J Am Soc Nephrol 10: 43–50, 1999

Intrarenal Insulin-Like Growth Factor-1 Axis after Unilateral Nephrectomy in Rat

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Abstract. It has been suggested that insulin-like growth factor-1 (IGF-1) may play a role in early compensatory renal growth. Since IGF-1 action is influenced by IGF binding proteins (IGFBP), this study was conducted to characterize the changes in gene expression not only of IGF-1 and its receptor, but also of IGFBP in the hypertrophying kidney of adult and weanling rats 1 wk after removal of the other kidney. At this time, there were distinct age-dependent changes in the renal IGF-1 axis. In the mature kidney, IGF-1 mRNA levels fell without a change in kidney IGF-1 peptide content. Likewise, although IGFBP-2, -3, and -5 mRNA levels fell, membrane-associated IGFBP did not change. IGF-1 receptor mRNA levels and IGF-1 receptor number both fell. In the weanling kidneys, IGF-1 mRNA and peptide levels and IGF-1 receptor binding were unaltered. However, IGFBP-3, -4, and -5 mRNA levels were increased, as were plasma membrane-associated IGFBP. Although these changes in the intrarenal IGF-1 axis were distinct, it is difficult to conceive how in either the mature or immature rat they could contribute to the ongoing compensatory renal growth that occurs 1 wk after loss of kidney mass unless IGF-1 were acting in a synergistic manner with other growth promoters.

Loss of renal mass is followed by compensatory renal growth, a process thought to be mediated in part through the action of local growth factors. Among them, it has been suggested that insulin-like growth factor-1 (IGF-1) may play a predominant role. This growth factor has the property of promoting renal growth and increasing renal blood flow and GFR (1). Insulin-like growth factor-1 has been implicated as a mediator of compensatory renal growth because of reports describing an increase in kidney IGF-1 levels in mature rats during the first few days after a unilateral nephrectomy (2,3). The mechanism responsible for this increase in kidney IGF-1 in early compensatory renal growth remains to be identified. Some studies report an increase of kidney IGF-1 mRNA levels (4,5), but others show no change (6,7). Furthermore, circulating plasma IGF-1 levels are unchanged, and there is no increase in kidney IGF-1 receptor binding to account for the increase in kidney IGF-1 peptide. A potential mechanism is increased trapping within the kidney by local IGF binding protein (IGFBP).

IGFBP are present in the circulation and are produced in tissues throughout the body, including the kidney (8,9). To date, six IGFBP have been identified. They have a similar or higher affinity for IGF-1 than the IGF-1 receptor, and by forming complexes with IGF-1, they affect the delivery of IGF-1 to the cells and modulate its bioactivity (8). In the circulation, >98% of the IGF-1 is complexed to the IGFBP, and these complexes serve as a slow-release storage form of the growth factor. At a cellular level, the IGFBP are generally inhibitory, but in some cell types they may enhance the action of IGF-1 (8,10,11) or even exhibit IGF-1-independent actions (12,13). Despite their important role in modulating IGF-1 action, relatively little is known about the response of kidney IGFBP to physiologic and pathologic changes.

Studies of the response of the weanling rat to loss of one kidney have also been reported. Unlike the adult rat that is growth hormone (GH)-dependent, the weanling is GH-independent (14). Also, there is an increase in kidney IGF-1 mRNA levels during the first 48 h after a uninephrectomy in the weanling (7). Whether there is a concomitant increase in the IGF-1 peptide or whether there are changes in the IGFBP profile or GH receptor expression is not known. Although it seems that IGF-1 may play a role in inducing renal enlargement during the early rapid phase of renal growth, it is unclear whether it plays a role at a later stage. Accordingly, we have examined the response of the kidney IGF-1 system in rats 1 wk after the removal of a single kidney. At this time, the rapid phase of compensatory renal growth is complete and a phase of slower compensatory growth ensues (14,15).

Materials and Methods

Animals

Male weanling (3 to 4 wk old) and adult (8 wk old) Sprague Dawley rats were anesthetized with ketamine (60 mg/kg, intraperitoneally) and xylazine (12 mg/kg) and underwent a left kidney nephrectomy or a sham operation. Animals were pair-fed standard laboratory rat chow (20% protein) with free access to water. Seven days later, rats were anesthetized, and kidneys were removed and stored at −80°C for mRNA analysis. For membrane preparation and measurement of kidney IGF-1 levels, a second group of rats prepared as above was used. At sacrifice, kidneys were flushed with phosphate-buffered

Received November 12, 1997. Accepted July 6, 1998.
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1046-6673/1001-0043$03.00/0
Journal of the American Society of Nephrology

saline (37°C) until the effluent was bloodless so as to remove serum IGFBP and stored at −80°C. Preliminary experiments showed no difference in gene expression between sham-operated and unoperated control rats after 1 wk; thus, the study is confined to comparisons between sham-operated and uninephrectomized (UNx) rats.

Preparation of Radiolabeled Probes

Labeled antisense IGF-1 and IGF-1 receptor riboprobes used for the solution hybridization protection assay were synthesized by using ^{32}P-CTP (16). Briefly, the IGF-1 riboprobe was designed to detect both the Ea and Eb IGF-1 mRNA. The IGF-1 receptor riboprobe contained 280 bases complementary to the IGF-1 receptor mRNA. For Northern blot analysis, the cDNA probes were labeled with 50 μCi [^{32}P]dCTP (3000 Ci/mmol, Amersham, Arlington Heights, IL) by a random primer method (DNA labeling kit 70200, United States Biochemical Corp., Cleveland, OH). Radiolabeled cDNA probes were purified by centrifugation through a G-50 Sephadex column.

The cDNA probes for IGFBP-1 to -5 were derived from rat cDNA (17), gifts of Dr. S. Shimasaki (Whittier Institute, La Jolla, CA). The IGFBP-1 probe was composed of 407 bp corresponding to the coding region spanning nucleotide position 486 to 892 in the rat IGFBP-1 cDNA sequence. The IGFBP-2 probe consisted of 342 bp spanning nucleotide position 670 to 1011. The IGFBP-3 probe consisted of 636 bp spanning nucleotide position 196 to 831. The IGFBP-4 probe consisted of 388 bp spanning nucleotide position 487 to 840. The IGFBP-5 probe consisted of 259 bp spanning nucleotide position 856 to 1114.

Northern Blot Analysis

Total kidney RNA was isolated by an acid guanidinium thiocyanate-phenol chloroform single-step technique and size-separated by formaldehyde agarose gel electrophoresis as described previously (16). Briefly, RNA was denatured with 0.55 M formaldehyde, and 20 μg was loaded into each lane and electrophoresed on a denaturing agarose gel containing 0.55 M formaldehyde. RNA was transferred onto nitrocellulose filters and ultraviolet-crosslinked (UV 1800 Stratalinker, Stratagene, La Jolla, CA). Prehybridization of the filters was carried out as before (16). The size and amount of IGFBP mRNA were determined by hybridizing the mRNA on the Northern blots to ^{32}P-labeled cDNA probes (approximately 2.5 million cpm/ml) of rat IGFBP-1, -2, -3, -4, and -5 at 42°C overnight. Washed filters were then exposed to Kodak XAR-5 films (Kodak, Rochester, NY) at −70°C for 1 to 7 d. The mRNA abundance for each IGFBP was quantified by scanning densitometry (Ultrascan XL, Pharmacia LKB Biotechnology, Alameda, CA). Readings of the 18S rRNA signal obtained from photographic negatives of the nitrocellulose filters were used for RNA loading corrections.

Solution Hybridization Ribonuclease Protection Assay

Protection assays were performed as before (16). Briefly, total RNA (20 μg) was hybridized with [α-^{32}P]CTP-labeled antisense IGF-1 or IGF-1 receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes overnight. After hybridization, the mixture was RNase digested, followed by addition of proteinase K, and the protected hybrids were ethanol-precipitated and size-separated on a 5% polyacrylamide/8 M urea denaturing gel. Protected bands were quantified by densitometry of the autoradiogram. For IGF-1, we quantified the Ea IGF-1 mRNA transcripts only, because it accounted for >95% of the IGF-1 mRNA signal in the kidney.

Reverse Transcription-PCR

Reverse transcription-PCR was performed according to Ausubel *et al.* with minor modifications (18). Kidney GH receptor mRNA and 18S rRNA levels were measured simultaneously by using a Gene Amp RNA-PCR kit (Perkin Elmer Cetus, Norwalk, CT). Random hexamers were used to reverse transcribe total RNA. The primer set for the GH receptor amplified a 185-bp DNA fragment that includes 26 bases in exon 9, and 159 bases in exon 10 that correspond to the intracellular domain of the rat GH receptor cDNA sequence (19). The GH receptor mRNA was amplified by 28 PCR cycles, and as an internal amplification control 18S rRNA was amplified in the same assay tube for the last 10 cycles by adding specific 18S rRNA primers. The 18S rRNA primer amplified 140 bases spanning nucleotide positions 107 to 246 in the human cDNA sequence (20). A 5% polyacrylamide/8 M urea gel was used to separate the PCR products. Signals were analyzed by quantitative autoradiography.

Preparation of Crude Kidney Membranes

Crude plasma membranes were isolated as described previously (16). In brief, kidney homogenates prepared in 8% wt/wt sucrose and submitted to differential centrifugation. Crude membranes were collected after a 47,000 × *g* centrifugation, washed, and resuspended in calcium-free Krebs-Ringer Hepes buffer (KRH, pH 7.4), and stored at −80°C. Membrane protein content was determined using the Bio-Rad method (Bio-Rad Laboratories, Richmond, CA).

^{125}I-IGF-1 Receptor Binding Assay

Binding of IGF-1 to IGF-1 receptors in crude membranes prepared from saline-perfused kidneys was determined as before with recombinant human IGF-1, a gift from Genentech, Inc. (South San Francisco, CA) radiolabeled with ^{125}I-Na (<1 iodine atom/molecule) according to the chloramine T method (16). Binding of ^{125}I-IGF-1 to crude membrane preparation (60 μg of protein) was carried out in calcium-free KRH containing 0.1% bovine serum albumin, 2 mM bacitracin, 5 mM *N*-ethylmaleide, 10^{-11} M ^{125}I-IGF-1, and with or without various concentrations of unlabeled IGF-1. The membrane suspension was then incubated at 4°C overnight. Membrane-bound radioactivity was separated by centrifugation and then counted. Nonspecific binding was defined as the counts of ^{125}I-IGF-1 associated with membranes in the presence of 10^{-6} M of unlabeled IGF-1, and was subtracted from total binding to yield specific binding. Data from competition curves were subjected to Scatchard analysis to determine binding affinity and receptor number. In an earlier study, we established by means of crosslinking experiments that specific binding is almost entirely due to binding to the IGF-1 receptor (16).

Western Ligand and Immunoblot

Plasma membranes were prepared from saline-perfused whole kidney and assayed by Western ligand blotting (16). In short, 60 μg of kidney membrane protein were electrophoresed in 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions. Electroblotting of the separated proteins onto nitrocellulose filters was performed in a semidry transfer cell (Bio-Rad Laboratories). The separated proteins were incubated in a buffer containing 1×10^6 cpm of ^{125}I-IGF-1 and ^{125}I-IGF-2 overnight at 4°C. Filters were washed and visualized by autoradiography. Western immunoblots were prepared with a polyclonal rabbit antihuman IGFBP-1 (Upstate Biotechnology, Lake Placid, NY) as before (21).

RIA of Serum and Kidney IGF-1

IGF-1 was measured by RIA in acid ethanol extracts prepared from serum- and saline-perfused kidney (16). In brief, kidney IGF-1 was extracted by homogenizing the tissue in 1 M acetic acid and centrifuged at 10,000 × *g* for 5 min at 4°C. The supernatant was saved for RIA. Serum was extracted with acid ethanol. IGF-1 content was measured using a commercial RIA kit (Nichols Institute, San Juan Capistrano, CA). Measurement of recovered IGF-1 added to kidney homogenate of sham control and UNx kidneys averaged 96 ± 4 and 94 ± 3%, respectively, and from serum 91 ± 3 and 101 ± 3%, respectively.

Statistical Analyses

Two-tailed unpaired *t* test was used to assess differences between sham-operated controls and UNx rats. Differences were considered significant at $P < 0.05$. Results are expressed as the mean ± SEM of five rats per group unless otherwise stated. Densitometry readings of the mRNA of interest were corrected for the corresponding GAPDH mRNA or 18S rRNA reading. The average of the control values was then assigned an arbitrary unit of 1, and all readings were expressed relative to this unit.

Results

Body weights of UNx and sham-operated rats were similar at the beginning and end of the 7-d study period. As expected, the weight of the remaining kidney increased significantly in the UNx rats ($P < 0.01$); on average, the percent increase was greater in the immature rats (Table 1).

Adult Rats

Because kidney IGF-1 expression is regulated partly through the GH receptor, we measured GH receptor mRNA levels in kidneys 7 d after surgery. At this time, relative GH receptor mRNA levels were similar in the UNx and sham-operated control rats (0.83 ± 0.08 *versus* 1.0 ± 0.06 arbitrary units). In contrast, kidney IGF-1 receptor and IGF-1 mRNA levels were reduced significantly after uninephrectomy, averaging 56 and 63% of the respective control values ($P < 0.01$). These results are shown in Figure 1.

Specific binding of tracer ^{125}I-IGF-1 to kidney plasma membranes from kidneys of UNx rats was approximately 27% lower than binding to membranes from sham-operated controls

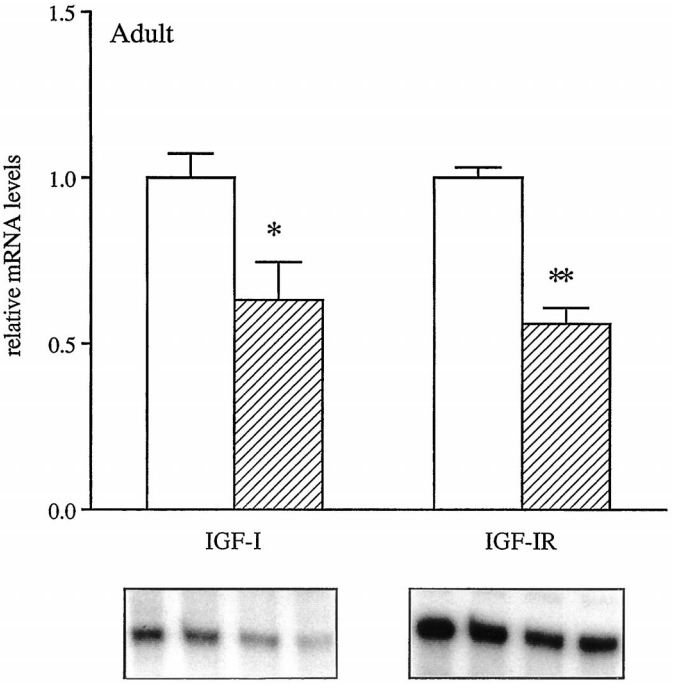

Figure 1. Relative insulin-like growth factor-1 receptor (IGF-1R) and IGF-1 mRNA levels in kidneys from adult sham-operated control (□) or uninephrectomized (▨) rats 7 d after surgery. IGF-1R, IGF-1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were determined simultaneously in solution hybridization-RNase protection assays. Autoradiograms were quantified by densitometry, and the IGF-1R signal was corrected for GAPDH. The average of the control values was then assigned an arbitrary unit of 1, and all readings were expressed relative to this unit. Representative autoradiograms are shown. *$P < 0.05$; **$P < 0.01$.

(8.5 ± 0.3 *versus* 11.6 ± 0.5%/60 μg membrane protein; $P < 0.01$). This difference in receptor binding between the two groups is readily apparent from competitive displacement curves shown in Figure 2. When the values were expressed as a percentage of maximal bound, the curves were superimposable, indicating that the receptor affinities are comparable in the two groups (Figure 2, inset). The concentration of cold IGF-1 at which half-maximal binding of ^{125}I-IGF-1 occurred

Table 1. Body weights of experimental rats[a]

Group	Body Weight (g)		Right Kidney (g)	% Change
	Day 0	Day 7	Day 7	
Adult				
sham	190 ± 1	227 ± 3	0.95 ± 0.03	
UNx	195 ± 2	230 ± 3	1.27 ± 0.04[b]	34
Weanling				
sham	75 ± 1	112 ± 4	0.55 ± 0.02	
UNx	72 ± 2	111 ± 2	0.83 ± 0.02[b]	51

[a] UNx, uninephrectomized.
[b] *t* test, $P < 0.01$ sham *versus* UNx (5 rats/group).

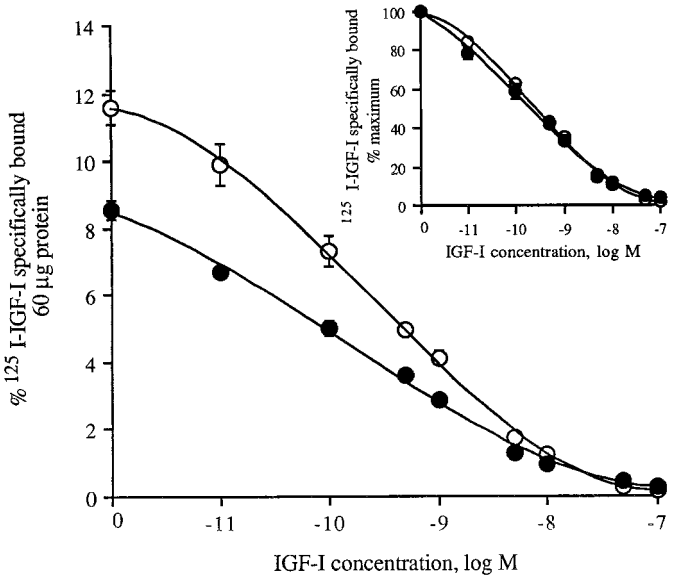

Figure 2. Competitive inhibition of ^{125}I-insulin-like growth factor-1 (IGF-1) binding to kidney plasma membrane from adult sham-operated control (○) or uninephrectomized (●) rats. Various concentrations of IGF-1 were added to membranes incubated with ^{125}I-IGF-1 (10^{-11}M). Data are corrected for nonspecific binding and are from three rats per group. In the inset, data are expressed as a percentage of maximal binding.

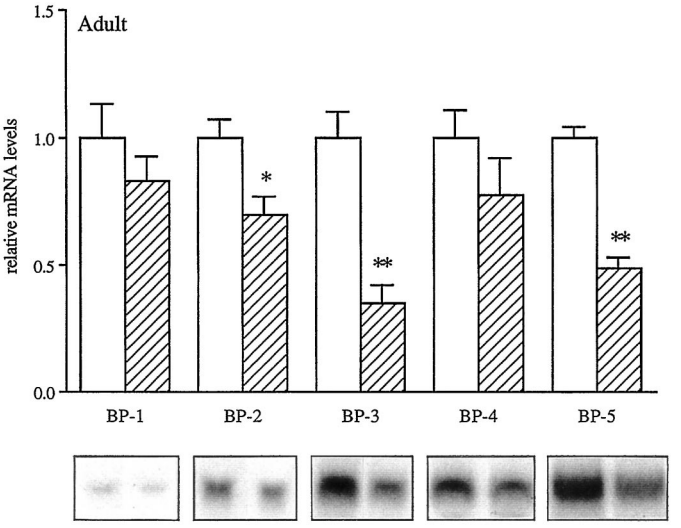

Figure 3. Relative mRNA levels of IGF-1 binding protein (BP)-1, -2, -3, -4, and -5 in kidneys from adult control (□) or uninephrectomized (▨) rats. mRNA levels were detected by Northern blot analysis and corrected for the 18S rRNA levels. Results were analyzed and expressed as described in Figure 1. Representative Northern blots are shown. $*P < 0.05$; $**P < 0.01$.

was the same in the two groups (0.96 nM). Thus, the difference in binding reflects a decrease in the number of IGF-1 receptors in the UNx group. This finding was confirmed by Scatchard analysis, which revealed two classes of receptors, *i.e.*, high-affinity low-capacity receptors and low-affinity high-capacity receptors. The affinity of the first class of receptor was similar in the UNx and control groups (0.081 ± 0.15 *versus* 0.085 ± 0.03 nM, respectively). In contrast, receptor number was lower in the UNx group (5.0 ± 0.22 *versus* 7.3 ± 0.66 pM/60 µg protein; $P < 0.05$). The affinity of the second class of receptors also did not differ between groups (2.7 ± 0.36 and 2.52 ± 0.12 nM). However, receptor number was 25% lower in the UNx group (113 ± 7 *versus* 140 ± 3 pM/60 µg protein; $P < 0.05$). Thus, receptor affinity was similar in the two study groups, whereas receptor number was lower in the UNx rats.

Kidney mRNA levels for IGFBP-2, -3, and -5 fell significantly after uninephrectomy, with an average reduction of 30, 65, and 51%, respectively, compared with controls (Figure 3). The IGFBP-1 and -4 mRNA levels were unaltered. Western ligand blot analysis for IGFBP demonstrated the presence of at least three plasma membrane-associated IGFBP of 24 and 29 to 31 kD in size; these did not differ between the UNx and control animals (Figure 4A). The 24-kD protein is consistent with the size of IGFBP-4. The 29- to 31-kD proteins are consistent with the size of IGFBP-1, -2, and -5, with IGFBP-1 being the largest of these proteins (8). The Western immunoblot showed that the IGFBP-1 levels were similar in the two groups (Figure 5, top). This finding is consistent with the unchanged IGFBP-1 mRNA levels.

Although serum IGF-1 concentration had a tendency to be higher in UNx compared with the control group (847 ± 73 *versus* 644 ± 51 ng/ml, respectively), this difference was not statistically significant. A similar trend was noted with regard to the kidney IGF-1 concentration (6.4 ± 0.7 *versus* 5.2 ± 0.45 ng/mg protein).

Weanling Rats

Relative growth hormone receptor mRNA levels were similar in kidneys of the experimental and sham-operated rats (0.89 ± 0.04 *versus* 1.0 ± 0.05 arbitrary units) 7 d after surgery. Unlike adult rats, kidney IGF-1 mRNA levels were similar in UNx and control weanling rats (0.93 ± 0.13 *versus* 1.04 ± 0.11 arbitrary units). On the other hand, as in the adult rats, there was a small (24%) but significant decrease in the IGF-1 receptor mRNA levels after uninephrectomy. These results are shown in Figure 6. IGF-1 binding to kidney plasma membrane receptors of UNx rats did not differ from that of controls (11.3 ± 0.53% *versus* 10.2 ± 0.46%/60 µg protein). This finding differs from the fall in receptor binding noted in adult UNx rats.

Northern blot analysis of the mRNA levels for IGFBP-1, -2, -3, -4, and -5 are shown in Figure 7. Compared with controls, IGFBP-3, -4, and-5 mRNA levels in the UNx group were increased significantly by 119, 62, and 45%, respectively. There were no differences in the IGFBP-1 and -2 mRNA levels. This pattern of change was distinctly different from that seen in the adult rats. Western ligand blot analysis of IGFBP associated with kidney membranes revealed three signals close to the 24- and 29- to 31-kD regions as seen in the adults. As demonstrated in Figure 4B, uninephrectomy was associated with a significant increase in the signal for all of these proteins. Densitometry readings showed a 57% increase in the 24-kD

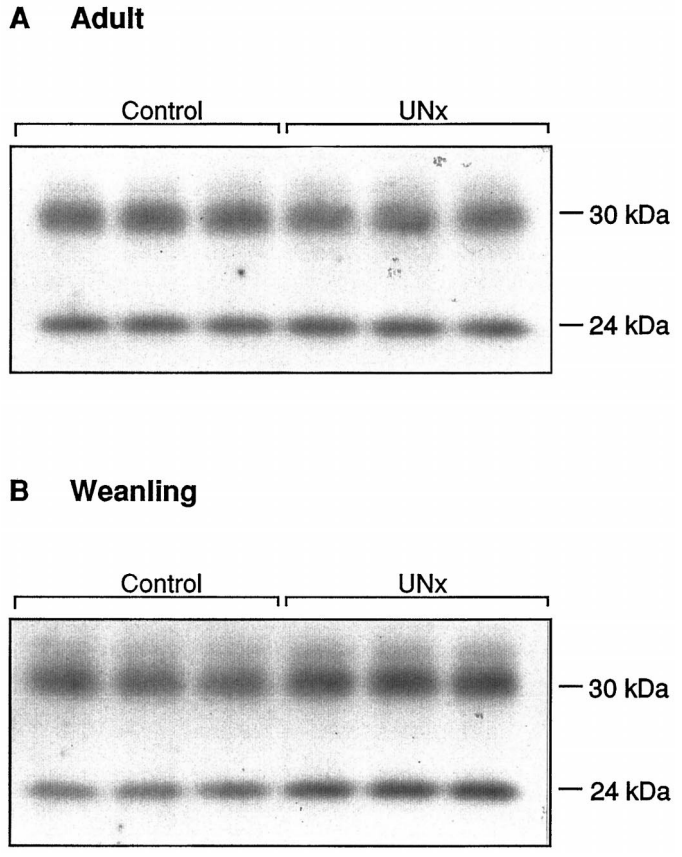

Figure 4. Western ligand blot of plasma membrane-associated insulin-like growth factor binding proteins. Membranes were prepared from saline-perfused kidneys from adult (A) and weanling (B) sham-operated control or uninephrectomized (UNx) rats.

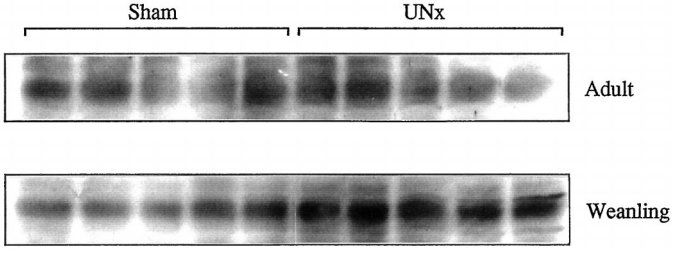

Figure 5. Western immunoblot of plasma membrane-associated insulin-like growth factor binding proteins with an antibody against IGF-1 binding protein. Membranes were prepared from kidneys from adult (top) and weanling (bottom) sham-operated control or UNx rats.

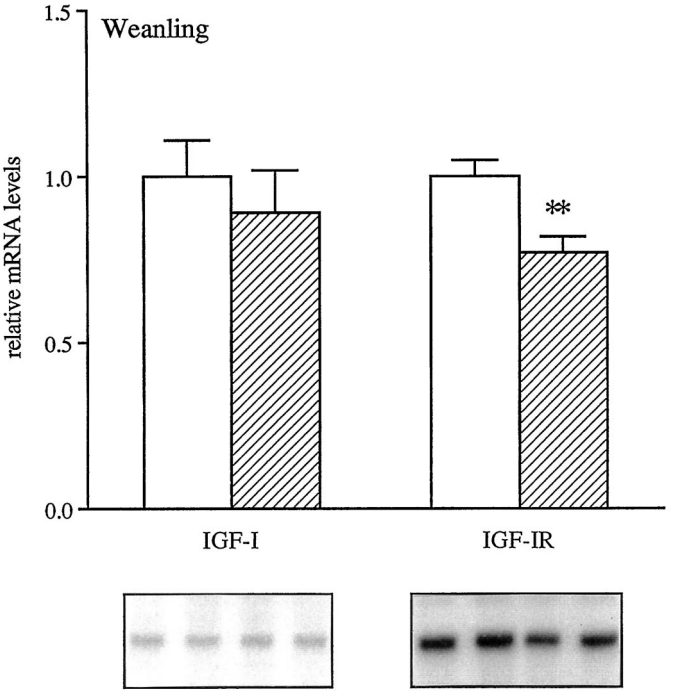

Figure 6. Relative IGF-1R and IGF-1 mRNA levels in kidneys from weanling sham-operated control (□) or UNx (▨) rats 7 d after surgery. Representative autoradiograms are shown. $^{**}P < 0.01$.

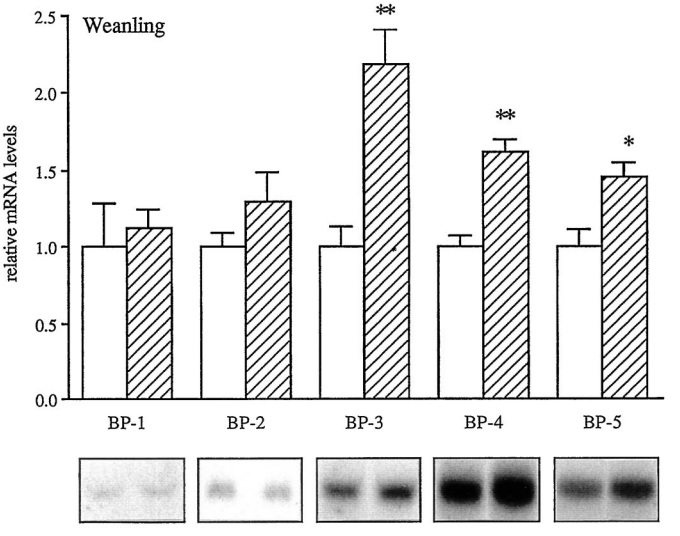

Figure 7. Relative mRNA levels of IGF-1 binding protein (BP)-1, -2, -3, -4, and -5 in kidneys from weanling control (□) or UNx (▨) rats. Representative Northern blots are shown. $^{*}P < 0.05$; $^{**}P < 0.01$.

protein signal (consistent with IGFBP-4) in the UNx rats compared with controls (1.76 ± 0.06 *versus* 1.12 ± 0.08 arbitrary densitometry units, $P < 0.01$). A smaller (23%) but still statistically significant increase in the 29-kD protein signal was also observed after uninephrectomy (1.77 ± 0.05 *versus* 1.44 ± 0.09 arbitrary densitometry units; $P < 0.05$). Consistent with the mRNA levels, the IGFBP-1 protein levels measured by Western immunoblot were similar in the two groups (0.6 ± 0.08 *versus* 0.61 ± 0.04 arbitrary densitometry units) (Figure 5, bottom). These findings, taken together with the changes in kidney mRNA levels noted above, suggest that the small increase in the 29-kD signal detected by the ligand blot and consistent with the size of IGFBP-1, -2, and –5 likely reflects an increase in IGFBP-5.

Serum IGF-1 levels were comparable in the UNx and control groups (765 ± 39 *versus* 700 ± 105 ng/ml). Similarly, tissue IGF-1 content did not differ between the two groups (9.4 ± 0.87 *versus* 7.4 ± 1.22 ng/mg protein).

Discussion

Because IGF-1 promotes renal growth, there has been intense interest in determining whether the intrarenal IGF-1 axis participates in compensatory renal growth after loss of renal mass. There is substantial evidence showing that in the adult rat whole kidney, IGF-1 levels increase early after a uninephrectomy, returning to basal levels after 4 d (2,3,5); others have shown a persistent increase in kidney IGF-1 levels, even as long as after a month (5). Of note, most of the reports fail to show a concomitant increase in IGF-1 gene expression (4,6,22). On the other hand, in weanling rats a transient increase in whole kidney IGF-1 mRNA level during the first 2 d after renal ablation has been described (14). Whether this change is accompanied by an increase in IGF-1 peptide is not known. Several explanations have been proposed to account for the paradoxical increase in kidney IGF-1 concentration in the adult rat in the presence of unchanged IGF-1 mRNA levels (3). First, elevated circulating IGFBP other then IGFBP-3 could facilitate the movement of IGF-1 into the kidneys because of their relatively small size (21). Second, there could be increased trapping of circulating or locally produced IGF-1 within the kidney due to increased kidney membrane-associated IGFBP or IGF-1 receptor number. Third, reduced cellular breakdown of internalized IGF-1 may be involved as occurs in K depletion (21). Finally, the disparity between mRNA and peptide levels could be caused by enhanced posttranscriptional mechanisms with increased generation of IGF-1. In this study, we have examined the age-related response of the intrarenal IGF-1 system to loss of one kidney. This system includes IGF-1, the IGF-1 receptor, the GH receptor, and the IGFBP. We were particularly interested in the IGFBP, because these proteins have profound modulatory effects on IGF-1 action (1,8), and little is known about the response of the IGFBP after renal ablation in the adult rat.

In both the mature and immature rats studied 1 wk after uninephrectomy, we observed significant changes in the kidney IGFBP profile. However, the changes differed between the two age groups. In the adult kidney, mRNA levels for IGFBP-2, -3, and -5 were significantly reduced. This was not accompanied by a change in kidney plasma membrane-associated IGFBP levels. A fall in kidney IGFBP mRNA levels after a uninephrectomy in adult rats has also been described by Hise *et al.* (22). They reported that, 6 d after loss of renal tissue, IGFBP-4 and -5 mRNA levels were reduced. In the weanling rat, in contrast to the response in adult rats, we found that kidney IGFBP-3, -4, and -5 mRNA levels increased 1 wk after loss of one kidney. This increase was attended by a significant increase in plasma membrane-associated IGFBP.

To evaluate the potential significance of these changes in the kidney IGFBP profile, the effect of IGFBP on IGF-1 action requires consideration (8). In general, circulating and tissue IGFBP act as IGF-1 carriers forming a reservoir from which the free IGF-1 is slowly released. Because the IGFBP limit the immediate bioavailability of IGF-1, they generally have an inhibitory effect on IGF-1 action. However, under selected conditions some IGFBP may actually enhance the mitogenic activity of IGF-1 on some cell types, including fibroblasts (10). There is also evidence that some IGFBP may have actions independent of IGF-1 (12,13). Regarding the kidney, there is little information on the function of IGFBP, although the marked anatomical variation of IGFBP gene expression in the kidney suggests diversity of function (9). We have studied the effect of IGFBP secreted by cultured rabbit proximal tubule cells on the mitogenic activity of IGF-1 and found that the overall effect is inhibitory (12). Similarly, recombinant IGFBP-3 inhibited IGF-1-induced mitogenesis in these cells and also in the opossum kidney proximal tubule cell line. On the other hand, there is a preliminary report describing IGFBP-2-enhanced IGF-1-stimulated DNA synthesis in Madin-Darby canine kidney cells, a cell line derived from the dog kidney distal nephron (11). Taken together, it appears that until the intricate interactions between IGF-1, the IGFBP, and the different cell types of the kidney are understood, the significance of the changes in renal IGFBP gene expression and protein levels that follow the loss of renal mass will remain obscure.

Regarding IGF-1, in the adult rat we found that 1 wk after the loss of one kidney, IGF-1 mRNA levels were reduced significantly in the remaining kidney. In contrast, kidney IGF-1 peptide levels were unchanged. This discrepancy may be explained by the finding that the levels of serum IGF-1, a major source of kidney IGF-1, did not differ significantly in the UNx and control rats. Kidney IGF-1 is derived both from the circulation and from local production; thus, changes in local production may have been masked by serum-derived IGF-1. Descriptions of kidney IGF-1 mRNA levels after loss of renal mass have varied. In contrast to our finding of low kidney IGF-1 mRNA levels, others have reported that kidney IGF-1 mRNA levels are unchanged (5,7) or even increased in the adult rat (4). On the other hand, Hise *et al.* noted a transient decrease in the IGF-1 mRNA level on the second day after uninephrectomy (22). These discordant findings may be explained by disparities in the age or gender of the animals, the use of pair-fed or *ad libitum*-fed rats as controls, or the use of the contralateral excised kidney from the same animals or kidneys from sham-operated rats as controls.

In the mature rat, we also found that kidney IGF-1 receptor mRNA levels were reduced by near half after a uninephrectomy. This was accompanied by a fall in plasma membrane IGF-1 receptor number. On the other hand, GH receptor mRNA levels did not differ from control values. In their study, Hise *et al.* noted a significant reduction in kidney IGF-1 receptor mRNA levels 1 d after uninephrectomy that returned to control values the next day (22). Mulroney *et al.* reported that IGF-1 receptor mRNA levels fell during the 48-h period after uninephrectomy, whereas receptor binding was essentially unchanged (14). Taking our findings together, the lack of change in kidney IGF-1 peptide levels and the decrease in IGF-1 receptor binding argues against a role for IGF-1 as a mediator of compensatory growth of the whole kidney in the mature rat 7 d after the loss of one kidney. Because there is considerable anatomical heterogenicity in the expression of the components of the IGF-1 axis (9), changes limited to specific nephron segments may have been missed in this study. Thus, it

is conceivable that although IGF-1 is not a mediator of compensatory growth of the whole kidney, it may mediate growth in specific nephron segments.

In the weanling rat, in contrast to the adult, kidney IGF-1 mRNA levels were unchanged 7 d after uninephrectomy. Serum and kidney IGF-1 peptide levels were also unaffected. GH receptor mRNA levels were unchanged, but there was a small (24%) statistically significant fall in kidney IGF-1 receptor mRNA levels. Binding to plasma membrane IGF-1 receptors was unaltered. Studying weanling rats during the 48-h period after uninephrectomy, Mulroney *et al.* noted an increase in kidney IGF-1 receptor and IGF-1 mRNA levels (14). They found that IGF-1 receptor binding increased in kidney cortex but not in medulla. Kidney IGF-1 peptide levels were not measured. These researchers subsequently showed that the onset of the increase in IGF-1 receptor and IGF-1 mRNA levels precedes the expression of the early intermediate genes c-fos and c-jun and the start of DNA synthesis that occurs in the compensating weanling kidney (23). This proliferative response subsides after 72 h and is followed by a slower hypertrophic phase of compensatory growth. Taking all of this together, they postulated that IGF-1 may play a role in the early rapid hyperplastic phase of growth in the immature rat kidney. Our data suggest that IGF-1 does not play a major role in the later hypertrophic phase and is comparable to our earlier conclusion regarding the adult rat in which compensatory renal growth occurs predominantly by cellular hypertrophy (24).

From the present study, it is apparent that 1 wk after loss of one kidney, there are marked changes in the renal IGF-1 axis. These changes differ in mature and immature rats, even though at this time in the course of compensatory renal growth, enlargement of the kidney in both age groups is largely due to cellular hypertrophy. In the mature rat kidney, IGF-1 mRNA levels fell without a concomitant change in kidney IGF-1 peptide content. Similarly, although IGFBP-2, -3, and -5 mRNA levels fell, membrane-associated IGFBP did not change. On the other hand, IGF-1 receptor mRNA levels and IGF-1 receptor binding both fell significantly. In the immature rat, there was no change in IGF-1 mRNA and peptide levels or in IGF-1 receptor binding. IGFBP-3, -4, and -5 mRNA levels were increased, as were plasma membrane-associated IGFBP. Taken together, it is difficult to perceive how these changes in the intrarenal IGF-1 axis in the adult and weanling rats might directly contribute to the adaptive kidney growth that is ongoing 1 wk after loss of a kidney. On the other hand, it is conceivable that the IGF-1 axis may act in a synergistic manner with other growth factors.

Acknowledgments

This study was funded by the Department of Veterans Affairs Research service. Dr. Fervenza was supported by the Satellite Dialysis Center Fund and Conselho Nacional de Pesquisas (Brazil).

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