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

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Running title: Relaxin-induced natriuresis in the male rat

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Abstract

Pregnancy is associated with profound changes in renal haemodynamics 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 non-pregnant female and male rats. However, its effects on renal electrolyte handling are unknown. Accordingly, the influence of short (2 h) and long (7 days) term infusion of relaxin on renal function were determined in the male rat. Short term infusion of recombinant human relaxin (rhRLX) at 4 µg h⁻¹ 100g bwt⁻¹ induced a significant increase in effective renal blood flow (ERBF) within 45 mins, 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 100g bwt⁻¹ $P < 0.01$). GFR and urinary excretion of electrolytes were unaffected. After 7 days infusion of rhRLX at 4 µg h⁻¹, ERBF (1.4 ± 0.2 vs 2.5 ± 0.4 ml min⁻¹ 100g bwt⁻¹ $P < 0.05$), urine flow rate (3.1 ± 0.3 vs 4.3 ± 0.4 µl min⁻¹ 100g bwt⁻¹ $P < 0.05$) and urinary sodium excretion (0.8 ± 0.1 vs 1.2 ± 0.1 µmol min⁻¹ 100g bwt⁻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 do not involve changes in plasma aldosterone or atrial natriuretic peptide concentrations.

Key words: relaxin, kidney, sodium excretion, renal blood flow
Introduction

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 foetus 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 localised to the brain, kidney, uterus and testes (22) supporting the notion that relaxin may exert other actions in the pregnant or non-pregnant 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 hours, and up to 5 days, produces an increase in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in both non-pregnant female (11, 13) and male (12) rats. These studies show that relaxin is able to induce changes in renal haemodynamics which reflect those seen during pregnancy in both humans (40) and rats (6). Furthermore, Danielson and Conrad (11) have recently reported that these haemodynamic effects are dose-dependent. At infusion rates which raised the plasma relaxin concentration to that seen in the rat at gestational day 11 (approximately 20 ng ml\(^{-1}\)), which coincides with maximal dilatation 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 (approximately 80 ng ml$^{-1}$) when renal haemodynamics are returning to pre-pregnant 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 which produced haemodynamic effects, relaxin administration was also associated with a fall in plasma osmolality and plasma sodium concentration (12, 13) in a dose-dependent manner (11). Furthermore, administration of relaxin neutralising antibodies abolished the fall in plasma osmolality and sodium in pregnant rats (29). The pregnant relaxin knockout mouse (rlx$^{-/}$) has elevated plasma osmolality by comparison with wild type mice, providing further evidence of relaxin’s role 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. 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). The potential role of relaxin 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 naïve kidney is able to respond to relaxin. Furthermore, as the haemodynamic effects of relaxin have been shown to be time as well as dose-dependent (11), we assessed renal excretory function over both a
short (2 hour) period of infusion and after 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 renal function study as described previously (1). Briefly, animals were anaesthetised with Intraval (100 mg kg\(^{-1}\) body weight, i.p., thiopentone sodium BP, Rhone-Poulenc Rorer Limited, Nenagh, Co Tipperary, Ireland) and cannulae were inserted into an external jugular vein, carotid artery and the bladder. Euvolaemic fluid replacement of spontaneous urine output was achieved using a servo-controlled fluid replacement system, as described previously (1). Briefly, urine flow rate is determined gravimetrically and this information is transmitted via a computer to an adjustable pump. A programme developed at the University of Manchester (8) allows the infusion rate of the pump to be automatically adjusted to precisely replace intravenously the volume of fluid lost as urine. Clearance markers (\(^{3}\)H inulin, 4 µCi h\(^{-1}\), Amersham International plc, Little Chalfont, Bucks, UK and para-aminohippuric acid (PAH) 2 mg h\(^{-1}\), Sigma, in 0.9% saline) for the determination of glomerular filtration rate and effective renal plasma flow are delivered via a second, slow, constant infusion pump (1 ml h\(^{-1}\)).

Following 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 hour equilibration
period, animals were randomly divided into 2 groups. Both groups continued to receive a saline infusion for a 1 hour control period after which half of the rats received recombinant human relaxin (rhRLX, \( n = 7 \), a gift from Dr E Unemori, Connetics Corp., Palo Alto, CA, USA) at 4 \( \mu \text{g h}^{-1} 100\text{g body weight}^{-1} \) while the remaining rats (\( n = 6 \)) received saline alone for the next 2 hours. 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 ng ml\(^{-1}\)) (13, 38). Urine samples were collected every 15 mins 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 atrial natriuretic peptide concentration.

**Renal response to 7 days relaxin administration**

Thirteen male Sprague Dawley rats were implanted subcutaneously, under isoflurane anaesthesia, with an osmotic minipump (delivery rate 1\( \mu \text{l h}^{-1} \), Alzet model 2001, Durect Corporation, Cupertino, CA, USA) loaded with either vehicle (20 mM sodium acetate, \( n = 7 \)) or rhRLX (4\( \mu \text{g h}^{-1} \), \( n = 6 \)). Animals were allowed to recover and 7 days later renal function was studied using the method described above. Animals received a bolus dose of \(^3\text{H} \) inulin (4 \( \mu \text{Ci} \)) and PAH (2 mg) at the beginning of the infusion. Following a 3 hour equilibration period, all rats received an infusion of 0.9% saline containing \(^3\text{H} \) inulin (4 \( \mu \text{Ci h}^{-1} \)) and PAH (2 mg h\(^{-1} \)) for a further 3 hours. Urine samples were collected every 15 mins 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 atrial natriuretic peptide concentrations.

**Analysis**

Osmolality was determined in plasma and urine samples by freezing point depression (Roebling osmometer, LH Roebling, Berlin, Germany). Sodium and potassium concentrations were measured by flame photometry (Corning 480, Corning Ltd, Halstead, Essex, UK), calcium and magnesium concentrations were measured by atomic absorption spectrophotometry (model 3100, Perkin Elmer, Beaconsfield, Bucks, UK) and chloride concentration was measured using a chloride meter (Corning Analyser 925, Corning Ltd, Halstead, Essex, UK). $^3$H inulin activity was determined using a 1900CA Tri-Carb Liquid Scintillation Analyser $\beta$-counter (Canberra Industries, Meriden, CT, USA). PAH concentration was determined by standard colorimetric assay. Plasma aldosterone concentration was determined using a commercial radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corporation, Caernarfon, Gwynedd, UK). The intra-assay coefficient of variation was 3%. Plasma atrial natriuretic peptide concentration was determined following SepPak extraction using a commercial radioimmunoassay kit (Peninsula Laboratories Inc., San Carlos, CA, USA). The intra-assay coefficient of variation was 5.9 

**Statistical analysis**

Data are presented as the mean ± SEM. Statistical analysis of renal data was by repeated measures ANOVA, with $P \leq 0.05$ considered significant (SPSS for Windows, version 11.5.0, SPSS UK Ltd, Surrey, UK). As all measured renal parameters in the 7 day rhRLX
administration study remained stable in both groups over the 3 hour collection period, data have been combined and are presented as a single mean ± SEM value for the 3 hours. Body weights, plasma electrolytes, plasma aldosterone and plasma ANP concentrations were compared using independent samples t-test.

**Results**

**Renal response to acute relaxin administration**

Body weight did not differ between the vehicle treated and rhRLX treated rats (vehicle, \( n = 6 \), 226.4 ± 17.7 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 2h rhRLX infusion period.

rhRLX infusion induced a significant increase in effective renal blood flow (\( F_{1,11} = 21.6, P = 0.01 \)) within 45 mins of the start of infusion (Fig. 1a). Blood flow continued to increase over the 2 h period of rhRLX administration (\( F_{3,32} = 3.6, P = 0.023 \)), reaching a peak of 8.02 ± 1.04 ml min\(^{-1}\) 100g body weight\(^{-1}\) after 90 mins, compared with a flow rate of 2.10 ± 0.39 ml min\(^{-1}\) 100g body weight\(^{-1}\) in the vehicle treated animals at the same time point. Despite this increase in renal blood flow, glomerular filtration rate 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 atrial natriuretic peptide
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\(^{-1}\), \( P = 0.30 \)).

**Renal response to 7 days 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.

Effective renal blood flow was significantly higher (\( P = 0.02 \), Fig. 3a) in rhRLX treated rats but glomerular filtration rate 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\(^{-1}\) 100g body weight\(^{-1}\), \( P = 0.05 \)) and clearance (vehicle, \( n = 7 \), 11.1 ± 1.6 vs rhRLX, \( n = 6 \), 16.0 ± 1.5 µl min\(^{-1}\) 100g body weight\(^{-1}\), \( P = 0.047 \)) in the rhRLX group. In contrast, urinary potassium excretion did not differ in the rhRLX treated group (\( P = 0.25 \)) (Fig 4b); clearance and fractional excretion of potassium were similarly unaffected (Table 3). Consequently the urinary sodium to 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 ± 179 vs rhRLX, \( n = 6 \), 285 ± 141
pmol l⁻¹, \( P = 0.58 \). Plasma atrial natriuretic peptide 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⁻¹, \( P = 0.37 \)).

7 days of rhRLX treatment has no significant effect on the renal handling of divalent ions, although there was a tendency towards higher urinary calcium excretion (vehicle, \( n = 7, 1.7 \pm 0.4 \) vs rhRLX, \( n = 6, 2.9 \pm 0.8 \) nmol min⁻¹ 100g body weight⁻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 100g body weight⁻¹, \( P = 0.13 \)), clearance and fractional excretion did not differ between rhRLX and vehicle treated groups (Table 2). There was no significant difference 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, \( n = 6, 0.2 \pm 0.07 \), \( P = 0.28 \)).

**Discussion**

The main outcome of this study was that long term (7 days) rhRLX administration increased urine flow rate and sodium excretion. This was associated with a reduction in plasma osmolality and plasma sodium concentration. Both long and short term (2 h) rhRLX administration induced an increase in effective renal blood flow in male rats, the latter within 45 mins of the start of acute infusion, confirming earlier observations by Danielson et al. (12). However, in marked contrast to earlier reports (11-13), neither acute nor longer term rhRLX infusion had any effect on glomerular filtration rate in anaesthetised male rats in this study.
The haemodynamic 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 approximately 0.15 µg h⁻¹. The peak response observed at a dose of 0.4 µg h⁻¹ was comparable with their earlier observations using 4.0 µg h⁻¹ (12, 13). The latter administration rate was shown to induce a circulating relaxin concentration comparable with that seen in the pregnant rat at gestational day 11 (approximately 20 ng ml⁻¹), which coincides with maximal dilatation of the renal vasculature (9, 37). At the highest dose studied, 40 µg h⁻¹, renal function was unaffected (11). The infusion rates used in the current study (4 µg h⁻¹ 100g body weight⁻¹ for the short term infusion and 4 µg h⁻¹ 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 haemodynamics. rhRLX infused at 4.0 µg h⁻¹ into conscious female rats induced an increase in GFR within 1h and ERPF within 2h. By collecting urine samples every 15 mins, rather than once per hour, we were able to observe an effect on ERBF within 45 mins of the start of infusion. ERBF continued to increase over the remainder of the infusion period, reaching a peak at the end of the 2h 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 ET₉ receptor, and nitric oxide. Relaxin-induced increases in ERPF and GFR were prevented by co-administration of either the ET₉ receptor antagonist RES-701-1 (12) or the nitric oxide synthase inhibitor 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 ETB receptors, which mediate release of nitric oxide and prostacyclin, but not vascular smooth muscle ETB 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).

ETB receptors are expressed on both the pre-glomerular vessels (14) and mesangial cells (43) where they act to provide vasodilatory tone (35). There is also evidence which suggests that endothelin-1-induced vasoconstriction of the post-glomerular 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 of rhRLX on GFR in the current study. One possibility is that the use of anaesthesia here, as opposed to the use of conscious animals in the Conrad studies (11-13), has 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 anaesthesia. Under these conditions, the vasodilatory effects of rhRLX may have been sufficient to increase ERBF, presumably by dilating both the afferent and efferent arterioles, in the absence of a significant change in GFR.

Despite the lack of effect on GFR, long term rhRLX administration was associated with a diuresis and natriuresis and a tendency towards increased calcium excretion. However, there were no changes in urinary magnesium or potassium excretion, 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 that these only become apparent after longer term (>2h) 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 porcine relaxin infusion, but this had returned to baseline level after 5 days. Urine output was not altered despite an increase in water intake (13). Hence the current 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 these actions result in water retention rather than loss. Systemic relaxin can influence central fluid homeostatic processes via 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 non-pregnant rats (39). Conversely, administration of relaxin-neutralising 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 atrial natriuretic peptide (ANP) secretion by the perfused rat heart (46) and there is a preliminary report 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
angiotensin II-induced sodium reabsorption in the proximal tubule, antagonising 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 prior to assessment of renal function, but we do not have any data for this period. On the basis of the 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 Na⁺:K⁺ 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 ovariectomised, 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 following neurohypophysectomy (3) and in adrenalectomised or aldosterone-suppressed rats (28). Hence, the natriuresis observed following 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, is it worth noting that acute rhRLX infusion had no effect on sodium excretion. Bolus (i.v.) injection of relaxin has been shown to induce a rapid (within 1-2.5 mins) and sustained increase in plasma oxytocin for 25 mins in anaesthetised 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 2h time scale 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 ET<sub>B</sub> receptor. The ET<sub>B</sub> 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<sup>+</sup>:K<sup>+</sup> ATPase activity in the inner medullary collecting duct (49) which would favour 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 following long term rhRLX administration were also associated with reductions in plasma osmolality and plasma sodium and potassium concentration. No such change was noted following 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-neutralising 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 anaesthetised 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 ET<sub>B</sub> receptor (12), which raises the possibility that the natriuresis was induced by ET<sub>B</sub>-mediated inhibition of Na<sup>+</sup>:K<sup>+</sup>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.

Acknowledgements

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References


Table 1. Body weight, mean arterial blood pressure (MAP), plasma osmolality ($P_{Osm}$) and plasma electrolyte concentrations at renal function study in rats previously treated with recombinant human relaxin (rhRLX, 4µg h$^{-1}$, $n = 6$) or vehicle (20 mM sodium acetate, $n = 7$) for 7 days via osmotic minipump. Statistical analysis was by independent samples $t$-test. * $P < 0.05$ vehicle vs rhRLX.

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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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<td>MAP (mmHg)</td>
<td>101 ± 7</td>
<td>109 ± 10</td>
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<td>$P_{Osm}$ (mOsm Kg$^{-1}$)</td>
<td>303.6 ± 3.5</td>
<td>*292.1 ± 3.4</td>
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<td>$P_{Na}$ (mmol l$^{-1}$)</td>
<td>150.0 ± 1.6</td>
<td>*142.5 ± 2.0</td>
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<td>$P_{K}$ (mmol l$^{-1}$)</td>
<td>3.8 ± 0.07</td>
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<td>$P_{Cl}$ (mmol l$^{-1}$)</td>
<td>106.3 ± 2.9</td>
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<td>$P_{Ca}$ (mmol l$^{-1}$)</td>
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<td>$P_{Mg}$ (mmol l$^{-1}$)</td>
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Table 2. Renal clearance (C, expressed as µl min⁻¹ 100g body weight⁻¹) and fractional excretion (FE, expressed as % of filtered load) of sodium, potassium, calcium and magnesium in rats previously treated with recombinant human relaxin (rhRLX, 4µg h⁻¹, n = 6) or vehicle (20 mM sodium acetate, n = 7) for 7 days via osmotic minipump. Statistical analysis was by independent samples t-test. * P < 0.05 vehicle vs rhRLX.

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<td>CNa</td>
<td>5.1 ± 0.9</td>
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<td>0.9 ± 0.2</td>
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</table>
Figure legends

Figure 1  Effect of acute recombinant human relaxin (rhRLX) administration on effective renal blood flow (ERBF), glomerular filtration rate (GFR) and urine flow rate (UV) in anaesthetised rats. After a 3h equilibration period, control urine samples were collected for 1 h before the infusate was switched to rhRLX at 4 µg h⁻¹ 100g body weight⁻¹ (broken line, n = 7) or vehicle (0.9% saline, solid line, n = 6) for a further 2 h. Data are shown as mean ± SEM for each 15 min sample period. Statistical analysis was by repeated measures ANOVA. ERBF increased with time in the rhRLX-treated group (F₃,₃₂ = 3.4, P = 0.029) and was significantly higher than that in the vehicle treated group (F₁,₁₁ = 21.6, P = 0.001). GFR remained stable over time (F₄,₃₃ = 1.4, P = 0.25) and did not differ between rhRLX and vehicle-treated rats (F₁,₉ = 1.0, P = 0.34). UV tended to fall over time (F₃,₃₆ = 2.6, P = 0.065), but did not differ between rhRLX and vehicle-treated rats (F₁,₁₁ = 0.5, P = 0.49).

Figure 2  Effect of acute recombinant human relaxin (rhRLX) administration on the urinary excretion of sodium (UNaV) and potassium (UKV) in anaesthetised rats. After a 3h equilibration period, control urine samples were collected for 1 h before the infusate was switched to rhRLX at 4 µg h⁻¹ 100g body weight⁻¹ (broken line, n = 7) or vehicle (0.9% saline, solid line, n = 6) for a further 2 h. Data are shown as mean ± SEM for each 15 min sample period. Statistical analysis was by repeated measures ANOVA. UNaV (F₁,₁₁ = 1.5, P = 0.24) and UKV (F₁,₁₁ = 0.84, P = 0.38) did not differ between rhRLX and vehicle-treated rats.
Figure 3  Effect of 7 day recombinant human relaxin (rhRLX) administration on effective renal blood flow (ERBF), glomerular filtration rate (GFR) and urine flow rate (UV) in anaesthetised rats. Animals received either vehicle (20 mM sodium acetate, open bars, \( n = 7 \)) or rhRLX (4µg h\(^{-1} \), solid bars, \( n = 6 \)) for 7 days prior to 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 the mean ± SEM for the 3 h collection period. Statistical comparisons were by independent samples \( t \)-test. * \( P < 0.05 \) vehicle vs rhRLX.

Figure 4  Effect of 7 day recombinant human relaxin (rhRLX) administration on urinary sodium (UNaV) and potassium (UKV) excretion and the ratio of sodium : potassium excretion (UNaV : UKV ratio) in anaesthetised rats. Animals received either vehicle (20 mM sodium acetate, open bars, \( n = 7 \)) or rhRLX (4µg h\(^{-1} \), solid bars, \( n = 6 \)) for 7 days prior to 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 the mean ± SEM for the 3 h collection period. Statistical comparisons were by independent samples \( t \)-test. * \( P < 0.05 \) vehicle vs rhRLX.
Figure 1

(A) Relaxin

(B) Relaxin

(C) Relaxin
Figure 2

(A) Relaxin

Time (mins)

\( U_{NaV} \) (µmol min\(^{-1}\) 100g bwt\(^{-1}\))

0.0 0.5 1.0 1.5 2.0 2.5 3.0

(B) Relaxin

Time (mins)

\( U_{Kv} \) (µmol min\(^{-1}\) 100g bwt\(^{-1}\))

0.0 0.5 1.0 1.5 2.0
Figure 3

(A) ERBF (ml min⁻¹ 100g bwt⁻¹) *

(B) GFR (ml min⁻¹ 100g bwt⁻¹)

(C) UV (µl min⁻¹ 100g bwt⁻¹) *
Figure 4

(A) 

Vehicle Relaxin 

0.0 

0.5 

1.0 

1.5 

\( \text{UNa} V \) (\( \text{\mu mol min}^{-1} 100\text{g bwt}^{-1} \)) 

Vehicle Relaxin 

(B) 

Vehicle Relaxin 

0.0 

0.2 

0.4 

0.6 

0.8 

1.0 

\( \text{UKV} \) (\( \text{\mu mol min}^{-1} 100\text{g bwt}^{-1} \)) 

(C) 

Vehicle Relaxin 

0.0 

0.4 

0.8 

1.2 

1.6 

\( \text{UNa} V : \text{UKV ratio} \) 

Vehicle Relaxin