Renal expression of parathyroid hormone-related protein (PTHrP) and PTH/PTHrP receptor in a rat model of tubulointerstitial damage

RAQUEL LARGO, DULCENOMBRE GÓMEZ-GARRE, SOLEDAD SANTOS, CARLOS PEÑARANDA, JULIA BLANCO, PEDRO ESBRIT, and JESÚS EGIDO

Renal Research Laboratory and Metabolic Research Unit, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, and the Department of Pathology, Hospital Clínico, Universidad Complutense, Madrid, Spain

Renal expression of parathyroid hormone-related protein (PTHrP) and PTH/PTHrP receptor in a rat model of tubulointerstitial damage.

Background. PTHrP, which appears to act as a growth/differentiation factor in a variety of tissues, is present in the kidney; however, its role is unclear.

Methods. The expression of PTHrP and the PTH/PTHrP receptor were investigated by reverse transcription-polymerase chain reaction (RT–PCR) and immunohistochemistry in the remnant kidney of uninephrectomized (UNX) rats after protein overloading [1 g/day of bovine serum albumin (BSA)].

Results. Compared with UNX-control rats, proteinuria in BSA-overloaded animals was detected within the first 24 hours and increased during the entire study period (28 days). Kidney examination by light microscopy showed no significant renal lesions at day 1 of BSA treatment, whereas at days 8 and 28, tubular lesions, infiltration of mononuclear cells, and mesangial expansion were observed. PTHrP mRNA expression in the renal cortex was already increased at day 1 (fourfold) and plateaued between days 8 and 28 (12- and 15-fold, respectively) in BSA-overloaded animals compared with UNX-control rats. At day 8, immunohistochemical analysis with two different anti-PTHrP antibodies showed a dramatic increase of PTHrP staining in the damaged proximal and distal tubules from BSA-overloaded rats with respect to UNX-control rats. Moreover, intense PTHrP immunostaining was also observed in glomerular mesangial and endothelial cells in BSA-overloaded rats, but not in the UNX-control rats. A reciprocal decrease of PTH/PTHrP receptor mRNA and immunostaining, without significant changes in the cellular localization (proximal and distal tubule, and glomerular mesangial and epithelial cells) of the PTH/PTHrP receptor positivity was found to occur in the renal cortex of BSA-overloaded rats. At day 8, coinciding with the up-regulation of PTHrP, an increase in the angiotensin converting enzyme and proendothelin-1 gene expression was observed in the renal cortex of BSA-overloaded rats compared with UNX-control rats.

Conclusions. These results indicate that PTHrP can be added to the group of genes that are up-regulated in proximal tubular cells in response to intense proteinuria. Our results, together with previous findings, suggest that the vasoactive hormones angiotensin II and endothelin-1 could participate in the PTHrP production in the renal cortex of BSA-overloaded rats. Further experiments are required to clarify the mechanisms of PTHrP up-regulation and its possible role in the response to renal damage in this animal model.

Parathyroid hormone-related protein (PTHrP) is the main factor responsible for humoral hypercalcemia of malignancy [1]. The N-terminal region of PTHrP has a structural resemblance to PTH and binds to a common PTH/PTHrP receptor, which accounts for the similar bioactivities of both PTH and PTHrP in a variety of tissues [2]. However, in contrast to PTH, PTHrP is produced by many nonmalignant fetal and adult tissues, and current evidence points to PTHrP as an autocrine/paracrine regulator of several cell functions, including cell growth/differentiation [2]. PTHrP has been shown to be abundant in: the intrarenal arterial tree, including afferent and efferent arterioles; macula densa; and various nephron segments, including the glomeruli and proximal, distal, and collecting tubules [3, 4].

In the kidney, the physiological role of PTHrP is unclear. PTHrP and the PTH/PTHrP receptor are expressed in vascular smooth muscle cells (VSMCs), in which PTHrP exerts a vasorelaxant activity [5]. Indeed, various vasoconstrictors, including angiotensin II (Ang II), endothelin-1 (ET-1), and thrombin induced PTHrP gene expression in rat VSMCs [6, 7]. Recent studies suggest that PTHrP is a regulatory factor of the renal vascular tone through complex mechanisms [4, 5, 8]. PTHrP acts as an early response gene product and/or has growth regulatory properties in a variety of cell types, including renal tubular and mesangial cells [2, 3, 9–12]. Furthermore, PTHrP increases in vivo in response to either renal ischemic injury or chronic cyclosporine-induced nephrotoxicity and in an in vitro model that mimics the ischemic tubular damage [3, 13, 14]. In the ischemic model, the observed increase in PTHrP mRNA was accompanied by
a decreased PTH/PTHrP receptor mRNA expression [3]. However, it is currently unknown whether changes in PTHrP and/or the PTH/PTHrP receptor occur in vivo as a general response to renal damage.

In this study, we examined the renal PTHrP and PTH/PTHrP receptor gene expression in a rat model of intense proteinuria and tubulointerstitial nephropathy induced by protein overload [15]. It has previously been reported that the presence of nephrotic-range proteinuria in both human and experimental renal diseases correlates with the rate of progression to end-stage renal disease [16]. In addition, the decline in glomerular filtration rate shows a higher correlation with tubulointerstitial lesions than with glomerular lesions [16].

METHODS

Animal model

Female Wistar rats weighing 100 to 150 g were fed standard rat chow ad libitum and were given free access to water. The animals underwent unilateral left nephrectomy five days before the initiation of the study. Since day 0, experimental rats received intraperitoneally 1 g/day of bovine serum albumin (BSA; Catalog number A-4503; Sigma, St. Louis, MO, USA). A group of uninephrectomized (UNX) rats received the same volume of saline and served as controls. Animals were individually housed in metabolic cages with free access to tap water, and 24-hour urine was collected for protein measurement by the sulfosalicylic acid method [17].

On days 1, 8, and 28, animals were anesthetized with sodium pentobarbital (5 mg/100 g body wt). Then kidneys were perfused with cold saline, removed, and processed for light microscopy examination and immunohistochemistry. Pieces of renal cortex were snap frozen in liquid nitrogen and were stored at −70°C until RNA extraction.

Processing of renal tissue

Renal tissue samples for light microscopy examination and immunohistochemical studies were fixed in 4% buffered paraformaldehyde, dehydrated by graded concentrations of ethanol and xylene, and then were embedded in paraffin.

Histologic studies

Kidney sections (4 μm) were mounted on 3-aminopropyltriethoxy-silane (Sigma) treated slides and were stained with hematoxylin-eosin and Masson’s trichrome. Tubulointerstitial injury, defined as tubular dilation and/or atrophy, interstitial fibrosis, and inflammatory cell infiltrate, as well as glomerular damage, were graded using a semiquantitative scale from 0 to 4 according to the following criteria: 0 = no changes; 1 = focal changes that involved 1–25% of the sample; 2 = changes affecting 26%–50% of the sample; 3 = changes involving 51%–75% of the sample; and 4 = changes affecting more than 75% of the sample. Two independent observers performed all of the histologic studies in a blinded fashion.

RNA extraction and reverse transcription–polymerase chain reaction (RT–PCR)

For each animal, total RNA was extracted from the renal cortex by the acid guanidinium-phenol-chloroform method [18]. RNA yield and protein contamination were assessed by ultraviolet absorbance at 260 and 280 nm, respectively.

Total RNA from the renal cortex of 12 rats in each group was pooled in four groups of three arbitrarily selected samples per group. Pooled RNA was reverse transcribed, and the resulting cDNA was amplified with a commercial kit (Access RT–PCR System®; Promega, Madison, WI, USA) using specific primers for rat PTHrP, the PTH/PTHrP receptor, angiotensin converting enzyme (ACE), and preproET-1, which were designed according to the published sequences [14, 19–21]. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified using specific primers [21] as a constitutive control. Primer sequences and the sizes of the corresponding PCR products are detailed in Table 1.

The RT–PCR reaction mixture consisted of total RNA (PTHrP, ACE, preproET-1, and GAPDH: 100 ng; PTHrP receptor: 5 ng), 1 mM MgSO₄, 200 μM of each dNTP, 1 μM of each specific primer, 0.1 U/μL of avian myeloblastosis virus reverse transcriptase, 0.1 U/μL of thermus flavus DNA polymerase, and 2 μCi [α³²P]dCTP (>3000 Ci/mmol; Amersham, Buckinghamshire, UK). The reaction mixture was overlaid with mineral oil, and the tubes were placed on a Thermal Cycler® (Perkin-Elmer, Emeryville, CA, USA). Total RNA and the primers were preincubated for two minutes at 95°C. Each PCR cycle consisted of 30 seconds at 94°C, one minute at the corresponding annealing temperature (Table 1), and two minutes at 68°C. Afterwards, we performed a final extension of seven minutes at 68°C. We carried out preliminary experiments to determine the number of cycles that provided submaximal amplification of each gene studied (data not shown). Aliquots of PCR products were run on 6% polyacrylamide gels. Then the gels were dried and exposed to X-OMAT AS films (Eastman Kodak, Rochester, NY, USA). The resulting autoradiograms were quantitated using densitometric scanning (Molecular Dynamics, Sunnyvale, CA, USA).

Immunohistochemistry of PTHrP and PTHrP receptor

Immunostaining for PTHrP was performed with an affinity-purified rabbit anti-PTHrP antibody (Ab-2; Oncogene, Uniondale, NY, USA) that recognizes the sequence (38–64) of human and rat PTHrP, and rabbit anti-
Table 1. Polymerase chain reaction (PCR) primer sequences and PCR reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Fragment size (bp)</th>
<th>T (°C)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTHrP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-TGGTGTTCCTGCTCAGCTA-3'</td>
<td>266</td>
<td>61</td>
<td>30</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-CCTCGTGTCTGAGCCCAA-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH/PTHrP receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-AGGTTGTTCCAGGCACA-3'</td>
<td>320</td>
<td>64</td>
<td>30</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-CAAACCTTGCTGAGG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-GTCCTGACAGGAGGTTTGCAG-3'</td>
<td>317</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-GCGGCGCTCCAGGCAAACAGC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PreproET-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-AGCTCTTCCTCTGCTGG-3'</td>
<td>409</td>
<td>63</td>
<td>30</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-TCTTTGTACCCATTCTGATGC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-AATGCATCGTGCCACCA-3'</td>
<td>516</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-GTAGCCATATTCCATT-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are: bp, base pairs; T, annealing temperature.

Evolution of proteinuria in uninephrectomized (UNX)-serum C7 that detects the sequence (107–111) of either intact PTHrP or the C-terminal peptide PTHrP (107–139) [22]. For PTH/PTHrP receptor staining, an affinity-purified antibody specific for the extracellular domain of the rat PTH/PTHrP receptor (Ab VII; Babco, Richmond, CA, USA) was used. Immunostaining was carried out by the avidin-biotin-peroxidase complex method [23]. Briefly, the slides were deparaffinized and rehydrated by ascending ethanols. Then the sections were immersed in 3% H2O2 for five minutes, followed by a 30-minute incubation in trypsin (0.1% trypsin in phosphate-buffered saline, wt/vol). The tissue sections were incubated with either 5% normal swine serum (PTHrP) or 1.5% normal goat serum (PTH/PTHrP receptor) for 30 minutes to reduce nonspecific staining. The sections were then treated with either 4 μg/ml of anti-PTHrP antibody Ab-2 or 150-fold dilution of antiseraum C7 or 5 μg/ml of antibody Ab VII for two hours. Some tissue sections were treated with nonimmunogenic rabbit IgG as negative controls. After washing with phosphate-buffered saline solution, tissue sections were incubated with biotinylated swine or goat antirabbit IgG (Dako, Glostrup, Denmark) at 200-fold dilution for 30 minutes, sequentially followed by incubation with avidin-biotin-peroxidase complex (Vector, Burlingame, CA, USA) and with 3,3′-diaminobenzidine (Sigma) as the chromogen. The tissue sections were counterstained with Mayer’s hematoxylin (Sigma). Immunostaining was graded as follows: (−) no staining, (+) mild staining, (+ +) moderate staining, and (+++) intense staining. Two independent observers evaluated the immunohistochemical results in a blinded fashion.

Statistical analysis

Results are expressed as mean ± SEM. Comparisons between two groups were done using the unpaired t-test or the nonparametric Kruskal–Wallis test, when appropriate. The interobserver variations in histologic and immunohistochemical evaluations were assessed by Spearman correlation coefficient. Differences were considered significant when P < 0.05.

RESULTS
Evolution of proteinuria

In this study, in BSA-overloaded rats, proteinuria was detected within the first 24 hours, was increased during the following week, and then remained unchanged up to the end of the study on day 28 (Table 2). No changes in the proteinuria were found in rats that did not receive BSA (Table 2).

Morphological lesions

On light microscopic examination, no significant renal lesions were observed at day 1 in BSA-overloaded rats. However, on days 8 and 28 after BSA overload, the observed changes in the rat kidney included marked interstitial infiltrate, tubular atrophy and/or vacuolization, and protein casts within the proximal and distal tubules. Only occasional tubular brush border loss or basement membrane detachment was observed in all of the examined sections. BSA-overloaded rats also showed glomerular lesions consisting of mesangial hypercellularity and
Table 3. Semiquantification of morphological lesions of UNX-control and BSA overloaded rats

<table>
<thead>
<tr>
<th>Day 8</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubulointerstitium</td>
<td>Glomeruli</td>
</tr>
<tr>
<td>UNX-control</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>BSA-overloaded</td>
<td>2.5 ± 0.4</td>
</tr>
</tbody>
</table>

Morphological lesions were quantified as described in the Methods section. At least seven animals from each group were analyzed by two independent observers without significant over- or underestimation between them. Results are mean ± SEM. *P < 0.05 with respect to UNX-control rats at the same time period.

matrix expansion. Semiquantitation of the morphological lesions observed in the kidney of the experimental animals showed a significant increase in tubulointerstitial and glomerular lesions in BSA-overloaded rats with respect to UNX-control rats (Table 3). These renal lesions were slightly, but not significantly, higher at day 28 than at day 8 after BSA overload (Table 3). There was a significant correlation between the observers' semiquantitation of the different variables studied (Spearman correlation coefficients varied from 0.867 to 0.991, P < 0.01).

**PTHrP and PTH/PTHrP receptor expression in the renal cortex**

We found that PTHrP gene expression was increased in the renal cortex of BSA-overloaded rats compared with that of UNX-control animals at all time periods studied. Densitometric analysis of the PCR products showed that PTHrP mRNA was already increased fourfold at day 1 and reached a plateau (approximately 12-fold to 15-fold vs. UNX control) between days 8 and 28, P < 0.05 (Fig. 1). The levels of increased PTHrP gene induction were confirmed by serially diluting the amount of the PCR product used to run on the polyacrylamide gel. The dilution that provides a band intensity similar to that seen from the undiluted PCR product from the control animals determines the fold induction in BSA-overloaded rats. Using this approach, we found that BSA-overloaded rats at day 8 had 10- to 15-fold enhanced PTHrP gene expression compared with that of UNX-control rats, consistent with data in Figure 1.

Because the maximal increase in PTHrP gene expression in the renal cortex of BSA-overloaded rats, during
the period of study, was already seen at day 8, we explored whether there was also an alteration of the PTH/PTHrP receptor mRNA expression in the kidney cortex of these animals at this time period. As shown in Fig. 2, PTH/PTHrP receptor mRNA was decreased approximately twofold in BSA-overloaded rats compared with UNX-control animals, determined both with serial dilutions of PCR products (data not shown) and by densitometric analysis of the undiluted PCR products (Fig. 2B). GAPDH gene mRNA was found to be unchanged in the rat renal cortex after BSA overload (data not shown).

**Immunohistochemical detection of PTHrP and the PTH/PTHrP receptor in the rat kidney**

To localize the source of PTHrP and the PTH/PTHrP receptor in the rat kidney, immunohistochemistry was performed on paraffin-embedded kidney tissue sections from UNX-control and BSA-overloaded animals at day 8. We used two anti-PTHrP antibodies recognizing the middle and C-terminal regions of PTHrP, respectively, and compared the results obtained with both antibodies in consecutive sections for each tissue sample. Spearman correlation coefficients for interobserver variation in the semiquantitation of PTHrP staining varied from 0.723 to 0.996, demonstrating the lack of significant overestimation or underestimation between observers or between semiquantitation of both antibodies taken by the same observer. Staining was scored as described in the Methods section.

**Table 4. Semiquantification of PTHrP immunostaining in different rat kidney areas in UNX-control and BSA-overloaded rats**

<table>
<thead>
<tr>
<th>Area</th>
<th>UNX-control</th>
<th>BSA-overloaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubules</td>
<td>+</td>
<td>++/+++</td>
</tr>
<tr>
<td>Proximal</td>
<td>+</td>
<td>++/+++</td>
</tr>
<tr>
<td>Distal</td>
<td>+</td>
<td>++/+++</td>
</tr>
<tr>
<td>Glomerular</td>
<td>+</td>
<td>++/+++</td>
</tr>
<tr>
<td>Mesangial</td>
<td>–</td>
<td>+/+</td>
</tr>
<tr>
<td>Epithelial</td>
<td>++/++</td>
<td>+/+</td>
</tr>
<tr>
<td>Endothelial</td>
<td>–</td>
<td>++/+++</td>
</tr>
<tr>
<td>Vessels</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

PTHRP was immunostained with anti-PTHRP antibody Ab-2. At least five animals from each group were analyzed by two independent observers, without significant over- or underestimation between observers or between semiquantitation of both antibodies taken by the same observer. Staining was scored as described in the Methods section.

**Fig. 2. PTH/PTHrP receptor mRNA expression in the renal cortex of UNX-control and BSA-overloaded rats.** (A) Autoradiography showing two different pools of three animals. (B) Ratio of PTH/PTHrP receptor to GAPDH mRNA is expressed in arbitrary units. Data are mean ± sem. *P < 0.05 vs. UNX-control animals. Lanes 1 and 2 are from UNX-control rats. Lanes 3 and 4 are from BSA-overloaded rats.
Fig. 3. Immunolocalization of PTHrP by Ab-2 (A, C) and C7 antibody (B, D) in the kidneys from UNX-control and BSA-overloaded rats. (A and B) Kidney sections of a representative UNX-control animal, showing glomerular staining confined to epithelial cells and moderate staining in both proximal and distal tubules. (C and D) Glomeruli from BSA-overloaded rats showing an increase in the staining for PTHrP, which was predominantly present in mesangial and endothelial cells. A marked increase in the intensity of the staining in proximal and distal tubules was observed in these animals. (Original magnification, ×130). Publication of this figure in color was made possible by a grant from Fondo de Investigación Sanitaria (FIS).

Fig. 4. Immunolocalization of PTH/PTHrP receptor (Ab VII) in the kidney from UNX-control (A) and BSA-overloaded rats (B). In both groups of rats, the PTH/PTHrP receptor staining was localized in glomeruli, in brush border of proximal tubules (arrows) and in the basolateral membranes (arrowheads) (original magnification, ×100). Publication of this figure in color was made possible by a grant from Fondo de Investigación Sanitaria (FIS).

In both groups of rats (UNX control and BSA overloaded), a similar pattern of PTH/PTHrP receptor immunostaining, which was weaker in BSA-overloaded rats, was observed in glomeruli and in the basolateral and
ACE and preproET-1 mRNA expression in the renal cortex

Because Ang II and ET-1 have been reported to induce PTHrP gene expression in cultured rat VSMCs [6, 7], we examined the mRNA expression of ACE, the enzyme that controls the conversion of Ang I into Ang II, and the preproET-1 in the renal cortex of BSA-overloaded and UNX-control rats.

As shown in Figure 5A, we found the presence of a single band of the corresponding predicted sizes in the renal cortex of both UNX-controls and BSA-overloaded rats. Densitometric analysis of the bands showed a significant increase in both ACE and preproET-1 mRNA levels in BSA-overloaded rats with respect to UNX-control rats at day 8 (Fig. 5 B and C, respectively).

DISCUSSION

The physiological role of PTHrP is still unclear, but there is growing evidence for its involvement in the control of cell growth and/or differentiation as an autocrine/paracrine factor in a variety of cells [2]. PTHrP is present in normal fetal and adult kidney, in which it binds to an abundant PTH/PTHrP receptor [2, 3, 24]. Recent data suggest that PTHrP could be involved in the regenerative process following renal damage [3, 13, 14].

In this study, PTHrP mRNA was found to be dramatically increased in the renal cortex of rats with tubulointerstitial nephropathy induced by BSA overload. Two anti-PTHrP antibodies directed at both midregion and C-terminal domains of PTHrP revealed positive immunostaining in proximal and distal tubular cells and in both glomerular visceral and parietal epithelial cells in UNX-control rats, which is in agreement with previous findings [3, 4, 14]. After BSA overload, PTHrP positivity was more intense in both proximal and distal tubules and in glomeruli. Interestingly, in the glomerulus of BSA-overloaded rats, PTHrP staining appeared in mesangial and endothelial cells. Our results differ from those of a previous study in which PTHrP up-regulation, after acute renal ischemia, was found to occur in the glomeruli but in only epithelial cells [3]. These discrepancies might be accounted for by the different mechanisms underlying the glomerular response in different models of renal damage.

In addition, we found PTH/PTHrP receptor immunostaining mainly on the luminal and basolateral membranes of the tubules and in glomerular epithelial and mesangial cells in both UNX-control and BSA-overloaded rats. In this regard, adenylate cyclase-coupled PTH/PTHrP receptors have previously been characterized in the rabbit glomerulus, and PTH/PTHrP receptor mRNA has been localized in the glomerular podocytes by in situ hybridization [24, 25]. Therefore, the increased PTHrP positivity in the glomeruli of BSA-overloaded rats. In this regard, adenylate cyclase-coupled PTH/PTHrP receptors have previously been characterized in the rabbit glomerulus, and PTH/PTHrP receptor mRNA has been localized in the glomerular podocytes by in situ hybridization [24, 25]. Therefore, the increased

Fig. 5. Gene expression of ACE and preproET-1 in renal cortex of UNX-control and BSA-overloaded rats at day 8. Total RNA from the renal cortex of 12 rats in each group was pooled in 4 groups of 3 arbitrarily selected samples per group. (A) Autoradiography of two representative pools of each group. Arrows indicate the expected size for each PCR product. (B and C) Densitometric analysis of mRNA expression of ACE and preproET-1 in the renal cortex of UNX-control and BSA-overloaded rats. Ratio of ACE and preproET-1 to GAPDH mRNA is expressed in arbitrary units. Data are mean ± SEM. *P < 0.05 vs. UNX-control animals.
gene expression was observed in the renal cortex of BSA-overloaded rats. This work has partially been supported by grants from Fondo de Investigación Sanitaria of Spain (96/2021, 96/1167), Ministerio de Educación y Ciencia (C.E.C.) (PM 94/211, PM 95/93, PM 97/85, SAF 97/55), Comunidad Autónoma de Madrid (CAM) (97/884/0003) and EU Concerted Action Grant, BMH4-CT98-3631 (DG 12SSMI), and Fundación Ildefonso Álvarez de Toledo. We are grateful to Dr. Jesús Jiménez for his kind help in the statistical analysis. R. Largo, D. Gómez-Garre, and C. Peñaranda are fellows from M.E.C., CAM, and Fundación Conchita Rábago, respectively. Publication of Figures 3 and 4 in color was made possible by a grant from Fondo de Investigación Sanitaria (FIS).

Reprint requests to Dr. Jesús Egido, Laboratorio de Nefrología, Fundación Jiménez Díaz, Avda. Reyes Católicos, 2, 28040 Madrid, Spain. E-mail: egido@unifjcd.es

APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; Ang II, angiotensin II; BSA, bovine serum albumin; ET-1, endothelin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NF-κB, nuclear factor κB; PTHrP, parathyroid hormone-related protein; RT-PCR, reverse transcription–polymerase chain reaction; TGF-β, transforming growth factor-β; UNX, uninephrectomized; VSMCs, vascular smooth muscle cells.

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