Influence of dietary protein on glomerular angiotensin II-receptor binding in normotensive and spontaneously hypertensive rats

D. L. VAUGHAN and G. I. RUSSELL

Renal Research Laboratories, School of Postgraduate Medicine and Biological Sciences, University of Keele, Staffordshire, U.K.

(Received 17 October 1991/24 January 1992; accepted 26 February 1992)

1. The binding of angiotensin II to glomerular receptors was studied in spontaneously hypertensive and normotensive Wistar-Kyoto rats in response to 7, 16 and 32% isocaloric, isonatraemic protein diets.

2. Increased dietary protein elevated the systemic angiotensin II levels of both spontaneously hypertensive and Wistar-Kyoto rats \([F_{\text{shl}}(2,26) = 4.758, P<0.05; n=36]\), and this was not associated with changes in either systemic blood pressure or cortical renin activity.

3. Furthermore, no significant changes in the affinity or density of angiotensin II receptors were associated with changes of dietary protein intake in either strain.

4. These results indicate a dissociation between the systemic renin-angiotensin system and the tissue renin–angiotensin system in response to protein intake.

INTRODUCTION

High dietary protein is deleterious to renal function in both clinical and experimental states. A high protein diet has been shown to increase renal blood flow [1-4] and cause hyperfiltration, associated with increased glomerular intraglomerular capillary pressure [5, 6]. The mechanisms for this remain unclear but may be hormonal.

Stimulation of the renin–angiotensin system has been associated with increased dietary protein [7-9], with reported increases in plasma renin activity [10]. In addition, the effects of dietary protein on mean blood pressure are variably reported. Some studies indicate a decrease in mean arterial pressure in response to a high protein diet [8], thought to be induced by increasing sodium excretion [11-13], whereas others report an increase in blood pressure regardless of sodium intake with increased dietary protein [14]. Indeed, it has been noted that the effect of high dietary protein upon blood pressure is dependent upon experimental manipulation and the strain of rat used [15-17].

The aim of this study was to confirm the observed effects of dietary protein on the renin–angiotensin system, and to further investigate the influence of dietary protein on angiotensin II (ANG II) receptors within the glomeruli of normotensive rats (Wistar–Kyoto rats, WKY). Moreover, these studies have been extended to a hypertensive model (spontaneously hypertensive rats, SHR).

METHODS

Animals

Age-matched (15-20-week-old) female WKY and SHR (Olac Ltd, Bicester, Oxon, U.K.) were used throughout the study. Six experiments were carried out on groups of three animals from both strains for each protein diet.

Protocol

The groups of three animals were randomly allocated to high (32%), medium (16%) and low (7%) isocaloric, isonatraemic crude protein/fixed sodium diets (Special Diet Services Ltd, Witham, Essex U.K.). Each animal was housed separately in standard cages at 21°C and was 'pair-fed' [18] 16 g of the diet, mixed with distilled, deionized water, which served to minimize food wastage. Animals were given free access to distilled deionized water throughout the total feeding period of 14 days, during which both food and water consumption and weight gain were monitored.

Two animals from each group of three were placed in metabolic cages for the final 2 days of the experimental protocol and urine was collected to establish urinary volume and urinary sodium, potassium and protein concentrations. Blood samples were taken via the tail vein for the determination of plasma sodium, potassium and creatinine concentrations. A carotid cannula was implanted in the remaining rat under halothane anaesthesia. This enabled representative arterial blood pressure to be
recorded and blood samples for determination of plasma ANG II concentrations to be taken from conscious animals at least 15 h after surgery.

The glomeruli from all three rats were used to study the binding of ANG II to receptors.

Isolation of glomeruli

Preparations of glomeruli were obtained using modifications of the mechanical sieving techniques described by Fong & Drummond [19] and Misra [20]. Excised kidneys were decapsulated, bisected and the outer cortex sliced from the medulla while immersed in phosphate-buffered saline (PBS), pH 7.4, maintained at 4°C. The cortex was pushed through 250μm and 100μm nylon sieves and then forced through a 23 gauge needle to remove the Bowman's capsule and disperse contaminant tissue. Glomeruli were recovered from a 75μm sieve. The harvested glomerular suspension was centrifuged at 200g for 1.5 min and was assessed for purity using light microscopy, repeating previous steps if necessary to establish purity greater than 85%.

ANG II radioreceptor assay

Binding of ANG II to glomerular receptors was studied over a concentration range of 0.07–13 nmol/l of 125I-labelled ANG II ([iodotyrosine-3,125I]-[Ile3]ANG II; specific activity 2000 Ci/mmol) (Amersham International p.l.c., Amersham, Bucks, U.K.) and unlabelled ANG II (Hypertensin, Ciba-Geigy, Horsham, West Sussex, U.K.) and unlabelled ANG II (Hypertensin, Ciba-Geigy, Horsham, West Sussex, U.K.). Freshly prepared glomeruli (70–700 g/ml) were incubated in a media containing 125I-labelled ANG II, 2% BSA, 5 mmol/l dithiothreitol, 0.1 mmol/l phenylmethylsulphonyl fluoride and 0.05 mol/l NaH2PO4, pH 7.5 (10 mg of cortex/ml of media) and renin was retained in the supernatant [25]. ANG I was generated for 1 h in the presence of 10 µl of cortical supernatant, 100 µl of binephrectomized rat plasma, 10 µl of dimercaprol, 10 µl of 8-hydroxyquinoline and 1380 µl of maleate buffer, pH 6 (Dupont Medical Products Department, Stevenage, Herts, U.K.). Renin activity was measured by the radioimmunoassay of ANG I, and was expressed in ng h−1 ml−1.

Plasma ANG II concentration

The plasma ANG II concentration was measured by radioimmunoassay of ANG II extracted from plasma using Sep-Pak C18 cartridges (Waters Associates, Milford, MA, U.S.A.) with an elution solvent of 80% (v/v) methanol [24]. 125I-labelled ANG II extraction was found to be consistently greater than 95%. Cross-reactivity of the ANG II antibody used in the radioimmunoassay was found to be 1.5–2% with angiotensin I (ANG I), but 100% with angiotensin III. Cross-reactivity with other peptides was minimal.

Cortical tissue renin activity

Cortical tissue was homogenized in media containing 0.1 mol/l NaCl, 0.05 mol/l Na2HPO4, 5 mmol/l EDTA (tetrasodium salt), 5 mmol/l hydroxyquinoline, 2 mmol/l phenylmethylsulphonyl fluoride and 0.05 mol/l NaH2PO4, pH 7.5 (10 mg of cortex/ml of media) and renin was harvested in the supernatant [25]. ANG I was generated for 1 h in the presence of 10 µl of cortical supernatant, 100 µl of binephrectomized rat plasma, 10 µl of dimercaprol, 10 µl of 8-hydroxyquinoline and 1380 µl of maleate buffer, pH 6 (Dupont Medical Products Department, Stevenage, Herts, U.K.). Renin activity was measured by the radioimmunoassay of ANG I, and was expressed in ng h−1 ml−1.

Statistical analysis

Data were reported as means ± SEM unless otherwise indicated. n values refer to the number of experimental readings obtained. The significance of differences within each strain in response to diet in normally distributed data were analysed using one-way analysis of variance. Data which were not normally distributed were tested for significance using a non-parametric Kruskal–Wallis test.

The significance of differences between rat strains and diet was tested by two-way analysis of variance. Differences were considered significant at the 5% level.

RESULTS

Glomerular ANG II binding

There was no significant difference in the binding characteristics of ANG II receptors between the different rat strains [F0.05(1,26) = 2.177, P > 0.05; n = 36] or diets [F0.05(2,26) = 1.398, P > 0.05; n = 36] (Fig. 1). ANG II receptor densities were 766 ± 120, 945 ± 142 and 944 ± 230 fmol/mg of protein in SHR and 609 ± 52, 753 ± 156 and 832 ± 70 fmol/mg of protein in WKY fed high, medium and low protein diets, respectively.
All experimental groups, irrespective of strain \([F_{0.05}(1,26) = -0.2096, P > 0.05; n = 36]\) or diet \([F_{0.05}(2,26) = 0.5241, P > 0.05; n = 36]\), had similar ANG II affinity constants: 0.32±0.04, 0.28±0.01 and 0.33±0.01 (nmol/l)\(^{-1}\) for SHR on high, medium and low protein diets, respectively, and 0.28±0.02, 0.30±0.05 and 0.32±0.03 (nmol/l)\(^{-1}\) for WKY on high, medium and low protein diets, respectively.

**Plasma ANG II concentration**

Similar plasma ANG II concentrations were found in both rat strains when receiving the same diet (Fig. 2). Animals on medium and low protein diets had similar plasma levels of ANG II: 6.7±0.4 and 6.4±0.1 pg of ANG II/ml of plasma, respectively, in SHR and 12.3±3 and 18±2 pg of ANG II/ml of plasma, respectively in WKY. The high protein diet stimulated a large increase in plasma ANG II concentration in both strains compared with the other diets \([F_{0.05}(2,26) = 4.758, P < 0.05; n = 36]\).

**Cortical tissue renin activity**

There was no significant difference in tissue renin activity between the two strains \([F_{0.05}(1,27) = 0.0053, P > 0.05; n = 36]\) or in response to dietary protein \([F_{0.05}(2,27) = 1.9480, P > 0.05; n = 36]\). SHR showed activities of 3822±846, 2575±224 and 2432±322 ng of ANG I h\(^{-1}\) mg\(^{-1}\) of cortex and WKY exhibited values of 2988±505, 4810±1174 and 2565±305 ng of ANG I h\(^{-1}\) mg\(^{-1}\) of cortex when fed high, medium and low protein diets, respectively. No correlation was found between tissue renin activity and plasma ANG II concentration \((r = -0.1115, P > 0.05; n = 21)\).

**Blood pressure**

It was confirmed that SHR had higher mean blood pressures than WKY \([F_{0.05}(1,18) = 6.945, P < 0.05; n = 36]\). However, dietary protein had no effect on blood pressure in this study. Conscious direct blood pressure in SHR on high, medium and low protein diets were 153±2, 144±7 and 149±11 mmHg, and in WKY were 118±3, 118±5 and 118±3 mmHg, respectively \([F_{0.05}(2,18) = 0.033, P > 0.05; n = 36]\).

**Water and electrolyte balance**

A higher protein content in the diet was associated with increased total water consumption in both strains \([F_{0.05}(2,102) = 115.51, P < 0.01; n = 108]\), and was reflected in the total diuresis \([F_{0.05}(2,59) = 7.143, P < 0.01; n = 72]\). All animals showed reduced urinary sodium and potassium concentrations in response to increased dietary protein \([F_{0.05}(2,61) = 25.37, P < 0.01; n = 72\) and \([F_{0.05}(2,26) = 19.74, P < 0.01; n = 72]\), respectively. Total excretion of sodium and potassium was found to be equal between strains and diet and no significant differences in plasma sodium and potassium...
concentrations were noted. SHR had higher plasma creatinine \([F_{0.05}(1,59) = 8.809, P < 0.01; n = 72]\) and lower urinary creatinine \([F_{0.05}(1,59) = 6.0923, P < 0.05; n = 72]\) concentrations than WKY; this was not influenced by dietary protein. No differences in urinary protein concentration were evident between either strain or diet; however, the total amount of protein excreted in 24 h was significantly higher in animals on high protein diets \([F_{0.05}(2,54) = 4.055, P < 0.05; n = 72]\), (see Table 1).

Weight gain

SHR were initially heavier than WKY when allocated to diets. SHR placed on high, medium and low protein diets weighed 200.6 ± 15.9, 203.9 ± 9.9 and 200.8 ± 15.5 g (± SD, \(n = 54\)), respectively. WKY weighed 186.9 ± 14.7, 192.2 ± 13.1 and 190.7 ± 15.9 g (\(n = 54\)), respectively. Dietary protein had a significant influence on the final weight on both strains \([F_{0.05}(2,102) = 5.594, P < 0.05; n = 108]\). SHR gained 2.3 ± 2.5, 4.2 ± 2.6 and 2.3 ± 2 g during the 14 day feeding period on high, medium and low protein diets, respectively, whereas WKY gained 2.1 ± 2.1, 10.2 ± 1.4 and 9.2 ± 2.1 g, respectively.

**DISCUSSION**

We have confirmed that dietary protein intake influences the systemic renin-angiotensin system and at a level of intake that is not overly excessive, as in some previous studies [18, 26]. An approximate 10-fold increase in plasma ANG II concentration occurred in response to a high (32%) protein diet compared with medium (16%) and low (7%) protein diets. This was seen in both normotensive (WKY) and hypertensive (SHR) rat strains. However, there was no significant difference in the glomerular ANG II receptor number or affinity in response to the differing protein diets for either strain. This is in contrast to other work [27], where differences between normotensive and hypertensive rats on normal diets were noted for glomerular receptor number and affinity. These differences between studies may be related to the normotensive control used. The control strain for SHR remains controversial [28–30].

It is known that glomerular ANG II receptors can be modulated by changes in dietary sodium. Bellucci & Wilkes [31] demonstrated that specific binding of ANG II to glomerular receptors varied depending on sodium intake, whereas receptor affinity was not affected. Moreover, they demonstrated a significant inverse relationship between plasma ANG II concentration and glomerular ANG II receptor density and concluded that glomerular receptor regulation was related to circulating hormone and not directly to sodium intake. These findings emphasize the importance of ensuring a controlled intake of sodium in the study diets.

The speed with which receptors may be up/down regulated in response to a dietary stimulus is also an important consideration. Receptor modulation is known to occur rapidly in response to both denervation [32] and angiotensin-converting enzyme inhibition [33]. Two weeks of increased/decreased sodium intake is sufficient to produce changes in both circulating ANG II concentrations and glomerular ANG II receptors. Likewise, 2 weeks of increased dietary protein intake is sufficient to produce changes in blood pressure, a decreased renovascular resistance, an increased renal plasma flow and an elevated plasma renin concentration [14, 26]. Any anticipated changes in glomerular ANG II receptor characteristics in response to the diet would be evident within the first 14 days of feeding.

In this study, significant changes in plasma ANG II concentration were found in response to changes in dietary protein, but no significant difference was seen in glomerular receptor density. The mechanisms are ill understood, but glomerular ANG II may not be modulated solely by systemic levels of

**Table 1. Water and electrolyte balance in SHR and WKY. Values are means ± SEM (\(n = 12\)). Abbreviations: Ur., urinary; Pl., plasma; Cr, creatinine; Pr, protein. Statistical significance between diets (one-way analysis of variance): \(*P < 0.05, \**P < 0.001.\)**

<table>
<thead>
<tr>
<th></th>
<th>High protein</th>
<th>Medium protein</th>
<th>Low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water consumption (ml)**</td>
<td>46.5 ± 3.5</td>
<td>36.1 ± 2.2</td>
<td>25.4 ± 1.8</td>
</tr>
<tr>
<td>24 h Ur. volume (ml)**</td>
<td>13.9 ± 2.9</td>
<td>7.7 ± 1.3</td>
<td>6.2 ± 1</td>
</tr>
<tr>
<td>24 h Ur. Na+ excretion (mmol)</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>24 h Ur. K+ excretion (mmol)</td>
<td>2.8 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>24 h Ur. Cr excretion (mmol)</td>
<td>13.9 ± 6.2</td>
<td>18.2 ± 5.5</td>
<td>15 ± 4.1</td>
</tr>
<tr>
<td>Ur. Na+ concn. (mmol/l)**</td>
<td>61.9 ± 8.8</td>
<td>115.8 ± 16.8</td>
<td>225.2 ± 37.7</td>
</tr>
<tr>
<td>Ur. K+ concn. (mmol/l)**</td>
<td>224.9 ± 18</td>
<td>362.5 ± 48.4</td>
<td>643.4 ± 93</td>
</tr>
<tr>
<td>Ur. Cr concn. (mmol/l)</td>
<td>2.1 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>Ur. Pr concn. (mmol/l)</td>
<td>139.5 ± 7.2</td>
<td>138.6 ± 0.8</td>
<td>139.5 ± 0.7</td>
</tr>
<tr>
<td>Pl. Na+ concn. (mmol/l)**</td>
<td>11 ± 1.6</td>
<td>11.8 ± 1.5</td>
<td>9.1 ± 0.8</td>
</tr>
<tr>
<td>Pl. K+ concn. (mmol/l)</td>
<td>104.9 ± 15</td>
<td>105.8 ± 14.5</td>
<td>95.2 ± 14.5</td>
</tr>
<tr>
<td>Pl. Cr concn. (mmol/l)</td>
<td>0.42 ± 0.07</td>
<td>0.44 ± 0.06</td>
<td>0.63 ± 0.13</td>
</tr>
<tr>
<td>24 h Ur. Pr excretion (mg)</td>
<td>17.3 ± 5.3</td>
<td>10.3 ± 6.9</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water consumption (ml)**</td>
<td>51.1 ± 3.4</td>
<td>45.1 ± 3.5</td>
<td>30.9 ± 3</td>
</tr>
<tr>
<td>24 h Ur. volume (ml)</td>
<td>9.2 ± 1.6</td>
<td>7.4 ± 1.9</td>
<td>5.2 ± 1.4</td>
</tr>
<tr>
<td>24 h Ur. Na+ excretion (mmol)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>24 h Ur. K+ excretion (mmol)</td>
<td>2.1 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>24 h Ur. Cr excretion (mmol)</td>
<td>18.1 ± 5.5</td>
<td>19.8 ± 6.0</td>
<td>15.6 ± 5.1</td>
</tr>
<tr>
<td>Ur. Na+ concn. (mmol/l)**</td>
<td>77.2 ± 13.8</td>
<td>92.5 ± 20.3</td>
<td>254.7 ± 48.3</td>
</tr>
<tr>
<td>Ur. K+ concn. (mmol/l)**</td>
<td>271.8 ± 32</td>
<td>340 ± 50.9</td>
<td>621.5 ± 112</td>
</tr>
<tr>
<td>Ur. Cr concn. (mmol/l)</td>
<td>2.3 ± 0.5</td>
<td>2.9 ± 0.9</td>
<td>5.2 ± 1.7</td>
</tr>
<tr>
<td>Ur. Pr concn. (mmol/l)</td>
<td>137.9 ± 0.8</td>
<td>139.8 ± 0.3</td>
<td>140.6 ± 0.6</td>
</tr>
<tr>
<td>24 h Ur. Pr excretion (mg)</td>
<td>4.0 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>4.0 ± 2.1</td>
</tr>
</tbody>
</table>
ANG II [33]. The tissue renin–angiotensin system may be a modulator of receptors independent of circulating hormone [37]. In the present study, cortical renin activity was used as an index of the intrarenal renin–angiotensin system. There were no significant differences in the cortical renin activity between either the three diets or the two strains and, moreover, a relationship between glomerular receptor density and cortical renin activity was not established. However, the situation may be even more complex. It has been demonstrated [38] that tissue ANG II is more closely related to ANG II binding than renin activity in the adrenal gland. It is possible, therefore, that glomerular ANG II–receptor binding relates to local ANG II more than renin activity in the adrenal gland. It is possible that glomerular ANG II receptor binding is associated with local ANG II activity in the glomeruli. In some tissues and regulation of these may be different. However, it is still not clear whether there are two types in the kidney [41–44], although it is suggested that only one type of ANG II receptor is present in the glomeruli [45, 46].

The findings of this study are complementary to those of Puller & Hostetter [9], who reported an increase in plasma renin activity with no change in systemic arterial blood pressure in response to a high protein diet. Neither the affinity or density of mesenteric artery ANG II receptor was associated with the observations. ANG II within the kidney has well-known effects on the glomerulus, efferent arteriole and tubules together with intrahormonal influences. A high protein diet is well recognized to increase plasma flow and glomerular filtration rate, the opposite of the direct effect of ANG II in the glomerulus. A significant increase in the urine output was seen in rats on a high protein diet, although this may reflect a higher osmolar load from increased urea excretion. The difference in urinary electrolyte concentration was a reflection of water consumption and urinary volume, as indicated by the total amount of excreted sodium and potassium. Equivalent 24 h electrolyte excretion verified the equal intake of both potassium and sodium in the diets. Total excretion of protein was higher in the hypertensive rats and increased in rats on a higher protein diet, an effect which was not seen in normotensive rats.

All animals gained weight throughout the study. However, WKY on a high protein diet did not gain as much weight as WKY on the other diets, despite the similar caloric intake. The reasons for this are not apparent, but are not believed to be due to a wasting phenomenon. The difference in weight gain was not evident in SHR on varying the dietary protein intake. SHR had, as expected, significantly higher blood pressure than their normotensive controls. They also had significantly higher plasma and urinary creatinine concentrations compared with WKY owing to their greater muscle and activity. None of the above was influenced by their dietary regimen.

Therefore, in conclusion, increasing protein in the diet resulted in an elevation of plasma ANG II concentration, but no change in tissue renin activity or ANG II receptor number or affinity. Further, there were no changes in direct mean arterial blood pressure in conscious rats. This was true of both normotensive and hypertensive rat models.

ACKNOWLEDGMENTS

This study was supported by The National Kidney Research Fund. We thank Dr M. Jones for help and advice regarding statistics, and Miss K. Chesworth for preparing, administering and monitoring consumption of the diets throughout this study.

REFERENCES
