Influence of Recombinant Human Relaxin on Renal Hemodynamics in Healthy Volunteers

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Maternal renal hemodynamic adaptation to human pregnancy is one of the most dramatic of all physiologic changes, but the factors that are responsible have remained elusive. In rat pregnancy, there are comparable renal hemodynamic changes, and in this species there is comprehensive evidence that the ovarian hormone relaxin (RLX) is responsible. This study investigated the renal effects of recombinant human RLX (rhRLX) in humans. Eleven volunteers (six male, five female) received intravenous infusions of rhRLX over 5 h at an infusion rate that was chosen to sustain serum concentrations that are comparable to early pregnancy. The renal clearances of inulin and para-aminohippurate were used to measure GFR and renal plasma flow, respectively. Irrespective of gender, renal plasma flow was increased by 47% compared with baseline levels (P < 0.0001), but no significant change was observed in GFR. There were no side effects or adverse reactions of rhRLX given as an intravenous infusion, and the data suggest that RLX indeed may be one of the elusive renal vasodilatory factors in human pregnancy. Further work is necessary to elucidate the complimentary factors that permit the concomitant increase in GFR during pregnancy.

Newbury, UK) was given over 5 min followed by a sustaining infusion of inulin (70 ml), PAH (38 ml), and normal saline (340 ml) at 60 ml/h. After 60 min of equilibration, the volunteer voided, and then three 20-min urine collections were obtained each with midpoint blood sampling. An intravenous bolus of rhRLX (0.2 μg/kg) then was administered over 5 min followed by a sustaining infusion (0.5 μg/kg per h) to maintain serum RLX levels comparable to early pregnancy. After an additional 4-h equilibration period, three 20-min clearances were collected (Figure 1). Previous work demonstrated that the PAH/inulin protocol described here is not associated with changes in RPF and GFR over a 6-h period in nonpregnant individuals (11). For minimization of dead space error in the urine collections, an adequate diuresis was maintained throughout by ad libitum oral water intake.

Blood samples were collected into plain glass tubes, centrifuged within 3 h, and frozen at −80°C until analyzed. An ELISA was used to determine serum RLX levels (12). The metabolic clearance rate (MCR) of rhRLX was calculated from the following equation: MCR = (infusion rate of rhRLX)/(steady-state levels of rhRLX) − preinfusion levels.

Plasma electrolytes and osmolality were determined on fresh specimens within 4 h using methods that were described previously (3). Serum progesterone was measured by Freeman Hospital Laboratories (Newcastle Upon Tyne, UK) using a quantitative immunometric assay (ADVIA Centaur Progesterone, Bayer, Newbury, UK). GFR and RPF were calculated from the mean of the three inulin and PAH renal clearances, respectively. The renal extraction rate of PAH was assumed to be 0.92 (13). Analytical procedures and across-batch coefficients of variation for inulin and PAH have been described elsewhere (4).

Each volunteer acted as his or her own control. Study size was calculated to identify a difference of 15% in RPF between study occasions with a power of 95% at the 5% significance level. We determined sample size using GraphPad StatMate version 2.00 for Windows (GraphPad Software, San Diego, CA, www.graphpad.com). The results were analyzed using paired or unpaired t test as appropriate and repeated measures two-way ANOVA. All P values are two tailed, data are reported as mean ± SE, and significance was taken as P < 0.05.

Results

Sustained serum RLX levels akin to pregnancy (>1 ng/ml) were achieved (Figure 2) without any apparent side effects or adverse reactions. The MCR of rhRLX was calculated to be 256 ± 14 ml/h per kg. RPF was increased significantly during rhRLX infusion (P < 0.0001), but this was not accompanied by any significant increment in GFR (P = 0.67; Table 1) or decrement in P<sub>osm</sub> (P = 0.38; Table 2). For RPF, repeated measures two-way ANOVA (gender × treatment) demonstrated that the main effect of treatment (rhRLX versus baseline) was significant [F(1,9) = 47.98 P < 0.0001], the effect of gender was NS [F(1,9) = 0.02 P = 0.90; Figure 3], and there was no significant interaction between the two factors [F(1,9) = 0.09; P = 0.8].

Two female volunteers were studied in the follicular phase of the menstrual cycle (serum progesterone <5 mmol/L); the remaining three female volunteers were studied in the luteal phase (serum progesterone >20 mmol/L). The mean percentage increase over baseline in RPF during rhRLX infusion in the follicular and luteal phase studies were 66 and 42%, respectively. There were too few studies to allow meaningful statistical analysis of the effect of menstrual phase.

An additional single infusion study demonstrated that comparable renal effects were evident after only 30 min of sustaining rhRLX infusion (Figure 4A). In another, RPF remained elevated when the rhRLX infusion was continued for an additional two hours, or six hours in total. (Figure 4B).

Urinary flow rate was unchanged with rhRLX infusion, and plasma sodium concentrations (P<sub>Na</sub>) did not decrease. Small increases in the clearance, fractional excretion, and urinary excretion of sodium were observed (Table 2).
neutralizing or eliminating endogenous, circulating RLX (5). Therefore, it was surprising that, in this study in humans, short-term intravenous infusion of rhRLX (14) failed to increase GFR despite a marked increase in RPF during chronic rhRLX administration to rats, it is likely that similar changes occur for two reasons. First, the increases in GFR and RPF, as well as the fall in filtration fraction, are remarkably similar to the pregnant state (1). Indirect evidence from gravid women suggested a similar constellation of changes in glomerular function (2–4). Although the micropuncture technique has not been applied to the investigation of glomerular dynamics during chronic rhRLX administration to rats, it is likely that similar changes occur for two reasons. First, the increases in GFR and RPF, as well as the fall in filtration fraction, are remarkably similar to the pregnant state (1). Indirect evidence from gravid women suggested a similar constellation of changes in glomerular function (2–4).

To summarize, in the setting of chronic administration of RLX to conscious, nonpregnant rats or during rat pregnancy, when endogenous RLX circulates, the hormone mediates increases in both RPF and GFR. In humans, data are available only for creatinine clearance; nevertheless, the results are consistent with the findings in rats, insofar as they suggest that chronic exposure to RLX mediates increases in GFR.

Renal micropuncture studies in midterm pregnant rats indicated that the gestational increase in RPF and GFR is mediated by a fall in both the afferent and the efferent arteriolar resistance without a change in glomerular hydrostatic pressure (1). Indirect evidence from gravid women suggested a similar constellation of changes in glomerular function (2–4). Although the micropuncture technique has not been applied to the investigation of glomerular dynamics during chronic rhRLX administration to rats, it is likely that similar changes occur for two reasons. First, the increases in GFR and RPF, as well as the fall in filtration fraction, are remarkably similar to the pregnant state (1). Indirect evidence from gravid women suggested a similar constellation of changes in glomerular function (2–4).

Discussion

In this study, we investigated the renal effects of short-term intravenous infusion of rhRLX in normal humans. The plasma concentrations of rhRLX that were reached were comparable to the peak values that were observed during early pregnancy in women when RPF and GFR peak (10). There were three major findings: rhRLX (1) markedly increased RPF but without changing GFR in both male and female human volunteers, (2) produced a modest but significant natriuresis, and (3) failed to reduce plasma sodium concentration or osmolality.

Previous work in conscious, nonpregnant rats demonstrated that chronic subcutaneous administration of either porcine RLX or rhRLX for 2 to 5 d produced 20 to 40% increases in both RPF and GFR irrespective of gender (14–16). These findings mimicked the renal vasodilatation and hyperfiltration that were observed during midterm pregnancy in this species (17). In fact, neutralization of endogenous, circulating RLX with mAb or elimination of circulating RLX by ovariectomy prevented the renal changes during midterm pregnancy in conscious rats (5).

In the clinical trial of rhRLX administration to patients with scleroderma, there was a modest but significant increase in the predicted creatinine clearance by 10 to 15% throughout the 28-wk treatment, suggesting an increase in GFR (18). However, this trial was not designed to investigate renal outcomes; therefore, the true extent of any changes in renal function that were caused by chronic administration of rhRLX to humans remains unknown. The increase in creatinine clearance that typically is observed during the first trimester of human pregnancy was markedly subdued in women who did not have ovarian function and, hence, circulating RLX, and became pregnant by egg donation, in vitro fertilization, and embryo transfer (9).

Table 1. Hemodynamic measurements before and after 4-h intravenous infusion with rhRLX in healthy human volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>rhRLX</th>
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</thead>
<tbody>
<tr>
<td>RPF (ml/min per 1.73 m²)</td>
<td>983 ± 133</td>
<td>1403 ± 165&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>117.7 ± 9.7</td>
<td>115.6 ± 7.8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>114.7 ± 1.7</td>
<td>117.0 ± 3.0</td>
</tr>
<tr>
<td>PR (per min)</td>
<td>68 ± 1.8</td>
<td>67 ± 1.9</td>
</tr>
</tbody>
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<sup>a</sup>Data are mean ± SEM. MAP, mean arterial pressure; PR, pulse rate; rhRLX, recombinant human relaxin; RPF, renal plasma flow.

<sup>b</sup>P < 0.0001 baseline versus RLX.

Table 2. Renal handling of electrolytes before and after 4-h intravenous infusion with rhRLX in healthy human volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>rhRLX</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;oxm&lt;/sub&gt; (mmol/L)</td>
<td>284.9 ± 1.3</td>
<td>286 ± 0.8</td>
</tr>
<tr>
<td>P&lt;sub&gt;Na&lt;/sub&gt; (mmol/L)</td>
<td>138.9 ± 0.7</td>
<td>139.8 ± 0.5</td>
</tr>
<tr>
<td>C&lt;sub&gt;Na&lt;/sub&gt; (mmol/ml per 1.73 m²)</td>
<td>1.43 ± 0.2</td>
<td>1.73 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FE&lt;sub&gt;Na&lt;/sub&gt; (% filtered load)</td>
<td>1.18 ± 0.1</td>
<td>1.47 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>U&lt;sub&gt;Na&lt;/sub&gt; (mmol/ml per 1.73 m²)</td>
<td>0.19 ± 0.03</td>
<td>0.27 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P&lt;sub&gt;k&lt;/sub&gt; (mmol/L)</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;k&lt;/sub&gt; (mmol/ml per 1.73 m²)</td>
<td>26.2 ± 3.4</td>
<td>20.4 ± 3.0</td>
</tr>
<tr>
<td>FE&lt;sub&gt;k&lt;/sub&gt; (% filtered load)</td>
<td>23.5 ± 3.6</td>
<td>18.6 ± 3.6</td>
</tr>
<tr>
<td>P&lt;sub&gt;Cl&lt;/sub&gt; (mmol/L)</td>
<td>104.8 ± 0.6</td>
<td>106.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;Cl&lt;/sub&gt; (mmol/ml per 1.73 m²)</td>
<td>1.92 ± 0.4</td>
<td>1.98 ± 0.4</td>
</tr>
<tr>
<td>U&lt;sub&gt;Cl&lt;/sub&gt; (mmol/ml per 1.73 m²)</td>
<td>0.20 ± 0.04</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
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<sup>a</sup>Data are mean ± SEM. Statistical analysis was by paired t test. C, renal clearance. FE, fractional excretion; U, urinary excretion rate.

<sup>b</sup>P < 0.05 baseline versus rhRLX.
enhancement of RPF. This unexpected finding likely is attributable to offsets changes in glomerular dynamics. Specifically, the increase in GFR that normally accompanies a rise in RPF may have been opposed by a decline in glomerular hydrostatic pressure. In theory, this could arise if RLX has a more pronounced effect on the efferent rather than the afferent arteriole, which reduces renal vascular resistance, thereby increasing RPF, but at the same time decreases glomerular hydrostatic pressure. It is possible that a longer duration of rhRLX infusion may permit a concomitant increase in GFR. Indeed, as previously mentioned, tantalizing preliminary data from the clinical trial of rhRLX administration to patients with scleroderma suggested that during chronic infusion of the hormone for 28 wk, the predicted creatinine clearance increased (18). Therefore, unlike rats, a longer exposure to RLX may be needed in humans to elicit a more complete relaxation of the afferent arteriole, thereby preventing a fall in glomerular hydrostatic pressure.

Another possibility, perhaps again related in part to the short-term nature of the rhRLX administration in this study, is that modest plasma volume expansion is needed to permit an increase in GFR. First, a reduction in plasma osmolality was observed in conscious rats after administration of rhRLX for 6 h, the earliest time point studied (14), but no decline was observed in humans after 5 h of infusion (our study). The reduction in plasma osmolality in nonpregnant rats by RLX mimics the pregnant condition and ultimately occurs by repletion of solute-free water that is distributed to all of the body fluid compartments in proportion to their initial size, including the vascular compartment. Second, relative to body weight, the analogous studies in nonpregnant female rats used substantially higher infusion rates of sodium and attendant ions (Ringer’s solution and sodium PAH), which likely produced a volume-replete state relative to this investigation in humans (14). Although the precise nature of the putative link between volume repletion and the increase in GFR is uncertain, increased extracellular fluid and vascular volume can modify vasoconstrictor influences such as renal sympathetic nerve and tubuloglomerular feedback activities that impinge on the afferent arteriole. Therefore, volume repletion may be critical to the increase in GFR by permitting full relaxation of the afferent arteriole during acute rhRLX administration. In contrast, RLX may be equally efficient at relaxing efferent arteriolar tone in rats and humans because the hormone is a functional angiotensin II (AngII) antagonist (16, 19).

These arguments also are consistent with the lack of an increase in GFR despite a rise in RPF that was observed during acute or chronic infusion of rhRLX to rats that were studied under thiopental sodium anesthesia and after acute surgery for placement of vascular and bladder catheters (20). Under these stressful conditions, activation of the sympathetic nervous system and tubuloglomerular feedback activities likely occurred and may have prevented sufficient afferent arteriolar dilation by rhRLX to permit a concomitant increase in GFR. In future studies, it would be interesting to test whether GFR fails to increase in conscious rats during an acute rhRLX infusion protocol that minimizes alterations in vascular volume (e.g., by administering only inulin and 5% dextrose) and, conversely, whether GFR increases in humans during an acute rhRLX infusion protocol that increases vascular volume (e.g., by simultaneously administering a sodium load).

In the same vein, although the majority of plasma volume expansion occurs in the second half of pregnancy, there is some increase in early pregnancy that also may be critical to the hyperfiltration that accompanies the renal vasodilation. It is interesting that when Chapman et al. (21) studied serial changes in renal function during human pregnancy, their data showed that both GFR and RPF increase significantly by gestational week 6, but the increase in RPF is greater. Thus, the vascular compartment may need repletion after being vasodilated during early pregnancy, for an increase in GFR to be manifested fully. In this regard, it would be interesting to sodium-restrict pregnant rats or nonpregnant rats that are chronically administered rhRLX and then determine whether GFR still increases and, if not, whether the increase in RPF persists.

Other possible mechanisms that could explain the marked dissociation in RPF and GFR in this study are a decline in the ultrafiltration coefficient (Kf) and an increase in plasma oncotic pressure. The latter could be eliminated easily as a possibility by measuring plasma protein concentration, which determines the oncotic pressure. Unfortunately, plasma protein concentration was not measured in this study. Direct assessment of glomerular hemodynamics in humans is not possible, so changes in Kf cannot be determined; however, filtration pressure equilibrium is believed to be permissive for the RPF dependence of GFR (22). Renal micropuncture and/or modeling studies of glomerular hemodynamics suggest that rats but not humans are in filtration equilibrium (23). Therefore, the increase in GFR during the chronic infusion of rhRLX to nonpreg-
nant women (14) or during human pregnancy (2–4,21,24) may depend on an increase in $K_{f}$ possibly through glomerular hypertrophy and increased capillary surface area (25). These anatomic changes are likely to evolve more slowly, thereby explaining the lack of an effect of acute rhRLX administration on GFR in humans.

During acute infusion of rhRLX, significant increases in the urinary excretion of sodium, as well as the renal clearance and fractional excretion of sodium, were noted. In contrast, there was a trend for a decline in these variables for potassium. Because GFR did not change, rhRLX altered the tubular handling of sodium and potassium. This effect of rhRLX on renal sodium excretion was reported previously in rats (16,20). In one study, a transient doubling of 24-h urinary sodium excretion was observed on day 2 but not day 5 of porcine RLX administration (16). Unfortunately, urine potassium was not measured in this study. In another, acute intravenous infusion of rhRLX did not affect renal electrolyte excretion, but after 7 d of administration, there was a virtual doubling of sodium excretion, as well as significant increases in both the renal clearance and fractional excretion of sodium (20). The renal excretion of potassium was not affected significantly, and the plasma concentrations of atrial natriuretic hormone and aldosterone were not significantly altered by the chronic RLX infusion, although they were measured in the blood that was obtained from anesthetized, surgically stressed rats. Whether circulating levels may have been different before experimentation, when the animals were conscious and unstressed, is unknown. Nevertheless, the dissociation between the renal tubular handling of sodium and potassium in our study in humans suggests the contribution of a distal tubular site of action. Because RLX is a functional AngII antagonist in the vasculature (16,19), perhaps it also antagonizes AngII–induced secretion of aldosterone or the influence of AngII on renal tubular sodium absorption. Moreover, RLX has been shown to exert its renal vasodilatory influence ultimately through ET and ETB stimulation of NO production (6,15,16,26). Conceivably, RLX may influence sodium reabsorption and potassium secretion in the collecting duct through ET activation of ETB receptors, thereby inhibiting Na$^{+}$–K$^{+}$–ATPase activity (27 and citations therein). Alternatively, increased NO may inhibit the amiloride-sensitive sodium channel in the collecting duct through cGMP and cGMP-dependent protein kinase (PKG), thereby directly impairing sodium absorption and indirectly potassium secretion by decreases in lumen negative potential (28). Although our results generally are consistent with those from rats, there is one important caveat: Time control studies were not conducted; therefore, we cannot be certain that the changes in renal handling of sodium and potassium were specific to rhRLX administration.

At first glance, the natriuretic property of RLX is difficult to reconcile with the overall sodium retention and volume expansion of pregnancy, although in one study, the natriuresis was transient (16). By virtue of decreasing systemic vascular resistance and increasing global arterial compliance (29), RLX may cause, in the long term, renal retention of sodium by stimulating sodium-retaining hormones such as AngII and aldosterone, as well as renal sympathetic nerve activity. These indirect sodium-retaining properties of RLX may be offset partly by direct inhibition of sodium absorption in the distal tubule, thereby preventing undue sodium retention and volume expansion.

In human pregnancy, $P_{\text{osm}}$ decreases by approximately 10 mOsm/L during the first trimester and is maintained thereafter (24). Comparable changes are seen in rats during pregnancy (17,30) and after administration of rhRLX to nonpregnant rats (14–16). In humans, increased serum RLX levels in the luteal phase of the menstrual cycle are associated with reduced $P_{\text{osm}}$ (8), and in egg recipient pregnancies that lack circulating RLX (9), the decrements in $P_{\text{osm}}$ during the first trimester are less than in normal pregnancy, although this effect may not persist (31). In this study, we failed to demonstrate any change in $P_{\text{osm}}$ after rhRLX infusion. Once again, the failure to observe a decrease in $P_{\text{osm}}$ may be due to the short duration of the rhRLX infusion. Indeed, small but significant reductions in $P_{\text{osm}}$ were observed in humans who had scleroderma and received rhRLX by subcutaneous insulin pump for several weeks (E. Unemori, BAS Medical, San Mateo, CA, personal communication, September 22, 2004). However, as few as 6 h of administration to nonpregnant rats by subcutaneous osmotic minipumps was needed to reduce significantly the $P_{\text{osm}}$ (14).

This apparent discrepancy in the time required to reduce plasma osmolality by rhRLX may represent a true species difference.

Acknowledgments

The work received financial support from a National Institutes of Health grant awarded to K.P.C. (RO1 DK63321) and BAS Medical (San Mateo, CA).

These data were presented in abstract form at the Annual Scientific Meeting of the Society for Gynecological Investigation in Los Angeles, CA, March 23 to 26, 2005.

We acknowledge the meticulous laboratory support of L. Shiells and M. Kirkley and the generosity and altruism of all of the volunteers. This study would not have been possible without the generous donation of rhRLX and a contribution toward funding from BAS Medical.

References

6. Conrad KP, Novak J: Emerging role of relaxin in renal and

See the related editorial, “An Emerging Role for Relaxin as a Renal Vasodilator,” on pages 2960–2961.