Relaxin-induced changes in renal sodium excretion in the anesthetized male rat

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Bogzil, Alsadek H., Rachel Eardley, and Nick Ashton. Relaxin-induced changes in renal sodium excretion in the anesthetized male rat. Am J Physiol Regul Integr Comp Physiol 288: R322–R328, 2005. First published September 23, 2004; doi:10.1152/ajpregu.00509.2004.—Pregnancy is associated with profound changes in renal hemodynamics and electrolyte handling. Relaxin, a hormone secreted by the corpus luteum, has been shown to induce pregnancy-like increases in renal blood flow and glomerular filtration rate (GFR) and alter osmoregulation in nonpregnant female and male rats. However, its effects on renal electrolyte handling are unknown. Accordingly, the influence of short (2 h)- and long-term (7 day) infusion of relaxin on renal function was determined in the male rat. Short term infusion of recombinant human relaxin (rhRLX) at 4 μg·h⁻¹·100 g body wt⁻¹ induced a significant increase in effective renal blood flow (ERBF) within 45 min, which peaked at 2 h of infusion (vehicle, n = 6, 2.1 ± 0.4 vs. rhRLX, n = 7, 8.1 ± 1.1 ml·min⁻¹·100 g body wt⁻¹, P < 0.01). GFR and urinary excretion of electrolytes were unaffected. After a 7-day infusion of rhRLX at 4 μg/h, ERBF (1.4 ± 0.2 vs. 2.5 ± 0.4 ml·min⁻¹·100 g body wt⁻¹, P < 0.05), urine flow rate (3.1 ± 0.3 vs. 4.3 ± 0.4 μl·min⁻¹·100 g body wt⁻¹, P < 0.05) and urinary sodium excretion (0.8 ± 0.1 vs. 1.2 ± 0.1 μmol·min⁻¹·100 g body wt⁻¹, P < 0.05) were significantly higher; plasma osmolality and sodium concentrations were lower in rhRLX-treated rats. These data show that long-term relaxin infusion induces a natriuresis and diuresis in the male rat. The mechanisms involved are unclear, but they do not involve changes in plasma aldosterone or atrial natriuretic peptide concentrations.

Relaxin; kidney; renal blood flow; aldosterone; atrial natriuretic peptide

Relaxin is a member of the insulin and insulin-like growth factor family of peptide hormones. It is secreted by the corpus luteum of the ovary during pregnancy in humans and the rat; in other species, such as the guinea pig, the uterus is the principal source of relaxin, whereas the placenta is the main source in the rabbit (37). Its best known role is to stimulate an increase in the length of the interpubic ligament and remodel the cervix, facilitating the passage of the fetus at birth (4). However, there is now increasing evidence which suggests that relaxin exerts other effects, in both females and males. Relaxin gene expression has been identified in a number of tissues, including the brain, uterus, prostate gland, and kidney of the rat (20). The recently identified relaxin receptors LGR7 and LGR8 have also been localized to the brain, kidney, uterus, and testes (22), supporting the notion that relaxin may exert other actions in the pregnant or nonpregnant rat.

One such action of relaxin appears to be the mediation of the profound renal vasodilatation that accompanies pregnancy. In a series of studies, Conrad and colleagues have shown that relaxin administration for as little as 2–6 h, and up to 5 days, produces an increase in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in both nonpregnant female (11, 13) and male (12) rats. These studies show that relaxin is able to induce changes in renal hemodynamics that reflect those seen during pregnancy in both humans (40) and rats (6). Furthermore, Danielson and Conrad (11) have recently reported that these hemodynamic effects are dose dependent. At infusion rates that raised the plasma relaxin concentration to that seen in the rat at gestational day 11 (~20 ng/ml), which coincides with maximal dilation of the renal vasculature (9, 37), there was a dose-dependent increase in ERPF and GFR. At higher infusion rates, which produced a plasma relaxin concentration comparable with that seen in late pregnancy (~80 ng/ml) when renal hemodynamics are returning to prepregnant levels, relaxin had no effect on ERPF or GFR (11).

Relaxin has also been shown to induce changes in extracellular fluid composition similar to those seen in pregnancy. At infusion rates that produced hemodynamic effects, relaxin administration was also associated with a fall in plasma osmolality and plasma sodium concentrations (12, 13) and male (12) rats. These studies show that relaxin is involved in regulating extracellular fluid composition (50). However, apart from brief mention of an increase in urinary sodium excretion after 2 days of relaxin infusion (13), there have been no published reports of the effects of relaxin on renal electrolyte excretion. Yet pregnancy in both humans and rats is associated with profound changes in renal electrolyte handling.

Both pregnant women (2) and rats (17) display an increase in distal tubular sodium reabsorption, yet despite this, a disproportionate increase in water reabsorption results in a fall in plasma sodium concentration (36). Urinary potassium excretion falls in pregnant women (40), whereas calcium excretion increases in both women (21) and rats (19). Relaxin’s potential role in mediating some or all of these effects is unknown. Accordingly, the aim of this study was to determine the effect of relaxin administration on renal electrolyte handling by the male rat. Male rats were chosen to ascertain whether the naive kidney is able to respond to relaxin. Furthermore, as the hemodynamic effects of relaxin have been shown to be time as well as dose dependent (11), we assessed renal excretory function after both a short (2 h) period of infusion and a
longer-term exposure (7 days). As relaxin has been shown to stimulate atrial natriuretic peptide (ANP) release (46), we also measured the plasma concentration of ANP and aldosterone, as both hormones influence renal salt and water handling.

**METHODS**

**Renal response to acute relaxin administration.** Thirteen male Sprague-Dawley rats (180–250 g) were prepared for the renal function study as described previously (1). Briefly, animals were anesthetized with Intralnal Sodium (100 mg/kg body wt ip; thiopental sodium BP, Rhone-Poulenc Rorer, Nenagh, Ireland), and cannulas were inserted into an external jugular vein, carotid artery, and the bladder. The study was conducted in accordance with the “APS Guiding Principles for Research Involving Animals and Human Beings.”

Euvolemic fluid replacement of spontaneous urine output was achieved using a servo-controlled fluid replacement system, as described previously (1). Briefly, urine flow rate was determined gravimetrically and this information was transmitted via a computer to an adjustable pump. We used a program developed at the University of Manchester (8) that allows the infusion rate of the pump to be automatically adjusted precisely to replace intravasally the volume of fluid lost as urine. Clearance markers \[^{3}H\]inulin (4 μCi, Amer sham International, Little Chalfont, United Kingdom) and PAH (2 mg/h, Sigma) for the determination of glomerular filtration rate and effective renal plasma flow were delivered in 0.9% saline via a second, slow, constant-infusion pump (1 ml/h).

After surgery, a bolus dose of \[^{3}H\]inulin (4 μCi) and PAH (2 mg) was injected via the venous cannula, and the servo-controlled infusion was initiated. After a 3-h equilibration period, animals were randomly divided into two groups. Both groups continued to receive a saline infusion for a 1-h control period, after which half of the rats received recombinant human relaxin (rhRLX, n = 7, a gift from Dr. E. Unemori, Connexis, Palo Alto, CA) at 4 μg·h\(^{-1}\)·100 g body wt\(^{-1}\), while the remaining rats (n = 6) received saline alone for the next 2 h. This infusion rate has previously been shown to increase plasma relaxin to a concentration comparable with that seen in pregnancy at 12–14 days gestation (20–40 mg/ml) (13, 38). Urine samples were collected every 15 min and a blood sample (0.5 ml) was taken midway through each hour. A terminal trunk blood sample was taken at the end of the experiment for the determination of plasma ANP concentration.

**Renal response to 7-day relaxin administration.** Thirteen male Sprague-Dawley rats were implanted subcutaneously, under isoflurane anesthesia, with an osmotic minipump [delivery rate 1μl/h (Alzet model 2001, Durect, Cupertino, CA)] loaded with either vehicle (20 mM sodium acetate, n = 7) or rhRLX (4 μg/h, n = 6). Animals were allowed to recover, and 7 days later, renal function was studied using the method described above. Animals received a bolus dose of \[^{3}H\]inulin (4 μCi) and PAH (2 mg) at the beginning of the infusion. After a 3-h equilibration period, all rats received an infusion of 0.9% saline containing \[^{3}H\]inulin (4 μCi/h) and PAH (2 mg/h) for a further 3 h. Urine samples were collected every 15 min, and a blood sample (0.5 ml) was taken midway through each hour.

A terminal trunk blood sample was taken at the end of the experiment for the determination of plasma aldosterone and ANP concentrations.

**Analysis.** Osmolality was determined in plasma and urine samples by freezing point depression (Roebeling osmometer, LH Roebeling, Berlin, Germany). Sodium and potassium concentrations were measured by flame photometry (Corning 480, Corning, Halstead, United Kingdom), calcium and magnesium concentrations were measured by atomic absorption spectrophotometry (model 3100, PerkinElmer, Beaconsfield, United Kingdom), and chloride concentration was measured using a chloride meter (Corning Analyser 925, Corning). \[^{3}H\]inulin activity was determined using a 1900CA Tri-Carb Liquid Scintillation Analyser β-counter (Canberra Industries, Meriden, CT).

PAH concentration was determined by standard colorimetric assay. Plasma aldosterone concentration was determined using a commercial radioimmunoassay kit (Coat-a-Count, Diagnostic Products, Caernarfon, United Kingdom). The intra-assay coefficient of variation was 3%. Plasma ANP concentration was determined after SepPak extraction using a commercial radioimmunoassay kit (Peninsula Laboratories, San Carlos, CA). The intra-assay coefficient of variation was 5.9%.

**RESULTS**

**Renal response to acute relaxin administration.** Body weight did not differ between the vehicle- and rhRLX-treated rats (vehicle, n = 6, 226.4 ± 17.7 g vs. rhRLX, n = 7, 189.9 ± 5.7 g, P = 0.097). Mean arterial blood pressure (vehicle 121 ± 12 vs. rhRLX 98 ± 6 mmHg, P = 0.171), plasma osmolality, and plasma electrolyte concentrations (data not shown) were unaltered at the end of the 2-h rhRLX infusion period.

rhRLX infusion induced a significant increase in effective renal blood flow (ERBF) (F\(_{1,11}\) = 21.6, P = 0.01) within 45 min of the start of infusion (Fig. 1A). Blood flow continued to increase over the 2-h period of rhRLX administration (F\(_{2,12}\) = 3.6, P = 0.023), reaching a peak of 8.02 ± 1.04 ml·min\(^{-1}\)·100 g body wt\(^{-1}\) after 90 min, compared with a flow rate of 2.10 ± 0.39 ml·min\(^{-1}\)·100 g body wt\(^{-1}\) in the vehicle-treated animals at the same time point. Despite this increase in renal blood flow, GFR did not differ between vehicle and rhRLX-treated rats (F\(_{1,9}\) = 1.01, P = 0.34, Fig. 1B). Urine flow rate (F\(_{1,11}\) = 0.51, P = 0.49, Fig. 1C), sodium (F\(_{1,11}\) = 1.5, P = 0.24, Fig. 2A), potassium (F\(_{1,11}\) = 0.84, P = 0.38, Fig. 2B), calcium (F\(_{1,11}\) = 0.76 P = 0.41), and magnesium (F\(_{1,11}\) = 2.41 P = 0.14) excretion (data not shown) were also unaffected by rhRLX infusion. Plasma ANP concentrations did not differ between the two groups (vehicle, n = 6, 6.2 ± 1.3 vs. rhRLX, n = 7, 9.3 ± 2.5 pmol/l, P = 0.30).

**Renal response to 7-day relaxin administration.** Body weight and mean arterial blood pressure did not differ between the vehicle-treated and rhRLX-treated rats (Table 1). After rhRLX administration for 7 days, plasma sodium and potassium concentrations and plasma osmolality were significantly lower (P < 0.05) by comparison with vehicle-treated rats (Table 1). Plasma concentrations of chloride, calcium, and magnesium were unaltered.

ERBF was significantly higher (P = 0.02, Fig. 3A) in rhRLX-treated rats, but GFR did not differ from that of vehicle-treated animals (Fig. 3B). This was associated with significant increases in urine flow rate (P = 0.04, Fig. 3C) and urinary sodium excretion (P = 0.034, Fig. 4A) in rhRLX-treated rats. Sodium clearance (P = 0.022) and fractional excretion of sodium (P = 0.046) were significantly higher in the rhRLX-treated group by comparison with vehicle-treated rats (Table 2). This was mirrored by significant increases in urinary chloride excretion (vehicle, n = 7, 1.2 ± 0.2 vs. rhRLX, n = 6, 1.6 ± 0.1 μmol·min\(^{-1}\)·100 g body wt\(^{-1}\), P =
potassium excretion rate ratio was significantly higher in the rhRLX-treated group ($P = 0.016$, Fig 4C). However, plasma aldosterone concentrations did not differ between the two groups (vehicle, $n = 7, 416 \pm 179$ vs. rhRLX, $n = 6, 285 \pm 141$ pmol/l, $P = 0.58$). Plasma ANP concentrations were also comparable between the two groups (vehicle, $n = 6, 2.6 \pm 0.3$ vs. rhRLX, $n = 6, 2.2 \pm 0.3$, pmol/l, $P = 0.37$).

Seven days of rhRLX treatment had no significant effect on the renal handling of divalent ions, although there was a tendency toward higher urinary calcium excretion (vehicle, $n = 7, 1.7 \pm 0.4$ vs. rhRLX, $n = 6, 2.9 \pm 0.8$ nmol·min$^{-1}$·100 g body wt$^{-1}$, $P = 0.14$), clearance, and fractional excretion (Table 2). Magnesium excretion (vehicle, $n = 7, 12.7 \pm 0.6$ vs. rhRLX, $n = 6, 15.4 \pm 1.6$ nmol min$^{-1}$ 100 g body wt$^{-1}$, $P = 0.13$), clearance, and fractional excretion did not differ between rhRLX- and vehicle-treated groups (Table 2). No significant difference was observed in the ratio of urinary calcium-to-magnesium excretion between vehicle- and rhRLX-treated rats (vehicle, $n = 7, 0.13 \pm 0.03$ vs. rhRLX, $n = 6, 0.2 \pm 0.07$, $P = 0.28$).

Fig. 1. Effect of acute recombinant human relaxin (rhRLX) administration on effective renal blood flow (ERBF; A), glomerular filtration rate (GFR; B) and urinary flow rate (UV; C) in anesthetized rats. After a 3-h equilibration period, control urine samples were collected for 1 h before the infusate was switched to rhRLX at 4 $\mu$g·h$^{-1}$·100 g body wt$^{-1}$ (dashed line, $n = 7$) or vehicle (0.9% saline, solid line, $n = 6$) for an additional 2 h. Data are shown as means ± SE for each 15-min sample period. Statistical analysis was performed by repeated-measures ANOVA. ERBF increased with time in the rhRLX-treated group ($F_{3,32} = 3.4$, $P = 0.029$) and was significantly higher than that in the vehicle-treated group ($F_{1,11} = 21.6$, $P = 0.001$). GFR remained stable over time ($F_{4,33} = 1.4$, $P = 0.25$) and did not differ between rhRLX- and vehicle-treated rats ($F_{1,10} = 1.0$, $P = 0.34$). UV tended to fall over time ($F_{3,36} = 2.6$, $P = 0.065$) but did not differ between rhRLX- and vehicle-treated rats ($F_{1,11} = 0.5$, $P = 0.49$).

Fig. 2. Effect of acute rhRLX administration on the urinary excretion of sodium ($U_{\text{Na,V}}$; A) and potassium ($U_{\text{K,V}}$; B) in anesthetized rats. After a 3-h equilibration period, control urine samples were collected for 1 h before the infusate was switched to rhRLX at 4 $\mu$g·h$^{-1}$·100 g body wt$^{-1}$ (dashed line, $n = 7$) or vehicle (0.9% saline, solid line, $n = 6$) for an additional 2 h. Data are shown as means ± SE for each 15-min sample period. Statistical analysis was by repeated-measures ANOVA. $U_{\text{Na,V}}$ ($F_{1,11} = 1.5$, $P = 0.24$) and $U_{\text{K,V}}$ ($F_{1,11} = 0.84$, $P = 0.38$) did not differ between rhRLX- and vehicle-treated rats.
Table 1. Body weight, MAP, $P_{\text{Osm}}$ and plasma electrolyte concentrations at renal function study in rats previously treated with rhRLX or vehicle for 7 days

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>rhRLX</th>
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<tr>
<td>Body weight, g</td>
<td>355.7±15.4</td>
<td>360.5±20.4</td>
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<tr>
<td>MAP, mmHg</td>
<td>101±7</td>
<td>109±10</td>
</tr>
<tr>
<td>$P_{\text{Osm}}$, mosmol/kgH$_2$O</td>
<td>303.6±3.5</td>
<td>292.1±3.4*</td>
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<tr>
<td>$P_{\text{Na}}$, mmol/l</td>
<td>150.5±1.6</td>
<td>142.5±2.0*</td>
</tr>
<tr>
<td>$P_{\text{K}}$, mmol/l</td>
<td>3.8±0.07</td>
<td>3.4±0.15*</td>
</tr>
<tr>
<td>$P_{\text{Cl}}$, mmol/l</td>
<td>106.3±2.9</td>
<td>101.4±2.3</td>
</tr>
<tr>
<td>$P_{\text{Ca}}$, mmol/l</td>
<td>1.77±0.06</td>
<td>1.70±0.10</td>
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<tr>
<td>$P_{\text{Mg}}$, mmol/l</td>
<td>0.17±0.01</td>
<td>0.16±0.02</td>
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</table>

Values are means ± SE. Rats were previously treated with recombinant human relaxin (rhRLX; 4 µg/h, $n=6$) or vehicle (20 mM sodium acetate, $n=7$) for 7 days via osmotic minipump. MAP, mean arterial blood pressure; $P_{\text{Osm}}$, plasma osmolality; $P_{\text{Na}}$, $P_{\text{K}}$, $P_{\text{Cl}}$, $P_{\text{Ca}}$, $P_{\text{Mg}}$, plasma Na, K, Cl, Ca, and Mg concentration, respectively. Statistical analysis was done by independent samples t-test. *$P<0.05$ vehicle vs. rhRLX.

**DISCUSSION**

The main outcome of this study was that long-term (7 day) rhRLX administration increased urine flow rate and sodium excretion. This was associated with a reduction in plasma osmolality and plasma sodium concentration. Both long (7 day) and short-term (2 h) rhRLX administration induced an increase in ERBF in male rats, the latter within 45 min of the start of acute infusion, confirming earlier observations by Danielson et al. (12). However, in marked contrast to earlier reports (11–13), neither short- nor longer-term rhRLX infusion had any effect on glomerular filtration rate in anesthetized male rats in this study.

The hemodynamic effects of relaxin on the renal vasculature appear to be both dose and time dependent. Danielson and Conrad (11) recently reported that the threshold dose for renal vasodilatation, measured after 2 days of continuous rhRLX administration, was $\sim 0.15$ µg/h. The peak response observed at a dose of 0.4 µg/h was comparable with their earlier observations using 4.0 µg/h (12, 13). The latter administration rate was shown to induce a circulating relaxin concentration comparable with that seen in pregnant rats at gestational day 11 ($\sim 20$ ng/ml), which coincides with maximal dilation of the renal vasculature (9, 37). At the highest dose studied, 40 µg/h, renal function was unaffected (11). The infusion rates used in the current study (4 µg·h$^{-1}$·100 g body wt$^{-1}$ for the short-term infusion and 4 µg/h for the long-term infusion) fall within the range expected to induce a maximal vasodilatory effect on the rat kidney.

Danielson and Conrad (11) also reported the time course of relaxin-mediated changes in renal hemodynamics. rhRLX infused at 4.0 µg/h into conscious female rats induced an increase in GFR within 1 h and ERPF within 2 h. By collecting urine samples every 15 min, rather than one sample per period, we were able to observe an effect on ERBF within 45 min of the start of infusion. ERBF continued to increase over the remainder of the infusion period, reaching a peak of 8.02 ± 1.04 ml·min$^{-1}$·100 g body weight$^{-1}$ at the end of the 2-h infusion period. Despite this, we did not observe any change in GFR.

The vasodilatory effects of relaxin appear to be mediated by endothelin, via the ET$_B$ receptor, and nitric oxide. Relaxin-induced increases in ERPF and GFR were prevented by coadministering either the ET$_B$-receptor antagonist RES-701–1 (12) or the nitric oxide synthase inhibitor $N^\omega$-nitro-$L$-arginine methyl ester (l-NAME) (13). Similarly, RES-701–1 and l-NAME prevented the relaxin-induced reduction in myogenic reactivity observed in small renal arteries in vitro (30). These observations are consistent with the report (15) that relaxin selectively stimulates expression of endothelial and epithelial ET$_B$ receptors, which mediate release of nitric oxide and prostacyclin, but not vascular smooth muscle ET$_B$ receptors, which mediate vasoconstriction (27). A relaxin-induced, nitric oxide-dependent increase in coronary blood flow has also been reported in the isolated rat heart (5).

**Fig. 3.** Effect of 7 day rhRLX administration on effective renal blood flow (ERBF; $A$), glomerular filtration rate (GFR; $B$), and urine flow rate (UV; $C$) in anesthetized rats. Animals received either vehicle (20 mM sodium acetate, open bars, $n=7$) or rhRLX (4 µg/h, solid bars, $n=6$) for 7 days before renal function study. After a 3-h equilibration period, renal clearance measurements were made over 3 h, during which both vehicle- and rhRLX-treated rats were infused with 0.9% saline. Data are shown as means ± SE for the 3-h collection period. Statistical comparisons were made by independent samples t-test. *$P<0.05$, vehicle vs. rhRLX.
ETB receptors are expressed in both the preglomerular vessels (14) and mesangial cells (43), where they act to provide vasodilatory tone (35). Evidence also suggests that endothelin-1-induced vasoconstriction of the postglomerular vessels is mediated predominantly by ETB receptors (16). Hence, activation of endothelial ETB receptors by relaxin would be expected to increase both ERBF and GFR, as reported by Conrad and colleagues (11–13). Therefore, it is difficult to explain the lack of effect on GFR in the current study. One possibility is that the use of anesthetized animals here, as opposed to the use of conscious animals in the Conrad and colleagues’ studies (11–13), blunted the response to relaxin. The baseline GFR and ERBF levels reported herein are somewhat lower than those described by Danielson et al. (12) in conscious male rats, reflecting the cardioinhibitory effects of anesthesia. Under these conditions, the vasodilatory effects of rhRLX may have been sufficient to increase ERBF, presumably, by dilating both the afferent and efferent arterioles, without a significant change in GFR.

Despite the lack of effect on GFR, long-term rhRLX administration was associated with diuresis and natriuresis as well as a tendency toward increased calcium excretion. However, no changes in urinary magnesium or potassium excretion occurred, resulting in a significant increase in the urinary sodium-to-potassium excretion ratio. Short-term rhRLX infusion had no effect on renal electrolyte excretion or urine flow rate. This suggests that rhRLX had specific effects on renal handling of water, sodium, and, possibly, calcium, but these effects only become apparent after longer-term (>2 h) exposure to the peptide. Danielson et al. (13) have reported previously that 24-h sodium output was increased in female rats after 2 days of an infusion with porcine relaxin, but this increase had returned to baseline level after 5 days. Urine output was not altered despite an increase in water intake (13). Hence, our study is the first to describe in detail the effects of relaxin on renal electrolyte handling.

Relaxin is known to influence the central control of fluid balance, but its actions result in water retention rather than loss. Systemic relaxin can influence central fluid homeostatic processes via the the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), which are found in the anterior wall of the third ventricle and lack a blood-brain barrier (26). The SFO and OVLT mediate water drinking (23) and express high-affinity relaxin binding sites (32). Relaxin has been shown to stimulate drinking in both pregnant (31) and nonpregnant rats (39). Conversely, administration of relaxin-neutralizing antibodies to pregnant rats reduced fluid intake (51). Immunohistochemical studies have shown that relaxin induces Fos production in neurons of the SFO and OVLT and that ablation of the former inhibits relaxin-induced water drinking (42). Relaxin has also been shown to stimulate vasopressin secretion (18, 39), possibly via the OVLT (42). Although we did not measure circulating plasma vasopressin in the rhRLX-treated rats, the antidiuretic effect of any increase in concentration was clearly overcome following 7 days rhRLX administration.

Relaxin has also been shown to stimulate ANP secretion by the perfused rat heart (46), and one preliminary report noted

### Table 2. Renal clearance and fractional excretion of sodium, potassium, calcium, and magnesium in rats previously treated with rhRLX or vehicle for 7 days

<table>
<thead>
<tr>
<th>Substance</th>
<th>Vehicle</th>
<th>rhRLX</th>
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<tbody>
<tr>
<td>CNa</td>
<td>5.1±0.9</td>
<td>8.6±0.9*</td>
</tr>
<tr>
<td>FEna</td>
<td>0.9±0.2</td>
<td>1.4±0.1*</td>
</tr>
<tr>
<td>CK</td>
<td>245.4±8.2</td>
<td>258.4±17.2</td>
</tr>
<tr>
<td>FEnk</td>
<td>44.3±2.8</td>
<td>41.8±3.2</td>
</tr>
<tr>
<td>CcA</td>
<td>0.9±0.2</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>FEcA</td>
<td>0.16±0.03</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td>CMg</td>
<td>80.2±6.7</td>
<td>110.1±24.3</td>
</tr>
<tr>
<td>FEmg</td>
<td>14.1±0.9</td>
<td>16.5±2.3</td>
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</table>

Values are mean ± SE. Renal clearance (C) is expressed as μl·min⁻¹·100g body wt⁻¹, and fractional excretion (FE) is expressed as a percentage of filtered load of sodium (CNa, FEna); potassium (CK, FEnk); calcium (CcA, FEcA) and magnesium (CMg, FEmg) in rats previously treated with rhRLX (4 μg/kg/h, n = 6) or vehicle (20 mM sodium acetate, n = 7) for 7 days via osmotic minipump. Statistical analysis was done by independent samples t-test. *P < 0.05, vehicle vs. rhRLX.
that systemically administered relaxin caused depletion of ANP from rat cardiac myocytes (4). ANP induces diuresis and natriuresis in the rat by several different mechanisms, including increasing GFR, inhibiting ANG II-induced sodium reabsorption in the proximal tubule, antagonizing the actions of vasopressin in the cortical collecting ducts, inhibiting sodium reabsorption in the inner medullary collecting duct, and inhibiting angiotensin II-induced aldosterone secretion (25). However, we did not observe a difference in plasma ANP concentrations between either short- or long-term rhRLX-infused rats and their respective controls at the time of renal function assessment. An increase in circulating ANP may have affected renal handling of sodium over the week of rhRLX infusion before renal function was assessed, but we do not have any data for this period. On the basis of available evidence, it seems unlikely that ANP caused the observed diuresis and natriuresis either by a direct tubular action or by an increase in GFR in the current study. The increase in urinary sodium-to-potassium excretion ratio is characteristic of a reduction in aldosterone secretion; however, no such reduction in plasma aldosterone was observed in the long-term, rhRLX-treated rats.

Relaxin has been shown to stimulate oxytocin secretion, by increasing the firing rate of oxytocin neurons in the supraoptic nucleus, in ovariectomized, pregnant, and lactating rats (33, 41, 47). Oxytocin can exert a natriuretic action in the rat (10); it is required to restore renal sodium excretion after neurohypophysectomy (3) and in adrenalectomized or aldosterone-suppressed rats (28). Hence, the natriuresis observed after long-term rhRLX infusion could have been mediated, at least in part, by oxytocin. We did not measure circulating oxytocin in the current study, so we cannot be sure that plasma oxytocin was elevated. However, it is worth noting that acute rhRLX infusion had no effect on sodium excretion. Bolus (iv) injection of relaxin has been shown to induce a rapid (within 1–2.5 min) and sustained increase in plasma oxytocin for 25 min in anesthetized rats (18). If oxytocin contributed to the long-term effect of rhRLX on sodium output, it is reasonable to assume that it might also induce a natriuretic response within the 2-h timescale of the acute experiment. Clearly, further work is required to establish whether oxytocin contributes to the natriuretic actions of rhRLX observed in this study.

Relaxin may also influence sodium reabsorption in the loop of Henle and collecting duct via endothelin-1 activation of the ETB receptor. The ETB receptor is abundantly expressed in the thick ascending limb of the loop of Henle (34), the cortical collecting duct (45), and the inner medullary collecting duct (24), which have previously been implicated in altered renal electrolyte handling in pregnancy (19). Furthermore, endothelin-1 has been shown to inhibit Na⁺–K⁺-ATPase activity in the inner medullary collecting duct (49), which would favor the natriuresis seen here. The increase in fractional excretion of sodium, coupled with the lack of change in GFR, suggests that relaxin-induced a natriuresis by altering tubular handling of sodium. In this context, it is interesting to note that endothelin-1 excretion increases steadily throughout human pregnancy (7, 44) and correlates with changes in creatinine and osmolar clearances, urine flow, and sodium and potassium excretion rates (44). The diuresis and natriuresis observed after long-term rhRLX administration were also associated with reductions in plasma osmolality, plasma sodium, and potassium concentrations. No such change was noted after short-term rhRLX infusion, which may suggest a resetting of regulatory thresholds. Reductions in plasma osmolality have been reported previously in both male (12) and female (13) rats receiving either porcine relaxin or rhRLX for 2–5 days. Male rats receiving rhRLX for 5 days have also been reported to have lower plasma sodium concentration (12). Similar reductions in plasma osmolality and sodium concentration are seen in pregnancy and may be abolished by the administration of relaxin-neutralizing antibodies (29). It has also been suggested that relaxin induces the shift in the plasma osmolality threshold for vasopressin secretion seen in pregnancy (48).

In summary, this study has demonstrated that long-term (7 day) but not acute (2 h) rhRLX administration induces a diuresis and natriuresis in the anesthetized male rat. This was associated with a reduction in plasma osmolality and plasma sodium concentration, which are also seen in pregnancy (36). The underlying mechanisms are unclear but do not involve changes in GFR or circulating concentrations of ANP or aldosterone. Relaxin is known to activate the ETB receptor (12), which raises the possibility that the natriuresis was induced by ETB-mediated inhibition of Na⁺–K⁺-ATPase activity in the inner medullary collecting duct (49). This is consistent with the observed increase in fractional excretion of sodium, which suggests that the natriuresis arose through altered tubular rather than glomerular mechanisms.

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