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Relaxin in Human Pregnancy

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Abstract

Relaxin has beneficial effects upon the endometrium which are responsible for establishment of pregnancy. We have demonstrated that relaxin stimulates endometrial decidualization, the structural and biochemical changes in endometrial parenchymal cells, and the accompanying angiogenesis, modulation of matrix metalloproteinase activity and increased concentration in local immune cells which are required for implantation Our recent data also demonstrate that either too much or too little relaxin can be detrimental. Elevated circulating maternal relaxin concentrations (hyperrelaxinemia) are associated with premature birth. This is likely due to the effects of relaxin at the level of the cervix, via upsetting the balance in the maintenance of cervical connective tissue architecture. In addition, the absence of circulating relaxin during pregnancy in women may have negative consequences upon glucose metabolism.

Keywords

relaxin; rhesus monkey; endometrium; human pregnancy; preterm birth

The physiological roles of relaxin in women remain poorly understood. This is largely due to the paucity of *in vivo* studies in women or in suitable non-human primate models. Extensive data document dramatic species differences in the physiology of relaxin.[1] These differences include 1-sources of relaxin production, 2-patterns of circulating relaxin, 3-target tissues of relaxin actions and 4-amino acid homology. Thus, the assumption that conclusions from studies performed in other species are applicable to humans, without verification in a suitable human model, is unjustified. In addition, the establishment and maintenance of pregnancy is more complex in human and non-human primate species than in other species, with many redundancies and many more factors involved. In our studies, we have used several models of human pregnancy to elucidate actions of relaxin heretofore not recognized in human reproduction. Results from a rhesus monkey model of early human pregnancy, in which the actions of physiological concentrations of relaxin were studied, clearly demonstrate that relaxin has important, beneficial effects upon the endometrium which are responsible for establishment of pregnancy.[2] We have also demonstrated that elevated circulating maternal relaxin concentrations in women are deleterious, associated with premature birth.[3] Furthermore, our data also indicate that the absence of circulating relaxin during pregnancy in women may have negative consequences upon glucose metabolism. The effects of relaxin during human pregnancy may depend upon the amount of circulating hormone and the local endocrinological milieu.

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It is now clear that relaxin is a significant endometrial factor in women which is important in preparing the endometrium for early pregnancy. Maintenance of early pregnancy requires transformation of the endometrium into decidua. Decidualization refers to the collective structural and physiological changes which the endometrium undergoes to ensure maintenance of early pregnancy and its continuance. The end result is the formation of a functionally distinct tissue which develops from endometrial cell differentiation and influx of lymphoid cells. The morphologic changes which occur are independent of the trophoblast and do not require estrogen.[4] These changes include increased vascularization, and characteristic changes in morphological appearance and secretory products of the cells. Abundant data which support a role for relaxin in human endometrial decidualization have been generated in *in vitro* models.[5] Relaxin is an extremely potent stimulator of the secretion of various hormones/growth factors including insulin-like growth factor binding protein and prolactin, hallmarks of decidualization, in progesterone primed human endometrial stromal cells.[5]

In women, pregnancy can be established and maintained in the absence of circulating relaxin. Thus, local synthesis is necessary if relaxin plays a physiological role in human endometrial function. Relaxin protein and prorelaxin C peptide have been localized to human endometrium. However, this could be due to sequestration from circulating levels. Definitive evidence for relaxin synthesis in human endometrium required demonstration of relaxin specific mRNA and mature relaxin protein in the same cells. We assessed relaxin specific mRNA in cultured defined human endometrial cells using RT-PCR and determined relaxin protein in spent medium from these cultured cells by our sensitive, human relaxin specific radioimmunoassay. Relaxin specific mRNA and relaxin protein were detected in cultured cells (both stromal and glandular epithelial cell types) and media respectively, demonstrating definitive synthesis of relaxin in human endometrial cells.[6]

To verify that relaxin mRNA is expressed in human endometrium in situ, we identified relaxin mRNA by RT-PCR in endometrial tissues from 10 regularly menstruating women, 22–36 years old, taken from each woman at two time points during an ovulatory cycle 1) 11–12 days after the start of menses (proliferative phase) and 2) 8–9 days after an LH surge (secretory phase). Relaxin specific mRNA was detected from all 10 subjects in proliferative phase endometrial tissues and in 7 of the corresponding secretory phase samples.

Results from the early work of Hisaw and colleagues, using a rhesus monkey model, were the first to suggest a role for relaxin in endometrial vascularization and differentiation of endometrial stromal cells into predecidual cells.[7,8] However, these studies were severely limited since relaxin-containing tissue extracts were used, rather than pure relaxin protein.

To elucidate the role of relaxin in human endometrial function, we established an ovariectomized, steroid-primed, rhesus monkey (*Macaca mulatta*) model of early human pregnancy. In this model, the steroidal milieu of early pregnancy was recapitulated using estradiol and progesterone-filled silastic capsules, as described in detail.[2] Animals were randomized to either control (vehicle treatment) or relaxin treatment groups. Injection of human relaxin achieved circulating relaxin levels of 0.56 to 1.06 ng/ml, approximately the same as levels detected during early human pregnancy. This is a successful model for determination of the specific effects of relaxin upon various target organs, advantageous in that relaxin is the only variable; estrogen and progesterone levels are equivalent in both groups of monkeys. Thus, it can be definitively concluded that effects observed are due only to the action of relaxin. Many significant effects of relaxin upon the cervix, endometrium, myometrium and mammary gland were observed, changes seen in early pregnancy in women.

Relaxin caused dramatic changes in multiple morphological and biochemical aspects of endometrial function. The morphology of the endometrium of the control animals revealed the histological appearance of secretory phase endometrium, expected in view of the steroid hormone levels achieved. Endometrium of the relaxin-treated animals resembled a generally more decidualized morphology with larger stromal cells, greater cytoplasmic/nuclear ratios, and more periarteriolar cells. Quantitative morphometric analyses demonstrated that the endometrium of the relaxin-treated animals had significantly greater number of arterioles and a significantly greater number of total lymphocytes than endometrium from the control animals.[2] Relaxin selectively increased the number of neutrophils, CD56 positive (uterine NK) cells, and CD68 positive cells (macrophages) in the endometrium and had no effects upon CD3 positive cells (T lymphocytes).

The stimulatory effects of relaxin upon specific lymphocyte cell types are very important actions since uterine NK cells are critical for spiral artery remodeling and produce various cytokines, angiogenic factors and nitric oxide synthase, necessary for implantation and maintenance of pregnancy.[9] Evidence in uNK deficient mice demonstrating fetal loss associated with progressive changes in the maternal uterine arterioles, implicate uNK cells are required for pregnancy success.[10] Recent data demonstrate that human uNK cells control trophoblast invasion.[11] Endometrial uNK number is not regulated by estradiol *in vitro* and endometrial uNK cells do not contain receptors for estrogen receptor alpha or either isoform of progesterone receptor.[12] Thus relaxin may be the primary regulator of resident uNK cell number.

Estrogen and progesterone are essential regulators of endometrial development and required for successful pregnancy maintenance. In our rhesus monkey model, Relaxin significantly inhibits protein levels of endometrial estrogen receptor (ER) alpha, and has no effect upon ER beta.[2] Relaxin significantly inhibits protein levels of endometrial progesterone receptor (PR) B and PR A.[2] The ability of relaxin to inhibit endometrial levels of mature ER alpha, and both PRB and A proteins may explain the decline in human endometrial levels of ER and PR in the secretory phase of the cycle in women, at the time when circulating levels of relaxin are rising.

Endometrial connective tissue integrity, required for decidualization and early pregnancy maintenance, is regulated by a balance between the maintenance of type I collagen and its degradation. Relaxin significantly inhibits levels of endometrial proMMP-1 and proMMP-3 while it increases levels of the endogenous inhibitor TIMP-1. Relaxin is therefore a negative regulator of MMP expression in endometrium.[2] Although the roles of MMPs and TIMPs in early pregnancy are not well worked out, TIMPs appear to play an important role in maintaining the integrity of endometrial tissue and of blood vessels. In women, TIMPs are expressed maximally in decidualized stroma compared to endometrial levels in menstrual cycle, supporting the concept that the maternal decidua can control invasion of embryonic trophoblast. The significant stimulation of endometrial TIMP-1 by relaxin are especially important since neither estrogen nor progesterone regulate human endometrial TIMP-1.[13]

In addition, relaxin negatively regulates endometrial matrix metalloproteinase levels in human endometrial cells as it does in the rhesus monkey *in vivo*. We studied the effect of relaxin upon MMP expression from primary cultures of human endometrial cells in collaboration with Dr. Linda Tseng. Consistent with our *in vivo* data, relaxin significantly inhibits proMMP-1 expression from both glandular epithelial cells and from stromal cells taken during the secretory phase of the cycle.[6] That relaxin preserves endometrial connective tissue integrity supports our hypothesis that relaxin plays an important role in maintenance of early pregnancy. The integrity of connective tissue architecture requires a precise balance between the action of matrix metalloproteinases (MMPs), which degrade the

extracellular matrix, and the endogenous tissue inhibitors of metalloproteinases, which regulate the activity of the metalloproteinases. Relaxin has been shown to directly stimulate MMP activity and MMP protein and mRNA levels in various target organs from species including rat, pig and human.[1] Relaxin negatively regulates TIMP-1 levels as well.[1] In contrast, in endometrium, relaxin increases TIMP-1 and inhibits MMP-1 and MMP-3, thus negatively regulating MMPs.

That the effects of relaxin upon human endometrium are likely to be direct effects is suggested by data from our laboratory and others which demonstrate expression of the LGR7 relaxin receptor in endometrial stroma and glandular epithelial cells.

We have also utilized two human *in vivo* models for study of the actions of relaxin during human pregnancy. Our first model consisted of women who have highly elevated maternal circulating relaxin concentrations for the entire duration of gestation. Serum relaxin concentrations in pregnant patients who had controlled ovarian hyperstimulation are substantially higher than those of normal controls, even in singleton pregnancies. Mean relaxin levels in singleton pregnancies after controlled ovarian hyperstimulation followed by in vitro fertilization (*IVF*) are more than double the mean in normal non-stimulated patients with singleton pregnancies.[3] These highly elevated levels of circulating relaxin in women with singleton pregnancies after controlled ovarian hyperstimulation are maintained throughout the entire duration of the pregnancies. The reason for the higher relaxin concentrations in these patients is the larger number of corpora lutea produced, resulting in a larger mass of relaxin-secreting tissue. Only the relaxin secreted from corpora lutea is detected peripherally, that produced by endometrium, decidua and placenta is only detected locally. We hypothesized that hyperrelaxinemia caused by controlled ovarian hyperstimulation results in an increased rate of the risk of premature labor and preterm birth.[3] To test this hypothesis, we studied the risk of premature labor and preterm birth in two groups of women: 1) women achieving pregnancy after ovarian stimulation (n=114) and 2) women achieving pregnancy without treatment (n=37). Serum obtained at 6–12 weeks gestational age was assessed for relaxin using a specific human ELISA. The outcomes of the pregnancies were recorded. Hyperrelaxinemia was defined as levels greater than 3 standard deviations above the weighted mean of levels in normal unstimulated singleton pregnancies at 6–12 weeks gestation (1.18 ng/ml). A significant positive association was found between prematurity risk or premature delivery and elevated circulating maternal relaxin concentrations in women having singleton pregnancies following controlled ovarian hyperstimulation. An increase of 5 ng/ml in circulating maternal relaxin concentrations doubled the risk of prematurity. Levels greater than 16 ng/ml in women having controlled ovarian hyperstimulation singleton pregnancies and levels greater than 7 ng/ml in women who had multiple gestations predicted prematurity risk or premature delivery in 50% of the women. In summary, relaxin levels are increased in women destined to deliver prematurely. Relaxin affects the cervix to facilitate softening and ripening. Relaxin is a positive regulator of matrix metalloproteinases in human cervical fibroblasts in vitro. This may be the mechanism by which relaxin is related to prematurity.

Our second human pregnancy model consisted of women who have undetectable circulating relaxin concentrations throughout pregnancy. These women have premature ovarian failure who achieved singleton pregnancy via oocyte donation and thus have no corpora lutea and consequently no detectable circulating relaxin. We used these women and our model of hyperrelaxinemic women to address the significance of previous findings demonstrating that relaxin enhances insulin action. The affinity of insulin binding to adipocytes from either mature female rats or women is enhanced in the presence of relaxin, suggesting that relaxin may protect against gestational diabetes in pregnancy.[14] We therefore hypothesized that

circulating relaxin concentrations correlate with insulin response to glucose in pregnant women. In this study, three groups of women were enrolled:

1. Women with normal spontaneous singleton pregnancies who served as controls. In this group, circulating Relaxin concentrations were 0.92±0.08 [M±SE] ng/ml.
2. Aluteal women with no ovarian function who achieved singleton pregnancy via oocyte donation. In these women circulating concentrations of Relaxin were undetectable (< 35 pg/ml).
3. Hyperrelaxinemic women with singleton pregnancies following controlled ovarian hyperstimulation. In these women, relaxin levels were greater than the mean plus three standard deviations of the mean in the control group, i.e. greater than 1.79 ng/ml. At 28 weeks gestation, glucose tolerance tests after an overnight fast were performed in which a 100 gm glucose bolus was ingested. Serum samples were collected at baseline and at 1, 2 and 3 hours after glucose ingestion. Serum levels of insulin, glucose and relaxin were determined. Circulating relaxin levels negatively correlated with fasting insulin levels (i.e. fasting insulin levels were lower in the hyperrelaxinemic group). Insulin sensitivity was higher in women with hyperrelaxinemia and the absence of circulating relaxin was associated with decreased insulin sensitivity and increased insulin resistance. Relaxin actions have been shown to be antagonistic to those of progesterone. Certain actions of progesterone are beneficial, whereas certain actions of progesterone are deleterious. Progesterone promotes insulin resistance and relaxin may serve to counter-balance this negative effect of progesterone.

SUMMARY

Relaxin has pronounced effects upon endometrial function which support our hypothesis that relaxin is an important player in early pregnancy maintenance. These novel actions of relaxin include increased arteriole number, increased number of lymphocytes, neutrophils, macrophages and uNK cells, inhibition of ER alpha and PR isoforms, and inhibition of MMP-1 and stimulation of TIMP-1. In addition, we have shown that relaxin is synthesized by human endometrium and that several endometrial cell types express relaxin receptors. In concert, these findings demonstrate that relaxin promotes decidualization and is an important endometrial/decidual angiogenic factor. In women, elevated concentrations of maternal relaxin during pregnancy are associated with preterm birth and the lack of circulating relaxin is associated with increased insulin resistance.

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