The Renin-Angiotensin System and Compensatory Renal Hypertrophy in the Rat

Jean-Pierre Valentin, Leonardo A. Sechi, Chandi A. Griffin, Michael H. Humphreys, and Morris Schambelan

Angiotensin II (Ang II) may act as an angiogenic and growth promoting factor in different tissues. To assess the role of Ang II in compensatory renal growth following unilateral nephrectomy (UNX), we measured renin, angiotensinogen, and Ang II type 1 (AT1) receptor mRNA levels, as well as Ang II receptor density, in two groups of Sprague-Dawley rats 7 days after either sham operation or UNX. Half of each group received either no treatment or an angiotensin-converting enzyme inhibitor (100 mg/dL captopril in the drinking water, initiated at the time of the intervention). Following UNX, the ratio of kidney weight to body weight (KW/BW) in untreated animals was greater than in rats undergoing sham UNX (0.46 ± 0.01 vs 0.37 ± 0.01%, P < .01). Neither renal renin, nor renal or hepatic angiotensinogen mRNA levels, determined by slot blot hybridization, changed significantly after UNX. Ang II receptor density in glomeruli, determined using an 125I-Sar1-Ile8 Ang II in situ receptor binding assay on frozen kidney sections, did not change significantly after UNX, nor did renal AT1 receptor mRNA. In captopril-treated rats, KW/BW was greater in UNX than in sham operated rats (0.44 ± 0.01 vs 0.37 ± 0.01%, P < .01), similar to results in untreated animals. Renal and hepatic angiotensinogen mRNA levels were not affected by captopril treatment and did not change further in response to UNX. Captopril treatment increased renin mRNA in both sham operated and UNX rats as compared with untreated controls, but had no significant effect on Ang II receptor density and AT1 receptor mRNA; and no change was observed in either variable as a consequence of UNX. Thus, compensatory renal hypertrophy following UNX occurred in the absence of measurable changes in components of the renin-angiotensin system, and despite functionally significant inhibition of this system by captopril. These data do not support a critical role for Ang II in compensatory renal hypertrophy. © 1997 American Journal of Hypertension, Ltd. Am J Hypertens 1997;10:397-402

KEY WORDS: Angiotensin II, angiotensin converting enzyme inhibition, angiotensinogen mRNA, renin mRNA, AT1 receptor, angiotensin receptor antagonists, captopril, hypertrophy, kidney, uninephrectomy.
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ilateral nephrectomy results in growth of the remaining kidney through hypertrophy of the remaining kidney through hypertrophy, with only a small component of hyperplasia. The mechanisms responsible for this compensatory response are not known with certainty, although a number of biochemical and molecular changes have been observed that could be involved. Among the earliest of these are alterations in the levels of cyclic nucleotides and an increase in phospholipid incorporation into cortical membranes; protein kinase C activity also increases, as does renal polyamine content. Subsequently, an increase in RNA content occurs, which includes the expression of genes encoding for pre-rRNA constituents, structural proteins, transport proteins, and proto-oncogenes. Angiotensin II (Ang II) binds to specific cell surface receptors, activates intracellular signaling pathways associated with cell growth, and induces proliferation and hypertrophy of a variety of cells. The role of Ang II in cell growth has been studied extensively in vascular smooth muscle cells, where it has been implicated as a mediator of the abnormal growth of these cells in some models of hypertension. Among the earliest of these are alterations in the levels of cyclic nucleotides and an increase in urinary excretion of sodium and potassium, rats were placed in metabolic cages on the day preceding the intervention and on the last day of the experiment; urinary sodium and potassium concentrations in 24-h urine collections were determined by flame photometry (Instrumentation Laboratories Model 943, Lexington, MA). One week following UNX, rats were anesthetized with Inactin (Andrew Lockwood and Associates, Sturtevant, WI) and placed on a heated table to maintain rectal temperature at 37 ± 0.5°C. Rats underwent tracheostomy to allow spontaneous breathing and were prepared for acute experimentation as previously described. Briefly, catheters were inserted into a jugular vein for infusion of solutions and a carotid artery for continuous measurement of arterial pressure with a Statham P23ID pressure transducer (Gould Instruments, Oxnard, CA) connected to a polygraph (model 7D, Grass Instrument Co., Quincy, MA). After stabilization following completion of surgery, arterial pressure was recorded for 10 min, after which the efficacy of angiotensin converting-enzyme inhibition was assessed by determination of the pressor response to an intravenous bolus injection of 200 ng of Ang I (Sigma Chemical, St. Louis, MO). Arterial pressure was recorded for the next 10 min; the rats were then killed, and the kidneys removed and weighed.

**METHODS**

**Experimental Design** Male Sprague-Dawley rats (Bantin-Kingman, Fremont, CA) were housed in climate controlled conditions (20°C and 55% relative humidity with a 12-h light/dark cycle) and provided standard rat chow and drinking fluid ad libitum. Experiments were conducted according to two major experimental protocols.

**Experiment 1** Rats 3 months old, weighing 290 to 340 g, were anesthetized with pentobarbital (30 mg/kg intraperitoneally, Abbott Laboratories, Chicago, IL). The right kidney was exposed using a dorsal approach and either removed (UNX; n = 14) or gently manipulated and left in place (Sham UNX; n = 14); removed kidneys were weighed and processed as described below. The wound was closed and the animals allowed to recover. Among both the UNX and the sham UNX animals, half received no treatment, whereas the other half received an angiotensin converting enzyme inhibitor (captopril; Squibb Institute for Medical Research, Princeton, NJ) dissolved in the drinking water at a concentration of 100 mg/dL. Captopril treatment was initiated at the time of the intervention and maintained for the duration of the study. Seven days after either UNX or sham operation, the animals were killed by decapitation, and the kidneys were quickly removed, weighed, snap-frozen in liquid nitrogen, and stored at -80°C. Livers were also snap-frozen in liquid nitrogen.

**Experiment 2** The functional adaptation to UNX and the efficacy of angiotensin converting enzyme inhibition were assessed in 18 UNX rats, half of which received captopril, initiated 1 week before UNX. To determine 24-h water intake, urine volume, and the daily urinary excretion of sodium and potassium, rats were placed in metabolic cages on the day preceding the intervention and on the last day of the experiment; urinary sodium and potassium concentrations in 24-h urine collections were determined by flame photometry (Instrumentation Laboratories Model 943, Lexington, MA). One week following UNX, rats were anesthetized with Inactin (Andrew Lockwood and Associates, Sturtevant, WI) and placed on a heated table to maintain rectal temperature at 37 ± 0.5°C. Rats underwent tracheostomy to allow spontaneous breathing and were prepared for acute experimentation as previously described. Briefly, catheters were inserted into a jugular vein for infusion of solutions and a carotid artery for continuous measurement of arterial pressure with a Statham P23ID pressure transducer (Gould Instruments, Oxnard, CA) connected to a polygraph (model 7D, Grass Instrument Co., Quincy, MA). After stabilization following completion of surgery, arterial pressure was recorded for 10 min, after which the efficacy of angiotensin converting-enzyme inhibition was assessed by determination of the pressor response to an intravenous bolus injection of 200 ng of Ang I (Sigma Chemical, St. Louis, MO). Arterial pressure was recorded for the next 10 min; the rats were then killed, and the kidneys removed and weighed.

**Slot Blot Analysis of Renin, Angiotensinogen, and AT1 Receptor mRNA** Total cellular RNA was isolated from frozen tissue by a modification of the guanidinium thiocyanate method as described previously. The rat renin clone was a kind gift of Kevin Lynch, University of Virginia and the AT1 cDNA probe a kind gift of Kenneth Bernstein, Emory University. Angiotensinogen, renin, and angiotensin receptor mRNAs were quantitated by slot blot hybridization, as described previously. Autoradiographs were obtained by exposure to Cronex x-ray film (DuPont, Wilmington, DE) with an intensifying screen at ~80°C for 4 to 8 days and were scanned using a laser densitometer (LKB 2202 Ultrascan, Piscataway, NJ) for quantitation.

**In Situ Ang II Receptor Assay** The distribution and density of Ang II receptors was assessed by a modification of the in situ radiographic technique of Mendelsohn et al as previously described.
Statistical Analysis Values are expressed as mean ± SEM. Data resulting from scanner analysis are presented as arbitrary scanner units. Since no difference in any measured variable was observed between the left kidney removed at nephrectomy and either the left or right kidney removed at the end of the experiment in untreated sham UNX animals, the values from these kidneys were pooled together and considered as a control group. Student's t test for paired or unpaired comparisons was used to assess significance within and between groups. A P = .05 was considered the minimum level of significance.

RESULTS

Experiment 1 Effect of UNX in Normal Rats In sham UNX animals, the weights of the right and left kidney were identical, and similar to the left kidney weight removed at the time of nephrectomy (Table 1). UNX was associated with a 23.3% ± 2.3% (P < .0001) increase in kidney weight, whereas body weight increased by only 3.7 ± 2.1%. Consequently the ratio of single kidney weight to body weight (KW/BW) increased significantly (Table 1). Neither renal renin nor renal or hepatic angiotensinogen mRNA levels changed significantly after UNX. In control kidneys, specific binding of [125I]-Sar^1-Ile^8 Ang II occurred in glomeruli and medullary vascular bundles, as reported previously,^2^ and UNX had no effect on binding distribution. Neither glomerular Ang II receptor density nor renal AT, receptor mRNA level was altered significantly by UNX (Table 1).

Influence of Angiotensin Converting Enzyme Inhibition on the Response to UNX Captopril treatment markedly reduced the pressor response to injected Ang I (Table 2). In sham operated rats, the ratio of KW/BW was not affected by captopril treatment as compared with untreated animals (Table 1). Following UNX, kidney weight increased by 20.4 ± 1.2% (P < .0001) from a control value of 1.24 ± 0.04 g. One week after UNX, the weight of the remaining kidney in these captopril-treated rats (1.50 ± 0.05 g) was not different from the weight of the remaining kidney of untreated animals (1.53 ± 0.03 g). Body weight increased by only 5.9 ± 1.8%; as a consequence, KW/BW increased to the same extent as in untreated animals. Captopril treatment resulted in a four- to fivefold increase in renin mRNA levels and no change in either renal or hepatic angiotensinogen mRNA levels. AT, receptor mRNA levels and glomerular Ang II receptor density were minimally increased as a consequence of the treatment (+13% and +25%, P = NS and P = 0.064 respectively) (Table 1). Renin mRNA levels were slightly reduced after UNX as compared with sham UNX animals, but were still significantly greater than values from control and UNX untreated kidneys (Table 1). As was also observed in untreated animals, neither renal nor hepatic angioten-
TABLE 2. EFFECT OF UNILATERAL NEPHRECTOMY ON WATER INTAKE, ELECTROLYTE EXCRETION, AND BLOOD PRESSURE IN CONTROL AND CAPTOPRIL-TREATED RATS

<table>
<thead>
<tr>
<th>Water Intake, mL/24 h</th>
<th>UNaV, meq/24 h</th>
<th>UKV, meq/24 h</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day -1 Day 7</td>
<td>Day -1 Day 7</td>
<td>Day -1 Day 7 Baseline Post-Ang II</td>
</tr>
<tr>
<td>Unilateral nephrectomy, untreated rats (n = 9)</td>
<td>35.6 ± 1.1 34.2 ± 2.1</td>
<td>150 ± 1.1 151 ± 0.8</td>
<td>1.62 ± 0.29 1.42 ± 0.24</td>
</tr>
<tr>
<td>Unilateral nephrectomy, captopril-treated rats (n = 9)</td>
<td>38.4 ± 2.0 41.8 ± 2.4</td>
<td>161 ± 1.3 170 ± 2.1</td>
<td>1.47 ± 0.31 1.31 ± 0.19</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of data obtained in metabolic cages. Angiotensin converting enzyme inhibition was obtained by captopril. 100 mg/L in the drinking water. *P < 0.01 post-angiotensin II (Ang II; 200 ng intravenous bolus) versus baseline. MAP, mean arterial pressure; UV, urine volume; UNaV, and UKV, urinary excretion of sodium and potassium.

sinogen mRNA levels changed significantly after UNX. Moreover, neither the density of glomerular Ang II receptors nor the level of AT1 receptor mRNA was altered following UNX, similar to results in the untreated animals (Table 1).

**Experiment 2** The functional adaptation to UNX, evaluated by the capacity of the remaining kidney to increase electrolyte excretion, was preserved in captopril-treated animals as indicated by similar 24-h urinary excretion of sodium and potassium before and after UNX (Table 2). Because Ang II could have growth promoting activity during the first hours following UNX before the rats started to ingest the captopril, we evaluated compensatory growth in rats pretreated with captopril for 1 week before UNX. In this group, the ratio of KW/BW increased to the same extent as in untreated rats (from 0.37 ± 0.01 to 0.42 ± 0.01% and from 0.39 ± 0.01 to 0.43 ± 0.01%, respectively). Thus, despite functional inhibition of the renin-angiotensin system, compensatory renal hypertrophy took place to a similar extent in both captopril-treated and untreated animals.

**DISCUSSION**

Unilateral nephrectomy results in an increase in the weight of the remaining kidney primarily through a process of cell hypertrophy, with only a small component of hyperplasia.1-3 Ang II has an established role as a growth factor in a variety of tissue and cellular systems. It has been shown to cause hypertrophy of cultured quiescent rat aortic smooth muscle cells,4 as well as rat renal mesangial5 and proximal tubular6 cells in culture. In addition, the renin-angiotensin system has been implicated in several forms of renal growth. An increase in dietary protein intake leads to renal hypertrophy, accompanied by an increase in plasma renin activity and renal renin mRNA.7 In diabetic rats, a high dose of enalapril blocks the enhanced renal hypertrophy and microalbuminuria induced by UNX.24 Furthermore, angiotensin converting enzyme inhibition prevents the development of proteinuria and glomerulosclerosis after UNX when the treatment is initiated at the time of the intervention, but not when started later.25 Captopril has beneficial effects in preventing functional and structural glomerular damage in rats with nephron loss from renal ablation.26 Angiotensin converting enzyme inhibition also suppressed accelerated growth of glomerular cells of maturing kidneys both in vitro and in vivo after UNX; however, in that study, UNX was performed in young rats, so that the inhibitor could have affected hyperplasia rather than hypertrophy. Ang II may also contribute to renal growth indirectly, as it potentiates the mitogenic effect of epidermal growth factor in proximal tubular cells in culture.27 Ang II itself has a hypertrophic effect on cultured mesangial and proximal tubular cells28,29 that could be mediated via stimulation of transforming growth factor-β.30 Although the pattern of activation of early response genes in the kidney after UNX is a matter of some controversy,31-36 Ang II induces activation of proto-oncogene mRNA in cultured murine proximal tubule cells that resembles the pattern observed after UNX.30,37 Such observations provide further circumstantial evidence in favor of a role for Ang II in the hypertrophic response, although the relationship of these changes in proto-oncogene expression to the hypertrophy has been questioned.38

The results of this study provide evidence against a role for Ang II as a growth promoting factor in the development of compensatory renal hypertrophy in adult animals. Renal hypertrophy occurred in the absence of activation of the renal renin-angiotensin system and despite suppression of Ang II generation, achieved by chronic captopril treatment. UNX did not lead to a significant change in renin or angiotensinogen gene expression 7 days after UNX. These results are consistent with the observation of Rosenberg et al,31 who showed no change in renin mRNA level.
or plasma renin activity 7 days after UNX. Although activation of one or several components of the renin-angiotensin system could have occurred at earlier time points, this does not seem likely: in rats in which both tissue and circulating levels of Ang II were likely to have been reduced shortly after UNX by treatment with captopril, renal hypertrophy occurred after UNX, just as in untreated rats, suggesting that angiotensin-mediated responses in the period immediately following UNX were not important components of the subsequent hypertrophic response.

The efficacy of captopril treatment was confirmed by the marked attenuation of the pressor response to a bolus intravenous injection of Ang I. In addition, renal renin mRNA levels increased by four- to fivefold during captopril treatment, in agreement with a previous observation. Captopril treatment did not affect compensatory renal growth after UNX. These results are in contradiction to earlier studies showing a partial inhibition of compensatory renal growth following UNX by angiotensin converting enzyme inhibitors. Wight et al showed that, in adult Wistar rats, enalapril treatment reduced compensatory renal growth following UNX. Dworkin et al observed that both captopril and enalapril reduced compensatory renal growth in spontaneously hypertensive rats. However, UNX in these studies of Dworkin and associates was performed in 6-week-old rats, an age at which both hyperplasia and hypertrophy would be expected to occur. In the studies of Wight et al in adult rats, control animals undergoing UNX had increased renal protein and DNA content 1 week later so that the protein:DNA ratio was unchanged. In enalapril-treated rats, DNA content, used as a marker of hyperplasia, did not increase after UNX despite a large increase in protein content. Therefore, they concluded that converting enzyme inhibition interfered with hyperplasia but did not affect hypertrophy. This interpretation is somewhat at variance with most of the work examining compensatory renal growth, which has uniformly shown that this occurs primarily through a process of hypertrophy shown at best only a small (< 20%) component of hyperplasia (reviewed in references 1-3). Although we did not measure renal protein or DNA content, our data clearly show that functionally effective converting enzyme inhibition did not alter compensatory renal growth. The basis for the difference in our results from those of Wight and colleagues is not clear. Their treatment with enalapril was started 4 weeks before UNX, whereas in our Experiment 2 studies, captopril was administered 1 week before UNX. It is difficult to imagine that this difference in length of exposure could modify the results, or that the two different converting enzyme inhibitors used had different actions. Rats in the study of Wight and associates lost weight in the week following UNX, and it is possible that postsurgical hypercatabolism modified their results in some way. In our present study, as well as in previous work from this laboratory, rats gained weight after UNX. It is also possible that converting enzyme inhibitors do block the hyperplastic component of compensatory renal growth. If so, this component must have been sufficiently small in our studies so as not to be detected by the methods we used.

In summary, our results indicate that compensatory renal hypertrophy following UNX occurred despite inhibition of the renin-angiotensin system by captopril. These studies therefore do not support a critical role for Ang II in the development of compensatory renal growth following UNX.

ACKNOWLEDGMENT

The authors are grateful to Dr Zola P. Horovitz, Bristol-Myers Squibb Institute for Medical Research, Princeton, New Jersey, for the gift of the captopril used in these studies.

REFERENCES


