn, 1976) to follow possible changes during states of altered sodium handling.

The effect of converting enzyme blockade was assessed to determine whether this enzyme was necessary for the formation of intrarenal ANG II.
Methods

Animals and analytical methods

Male Sprague–Dawley rats of weight 200–250 g were fed on a diet containing Na+ (45 mmol/kg) and K+ (105 mmol/kg) (diet A). The animals were either anaesthetized with pentobarbitone (60 mg/kg, intraperitoneally) or killed by stunning and cervical dislocation, as indicated below.

Extraction, partial purification and radioimmunoassay of kidney angiotensin II. The method for measurement of kidney ANG II was described previously (Mendelsohn, 1976). In brief, the kidneys were rapidly removed, quickly frozen in liquid nitrogen, and homogenized in cold acid–ethanol. The solution was extracted by using ion-exchange resin and further purified with columns of immobilized ANG II antiserum before radioimmunoassay for ANG II. Recovery of ANG II was monitored by using 125I-labelled ANG II. The following minor modifications were used: kidneys were homogenized only once in acid–ethanol, since this was found to extract 95% (SD 11; n = 20) of the ANG II released by the original method. ANG II was extracted directly from the acid–ethanol extracts by using columns (0.6 cm Dowex resin, since this procedure was faster and gave better recovery of ANG II (76±2%, SD 8±3, n = 42).

Kidney renin concentration. This was determined by incubating kidney homogenates with sheep plasma renin substrate and measuring the angiotensin I (ANG I) released by radioimmunoassay. Approximately 100–150 mg of frozen kidney was removed from the upper pole of the right kidney, weighed and homogenized in distilled water (5 mg of tissue/ml) in a Polytron homogenizer. The homogenate was frozen and thawed three times and centrifuged at 1000 g for 30 min at 4°C. Incubation mixtures contained 10 μl of the kidney extract and 120 pmol of sheep renin substrate prepared by the method of Boucher, Menard & Genest (1967). 2,3-Dimercaprol (1.25 mmol/l) and phenylmethylsulphonylfuoride (4.6 mmol/l) were added to inhibit angiotensinases. The total volume was adjusted to 500 μl with sodium phosphate (50 mmol/l), pH 6.5, containing sodium chloride (154 mmol/l), disodium EDTA (10 mmol/l) and neomycin (0.2 g/l) (buffer A). After 30 min incubation at 37°C, the reaction was terminated by boiling for 15 min. Portions (10 μl) of the supernatant were used for radioimmunoassay of ANG I.

Angiotensin I radioimmunoassay. The ANG I radioimmunoassay was performed by using an antibody raised in rabbits to a copolymer of [Asp', Ile']ANG I (Schwarz–Mann, Orangeburg, New York, U.S.A.) and bovine serum albumin. The antibody contained one class of high-affinity sites of Kd 2.5 × 10^-10 l/mol (sd 0-9; n = 4) and binding-site concentration of 1-1 μmol/l (sd 0-2; n = 4) of the original antiserum. The tracer was monoiodinated, 125I-labelled ANG I (New England Nuclear Corp., Boston, Mass., U.S.A.). Standards of [Asp', Ile']-ANG I (Medical Research Council Research standard A, 71/328) were used in the range 10–150 fmol and separation of bound and free ANG I was performed by using dextran-coated charcoal.

Plasma renin activity. Rat blood collected from the trunk after decapitation was placed in heparinized tubes on ice, centrifuged at 1000 g for 15 min and the plasma separated and stored at -20°C. Incubation mixtures contained 50 μl of plasma, 185 μl of buffer A and the same concentrations of angiotensinase inhibitors as were used for the kidney renin assay. After incubation at 37°C for 4 h, the reaction was stopped by boiling. Portions (10 μl) of the supernatant solutions were used for radioimmunoassay of ANG I.

Urinary sodium and potassium. These were measured with a flame photometer (model 143, Instrumentation Laboratory Inc., Boston, Mass., U.S.A.) with a lithium internal standard and a model 144 dilutor.

Procedures designed to test the significance of ANG II detected in kidney

Perfusion of kidneys. This was performed with cold NaCl solution (154 mmol/l) or disodium EDTA (10 mmol/l) in NaCl solution as previously described (Mendelsohn, 1976).

Effect of a delay in freezing kidneys. One kidney was removed as quickly as possible and frozen while the contralateral renal artery was clamped, and the kidney was allowed to remain at body temperature for a further 1 min before freezing.

Effect of sodium deficiency on intrarenal ANG II

Groups of 10 rats were fed on a sodium-deficient diet which consisted of polished rice, casein and vitamin B supplements and contained Na+ (5 mmol/kg) and K+ (40 mmol/kg) (diet B). Distilled water was given as drinking fluid. Frusemide (2 mg) was injected intraperitoneally on days 1, 3, 5 and 7. A control group was given the same diet but
had the choice of isotonic sodium chloride solution (154 mmol/l: saline) or distilled water to drink and were injected with saline in place of frusemide. On day 8, the animals were killed by cervical dislocation, the kidneys rapidly removed and frozen in liquid nitrogen, and samples of blood and bladder urine were obtained.

**Sodium loading**

Groups of eight male rats weighing 120–150 g were individually housed in metabolic cages. Control animals were fed on diet A and given tap water to drink. The sodium-loaded group ate the same diet but drank saline in place of water. After 3 weeks, urinary Na⁺ and K⁺ excretion were measured during a 24 h urine collection and the animals were killed by stunning and decapitation.

**Converting enzyme inhibition**

Six male rats weighing 200–250 g were fed on diet A and injected intraperitoneally with the converting enzyme inhibitor SQ 20881, 1.82 mmol/kg (2.0 mg/kg), as a 1-82 mmol/l (2.0 mg/ml) solution in saline. Controls were injected with saline in place of the inhibitor. At 1 h later the animals were killed and blood and kidney tissue collected for renin and angiotensin assays.

**Results**

**Validation of the renin and angiotensin I assays**

**Angiotensin I radioimmunoassay.** The ANG I radioimmunoassay standard curve is shown in Fig. 1. Within-assay variation was assessed as described for the ANG II assay (Mendelsohn, 1976). The mean coefficient of variation so calculated in 20 consecutive assays was 4.7–6.3% with measurements in the range 25–150 fmol. The between-assay variation obtained by 58 consecutive measurements of a pool of ANG I gave a value of 2.28 pmol h⁻¹ ml⁻¹ (SD 0.30; coefficient of variation = 13.1%).

**Kidney renin assay.** The sheep renin substrate used for this assay was free from angiotensinase, contained activity less than 0.8 pmol of ANG I h⁻¹ ml⁻¹ for endogenous renin and was found to have an apparent $K_m$ of 0.6 μmol/l (SEM 0.2; $n = 3$) in its reaction with rat renin compared with 1.7 μmol/l (SEM 0.2; $n = 3$) obtained with rat renin substrate prepared in a similar manner. During incubation with samples containing the highest renin concentration, less than 3% of the substrate was consumed and with most samples this was less than 1%.

The recovery of exogenous ANG I added to incubations was 98 ± SD 6% ($n = 4$), suggesting that angiotensinase activity had been completely blocked.

The relationship between the mass of ANG I generated and time of incubation was linear, as was that with variation in enzyme concentration.

**Plasma renin activity.** The rate of generation of ANG I was linear during incubations up to 4 h. The recovery of exogenous ANG I added to the plasma incubate was 99 ± SD 15% ($n = 4$) of that predicted from the sum of endogenously generated and added ANG I. This result suggests efficient blockade of angiotensinases.

Between-assay variability was assessed by 11 consecutive measurements of a pool of rat plasma, which gave a value of 2.28 pmol h⁻¹ ml⁻¹ (SD 0.30; coefficient of variation = 13.1%).
TABLE 1. Effect on intrarenal angiotensin II of perfusion via the renal artery before processing the perfused kidneys

Perfusion solutions were NaCl solution (154 mmol/l) or EDTA (disodium salt; 10 mmol/l in the NaCl solution). Mean results for six perfusions are shown \( \pm \) SEM.

<table>
<thead>
<tr>
<th>Kidney angiotensin II (pmol/kidney)</th>
<th>Control Saline-perfused</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1</td>
<td>0.61 ± 0.15</td>
<td>0.88 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>EDTA-perfused</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>0.79 ± 0.14</td>
<td>0.53 ± 0.12</td>
</tr>
</tbody>
</table>

**Effect of various manoeuvres on intrarenal angiotensin II**

**Effect of perfusion of kidneys.** Perfusion of the kidneys with either NaCl solution or NaCl solution containing EDTA did not change intrarenal ANG II (Table 1).

**Effect of a delay in freezing the kidneys.** The ANG II concentration in kidneys removed as rapidly as possible was 0.69 pmol/g (SEM 0.11, \( n = 8 \)). This value was not different from that in the contralateral kidneys whose freezing was delayed, which contained 0.74 pmol/g (SEM 0.13, \( n = 8 \)) (\( P > 0.6 \), paired t-test).

**TABLE 2. Concentrations of angiotensin II in the left and right kidneys of eight rats and within- and between-rat variation**

Values are means \( \pm \) SD of four determinations.

<table>
<thead>
<tr>
<th>Kidney angiotensin II (pmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney</td>
</tr>
<tr>
<td>Right kidney</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>7</td>
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<tr>
<td>8</td>
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</tbody>
</table>

Between- and within-rat variation of intrarenal angiotensin II. To determine whether the variation of intrarenal ANG II between animals was due to methodological error or biological variation, the variation of ANG II concentration between the two kidneys of the same animal was compared with that between kidneys of different animals. One-way analysis of variance of these data revealed that the between-animal variance was much greater than
that within animals (Table 2), and suggests a biological source for the variance.

**Dietary sodium deprivation.** The effects of dietary sodium deprivation are shown in Fig. 2. Sodium-deprived animals had very low urinary Na\(^+\) concentrations, markedly elevated plasma renin activities and an increase in kidney renin concentration. Intrarenal ANG II was highly significantly increased in the sodium-deprived animals. There was a significant inverse linear relationship between kidney ANG II and urinary sodium concentration, as shown in Fig. 3. The regression equation relating these two parameters was: \( \text{y} = 856 (\pm 67) - 2.96 (\pm 0.81)x \); \( r = 0.651, \text{df} = 18, P < 0.01 \), where y is the kidney ANG II (fmol/g) and x the urinary Na concentration (mmol/l), and the values in parenthesis are standard errors of the regression parameters (Armitage, 1971).

**Sodium loading.** Saline-loaded rats had elevated urine flow rates and sodium concentrations without detectable change in endogenous creatinine clearance (Table 3). Plasma renin activity and kidney renin concentration were both reduced in

**TABLE 3. Effect of saline loading**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saline-loaded</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow rate (( \mu l/min ))</td>
<td>3.99 ± 0.68</td>
<td>6.80 ± 0.48</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Urine Na(^+) concentration (mmol/l)</td>
<td>91.3 ± 3.5</td>
<td>350 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine Na(^+) excretion (( \mu l/min ))</td>
<td>0.358 ± 0.056</td>
<td>2.33 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine clearance (ml min(^{-1}) kg(^{-1}))</td>
<td>5.36 ± 0.68</td>
<td>5.40 ± 0.58</td>
<td>&gt;0.95</td>
</tr>
<tr>
<td>Plasma renin activity (pmol h(^{-1}) ml(^{-1}))</td>
<td>3.31 ± 0.33</td>
<td>1.83 ± 0.24</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney renin concentration (pmol h(^{-1}) mg(^{-1}))</td>
<td>89.4 ± 8.5</td>
<td>40.4 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kidney ANG II (pmol/g)</td>
<td>0.751 ± 0.084</td>
<td>0.776 ± 0.100</td>
<td>&gt;0.8</td>
</tr>
</tbody>
</table>

\( \text{FIG. 3. Relationship between kidney ANG II and urinary sodium concentration (} U_{\text{Na}} \text{)} \) in sodium-deplete (○) and sodium-replete (●) rats.

\( \text{FIG. 4. Kidney ANG II, kidney renin concentration and plasma renin activity (PRA) in rats injected 1 h previously with the converting enzyme inhibitor SQ 20881 (1.82 mmol/kg) (SQ) or control animals given vehicle alone (CTL). Horizontal bars represent mean± sem (n = 6).} \)
the saline-loaded group. Kidney ANG II was not altered by this manoeuvre.

Converting enzyme blockade. The administration of SQ 20881 led to a marked increase in plasma renin activity without significant alteration of kidney renin concentration, as shown in Fig. 4. Kidney ANG II was markedly depressed in animals treated with the inhibitor.

Discussion

These experiments confirm the previous finding (Mendelsohn, 1976) of high local concentrations of ANG II in rat kidney. This material does not appear to be due to contamination by plasma ANG II since the concentration of ANG II in kidney exceeds that which could be accounted for by trapped blood (Mendelsohn, 1976) and is unaltered by removal of blood by perfusion of the kidneys. It seems unlikely that kidney ANG II is formed by the action of renal renin on plasma renin substrate after removal of the kidneys, because perfusion of the kidneys with either sodium chloride solution or with disodium EDTA to block converting enzyme additionally did not alter kidney ANG II concentration. In addition a deliberate delay in freezing the kidneys did not alter intrarenal ANG II concentrations, suggesting that generation of ANG II ex vivo was not an important source of the peptide.

Intrarenal ANG II concentration increased during sodium deprivation and was inversely related to urinary sodium concentration. It is possible that intrarenal ANG II plays a role in renal sodium conservation by actions on glomerular filtration rate (Blantz, Konnen & Tucker, 1976) mediated by specific receptors at that site (Sraer, Sraer, Ardaillou & Mimoune, 1974) as part of the tubulo–glomerular feedback system (Thurau, 1974).

The relationship between sodium balance and intrarenal ANG II is, however, non-linear since mild sodium loading did not alter intrarenal ANG II levels. Experiments with angiotensin receptor blockers or converting enzyme inhibitors support the concept that intrarenal ANG II is involved in renal sodium conservation during sodium depletion (Kimbrough, Vaughan, Carey & Ayers, 1977; Lohmeier, Cowley, Trippodo, Hall & Guyton, 1977).

Administration of the converting enzyme blocker SQ 20881 led to a marked fall in intrarenal ANG II. This finding indicates that intrarenal ANG II is rapidly turned over and is probably dependent on the activity of converting enzyme for its production. The enzyme, tonin (Boucher, Asselin & Genest, 1974), which cleaves ANG II directly from renin substrate and could provide an alternative pathway of ANG II formation in tissues, is not inhibited by the available converting enzyme blockers (Boucher, Demassieux, Garcia & Genest, 1977). The current finding that the inhibitor SQ 20881 decreases intrarenal ANG II concentrations therefore suggests that classical converting enzyme is important in production of intrarenal ANG II.

Acknowledgments

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References


