Response of Proximal Tubules to Angiotensin II Changes During Maturation

Jeffrey L Garvin, William H. Beierwaltes

Abstract—The rennin-angiotensin system changes with age, but it is unclear how renal responses to angiotensin II (Ang II) evolve as an animal matures. We hypothesized that Ang II exerts a greater effect on proximal nephron volume absorption (Jv), blood pressure (BP), renal blood flow (RBF), and renal vascular resistance (RVR) in young compared with adult rats. To test this hypothesis, we investigated the effects of Ang II on proximal nephron fluid absorption in response to 10^-11 mol/L Ang II in rats from three age groups: young (4 to 5 weeks old), intermediate (6 weeks old), and adult (7 weeks old). In proximal straight tubules from 7 young rats, Jv was 0.64±0.05 nL/mm per minute. Ang II in the bath increased Jv by 69±18% to 1.05±0.07 nL/mm per minute (P<0.05). In tubules from five intermediate-aged rats, Jv was 0.60±0.10 nL/mm per minute and increased by 34±5% to 0.83±0.16 nL/mm per minute after Ang II (P<0.02). In five adult rats, Jv was 0.69±0.10 nL/mm per minute and increased 20±6% to 0.85±0.13 nL/mm per minute after Ang II (P<0.05). Next, we tested whether the exaggerated effect of Ang II on proximal tubular Jv in young rats was due to Ang II-induced changes in cAMP. cAMP content of proximal tubules from eight young rats was 24.8±2.7 fmol/mm and fell by 29±7.2% (P<0.05) after treatment with Ang II. In contrast, cAMP content of proximal tubules from nine adults was only 9.8±2.5 fmol/mm, 40% of baseline in young rats, and was unchanged by Ang II (9.2±4.5 fmol/mm). We finally determined whether the increased sensitivity to Ang II in tubules of young rats is mimicked by renal hemodynamics. Eleven adult rats had BP of 115±7 mm Hg, RBF of 6.9±0.4 mL/mm per g kidney weight (kw), RVR of 36.8±2.0 RU, and plasma renin activity (PRA) of 11.2±2.3 ng Ang I/mL per hour. Seven young rats had BP of 98±7 mm Hg, 17 mm Hg lower than adults (P<0.025). RBF was 4.9±0.23 mL/mm per g kw, and RVR was 20.3±1.9 RU, 20% greater than in adults (P<0.025). PRA was 9.2±2.2 ng Ang I/mL per hour. There were no differences between groups with regard to increased BP, decreased RBF, or increased RVR with graded doses of 8, 40, and 200 fmol Ang II/g body weight. Thus, Ang II increased Jv more in young rats but had a lesser effect in adults. This was coupled with a greater effect of Ang II on tubular cAMP in young rats, but no differences in systemic or renal hemodynamic responses to Ang II between adults and young. We conclude that during adolescent development, Ang II may be an important factor in the regulation of salt and water metabolism, but not renal hemodynamics (Hypertension. 1998;31(pt 2):415-420.)

Key Words: cAMP ■ renal blood flow ■ blood pressure ■ transport ■ sodium excretion

Renin is a circulating enzyme that acts as the rate-limiting step in the formation of the potent vasoconstrictor Ang II. Renin is secreted primarily from the juxtaglomerular cells of the renal afferent arteriole in response to various stimuli to regulate the formation of angiotensin. Besides its effects on total peripheral resistance and organ perfusion, Ang II stimulates sodium retention via direct actions within the kidney on the resistance vessels and proximal tubule and also via indirect actions through the release of aldosterone, which acts on the distal nephron. Angiotensin appears to play a critical role in the development of the kidney. At birth, PRA in the rat is approximately 50 ng Ang I/mL per hour and continues to increase to a peak of 75 ng Ang I/mL per hour by 2 weeks of age. After this zenith, the PRA declines to a steady state of only about 10% of the previous peak (7 ng Ang I/mL per hour) by 4 weeks of age. Whereas the circulating renn peaks early, the tissue rennin does not peak until 3 to 4 weeks of age and declines to a constant level by 6 weeks. These dramatic changes suggest that Ang II may play a significant role in late renal development in the neonate.

Although age-related changes in the rennin-angiotensin system have been studied extensively, such investigations have primarily focused on the dramatic changes that occur during fetal development, or alternatively, those that occur with old age. Perhaps because circulating rennin levels stabilize during the rapid growth phase associated with adolescence (4 to 12 weeks of age in the rat), the influence of angiotensin on renal function during this period has largely been ignored. Although PRA does not change during adolescent development, this does not necessarily mean that changes in (or responses to) Ang II do not change. For instance, the quantity and types of Ang II receptors expressed in the kidney change with age, and
young animals show a smaller increase in urinary volume and sodium excretion after volume expansion than do adult rats. This is proposed to be due to an exaggerated response of the kidney to Ang II, perhaps the result of stimulation of nephron sodium absorption by Ang II.

To our knowledge, no one has yet investigated the separate roles of Ang II-induced stimulation of transport and vasoconstriction in Ang II-dependent enhanced sodium retention during rapid growth and maturation. We hypothesized that Ang II would elicit more profound responses from renal resistance vessels and proximal tubules from young rats than those from adult rats.

Methods

Young and adult male rats were obtained from Charles River Laboratories (Wilmington, MA). For the in vitro studies, we used young male rats weighing approximately 105 to 115 g, supplied to us as 4 to 5 weeks old, weighing 140 g. In our in vivo studies, we used adult male rats supplied to us as 8 weeks old, weighing approximately 250 g. These were compared with young rats supplied to us as 5 weeks old, which weighed 110 to 115 g. Rats were cared for according to institutional guidelines, and all procedures had been approved by the Institutional Animal Care and Use Committee.

Tubule Perfusion

Rats maintained on a 0.22% Na and 1% K diet (Ralston Purina) for 6 to 10 days were anesthetized with ketamine (100 mg/kg bw, Parke Davis), and the peritoneal cavity was opened via a midline incision. Ice-cold isotonic saline was poured over the left kidney while it was still perfused with blood, after which the kidney was removed. Segments were cut and placed in tubular perfusion solution on ice. Medullary rays were dissected from the slices, and individual proximal straight tubules were dissected from the rays. Tubules were perfused as described previously. The composition of the tubular perfusion solution (in mmol/L) was NaCl 114.0, KCl 4.0, NaH2PO4 2.5, MgSO4 1.2, Na2 citrate 1.0, NaHCO3 25.0, alanine 6.0, Ca lactate 2.0, glucose 5.5, and raffinose 5.0. The osmolality of all solutions was 295 ± 3 mOsm as measured by freezing-point depression. The pH of the bath was 7.4. Solutions were passed with 95% O2-5% CO2. Ang II (10^-10 mol/L) was added to the bath as indicated in the text.

Jv was measured using raffinose as a volume marker. Raffinose was measured in the perfusate and collected fluid using a previously described enzymatic assay with a continuous-flow ultramicrofluorometer. The perfusion rate (Vo) was calculated from the equation:

\[ Jv = \frac{Vo}{VL} = \frac{(CL/Co)VL}{VL} = CL/Co \]

where CL is the concentration of raffinose in the collected fluid. Co is the concentration of raffinose in the perfused fluid, and VL is the collection rate per unit of tubule length. The rate of Jv was calculated from the equation:

\[ Jv = \frac{Vo}{VL} \]

Angiotensin and Proximal Nephron Fluid Absorption

Because it has been postulated that proximal tubules of young animals reabsorb sodium more avidly than those of adult rats, we first examined the effect of 10^-10 mol/L Ang II on proximal nephron fluid absorption in three successive age groups: young, intermediate, and adult. Using the perfusion methods outlined above, we compared effects on Jv in proximal tubules by adding Ang II to the bath versus an untreated control period. We chose this concentration because it stimulates fluid absorption in isolated, perfused proximal tubules, and Ang II has been reported to stimulate transport in this segment in vivo.

Tubule Suspensions

Sprague-Dawley rats were injected with 0.1 mL of heparin (1000 USP/mL) and then anesthetized with ketamine (0.1 mg/kg). The abdominal cavity was exposed, and the aorta was cannulated below the left kidney, then clamped below the cannula and above the kidney. The left kidney was perfused with 37°C, type I collagenase (75 mg/mL, Sigma) containing 25 mmol/L mannitol in perfusion solution. Each kidney was perfused with 40 mL of this solution for 10 minutes, after which 150 mmol/L NaCl was poured on the kidney. The partially digested kidney was cut, minced, and stirred at 4°C for 10 minutes in perfusion solution. The suspension was then passed through a nylon mesh (25 μm) and diluted with perfusion solution.

Angiotensin-Induced Reductions in cAMP

Ang II acts, in part, via a decrease in cAMP in the proximal nephron. Because age-related changes in renal metabolism of cAMP have been reported and we found that the effect of Ang II on proximal tubular Jv was greater in young rats than in adults (see "Results"), we investigated whether this was due to a greater Ang II-induced decrease in cAMP. Using tubule suspensions, we collected loose proximal tubules under a dissecting microscope and transferred them to 1-mL tubes using a lacade. Tubes were incubated in 95 μL of perfusion solution containing 1 mmol/L nlobutylmethoxyamine at 37°C for 10 minutes before addition of 5 μL of vehicle or Ang II to yield 10^-10 mol/L. We tested the effects of Ang II on proximal tubules from young and adult rats. The reaction was stopped with 100 μL of methanol after 20 minutes, and the solution was stored at -80°C. cAMP levels were determined using a radioimmunoassay purchased from Biomedical Technologies. On the day of the assay, the samples were dried in a Savant dryer (Forma Scientific) and reconstituted in 11 μL of sodium acetate buffer. cAMP standards were prepared similarly to the samples.

Renal Hemodynamics

Hemodynamic responses to Ang II were compared in adult versus young male Sprague-Dawley rats. They were maintained on a diet containing 0.22% Na and 1% K for 8 to 10 days. At the conclusion of this period, rats fasted overnight but were allowed free access to water. Then they were anesthetized with ketamine (0.1 mg/kg IP) for the acute procedures outlined below.

Rats were given a tracheostomy using PE-240 tubing (Clay Adams/Becton Dickinson) to facilitate spontaneous breathing of room air. The femoral vein was catheterized with PE-50 tubing for delivery of drugs and a maintenance infusion of 100 μL/min 0.9% NaCl, and the femoral artery was catheterized with PE-50 tubing filled with heparinized saline connected to a Statham pressure transducer (Viggo-Spectramed) and a Gould recorder (Gould Instruments) for continuous monitoring of BP and sampling of arterial blood. When BP was stable, a 250-μL venous sample was collected in 50 μL of 3% EDTA for the determination of PRA. The rats then received a supplemental

Selected Abbreviations and Acronyms

<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>Ang I, II</td>
<td>Angiotensin I, II</td>
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<td>BP</td>
<td>blood pressure</td>
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<td>bw</td>
<td>body weight</td>
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<td>Jv</td>
<td>fluid absorption</td>
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<td>kw</td>
<td>kidney weight</td>
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<td>PRA</td>
<td>plasma renin activity</td>
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<td>RBF</td>
<td>renal blood flow</td>
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<td>RU</td>
<td>resistance units normalized to kidney weight</td>
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<td>RVR</td>
<td>renal vascular resistance</td>
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\[ Jv = \frac{Vo}{VL} = \frac{(CL/Co)VL}{VL} = CL/Co \]
bolus of 6% heat-inactivated bovine serum albumin (Difco Laboratories) of either 1.0 mL for adult rats or 0.5 mL for young rats. To measure RBF, a midventral incision was made in the abdominal cavity, and the left renal artery was dissected from the surrounding tissues. A noncannulating electromagnetic flow probe (Carolina Medical Electronics) with an internal circumference of 2.0 mm for adult rats or 1.5 mm for young rats was placed on the renal artery and allowed to stabilize. At the conclusion of the experimental protocol, the flowmeter was calibrated in situ by measuring graded flows after direct cannulation of the renal artery and gravimetric determination of blood flow over timed intervals. At the end of the experiment, all animals were killed, and the left kidney was decapsulated, dissected, and weighed to normalize measurements of RBF per gram of kidney weight.

Renal hemodynamic and systemic responses in vivo to graded bolus doses of Ang II (Sigma) were tested. Each dose was administered in a 200-μL volume of saline vehicle. Responses to the vehicle alone were first measured, and any detectable infusion artifact was corrected for by subtracting this amount (if any) from subsequent responses before analysis. Then we monitored the systemic pressor response and change in RBF after each of three graded doses of Ang II. In adult rats, we administered doses of 8, 40, and 200 fmol Ang II/g bw in a 200-μL bolus. We chose these doses because they bracket the concentration of Ang II used in isolated, perfused tubule experiments. Between doses, the rats were allowed to stabilize and return to baseline for 10 minutes. In young rats (whose body weight was only 44% that of adults) doses of Ang II were corrected by body weight to provide equivalent volume of distribution as the adult doses, based on preliminary studies. The actual doses delivered to young rats equaled 8, 40, and 200 fmol Ang II/g bw. RVR was calculated as the ratio of BP (assumed to be the same as renal perfusion pressure) to RBF. RVR was calculated as mm Hg per ml per minute per g of kidney weight, hereafter referred to as resistance units (RU). PRA was measured from a blood sample obtained from the femoral venous catheter. It was collected in potassium EDTA, separated by centrifugation, and frozen for later determination by radioimmunoassay for Ang I using the Gammacoat kit (Incastor Corp) as adapted from the original method of Haber et al.

Analysis
All values are reported as mean±SEM. For the in vitro studies, results were analyzed by unpaired or paired t-tests as appropriate. Baseline values from the whole animal studies were analyzed using a Student's unpaired t-test to compare responses in young versus adult rats. Differences in the response to graded doses of Ang II were analyzed using ANOVA with the Bonferroni correction. A value of P<.05 was considered statistically significant.

Results

Angiotensin and Proximal Nephron Fluid Absorption
We first examined the effect of 10−7 mol/L Ang II on proximal nephron fluid absorption in three successive age groups, including young (4–5 weeks old, 115±4 g bw), intermediate (6 weeks old, 140±3 g bw), and adult (7 weeks old, 166±4 g bw) rats. As shown in Fig 1, during the control period, proximal straight tubules from seven young rats absorbed fluid at a rate of 0.64±0.05 nL/mm per minute. After Ang II was added to the bath, fluid absorption increased by 69±18% to 1.05±0.07 nL/mm per minute (paired difference equals 0.41±0.08 nL/mm per minute, P<0.05). In comparison, proximal straight tubules from five rats in the intermediate-aged group absorbed fluid at 0.60±0.10 nL/mm per minute during the control period, and increased by 34±5% to 0.83±0.16 nL/mm per minute after Ang II was added to the bath (paired difference equals 0.23±0.06 nL/mm per minute; P<0.02). In the proximal straight tubules from five rats in the adult group, basal fluid absorption was 0.69±0.06 nL/mm per minute and increased 20±6% to 0.85±0.13 nL/mm per minute after treatment with Ang II (paired difference equals 0.16±0.07 nL/mm per minute, P<0.05). Thus, this concentration of Ang II increased fluid absorption more in the youngest rats and had a progressively lesser effect on fluid absorption in the older groups, while basal rates of fluid absorption did not differ (Fig 2). As a result of these progressive changes, further experiments were performed on rats from only the young and the adult groups.

Angiotensin-Induced Reductions of cAMP
Because the effect of Ang II on proximal tubular Jv was greater in young rats compared with adults, we investigated whether...
Proximal Tubules, Ang II, and Maturation

Figure 3. Changes in cAMP content of proximal straight tubules in young and adult rats in response to Ang II. *P < 0.05 vs no change.

The bolus administration of vehicle produced no remarkable infusion artifact. As shown in Fig 4, the systemic pressor response to graded doses of 8, 40, and 200 fmol/g bw were 5 ± 2, 14 ± 2, and 34 ± 3 mm Hg, respectively. Whereas there was no difference in the pressor response to Ang II between adult and young rats at the two lower doses, the response to the highest dose in young rats was 20% less than in the adults (P < 0.05). As with the adults, graded doses of Ang II decreased RBF in young rats by 3%, 15%, and 49% from baseline, respectively. Although absolute changes in RBF (in mL/mm per g kw) were somewhat less in the young rats, qualitative changes (expressed as percent change from baseline) were virtually the same as in the adults. The progressive increase in RVR with graded doses of Ang II was not different between adult and young rats (Fig 4). Overall, systemic and renal hemodynamic responses to graded doses of Ang II were very similar in both adult and young rats.

Discussion

We found that the response of proximal nephron fluid absorption to Ang II decreases as rats mature. This age-dependent response to Ang II was at least partially due to changes in cAMP metabolism, as basal cAMP was 60% greater in young rats and Ang II decreased cAMP by 30% in young rats but had no effect in adults. In contrast, we observed no age-related changes in the response to Ang II in either systemic BP or RVR, and no age-dependent differences in PRA. These observations suggest that in the rapid-growth, adolescent phase of development, Ang II may be an important factor in the evolving regulation of salt and water metabolism but not renal hemodynamics.

We and others have reported that Ang II stimulates fluid absorption by the proximal nephron. This stimulation is a combined result of enhanced sodium entry and exit processes. However, to our knowledge the data reported here are the first to show directly that Ang II stimulates fluid absorption to a greater extent in young animals than in adults. The differences in the response of the proximal nephron to angiotensin in adult compared with young rats is unlikely to be due to differences in circulating Ang II levels, as we found that the PRA was similar in adult and young rats.
Because we found an age-dependent effect of angiotensin on proximal nephron function, we next examined whether these differences might also be reflected in the reactivity of the renal vessels to Ang II. However, we observed no consistent differences between adult and young rats to graded doses of Ang II, either in the response of systemic BP or in RVR. This dissociation of tubular from vascular responses to Ang II in adult versus young rats suggests that despite higher basal RVR, the renal resistance vessels have largely matured by 6 weeks of age.

Young rats display a diminished capacity to excrete sodium and water in response to volume load compared with adult rats. This attenuated excretory response has been postulated to be due to stimulation of nephron transport by elevated Ang II levels. Our results support the hypothesis that young animals have a reduced ability to excrete a sodium load due to enhanced nephron transport. However, they also indicate that greater sensitivity to Ang II in the proximal nephron may account for part of this response, in addition to elevated Ang II levels. In fact, the greater sensitivity of the proximal nephron may be even more important, since in 4- to 6-week-old rats Ang II levels were not significantly different from those of adults, but the proximal nephron still showed an enhanced sensitivity to Ang II.

The fact that we found no age-dependent differences in the renal hemodynamic response to Ang II was somewhat surprising in light of our results concerning the proximal nephron. However, superficial nephrons are the last segment of the kidney to develop, while the vasculature for these nephrons is already present. Thus an age-related difference in the response of hemodynamic parameters may exist in animals younger than those we studied.

When Ang II binds to its receptor in the proximal tubule, the resulting receptor-ligand complex activates at least two classes of G proteins. One is an inhibitory G protein (Gi), which blocks adenylate cyclase. The inhibition of adenylate cyclase results in turn in a decrease in both cAMP levels and in protein kinase A activity. We found age-dependent changes in cAMP metabolism in response to Ang II with young animals, which displayed a greater ability to reduce cAMP levels in response to Ang II than did adult rats. Age-dependent changes in cAMP metabolism effected by other hormones have been reported previously. For instance, parathyroid hormone stimulates cAMP production to a greater extent in young rats than in adults.

There remains the question of what this exaggerated effect in young rats might be attributed to. A change in the amount of the Gi protein in the proximal tubular cells may account for our results. A decrease in Gi protein with age has been shown in vascular smooth muscle as well as in the kidney. Interestingly, a decrease in the stimulatory G protein Gs as well as Gi has been shown to occur in the kidney with age. However, changes in other enzymes associated with the cAMP second messenger cascade, such as protein kinase A or phosphodiesterase, may also be involved.

Although the differences in response to Ang II may be due to differences in receptor number and/or subtype, it should be noted that the number of receptors has been reported to decrease only 15% in rats of these ages. However, the superficial nephrons studied here are the last to develop and thus may be in a different developmental stage than the kidney as a whole. Consequently, variations in receptor density and type cannot be ruled out as an explanation for the results.

The second messenger cascade activated by Ang II is the protein kinase C cascade. We did not study whether the activation of protein kinase C is enhanced in young animals compared with adults. However, our preliminary data indicate that the total amount of protein kinase C is greater in young rats than in adult animals. Thus, it seems likely that the activation of this enzyme by Ang II would be greater in the former. Interestingly, the basal rates of fluid absorption did not vary between groups. Elevated levels of protein kinase C or some other factor that stimulates transport may account for the basal rates of fluid absorption being similar, but young animals have a much greater cAMP content.

In summary, we report that the Ang II stimulation of proximal nephron fluid absorption decreases as rats mature, and that this age-dependent response to Ang II was mirrored by changes in cAMP metabolism, as both basal and Ang II-decreased cAMP in proximal tubules was greater in young rats. These age-related responses to Ang II were not reflected in systemic BP, the renal vasculature, or PRA. We suggest that in the rapid-growth, adolescent phase of development, Ang II may be an important factor in the evolving regulation of salt and water metabolism, but not renal hemodynamics.

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