Ernest H. Starling Distinguished Lectureship of the Water and Electrolyte Homeostasis Section, 2010

Maternal Vasodilation in Pregnancy: The Emerging Role of Relaxin

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Running Head: Vasodilatory role of relaxin in pregnancy

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Abstract

Pregnancy is a unique physiological condition of profound maternal renal and systemic vasodilation. Our goal has been to unveil the reproductive hormones mediating this remarkable vasodilatory state, and the underlying molecular mechanisms. In addition to advancing our knowledge of pregnancy physiology, reaching this goal may translate into therapeutics for pregnancy pathologies such as preeclampsia and for diseases associated with vasoconstriction and arterial stiffness in non-pregnant women and men. An emerging player is the 6 kDa corpus luteal hormone relaxin, which circulates during pregnancy. Relaxin administration to rats and humans induces systemic and renal vasodilation regardless of sex, thus mimicking the pregnant condition. Immunoneutralization or elimination of the source of circulating relaxin prevents renal and systemic vasodilation in midterm pregnant rats. Infertile women who become pregnant by donor eggs – IVF – embryo transfer lack a corpus luteum and circulating relaxin, and they show a markedly subdued gestational increase in glomerular filtration rate. These data implicate relaxin as one of the vasodilatory reproductive hormones of pregnancy. There are different molecular mechanisms underlying the so-called rapid and sustained vasodilatory actions of relaxin. The former is mediated by Gαi/o protein coupling to phosphatidylinositol-3 kinase/Akt (protein kinase B)-dependent phosphorylation and activation of endothelial nitric oxide synthase, and the latter by vascular endothelial and placental growth factors, and increases in arterial gelatinase(s) activity. The gelatinases, in turn, hydrolyze big endothelin (ET) at a gly-leu bond to form ET₁.
which activates the endothelial ET<sub>B</sub> receptor/nitric oxide vasodilatory pathway.

**Key Words:** systemic hemodynamics, arterial compliance, renal circulation, artery, angiogenic growth factors, matrix metalloproteinase, endothelin, nitric oxide
Preamble

Being cognizant of the major scientific contributions of Dr. Starling and previous winners of the Starling Lectureship, I am humbled and honored to be this year’s recipient. The general topic I will address – maternal cardiovascular and renal adaptations to pregnancy – is a highly integrative and specialized niche at the cross-roads of renal, cardiovascular, reproductive and endocrine physiology, and as such, is but a tiny twig at the top of the physiological tree. In contrast, the fundamental discoveries of Dr. Starling and other prominent cardiovascular scientists of his caliber clearly make up the trunk of this great tree.

Of note, approximately 210,000,000 women worldwide become pregnant annually (74), and the vast majority successfully undergo the remarkable circulatory adaptations of pregnancy that I will discuss. Unfortunately, pregnancies do not always proceed normally, and as rough estimates, 5% or 10,500,000 experience the hypertensive disorders of pregnancy including preeclampsia (54); 9.6% or 20,160,000 deliver prematurely (7); and 9.5% or 19,950,000 deliver a neonate with low birth weight defined as < 2500 g at > 37 weeks gestation (26). These pregnancy pathologies are associated with maternal, fetal and neonatal morbidity and mortality, especially in developing countries with inadequate health-care delivery, and they are also associated with adverse health outcomes later in life for both mother and offspring. Clearly, however, I am preaching to the choir when I suggest that a complete understanding of pregnancy physiology may be prerequisite to understanding pathophysiology, or at the very least will facilitate investigation of pregnancy
Before beginning the scientific part of my talk, I acknowledge the wonderful mentorship and support that I have enjoyed throughout my career, and without which, my work would not be possible: Ulrich Luft enabled my first experience in a research laboratory at Lovelace Medical Foundation, when I was a high school student; Bill Haffner introduced me to preeclampsia while rotating through the Gallup Indian Medical Center as a medical student; as a medical intern at the University of Colorado Health Sciences Center, Bob Schrier indoctrinated me into the theory of arterial underfilling; Miklos Gellai taught me the chronically instrumented, conscious rat technique for measurement of renal function during my first postdoctoral fellowship at Dartmouth Medical School; during my second, Michael Dunn, then of Case Western Reserve University, introduced me to cell physiology; Jim Roberts at the University of Pittsburgh established a wonderfully nurturing environment for scientific creativity and productivity at the Magee-Women’s Research Institute; John Davison from the University of Newcastle upon Tyne inspired me to follow in his footsteps investigating the clinical physiology of pregnancy in women; Marshall Lindheimer of the University of Chicago provided indefatigable support from the beginning; and last, but not least, I acknowledge the invaluable mentorship and constant support of Jim Conrad and Heinz Valtin who are my biologic and academic fathers, respectively. I also am indebted to John F. Kennedy, Jr., who launched the space race to the moon, and unwittingly, I suspect, inspired a whole generation of Americans including me, passionate about mathematics, science
and medicine. Finally, none of my science would be possible without the steady and loving, support and understanding of Kris, Paul and Claire, who are what really matter to me.

**Rationale: Why Study Maternal Circulatory Adaptations to Pregnancy?**

First, as a physiologist, I am eager to unveil the hormonal and molecular mechanisms underlying the extraordinary vasodilatory phenomena of pregnancy. Second, having medical training, I am also motivated by the possibility that understanding of these fundamental mechanisms may provide novel insights into the pathophysiology of preeclampsia and intrauterine growth restriction, as well as potential therapeutic strategies for treatment and prevention of these pregnancy-specific pathologies, and diseases associated with vasoconstriction and arterial stiffness in non-pregnant women and men.

**Overview of the Maternal Systemic and Renal Circulations During Pregnancy**

Maternal systemic vascular resistance plummets during early pregnancy, initiating a chain of events that leads to marked increases in cardiac output and intravascular volume by at least 40% above pre-pregnant levels (8, 70). Furthermore, global arterial compliance (AC) rises in parallel with cardiac output, thereby preserving diastolic pressure and efficient ventricular-arterial coupling.
These changes are maximal by the end of the first or beginning of the second trimester, when the oxygen requirement of the nascent fetoplacental unit(s) is minimal. Consequently, the large increase in oxygen delivery secondary to the marked rise in cardiac output exceeds oxygen demand, resulting in narrowing of the arterial-mixed venous oxygen content difference (3). The anticipatory nature of these early gestational changes in systemic hemodynamics, i.e., in preparation for the rapid growth phase of the fetus and placenta in the second half of gestation, is underscored by the fact that they are also observed in the luteal phase of the menstrual cycle, albeit to lesser degrees (9, 68). This observation also suggests that corpus luteal factors contribute to the early systemic hemodynamic changes of pregnancy (see below). Another remarkable maternal cardiovascular adaptation to pregnancy is the attenuated systemic pressor response to vasoconstrictors including angiotensin II and norepinephrine (38, 55). This apparent vascular refractoriness to vasoconstrictors undoubtedly contributes to the profound vasodilation of pregnancy. Finally, conscious rats manifest similar changes in the systemic circulation during pregnancy, thus providing an experimental model in which to begin investigating mechanisms (12, 29, 40, 75).

Concurrent with systemic vasodilation, the maternal renal circulation also undergoes massive vasodilation and, in concert with that of other, mainly non-reproductive organ circulations, contributes to the overall decline in systemic vascular resistance during the first trimester (8, 14, 25). Vasodilation of the maternal kidneys and other organ circulations beginning early in gestation leads
to relative “arterial underfilling” that ultimately serves as a potent stimulus for renal sodium and water retention, and volume expansion (40, 71). As a result of kidney vasodilation, renal plasma flow (RPF) and glomerular filtration rate (GFR) increase by at least 50%, again by the end of the first or beginning of the second trimester. These gestational changes in renal function are also anticipated in the luteal phase (9, 14). Once again, conscious gravid rats demonstrate comparable alterations in the renal circulation, and they also display attenuated renal pressor responses to angiotensin II (11, 12, 19, 61). Finally, renal micropuncture studies in rats and indirect evidence from women link the gestational increase in GFR to the rise in RPF with no increase in glomerular hydrostatic pressure (6, 53, 69).

**Figure 1** illustrates a theoretical working model for the regulation of cardiac output in pregnancy.

**Overview of Relaxin**

Relaxin is a 6 kDa peptide hormone in the insulin-relaxin superfamily of structurally related hormones, which is produced by the corpus luteum and circulates during pregnancy in humans, non-human primates, rats and mice. Humans have three ligands (relaxin-1, -2, and -3), whereas rats and mice have two (relaxin-1 and -3) (73). Rat and mouse relaxin-1, and human relaxin-2 are true orthologs secreted by the corpus luteum during pregnancy. The relaxin/insulin-like family peptide receptor, RXFP1, binds relaxin-1, -2, and -3. Relaxin-3 also interacts with RXFP3, whereas RXFP2 preferentially and avidly
binds insulin-like hormone 3 (Insl-3), and relaxin only weakly at best. RXFP1 and RXFP2 are glycosylated GPCRs containing leucine rich repeats and a LDLa module in the extracellular domain (5, 73).

Relaxin is detectable in the circulation of women during the luteal phase, and if conception occurs, serum concentrations rapidly rise reaching a peak of approximately 1 ng/ml at the end of the first trimester. Thereafter, relaxin levels fall to an intermediate value of around 0.5 ng/ml throughout the remainder of gestation (reviewed in (72)). In rats, circulating relaxin is detectable on gestational day 8 or 9, and rises progressively reaching peak concentrations at the end of pregnancy of approximately 100 ng/ml (72). Thus, relaxin circulates in both species during pregnancy emanating from the corpus luteum, but the temporal pattern of change and serum concentrations are different (72).

**Relaxin Administration Mimics Maternal Vasodilation of Pregnancy**

The pattern of circulating relaxin in women parallels the vasodilation in the systemic and renal circulations observed during the luteal phase and in early pregnancy as discussed above. Previous reports that relaxin exerted morphological changes in endometrial blood vessels (18, 41), decreased arterial pressure in spontaneously hypertensive rats (SHR) (79), and increased coronary blood flow in isolated rat and guinea pig hearts (4) provided additional clues that relaxin may play a causal role in the maternal vasodilation of pregnancy.
Chronically instrumented conscious rats. Subcutaneous administration of recombinant human relaxin (rhRLX) by osmotic pump to non-pregnant female or male rats for 10 days reduced systemic vascular resistance by ~25% comparable to the magnitude of decline observed in early to midterm pregnant rats (13, 27, 29). The serum levels of rhRLX reached in these studies were comparable to the concentrations observed in early to midterm pregnant rats (72). Mean arterial pressure (MAP) did not fall significantly, because cardiac output rose in a reciprocal fashion due mainly to increases in stroke volume, again reminiscent of pregnancy. As measured by two different techniques (area method and stroke volume/pulse pressure), global AC also increased by about 25% (13, 27). Consistent with this last finding, the passive compliance of small renal arteries isolated from relaxin-administered rats and studied ex vivo was increased (13). Chronic administration of rhRLX to SHR reduced systemic vascular resistance, and increased cardiac output and global AC to a similar extent as observed in normotensive controls, i.e., ~25% (28). This finding is reminiscent of the vasodilatory effect of pregnancy in both rodents and women with chronic hypertension (2). Finally, short-term intravenous administration of rhRLX to conscious rats over several hours reduced systemic vascular resistance and increased cardiac output and global AC in angiotensin II-hypertensive rats, but not in SHR or normotensive control rats (28). (Chronic administration of relaxin in the angiotensin II model of hypertension was not tested.)
Subcutaneous administration of rhRLX by osmotic pump to non-pregnant female and male conscious rats for 5 days also reduced renal vascular resistance by ~25%, comparable to the magnitude of decline observed in early to midterm pregnant conscious rats (11, 21, 22). Once again, the serum concentrations of rhRLX reached in these studies were similar to those observed in early to midterm gravid rats. As a consequence of the reduced renal vascular resistance, both RPF and GFR also increased by approximately 25%. In association with renal vasodilation, myogenic constriction of small renal arteries isolated from the relaxin-treated rats was attenuated, thus mimicking the reduced myogenic constriction observed in small renal arteries from midterm pregnant rats (37, 60). Analogous to both midterm and late pregnancy in rats (12, 19, 61), the renal pressor response to an acute intravenous infusion of angiotensin II was attenuated in non-pregnant rats that were chronically administered relaxin (22). Last, short-term intravenous administration of rhRLX in conscious rats increased both RPF and GFR within 1-2 hours (20).

**Human studies.** Although human studies are limited through 2011, a Phase 1 clinical trial of short-term, 24-hr intravenous infusion of rhRLX in patients with stable congestive heart failure has been conducted (32). The hormone significantly increased cardiac index, and reduced systemic vascular resistance, pulmonary capillary wedge pressure, serum creatinine and BUN, with only modest decreases in systolic blood pressure analogous to the conscious rat studies as described above. Consistent with these studies in humans and rats, acute intravenous administration of rhRLX also increased cardiac index in a
swine model of myocardial ischemia-reperfusion injury (64). In other human trials designed to capitalize on the matrix-degrading attributes of relaxin (see below), rhRLX was administered for 6 months by insulin pump to patients with mild scleroderma with the goal of improving the skin manifestations of the disease (33, 48, 80). Although the primary (dermal) therapeutic endpoints were not reached, the hormone significantly increased predicted creatinine clearance by 10-15% throughout the study (and a modestly reduced systolic blood pressure). In another investigation, short-term intravenous infusion of rhRLX for 5 hours at a rate yielding serum levels of ~1.5 ng/ml (comparable to human pregnancy), increased RPF by 60% in both female and male subjects as early as 30 min after starting the infusion, but unexpectedly, GFR was unaffected (77).

**Figure 2** summarizes the effects of pregnancy and relaxin administration on the systemic and renal circulations in rats and humans. By and large, pregnancy and relaxin exert similar vasodilatory actions in both species. Parenthetically, relaxin administration also recapitulates the osmoregulatory changes of pregnancy (reviewed in (42)).

**Neutralization of Circulating Relaxin or Ovariectomy Prevents Maternal Circulatory Changes of Pregnancy**

**Chronically instrumented rats.** Administration of rat relaxin neutralizing antibodies every day beginning on gestational day 8 prevented the rise in cardiac output and global AC, and the fall in systemic vascular resistance at midterm
Using a similar protocol, rat relaxin neutralizing antibodies also precluded the gestational increases in RPF and GFR, and decrease in renal vascular resistance (57). An additional methodological approach was used to eliminate relaxin from the circulation of pregnant rats, i.e., ovariectomy and subsequent maintenance of pregnancy with exogenous estradiol and progesterone. The renal hemodynamic changes of pregnancy were also prevented by this maneuver (57). Moreover, myogenic constriction of small renal arteries isolated from the same gravid rats in which relaxin was either neutralized with specific antibodies or eliminated from the circulation by ovariectomy was relatively robust, resembling the virgin phenotype.

**Human pregnancy.** To our knowledge, there is only one pilot investigation in which the potential role of relaxin in the renal and osmoregulatory changes of human pregnancy was tested (78). In that study, infertile women who became pregnant by donor eggs – IVF – embryo transfer showed a subdued increase in 24-hr endogenous creatinine clearance and attenuated decrease in serum osmolality during the first trimester (later stages of pregnancy were not investigated). Because these women lacked a corpus luteum, there was no circulating relaxin, which was corroborated in the study by two different immunoassays. When considered in the context of the results from rat investigations as summarized above, an absence of circulating relaxin was implicated in the attenuated renal and osmoregulatory changes. Clearly, however, we could not exclude the possibility that deficiency of other corpus
luteal factors in these women contributed to the suboptimal maternal adaptations of pregnancy in this pilot study.

**Interim Summary**

The major take home messages from the first part of my lecture are: (i) pregnancy is a profoundly vasodilated state, (ii) relaxin is a potent vasodilator, and (iii) relaxin contributes to maternal vasodilation of pregnancy.

**Molecular Mechanisms of Relaxin Vasodilation: Sustained Vasodilatory Responses**

Plasma concentration and urinary excretion of cyclic guanosine 3',5'-monophosphate (cGMP) were reported to be increased during pregnancy in rats (10). Although its biochemical identity had not been yet conclusively identified, endothelium derived relaxing factor (EDRF) was postulated to be the first messenger, because cGMP was known to be a second messenger (66). Soon thereafter, however, EDRF was identified as nitric oxide (NO) (63), and increased biosynthesis of cGMP and nitric oxide (NO) were discovered to be increased in rat gestation, the latter based upon markedly enhanced urinary excretion and plasma levels of its major metabolites nitrate and nitrite (NOx), as well as detection of NO bound to hemoglobin by electron paramagnetic resonance spectroscopy (16, 17). The elevated urinary excretion of NOx was blocked by
the nitric oxide synthase inhibitor, L-nitroarginine methyl ester (L-NAME), and was also observed in pseudopregnant rats (16). Although the tissue(s) origin of increased NO production was not investigated, the vasculature was speculated to be one possible source.

The potential role of NO in mediating renal vasodilation of pregnancy was subsequently tested in chronically instrumented, conscious rats (19). Both L-NAME and L-NG-monomethyl arginine (L-NMMA) abolished renal vasodilation and hyperfiltration in midterm pregnant rats during short-term, low dose intravenous infusion, which only minimally affected RPF and GFR in virgin control rats. Renal vascular resistance is typically low compared to many other organ circulations, in part mediated by tonic activation of the endothelial endothelin ETB receptor and presumably through stimulation of NO synthesis [at least in conscious male rats (39)]. We reasoned, therefore, that this vasodilatory pathway might be accentuated during pregnancy, thus underlying gestational renal vasodilation and hyperfiltration. The specific ETB receptor antagonist, RES-701-1, when administered by short-term intravenous infusion at a dose that did not significantly affect RPF and GFR in virgin control animals, abolished renal vasodilation and hyperfiltration in midterm pregnant rats (15). In analogous studies, results using the mixed ETA/B antagonist, SB209670, supported these findings (15). Subsequent investigations further demonstrated that the endothelial ETB receptor and NO mediate renal vasodilation and hyperfiltration in conscious non-pregnant rats administered rhRLX for 5 days, and the same molecular intermediates also underlie the attenuation of myogenic constriction in
small renal arteries isolated from midterm pregnant and relaxin-infused non-pregnant rats (21, 22, 37, 60). Although the data are limited, there is no evidence for upregulation of endothelial NOS in the major zones of the kidney, in isolated small renal arteries or renal microvasculature of midterm pregnant rats, nor in the renal cortex of relaxin-treated non-pregnant rats (1, 59). Interestingly, recent evidence demonstrates increased neuronal NOSβ in the renal cortex of pregnant rats; however, the hemodynamic relevance of this finding has not been tested (76). Whether pregnancy or relaxin can upregulate the ET_B receptor is disputed, and thus requires further investigation (31, 47).

To substantiate the critical role of endothelin and the endothelial ET_B receptor, an inhibitor of endothelin converting enzyme (phosphoramidon) was tested (44). Unexpectedly, short-term intravenous administration at a rate sufficient to completely abrogate the slow pressor response to a large bolus of big ET, failed to block renal vasodilation and hyperfiltration elicited by chronic (5 day) relaxin administration in conscious rats. Because relaxin had been shown to increase matrix metalloproteinases (MMPs) in cultured fibroblasts prepared from several organs (62, 81, 82), a novel alternative pathway of endothelin formation was investigated, i.e., gelatinases MMP-2 and -9, which can hydrolyze big ET at a glycine-leucine bond to produce ET_{1-32} (34, 35). To this end, cyclic CTTHWGFTLC (cyclic CTT), a then newly discovered inhibitor of gelatinases (49), inhibited relaxin-induced renal vasodilation and hyperfiltration during short-term intravenous infusion (44). These results were corroborated using GM6001, a general inhibitor of MMPs, administered in the same fashion. To substantiate
these *in vivo* investigations, the myogenic constriction bioassay was again employed. MMP-2 neutralizing antibodies, tissue inhibitor of metalloproteinases (TIMP-2), cyclic CTT, and GM6001 all reversed the attenuated myogenic constriction of small renal arteries harvested from midterm pregnant and relaxin-administered non-pregnant rats; however, once again, phosphoramidon was ineffective (44).

Because regulation of endothelial NOS and ET$_B$ receptor expression by relaxin and pregnancy was unlikely (or disputed in the case of the ET$_B$ receptor), arterial gelatinase activity was investigated by zymography as a potential molecular locus of regulation (44). Both pro and active MMP-2 activities were upregulated in small renal and mesenteric arteries, and thoracic aortae isolated from midterm pregnant and relaxin-treated non-pregnant rats. Subsequently, increased MMP-2 mRNA and protein was identified as a basis for the increased activity (43). Other reports corroborated the increased expression of arterial MMP-2 during pregnancy or relaxin administration (23, 46, 51, 83). Unexpectedly, however, after 4 to 6 hr of relaxin administration, MMP-9 activity was upregulated in small renal and mesenteric arteries, while pro and active MMP-2 were unchanged (45). Consistent with these findings, MMP-9, but not MMP-2 neutralizing antibodies reversed the attenuated myogenic constriction of small renal arteries isolated from rats administered rhRLX for 4 to 6 hr (45). MMP-2 was observed in both vascular smooth muscle and endothelium in small renal and mesenteric arteries by immunohistochemistry, whereas MMP-9 was localized to smooth muscle (43, 45).
On the one hand, the expected attenuation of myogenic constriction was not observed in small renal arteries isolated from ET\(_B\) receptor deficient rats that were either pregnant or non-pregnant and chronically administered rhRLX, thus corroborating earlier findings using the ET\(_B\) receptor antagonists, RES-701-1 and SB209670 (37, 44, 51, 56, 60). On the other hand, enhanced MMP-2 activity was observed in small renal arteries isolated from the same rats. Taken together, these findings suggest that MMP-2 is upstream of the endothelial ET\(_B\) receptor in the relaxin vasodilatory pathway (44). Thus, arterial gelatinases are pivotal loci of regulation by relaxin and pregnancy. Presumably, increased gelatinase activity translates into more ET\(_{1-32}\) formation generated by the hydrolysis of big ET that, in turn, enhances the endothelial ET\(_B\) receptor-NO vasodilatory pathway. However, proof of this concept awaits measurement of ET\(_{1-32}\).

Using RXFP1 and RXFP2 receptor deficient mice, RXFP1 was identified as the relaxin receptor relevant to the vascular actions of relaxin, i.e., attenuation of myogenic constriction and increase in arterial compliance (30). In light of the prominent role of endothelium and NO in relaxin and pregnancy vasodilatory responses, it was surprising that RXFP1 mRNA and protein were predominantly expressed in smooth muscle with barely detectable levels in endothelium (unpublished). Based on this molecular evidence, a paracrine role for vascular endothelial growth factor (VEGF) was postulated to initiate the gelatinase-ET\(_B\) receptor-NO pathway in endothelium, subsequent to RXFP1 activation in
vascular smooth muscle by relaxin (51). Indeed, relaxin had been reported to stimulate VEGF expression in other cell types (reviewed in (51)).

In isolated rat and mouse small renal, and human subcutaneous arteries, 3-hr incubation with rhRLX in vitro attenuated myogenic constriction, which was restored to robust levels after addition of L-NAME, RES-701-1 or GM6001 to the bath (51). These findings are analogous to those in small renal arteries isolated from relaxin-infused or midterm pregnant rats and incubated with these inhibitors ex vivo (37, 60). Pre-incubation of isolated rat and mouse small renal, and human subcutaneous arteries with the VEGF receptor tyrosine inhibitor, SU5416, or specific VEGF neutralizing antibodies prevented rhRLX from attenuating myogenic constriction (51). In an effort to demonstrate specificity for VEGF, specific placental growth factor (PGF) neutralizing antibodies were also tested, but unexpectedly, these too precluded relaxin-induced attenuation of myogenic constriction (51). These in vitro studies were corroborated in vivo, insofar as chronic treatment with SU5416 prevented renal vasodilation and hyperfiltration in conscious rats after 4-6 hr and 3-5 days of rhRLX administration (51). As previously shown (vide supra), MMP-2 activity was increased in small renal arteries isolated from the control group of rats administered rhRLX (and DMSO vehicle instead of SU5416). Unexpectedly however, the increase in arterial MMP-2 activity elicited by rhRLX was not prevented by SU5416 (51). Contrary to our expectations, therefore, VEGF receptor tyrosine kinase activity is apparently not upstream of MMP-2 in the relaxin vasodilatory pathway.
The current working model of the sustained relaxin vasodilatory pathway is shown in Figure 3. Insofar as the molecular intermediates for relaxin-induced attenuation of myogenic constriction is comparable in rat and mouse small renal, and human subcutaneous arteries, there is evolutionary conservation of relaxin vasodilatory mechanisms. This contrasts with the actions of the hormone on reproductive tissues, which can vary considerably among species (72).

Molecular Mechanisms of Relaxin Vasodilation: Rapid Vasodilatory Responses

An emerging concept is that the vasodilatory mechanisms of relaxin appear to vary according to the duration of hormone exposure. Thus, in addition to the so-called sustained vasodilatory response as described above, which involves arterial MMP-9 and -2 after hour(s) and day(s) of relaxin exposure, respectively (Fig. 3), the hormone rapidly dilates select arteries across a range of species including humans within minutes of application (36, 52). The mechanism appears to involve endothelial Goi/o protein coupling to PI3 kinase, Akt (protein kinase B) and eNOS, but not VEGF receptor transactivation or increased intracellular calcium (52). A working model is depicted in Figure 4.

Perspectives and Significance

Implications of Relaxin Vascular Biology for Assisted Reproductive Technology (ART), Obstetrics and Medicine
In view of relaxin’s role in mediating maternal systemic and renal vasodilation during midterm pregnancy in conscious rats, and the markedly subdued changes in GFR and plasma osmolality during the first trimester in donor egg recipients who lack a corpus luteum and circulating relaxin, it is not unreasonable to hypothesize that there may be other maternal hemodynamic insufficiencies in women conceiving by donor eggs – IVF – embryo transfer. To my knowledge, maternal hemodynamics during pregnancy have not been investigated in these women. A complete understanding of them may shed light on emerging evidence that suggests an increased risk of adverse maternal and neonatal outcomes in ART pregnancies (67). If the hypothesis is borne out by investigations currently ongoing in our Clinical Research Center, then relaxin should perhaps be considered for inclusion in the hormonal regimen of donor egg recipients who lack the hormone. In contrast, less ovarian stimulation may be warranted in women utilizing their own eggs, thus producing fewer corpora lutea, and hence, more physiological circulating levels of relaxin.

A priori, it is logical to propose that relaxin administration to women with preeclampsia may be salutary because of the hormone’s vasodilatory attributes, which might improve organ perfusion (24). In light of the newly described role of VEGF and PGF in the vasodilatory pathway of relaxin (51), there may be mechanistic rationale as well. That is, relaxin administration might increase or potentiate local VEGF and PGF activity within the arterial wall effectively counteracting (or at least partially so) elevated circulating soluble VEGF receptor
1 (50), thereby improving endothelial cell function and health. Administration of rhRLX to pregnant women was reported to have a favorable safety profile (84).

Another recurrent theme throughout my lecture is that relaxin is vasodilatory irrespective of sex. One possible explanation is that relaxin also circulates in males, albeit at extremely low concentrations, although this is disputed. Another is that there is an arterial derived relaxin receptor-ligand system in both females and males (58). Regardless, relaxin’s unique systemic and renal vasodilatory actions occurring in both sexes as described above (Fig. 2), gave rise to the proposal that the hormone may be therapeutic in heart failure by reducing ventricular afterload and arterial stiffness, and antagonizing angiotensin II. Through these combined actions, relaxin may improve ventricular-arterial coupling, cardiac output and organ perfusion including the kidneys with potentially less clinically significant hypotension and worsening renal function as compared to other pharmaceutical agents (13, 80).
Acknowledgements

The work in the author’s laboratory would not have been possible without the invaluable contributions of many outstanding colleagues over the years, particularly collaborators Sanjeev G. Shroff PhD, Lee A. Danielson PhD, Laura J. Parry PhD, Jacqueline Novak PhD, Arun Jeyabalan MD, John M. Davison MD, and Jonathan T. McGuane, PhD (Postdoctoral Fellow). Dr. Conrad gratefully acknowledges the financial support of the National Institutes of Health (K11 HD00662, RO1 HD030325, RO1 DK063321, RO1 HL067937, and R21 HL093334, the 8th Mallinckrodt Scholar Award, New Mexico Heart Association Flinn Newly Independent Investigator Award, and a Grant-in-Aid from the American Heart Association (No. 0855090E).

Disclosures

The author discloses patents related to relaxin.
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82. **Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME, and Amento EP.** Relaxin induces an extracellular matrix-degrading phenotype in human


Figure Legends

Figure 1. Regulation of Cardiac Output During Pregnancy. In this theoretical model, profound maternal vasodilation (decreased ventricular afterload) in the first trimester initiates a large increase in cardiac output and relative “arterial underfilling”, the latter activating mechanisms that lead to volume retention (increased preload), thereby abetting the increase in cardiac output. See text for details and supporting references. Modified from Gilson GJ, Mosher MD, Conrad KP. Systemic hemodynamics and oxygen transport during pregnancy in chronically instrumented, conscious rats. *Am J Physiol.* 1992; 263: H1911-H1918.

Figure 2. Relaxin Administration Mimics the Renal and Systemic Vasodilation of Pregnancy. ?, not tested. +/-, chronic, but not acute relaxin administration increased GFR in humans. See text for details and supporting references.

Figure 3. Working Model of Relaxin Sustained Vasodilatory Response. The precise localization of VEGF and PGF in the relaxin vasodilatory pathway is currently unknown, but two possibilities are depicted (?): relaxin may increase expression of angiogenic growth factor(s) in the arterial wall and/or release them from the extracellular matrix via MMP-9 or -2. Inhibitors of pregnancy- and/or relaxin-induced vasodilation are shown in the boxes. ET, endothelin; MMP, matrix metalloproteinase; ECM, extracellular matrix; RBF, renal blood flow; GFR,
glomerular filtration rate; RXFP, relaxin/insulin-like family peptide receptors; SU5416, vascular endothelial growth factor receptor tyrosine kinase inhibitor; GM6001, a general MMP inhibitor; cyclic CTT, a specific peptide inhibitor of MMP-2; TIMP-2, tissue inhibitor of metalloproteinase; RES-701-1, a specific ET$_B$ receptor antagonist; SB209670, a mixed ET$_A$ and ET$_B$ receptor antagonist; L-NAME, L-N$^G$-nitroarginine methyl ester; L-NMMA, N$^G$-monomethyl-L-arginine. Note that RXFP2 knockout (in mice), STT (control peptide for cyclic CTT), heat inactivated TIMP-2, BQ-123 (a specific ET$_A$ receptor antagonist), phosphoramidon (an inhibitor of the classical endothelin converting enzyme), D-NAME and isotype-matched IgGs (controls for neutralizing antibodies) did not affect the sustained vasodilatory responses to relaxin. See text for details and supporting references. Modified from McGuane JT, Danielson LA, Debrah JE, Rubin JP, Novak J, Conrad KP. Angiogenic growth factors are new and essential players in the relaxin vasodilatory pathway in rodents and humans. Hypertension, in press.

Figure 4. Working Model of Rapid Stimulation of NO Production by Relaxin in Endothelial Cells. Relaxin activates RXFP1, leading to G$_{i3}$ activation and dissociation of the corresponding $\beta\gamma$ subunits (blocked by PTX), which in turn activates the class IB PI3K$_Y$. PI3K$_Y$ phosphorylates phosphatidylinositol lipids in inner leaflet of the cell membrane (not shown; blocked by LY294002 and wortmannin), which ultimately promotes phosphorylation of Akt (blocked by MK-2206) and eNOS, leading to NO generation (blocked by L-NAME/L-NMMA).
pathway shares common downstream effector components with the vascular endothelial growth factor (VEGF) pathway, which could explain the potentiation of rapid relaxin-induced vasodilation in the presence of SU5416. See text for details and supporting references. Modified from McGuane JT, Debrah JE, Sautina L, Rubin JP, Novak J, Segal MS, Conrad KP. Relaxin induces rapid dilation of rodent small renal and human subcutaneous arteries via PI3 kinase and nitric oxide. Endocrinology, in press.
Signal(s) of maternal and/or fetoplacental origin

- **↓ Afterload**
  - **↓** Total peripheral vascular resistance
  - **↑** Large artery compliance

- **↑** Sodium appetite and thirst, and renal retention of sodium and water

- **↑** Preload
  - **↓** Venous Compliance
  - **↑** Blood volume

- **↑** Cardiac Output

- **↑** Systemic oxygen transport

- **↑** Oxygen delivery to fetoplacental unit(s)

Figure 1
### Figure 2

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</tr>
<tr>
<td>Stroke Volume</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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</tr>
<tr>
<td>Systemic Vascular Resistance</td>
<td>↓</td>
<td>↓</td>
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<td>↓</td>
</tr>
<tr>
<td>Global Arterial Compliance</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
<td>↑</td>
</tr>
<tr>
<td>Ang II pressor response</td>
<td>↓</td>
<td>↓</td>
<td>?</td>
<td>↓</td>
</tr>
<tr>
<td>Glomerular Filtration Rate</td>
<td>↑</td>
<td>↑</td>
<td>+/-</td>
<td>↑</td>
</tr>
<tr>
<td>Renal Plasma Flow</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Renal Vascular Resistance</td>
<td>↓</td>
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<tr>
<td>Ang II renal vasoconstriction</td>
<td>?</td>
<td>↓</td>
<td>?</td>
<td>↓</td>
</tr>
<tr>
<td>Myogenic Constriction (ex vivo or in vitro)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum Osmolality</td>
<td>↓</td>
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</tr>
</tbody>
</table>
PREGNANCY/RELAXIN

VEGF & PGF 

- SU5416
- Neutralizing antibodies

Prepro-ET 

- Endothelial removal
- L-NAME/L-NMMA

Big ET 

- ECM release

RXFP1 

- RXFP1 knock-out mice

Vascular MMP-2 

- GM6001
- Cyclic CTT
- TIMP-2
- Neutralizing antibodies

Vascular MMP-9 

ET_{1-32} 

- ET_B receptor knock-out rats
- RES-701-1
- SB209670

Myogenic Constriction 
(Mouse, Rat and Human Small Arteries)

GFR 
(Conscious Rats)

RPF 

Figure 3
Figure 4