Relaxin Improves Renal Function and Histology in Aging Munich Wistar Rats

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Administration of recombinant human relaxin (rhRLX) to conscious, chronically instrumented rats increases GFR and effective renal plasma flow (ERPF) and decreases effective renal vascular resistance (ERVR) with no significant change in mean arterial pressure. The Munich Wistar albino rat shows progressive chronic nephrosis with age and therefore was used to determine the functional and histologic consequences of rhRLX on matrix remodeling in the kidney of older rats. RLX-infused rats showed increased GFR and ERPF with decreased ERVR. Furthermore, in a double-blinded examination, the renal histology showed a significant decrease in glomerular and tubular collagen deposition in the rhRLX-infused aged rats. During short-term rhRLX administration (24 h), gelatinase activity was found to be essential for renal vasodilation and hyperfiltration. Surprisingly, after 20 d, improved renal function was insensitive to the inhibition of gelatinase activity, suggesting that collagen degradation in these rats had permanently altered the matrix of the renal vasculature. In conclusion, long-term administration of rhRLX improves renal function and ameliorates renal pathology in an aging rat model. The biphasic action of rhRLX on the kidney indicates that, acutely, the vessels dilate, causing increased filtration and renal blood flow with decreased vascular resistance as a result of upregulation of gelatinase activity. Subsequently, the renal vessels undergo alteration in supporting matrix, showing increased blood supply even in the face of acute matrix metalloproteinase inhibition, most likely as a result of the inhibitory properties of RLX on collagen production or increased collagen breakdown.


Relaxin (RLX) has numerous biologic effects. Its role in the reproductive tract has been well documented (1). More recently, we have shown it to be a potent vasodilator in the kidney (2–5). In long-term instrumented conscious rats, we demonstrated that RLX stimulates nitric oxide (NO)-dependent renal vasodilation and hyperfiltration in vivo, and reduces myogenic reactivity of small renal arteries in vitro via endothelin (ET) and the endothelial ETB receptor subtype (5). We also have documented a role for the matrix metalloproteinases (MMP) in this phenomenon. Vascular gelatinase activity converts big ET to ET1–32, which potently activates the endothelial ETB receptor. Using a novel inhibitor of the gelatinases, cyclic CTHWGFTLC (CTT), we demonstrated that vascular gelatinase activity mediates renal vasodilatory action of RLX in rats (5). MMP-2 was specifically implicated in this phenomenon because an MMP-2–neutralizing antibody blocked the reduction in myogenic reactivity in isolated renal vessels that is typically caused by long-term RLX administration in vivo (5).

RLX is widely known for its “matrix-degrading” properties that are associated with the stimulation of MMP and inhibition of collagen expression (6–8). Specifically, RLX decreases synthesis and secretion of interstitial collagens, increases expression of the procollagenase (MMP-1), and decreases production of tissue inhibitor of metalloproteinases (TIMP) by human dermal fibroblasts (6). In a murine model of induced pulmonary fibrosis, RLX inhibits the formation of abnormal extracellular matrix (ECM) in vivo (7). In human lower uterine fibroblasts, RLX stimulates the production of MMP-2 (8). Long-term recombinant human RLX (rhRLX; BAS Medical, Palo Alto, CA) infusion upregulates MMP-2 expression in isolated rat arteries (5). Furthermore, RLX decreases renal fibrosis in a model of chemically induced papillary necrosis, seemingly independent of systemic hemodynamic changes (9). In two models of renal mass reduction, RLX decreased renal injury in the rat by at least two mechanisms: lowering BP and reducing glomerular and tubular sclerosis (10). In recent studies of the RLX gene knockout mouse, the kidney of male mice showed increased kidney weight and size with a build-up of collagen (11). Despite these intriguing correlative studies implicating RLX in the control of matrix remodeling, the mechanisms have not been established. Because of the significant renal vascular effects and matrix-remodeling properties of the hormone RLX and its connection with the metalloproteinases, especially gelatinase activity in the kidney, this study was designed to investigate a possible role for RLX and gelatinases in ameliorating age-related changes that are seen in the kidney.

Materials and Methods

Animal Preparation

Munich-Wistar male rats that were 3, 6, and 12 mo of age were purchased from Harlan Sprague Dawley (Frederick, MD) and provided...
followed by constant infusion (12 ml/min; Sage Instruments, Houston, TX) of the two reagents at 0.4 and 0.1 mg/min per 100 g body wt, respectively. Then, a silastic-covered stainless steel cannula was sewn into the abdominal aorta and inferior vena cava via the femoral artery and vein, respectively. Then, a silastic-covered stainless steel cannula was sewn into the urinary bladder with a purse-string suture and exteriorized through the ventral abdominal wall. For the infusion of metalloproteinase inhibitors, a catheter was implanted in the right carotid artery. All vascular catheters were tunneled subcutaneously and exteriorized between the scapulae.

Experimental Protocols

Effects on Renal Function during Chronic Infusion of rhRLX. Renal function and mean arterial pressure (MAP) were measured in a pair of age-matched, chronically instrumented conscious rats on day 0 (baseline) and 1, 3, 5, and 10 d of rhRLX/vehicle infusion. Before infusion of any fluids, blood was drawn for baseline blood chemistries. MAP/heart rate (HR) was measured by a Gould P23ID pressure transducer and Gilson ICT-2H Duograph (Middleton, WI). The obturator in the bladder catheter was removed and extended with a short piece of silastic tubing for collection of urine. A bolus of insulin (IN; Sigma, St. Louis, MO; 0.1 ml of a 50% stock solution/100 g body wt) and paraaminohippurate (PAH; Merck & Co., West Point, PA; 0.05 ml of a 4% working solution/100 g body wt) was given into the venous catheter followed by constant infusion (12 µl/min; Sage Instruments, Houston, TX) of the two reagents at 0.4 and 0.1 mg/min per 100 g body wt, respectively. After a 60-min equilibration, three baseline urine collections of 30 min each with midpoint blood collections of 300 µl each were obtained to measure the renal clearances of IN and PAH, which provide estimates of GFR and effective renal plasma flow (ERPF), respectively. After centrifugation and removal of plasma, all red cells were gently resuspended in Ringer’s solution and returned to the rat.

After these baseline measurements were made, an osmotic minipump (Alzet model 2ML1 or 2ML2; Durect Corp., Cupertino, CA) that contained rhRLX (4 µg/h) or vehicle was implanted subcutaneously in the hindquarters, under isoflurane anesthesia in each rat. Renal function in each pair of rats again was measured at 1, 3, 5, and 10 d during long-term infusion of rhRLX/vehicle using the aforementioned methods (Figure 1). After the last experiment, 1 ml of blood was collected for measurement of rhRLX and blood chemistries.

Influence of MMP on Renal Function in Rats Treated Chronically with rhRLX/Vehicle. Renal function and MAP were assessed in six pairs of age-matched conscious rats (12 mo) on days 2 through 5 and day 20 of rhRLX infusion using the aforementioned procedure. Infusion of RLX or vehicle was accomplished in this group of rats by replacing the 14-d Alzet minipump on day 13 with a primed 7-d pump to ensure continuous infusion for the full 20 d. After rhRLX/vehicle-influenced renal function was assessed, continuous infusion of the specific gelatinase inhibitor cyclic CTT at 1.0 µg/min or its control peptide STTH-WGFLS (STT) at 1.0 µg/min was administered via the carotid arterial catheter (5). Each pair of rats was randomly assigned to receive either cyclic CTT or STT on day 2 of rhRLX or vehicle administration. If they received cyclic CTT on day 2, then they were administered STT on day 5, and vice versa (Figure 2). Six 1-h renal clearances were obtained during the drug infusion as described. The general MMP inhibitor GM6001 (Iloasstat; rhRLX-treated rats, n = 4; vehicle-treated rats, n = 4) or its control, dilute DMSO (rhRLX, n = 4; vehicle, n = 4), was administered at 30 ng/min (5).

MMP-2 Expression in Small Renal Arteries Isolated from rhRLX- and Vehicle-Treated Rats. Kidneys were removed and processed using a validated technique that was described previously (5). Briefly, for gelatin zymography, the homogenates were prediluted in homogenizing buffer to equalize protein concentration with standard, combined with Novex Tris-Glycine SDS Sample Buffer (Invitrogen Co., Carlsbad, CA) and electrophoresed on Novex precast 10% Tris-Glycine gels that contained 0.1% gelatin. After staining/destaining, the gels were scanned using a Hewlett-Packard Scan Jet 5370C scanner and HP PrecisionScan Pro v1.4 computer program (Palo Alto, CA). The images were digitized by UN-SCAN-IT gel automated digitizing system v4.3 (Silk Scientific Corp., Orem, UT) (5).

Synthesis and Characterization of STT and CTT Peptides. Peptides were synthesized by a solid-phase Pioneer automated peptide synthesizer (PE-Biosystem, Inc., Framingham, MA) using the FMOC synthesis protocol (5).

Analytical Techniques

Plasma osmolality was measured with freezing-point depression osmometer (Advanced Instruments, Needham Heights, MA). Plasma and urine IN and PAH were assayed by standard techniques (3). The levels of rhRLX in serum were measured by a validated ELISA immunoassay (12). Microalbuminuria was assayed by Pyrogallol Red Reagent (Sigma-Aldrich, St. Louis, MO). The Vitros DT-60 photometer was used to measure plasma chemistries (Johnson/Johnson Diagnostics, Rochester, NY).

Histologic evaluation was performed for ultrastructural alteration of tissue. All histologic sections were reviewed by light microscopy. The degree of collagen deposition in the glomerulus and the interstitium was graded as follows: 0, no collagen deposition; 1+, mild; 2+ = moderate; 3+, moderately severe; and 4+, severe. The renal tissue was...
stained with two stains: Hematoxylin and eosin and Masson’s trichrome stain. The hematoxylin and eosin stain is used to look at general morphology of the glomerulus. The more specialized Masson’s trichrome stain dyes collagen a deep blue for easier quantification. All stains were evaluated in a double-blinded assessment.

Preparation of Drugs. PAH and IN were freshly prepared on the morning of the experiment. The rhRLX (provided by Elaine Unemori, BAS Medical, San Mateo, CA), provided as a 1.4-mg/ml solution in 20 mM sodium acetate (pH 5.0), was diluted in the same buffer. GM6001 (Chemicon International, Temecula, CA) was prepared as a 10-mg/ml stock solution in sterile, endotoxin-free DMSO and diluted in Ringer’s solution, yielding a final DMSO concentration of 0.025% for infusion. Endotoxin-free, sterile DMSO was diluted in Ringer’s solution as the vehicle control. Cyclic CTT and STT were prepared as 1.0-mM stock solutions. Stock solutions were aliquotted and stored at −20°C.

Statistical Analyses
Data are presented as mean ± SEM. The data for the three 30-min baseline renal clearances were averaged. Most of the data were analyzed using two-factor repeated-measures ANOVA. When significant main effects or interactions were observed, group means were compared by Tukey test. Blood chemistries were compared by unpaired t test. P < 0.05 was considered statistically significant.

Results
Three age groups—3, 6, and 12 mo—were selected to study the time course of the age-related changes and the effects of RLX administration. The 3-mo-old rats showed no significant decrease in renal function, abnormalities in blood chemistries, or significant renal pathology. Therefore, this age group was not investigated further except as comparison with older age groups.

Rats gained weight as they aged: Body weights for 3-, 6-, and 12-mo-old groups were 371 ± 5, 512 ± 8, and 591 ± 9 g, respectively. Therefore, renal function was normalized to 100 g body wt for comparison among the three age groups. Baseline GFR was significantly higher in the 3-mo-old group compared with the 6- and 12-mo-old groups (Figure 3). After 24 h of rhRLX infusion, there was a significant increase in GFR in all age groups over baseline. Days 3 and 5 renal function was not significantly different from day 10 values (one-factor repeated measures ANOVA); therefore, all results were averaged. After 10 d of infusion, the 6- and 12-mo-old groups continued to exhibit a lower GFR than the 3-mo-old group, but the oldest rats improved, showing the largest increase over baseline (Δ350 μl/min).

In Figure 4, effective renal plasma flow showed that the youngest rats had a significantly higher ERPF than the two older age groups. After 24 h of rhRLX infusion, ERPF increased significantly in all age groups compared with baseline. However, the greatest increase was observed in the 6- and 12-mo-old rats. After the 10-d infusion, the 12-mo-old group showed a further sizable increase in ERPF, thereby eliminating any difference in ERPF among the age groups. The oldest rats showed the most robust response to rhRLX infusion (Δ1260 μl/min). In all three groups, there was a significant decrease in effective renal vascular resistance (ERVR) after 10 d of infusion of rhRLX (Figure 5). Here again, the oldest age group showed the largest decrease in ERVR. Baseline MAP (105 ± 3, 109 ± 4, and 112 ± 3 mmHg, respectively) did not change significantly during the course of rhRLX infusion in the three age groups even after 10 d of infusion (103 ± 3, 112 ± 4, and 111 ± 3, respectively).

On histology, there was no statistical difference in collagen deposition in the glomerulus or the tubular interstitium of the 6-mo-old rats (data not shown). However, in the 12-mo-old rats, there is considerable alteration in collagen deposition in
both areas of the kidney. Using Fisher exact test, the 12-mo-old vehicle-treated rats had significantly more glomerular and tubulointerstitial collagen formation ($P \leq 0.001$ 3-mo-old baseline versus 6- and 12-mo-old baseline; $**P \leq 0.001$ versus baseline in all age groups; $^#P \leq 0.001$ 12-mo-old 10-d versus 1d rhRLX infusion.

Figure 4. Effect of rhRLX infusion on effective renal plasma flow (ERPF; $\mu$L/min per 100 g body wt) at baseline, 24 h of infusion, and 10 d of infusion in 3-, 6-, and 12-mo-old rats ($n = 6$ at each time point). $^*P \leq 0.001$ 3-mo-old baseline versus 6- and 12-mo-old baseline; $**P \leq 0.001$ versus baseline in all age groups; $^#P \leq 0.001$ 12-mo-old 10-d versus 1d rhRLX infusion.

Figure 5. Effect of rhRLX infusion (4 $\mu$g/min) for 10 d on effective renal vascular resistance (ERVR; mean arterial pressure [MAP]/ERPF per 100 g body wt) compared with vehicle infusion (R, rhRLX; V, vehicle). Each bar represents six animals in each age group ($^*P \leq 0.001$). Twelve-mo-old rats showed a significant decrease in ERVR compared with 3- and 6-mo-old rhRLX-infused groups ($^#P \leq 0.001$).

Figure 6. (A) Normal glomerulus with Masson’s trichrome staining after 20 d of infusion of rhRLX. (B) Normal tubular back-to-back staining with Masson’s trichrome after 20 d of infusion of rhRLX. (C) Sclerotic, disorganized glomerulus with Masson’s trichrome staining after 20 d of infusion of vehicle. (D) Distended tubules with cast material shown with Masson’s trichrome after 20 d of infusion of vehicle. Magnification, ×400.
Table 1. Effect of rhRLX versus vehicle infusion on collagen deposition in the glomerulus, tubules, and interstitium after 10 d treatment in the conscious rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grading</th>
<th>Glomerular Collagen Deposition</th>
<th>Tubular/Interstitial Collagen Deposition</th>
<th>Cast/Tubular Dilation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>rhRLX</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

*rhRLX, recombinant human relaxin. P < 0.001 using Fisher exact test. n = 12 animals for each group.

Table 2. Plasma and urine chemistry comparisons in conscious 6- and 12-mo-old rats after 10-d infusion of rhRLX or vehicle

<table>
<thead>
<tr>
<th>Rats</th>
<th>Treatment</th>
<th>Posm (mOsm/kg)</th>
<th>Sodium (mM)</th>
<th>Cholesterol (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>BUNa (mg/dl)</th>
<th>Proteinuria (mg/24 h)</th>
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</thead>
<tbody>
<tr>
<td>6 mo old</td>
<td>Baseline</td>
<td>292 ± 1.2</td>
<td>144 ± 2.0</td>
<td>50 ± 5</td>
<td>0.8 ± 0.2</td>
<td>12 ± 1.1</td>
<td>30 ± 9</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>290 ± 2.3</td>
<td>145 ± 1.3</td>
<td>50 ± 5</td>
<td>0.8 ± 0.2</td>
<td>12 ± 1.1</td>
<td>38 ± 5</td>
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<tr>
<td></td>
<td>rhRLX</td>
<td>277 ± 2.7b</td>
<td>138 ± 2.2b</td>
<td>55 ± 9</td>
<td>0.7 ± 0.3</td>
<td>9 ± 1.1b</td>
<td>32 ± 9</td>
</tr>
<tr>
<td>12 mo old</td>
<td>Baseline</td>
<td>291 ± 1.1</td>
<td>144 ± 2.0</td>
<td>55 ± 9</td>
<td>1.0 ± 0.3</td>
<td>15 ± 1.0</td>
<td>62 ± 8</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>293 ± 1.2</td>
<td>143 ± 1.2</td>
<td>50 ± 7</td>
<td>1.0 ± 0.3</td>
<td>16 ± 1.2</td>
<td>60 ± 6</td>
</tr>
<tr>
<td></td>
<td>rhRLX</td>
<td>278 ± 1.7b</td>
<td>138 ± 2.2b</td>
<td>76 ± 4b</td>
<td>0.9 ± 0.3</td>
<td>9 ± 1.1b</td>
<td>48 ± 9</td>
</tr>
</tbody>
</table>

*Posm, plasma osmolality; BUN, blood urea nitrogen.  
bP ≤ 0.01 using two-sided t test.

discussion

It is estimated that by the year 2050, 80 million people, one in every five Americans, will be more than 65 yr old. Half of the cases of chronic renal disease involving fibrosis occur in this age group. In humans, advancing age often is accompanied by decreased renal function. Anatomic changes include decrease in renal mass and size that occurs mainly in the cortex with loss of functioning of cortical glomeruli, narrowing of the renal arteries as a result of collagen formation, formation of afferent-efferent arteriovenous fistula as a result of this loss of glomeruli, and an increase in fibrosis in the cortex and renal pyramids (13). This fibrosis causes an increase in abnormal collagen deposition in the glomeruli and interstitium ECM, decreasing GFR and disrupting normal blood flow. The mechanisms underlying age-associated glomerular atrophy and tubulointerstitial fibrosis remain uncertain (14).

The aging of the human kidney is paralleled in the Munich Wistar albino rat (14,15), which demonstrates chronic renal nephropathy as described by Gray et al. (15), characterized by proteinuria, enlarged and sclerotic glomeruli with thickened basement membranes, and abnormal accumulation of collagen (15). Our study was designed to document the impact of rhRLX infusion on renal function and histology in this animal model. We hypothesized that both short- and long-term administration of RLX would improve renal function in the older rat. Furthermore, after long-term administration, RLX would reduce renal collagen deposition and improve renal histology.

Our study showed that 24 to 72 h of rhRLX infusion caused...
hyperfiltration and increased renal blood flow in all three age groups of rats, the largest increase occurring in the oldest age group. This acute improvement in renal function was reversed by an inhibitor of gelatinase, cyclic CTT. Isolation of the small renal arteries from 10-d rhRLX-infused rats showed a significant increase in pro- and active MMP-2 activity. Examination of Masson’s trichrome stain revealed a significant decrease in collagen deposition in the glomerulus, tubules, and interstitium in the 12-mo-old rats after 10 d of rhRLX infusion. After 20 d of infusion of rhRLX in the 12-mo-old rats, cyclic CTT (1.0 μg/min infusion) did not reverse the increase in GFR and ERPF. The results support our hypothesis that RLX works in a biphasic manner, causing an initial acute vasodilation and improvement in renal function with an upregulation of gelatinase activity in the renal vessels followed by a permanent structural alteration.

Several studies have demonstrated that RLX reduces fibrosis in experimental animal models (6–8). RLX-deficient knockout mice also demonstrate age-related progression of pulmonary, cardiac, and renal fibrosis (11). Our study suggests that in the aging rat kidney, RLX has the ability to increase renal function quickly (24 to 48 h) and reduce renal fibrosis significantly after 10 d of infusion.

The delicate balance between synthesis and degradation of ECM in the kidney is disrupted by the aging process. Reduced blood supply, lack of oxygen to the kidney as a result of increased ECM production versus degradation, and reduced filtering capacity of sclerotic glomeruli can stimulate the influx of inflammatory cells, leading to the release of cytokines and growth factors, including TGF-β (16). TGF-β has been shown to differentiate renal fibroblasts into activated myofibroblasts that express the marker α-smooth muscle actin. These activated cells increase matrix protein gene expression, leading to increased matrix production and resulting in tissue fibrosis (17).

Tubulointerstitial fibrosis is a widely recognized common pathway of all progressive renal disease regardless of the cause. Interstitial fibroblasts are important effector cells in renal fibrosis (18). RLX plays an important role in the downregulation of these fibroblasts, in vitro, resulting in decreased production of...

Figure 7. (A) Effect of gelatinase peptide inhibitor cyclic (c) CTT and the control peptide STT on GFR in conscious rats that received rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]) after the first 24 h. **P ≤ 0.005 versus all other rhRLX-infused groups; *P ≤ 0.001 versus vehicle. (B) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on ERPF in conscious rats that received rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]) after the first 24 h. **P ≤ 0.005 versus all other groups; *P ≤ 0.001 versus vehicle. (C) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on ERVR in conscious rats that received rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]) after the first 24 h. **P ≤ 0.005 versus all other groups; *P ≤ 0.001 versus vehicle. (D) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on MAP in conscious rats that received rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]) after the first 24 h. *P < 0.001 versus cCTT versus baseline.
TGF-β. Masterson et al. (19) recently showed that myofibroblast differentiation in vitro can be downregulated by RLX. Within 24 h, 25% of fibroblasts no longer exhibited α-smooth muscle actin, suggesting an RLX-mediated reversal of myofibroblast differentiation. This reversal caused a decrease in matrix production, increased collagenase synthesis, and an inhibition of collagen-I lattice contraction. RLX was found previously to abrogate the effects of TGF-β (6,9), recognized as a pivotal cause of glomerulosclerosis and tubulointerstitial fibrosis in renal disease by stimulation of matrix protein production (20) and increasing the contraction of surrounding ECM (21).

In our study, the oldest rats that showed the greatest decrements in renal function were the most affected by RLX infusion. That is, they demonstrated the greatest increase in GFR and ERPF after 24 to 48 h of rhRLX infusion. At least four possible pathways could explain these findings: (1) Increased action of the gelatinases affecting ECM and the vascular smooth muscle, (2) downregulation of the activating myofibroblasts in the first

**Figure 8.** (A) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on GFR in conscious rats that received 20 d of infusion of rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]). *P ≤ 0.001 versus vehicle. (B) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on ERPF in conscious rats that received 20 d of infusion of rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]). *P ≤ 0.001 versus vehicle. (C) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on ERVR in conscious rats that received 20 d of infusion of rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]). *P ≤ 0.001 versus vehicle. (D) Effect of gelatinase peptide inhibitor cCTT and the control peptide STT on MAP in conscious rats that received 20 d of infusion of rhRLX or vehicle for rhRLX (20 mM sodium acetate [pH 5.0]). *P < 0.001 versus cCTT versus baseline.

**Figure 9.** Effect of gelatinase peptide inhibitor GM6001 and control peptide DMSO on ERVR in conscious rats after 20 d of infusion of rhRLX or vehicle. *P ≤ 0.001 versus vehicle.

**Figure 10.** Representative gelatin zymogram of gelatinase activities in interlobar arteries from rhRLX-infused and vehicle-infused rats (10 d). STD, recombinant human matrix metalloproteinase-2 (MMP-2) active MMP-2 and pro-MMP-2 standards. *P ≤ 0.05 rhRLX versus vehicle-infused rats by densitometry.
24 h, (3) reduced TGF-β in the ECM from downregulation of myofibroblasts, or (4) increased production of NO (with an upregulation of NO synthase). The first three mechanisms would seem unlikely for the explanation of the quick 24- to 48-h reversal that we have documented.

Our study explored the possibility of vascular gelatinase upregulation. In the first 24 to 48 h, inhibition of gelatinase by cyclic CTT or GM6001 caused a complete reversal of renal hyperfiltration, vasodilatation, and decreased vascular resistance in the 6- and 12-mo-old rhRLX-infused rats, implicating that an upregulation of gelatinase expression as an important mediator. Indeed, pro- and active forms of MMP were increased in isolated small renal arteries by gelatin zymography.

Because we also were interested in the long-term effects of RLX infusion on renal histology and function, we continued the infusion for 10 d. The 12-mo-old rats showed a further increase in renal function during this 10-d infusion period. This may be explained by the significant decrease in renal collagen caused by the antifibrotic actions of RLX documented by histologic staining in this group as compared with the younger rats. Because the ECM was altered, the vessels may be free to dilate to their full extent. A second possibility is decreasing sclerosis of resistance arteries in glomeruli, leading to increased vasodilatation and hyperfiltration. The histology showed fewer inflammatory cells and less collagen deposition in the glomeruli and tubulointerstitial space as well as less tubular dilation containing cast material.

We further tested rhRLX infusion for 20 d in the 12-mo-old rats. To our surprise, the acute inhibition of gelatinase activity with cyclic CTT (1.0 μg/min) did not affect the increase of renal function that was induced by rhRLX. Because we verified the results with GM6001, these data suggest either that a greater concentration of MMP inhibitors is needed to reverse the RLX-induced vasodilation or that another mechanism of rhRLX action is responsible for the improved renal function after 20 d of infusion. This finding possibly may be explained by downregulation of myofibroblasts, changing the cytokine milieu, or an increased production of NO.

**Conclusion**

RLX infusion increases GFR and renal plasma flow and decreases renal vascular resistance in the aged Munich Wistar albino rat. This increase in the first 24 to 72 h is mediated by the action of vascular gelatinase activity. After 20 d of infusion of RLX, inhibition of this gelatinase did not abolish the increased renal function that was induced by RLX. This suggests a permanent change in the structure of the interstitium surrounding the glomerulus and tubules, possibly caused by the antifibrotic effect of rhRLX infusion. Further study is needed to examine the mechanistic details of this change. Because RLX improves renal function and ameliorates the pathologic nephropathy/fibrosis in this rat model, there is a possibility that this remodeling agent could be used to reverse the fibrotic condition of the human kidney in our older population as well as in other disease states with fibrotic injury, such as diabetes and polycystic kidney disease.

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**References**


