CHAPTER 4

Relaxin Physiology in the Female Reproductive Tract during Pregnancy

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Abstract

The characteristic functions of relaxin are associated with female reproductive tract physiology. These include the regulation of biochemical processes involved in remodeling the extracellular matrix of the cervix and vagina during pregnancy and rupture of the fetal membranes at term. Such modifications enable the young to move unimpeded through the birth canal and prevent dystocia. However, relaxin's physiological actions are not limited to late gestation. New functions for this peptide hormone in implantation and placentation are also emerging. Relaxin promotes uterine and placental growth and influences vascular development and proliferation in the endometrium. This chapter provides an overview of the current literature on relaxin physiology in the uterus, cervix and vagina of pregnant females and the impact on fetal health. It also outlines the potential mechanisms of relaxin action, particularly in the cervical extracellular matrix and uterine endometrium.

Introduction

A role for the peptide hormone relaxin in female reproductive tract physiology was first described by Hisaw in 1926. He injected serum from pregnant guinea pigs into virgin guinea pigs and observed a relaxation of the pubic symphysis.[1] This allows the two innominate bones to widen and facilitate passage of the fetus with its relatively large head through the pelvic girdle. These experiments generated the concept that relaxin was an important hormone for successful parturition and delivery of live young. It was not until the 1970s, when highly purified porcine and rat relaxin became available, that experiments could begin to investigate the physiological effects of relaxin treatment in the female reproductive tract (reviewed in ref. 2). Many studies used ovariectomized pigs and rats, with hormone replacement paradigms. In the 1980s, Sherwood and colleagues developed a monoclonal antibody to rat relaxin (MCA1) and used it to neutralize endogenous relaxin in pregnant rats. Both these approaches showed that in the absence of relaxin there were substantial delays in the onset of labor, prolonged duration of delivery and a greater incidence of neonate mortality at birth.[3-5] Relaxin gene knockout mice ($Rln1^{-/-}$) were developed by Zhao and colleagues in 1999,[6] and provided an equally valuable tool to further investigate the role of relaxin in reproductive tract physiology. The $Rln1^{-/-}$ females give birth to live young without any apparent sign of dystocia, despite having abnormal cervical and vaginal morphology and no elongation of the pubic symphysis.[6] This illustrates the complexity in the study of relaxin physiology. There is considerable variation in the sources and secretion of relaxin during pregnancy, as well as the localization of the receptors for relaxin. Many actions of relaxin are specific to certain species and to date, there are no obvious clinical conditions in pregnant women associated with

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relaxin deficiency.[7,8] However, relaxin treatment in pregnant animals clearly has several effects that could be perceived as beneficial for a successful pregnancy as well as facilitating the process of labor. This review outlines the current literature on relaxin physiology in the cervix, vagina and uterus of pregnant females, with an emphasis on the potential mechanisms of relaxin action in each tissue.

Relaxin Receptors

The receptor for relaxin is a leucine-rich repeat containing, heterotrimeric guanine nucleotide binding (G-protein)-coupled receptor (GPCR) known as LGR7,[9-11] recently assigned the nomenclature RXFP1.[12] Activation of LGR7 by its ligand stimulates a G_s-cAMP-protein kinase A-dependent signaling pathway.[13,14] Localization of relaxin receptors in the female reproductive tract initially relied on biotinylated porcine relaxin and radiolabeled ligand. Relaxin binding sites were found mainly in epithelial cells in the cervix and vagina of rats and pigs,[15,16] and the myometrium of the rat uterus.[17-19] Once the human, mouse and rat relaxin receptors (LGR7/Lgr7) were cloned,[9,11,20] researchers were able to analyze the expression and localization of Lgr7 more accurately. In the mouse, staining for Lgr7-specific β-galactosidase activity was identified underneath the basal layer of the vaginal epithelium and in the circular layer of the myometrium.[21,22] We have shown that Lgr7 mRNA is predominantly expressed in the myometrium compared with the endometrium or placenta of pregnant mice, with a down-regulation in myometrial Lgr7 at term.[23,24] The receptor is also highly expressed in the cervix of pregnant mice, but surprisingly, there is no surge in Lgr7 mRNA concentrations in the later stages of gestation to coincide with the dramatic changes in stromal extracellular matrix remodeling. Using in situ hybridization, we localized Lgr7 mRNA in the mouse cervix and vagina to the stromal tissue underlying the basal layer of epithelial cells (Fig. 1). Lgr7 was not highly expressed within the epithelium, which contradicts data from previous studies in the rat and pig.[15,16] However, the luminal epithelium is the predominant cell type in the human cervix and vagina (obtained from premenopausal hysterectomy patients) that expresses relaxin binding sites.[25] Relaxin also binds to the circular and longitudinal smooth muscle layers and vascular smooth muscle cells associated with blood vessels in the human cervix and vagina.[25]

The pattern of uterine LGR7 expression in primates and humans is very similar to the cervix and vagina but there is no consensus between studies on the predominant region expressing relaxin receptors. Immunoreactive LGR7 has been localized to the endometrial stromal and epithelial compartments in human and marmoset monkey uterus,[26,27] with more intense staining in the secretory phase of the cycle. Autoradiography studies contradict this finding and show ^{32}P-labeled human relaxin predominantly in the glandular and luminal epithelium (Fig. 2A in ref. 28). Furthermore, LGR7 mRNA expression is significantly higher in isolated human glandular epithelial and decidual cells compared with endometrial stromal cells.[29] Localization of biotinylated porcine relaxin in the uterus of hysterectomized women was similarly restricted to luminal and glandular epithelial cells,[25] although a few cells in the stromal extracellular matrix were also positive for relaxin binding sites. Both human relaxin binding sites and LGR7 mRNA in the endometrium are markedly up-regulated during the early secretory phase of the cycle (Fig. 2B in ref. 28). Only one study has identified LGR7 expression in human cultured myometrial cells,[26] although biotinylated porcine relaxin binding was shown in the myometrium of the marmoset monkey[30] and human.[25] Another proven target tissue for relaxin action in humans is the fetal membranes of late gestation. Initial studies with biotinylated porcine relaxin revealed prominent labeling in the amnion epithelial cells and placental villi projecting into the lacuna system, shown at high magnification to be in the syncytiotrophoblast cells.[25] The recent work of Lowndes et al[31] demonstrated specific LGR7 gene transcripts and immunoreactive LGR7 in the human decidua and chorionic cytotrophoblast, with very low expression of the receptor in the amniotic epithelium. The differences between studies may be explained by the variety of techniques used and issues associated with specificity of antibodies, biotinylated molecules and radiolabeled ligands, although all studies cited include appropriate negative controls. Another explanation is that these discrepancies may be due to the expression of various LGR7 splice variants which may or may not be functional.[32] Future LGR7

localization studies should include concurrent gene and protein analysis of the known full-length functional LGR7 variant to resolve the questions related to temporal and spatial expression of these receptors in the human uterus throughout the cycle and during pregnancy.

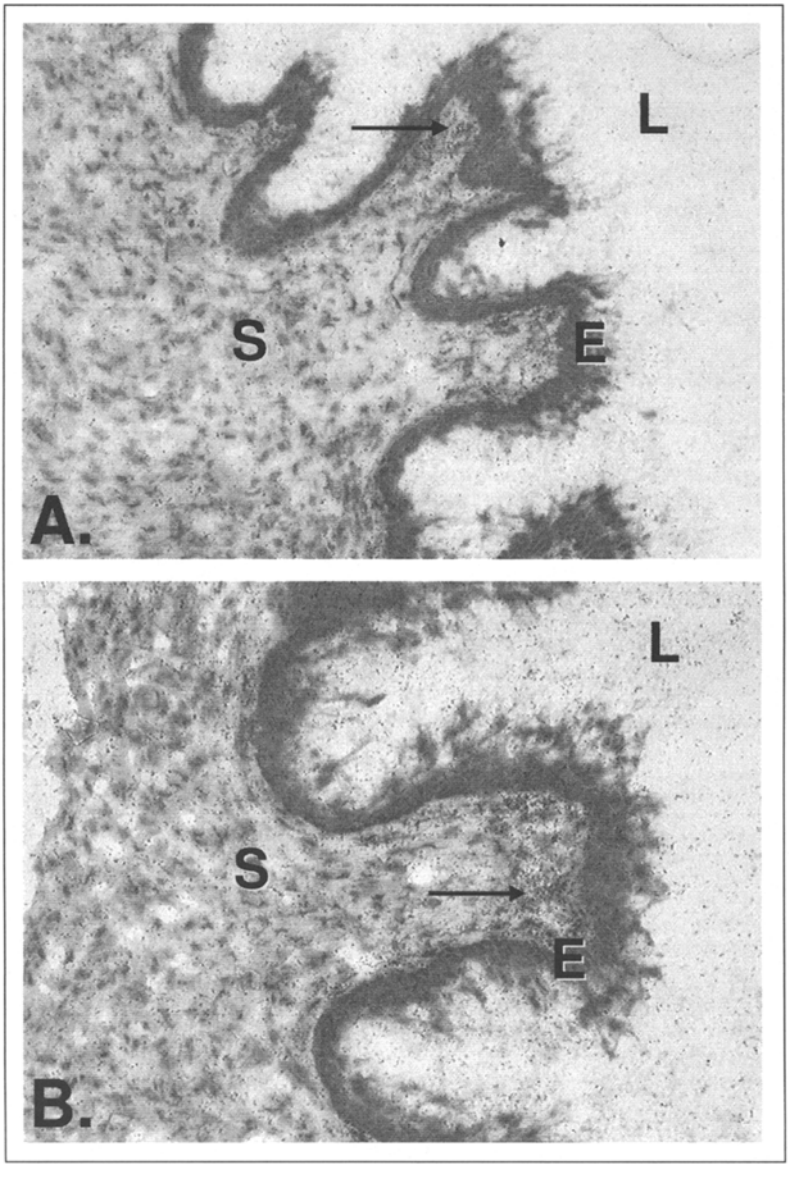

Figure 1. Localization of Lgr7 mRNA by in situ hybridization in the cervix (A) and vagina (B) of $Rln1^{+/+}$ mice on day 14.5 gestation. Positive hybridization signals (arrow) are present in the stromal tissue underlying the basal layer of epithelial cells. S, stroma; E, epithelium; L, lumen.

Effects of Relaxin on the Cervix

Several functional studies in rodents and pigs have demonstrated an important role for relaxin in the progressive softening of the cervix in the second half of gestation. There is a substantial reduction in cervical wet weight and extensibility in relaxin-deficient rats, with a higher incidence of neonate mortality.[3-5,33,34] Rln1$^{-/-}$ and Lgr7$^{-/-}$ mice also have abnormal cervical morphology but are able to give birth to live young.[6,21,22] The most obvious cervical phenotypes in pregnant Rln1$^{-/-}$ mice are the increased density of stromal extracellular matrix (particularly collagen) and a lack of epithelial proliferation (Fig. 3). These phenotypes are reversed by treatment with exogenous relaxin.[35] Morphological changes in the cervix induced by relaxin treatment are the increased area of luminal

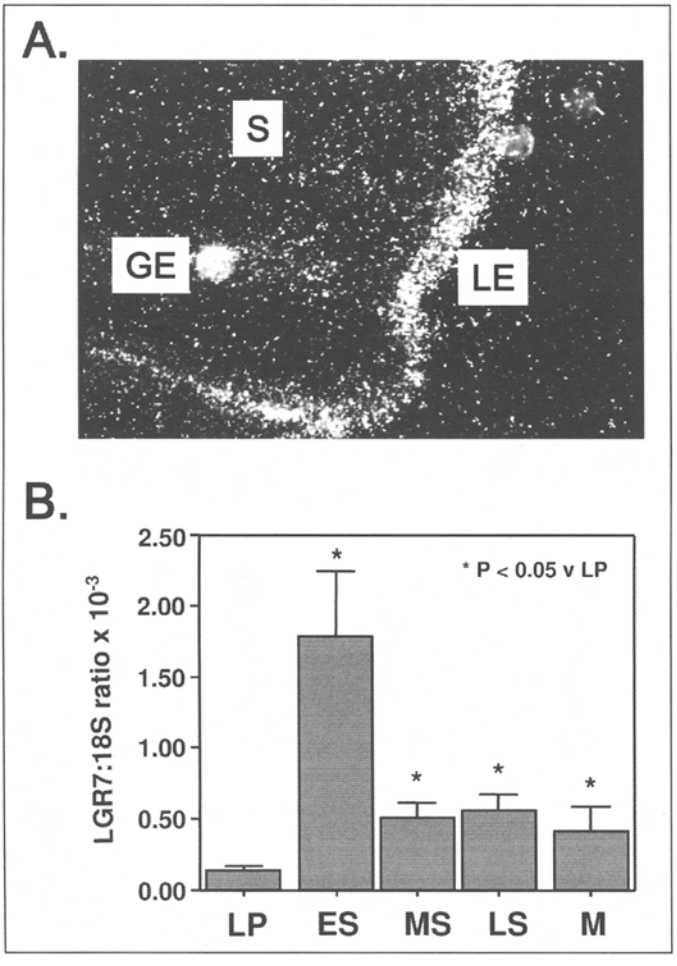

Figure 2. A) Autoradiographic localization of [^{33}P]-relaxin binding sites using photographic emulsion on a slide mounted section of human uterus obtained during the early to mid secretory phase. The dark field image shows silver grains in the glandular (GE) and luminal epithelial (LE) cells of the endometrium. B) LGR7 mRNA concentrations in the endometrium at five phases of the menstrual cycle. EP: early proliferative, ES: early secretory, MS: mid secretory, LS: late secretory, M: menstrual phase of the cycle. Adapted with permission from C.P. Bond et al. J Clin Endocr Metab; 89:3477-3485. ©2004 The Endocrine Society.

involutions and dispersal of collagen fibers, particularly in these involutions. There is also a marked proliferation of the epithelium, greater numbers of vacuolated epithelial cells and a large increase in the amount of a mucopolysaccharide lining the epithelium.[35] Similar findings were described in the ovariectomized rat and pig relaxin-replacement models, but in addition, relaxin increased the percentage hydration and glycosaminoglycan content in the cervix.[36,37]

Current theories on the mechanisms of relaxin action in the cervix focus predominantly on collagen dispersal and/or degradation. Relaxin treatment has little effect on cervical collagen content in pregnant ovariectomized rats or pigs.[36,38] Similarly, there is no difference in the percent-

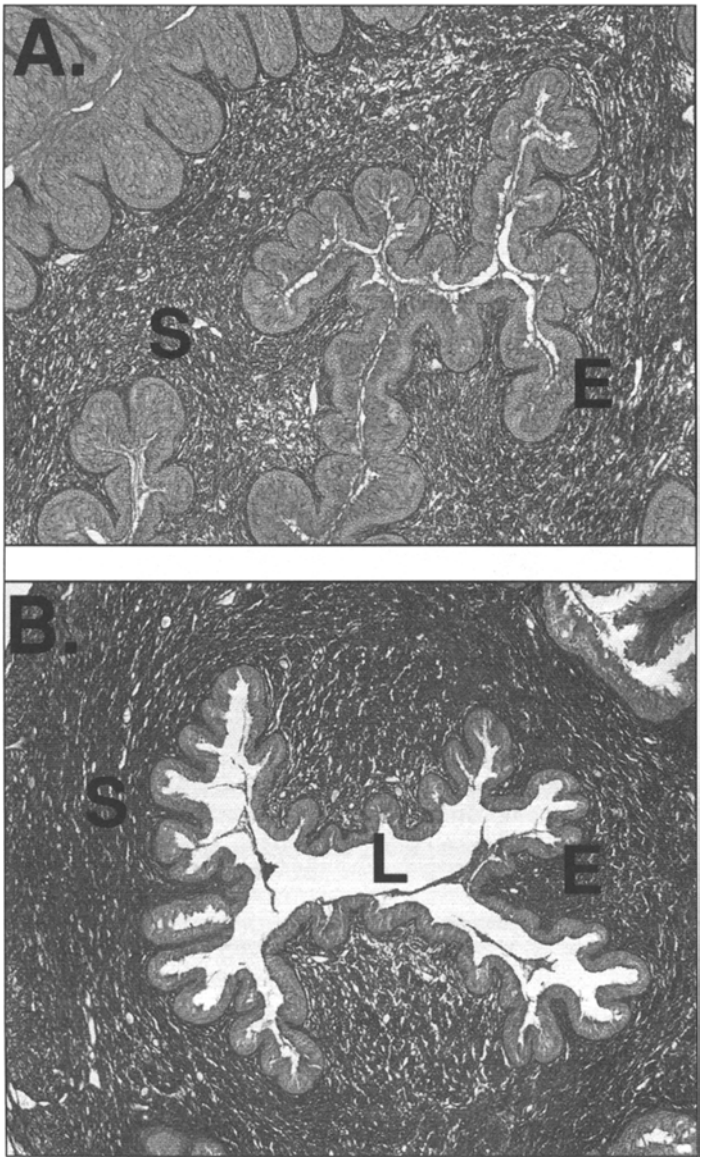

Figure 3. Collagen fibre density in the cervix of pregnant (A) $Rln1^{+/+}$ and (B) $Rln1^{-/-}$ mice on day 16.5 gestation. S, stroma; E, epithelium; L, lumen.

Table 1. The in vivo effects of relaxin deficiency and relaxin treatment on collagen and matrix metalloproteinase (MMP) gene expression in the cervix of late pregnant Rln1$^{-/-}$ mice

	Rln1$^{+/+}$		*Rln1$^{-/-}$ v Rln1$^{+/+}$*	*Rln1$^{-/-}$ + Relaxin*
	Gestation	*Term*		
Collagen I	↑	↓	↑ (day 18.5 only)	↓ on d18.5
Collagen III	↑	↓	↑ (day 18.5 only)	*Not measured*
TGFβ-1	↑	↓	↔	↔
MMP-13	↓	↓	↑ (highest at term)	↓ on d18.5
MMP-3	↑	↔	↑ (day 18.5 only)	*Not measured*
MMP-7	↓	↓	↑	*Not measured*
MMP-9	↓	↓	↑	*Not measured*
MMP-2	↑	↑	↔	↔

age hydroxyproline content in the cervix of late pregnant Rln1$^{+/+}$ and Rln1$^{-/-}$ mice.[39] These data are explained, in part, by the increased expression of α_1(I) and α_1(III) collagen in the cervix throughout gestation in Rln1$^{+/+}$ and Rln1$^{-/-}$ mice. The only difference between the genotypes was observed on day 18.5 gestation when α_1(I) collagen mRNA levels decrease significantly in Rln1$^{+/+}$ mice but remain high in Rln1$^{-/-}$ mice.[35] These data suggest that de novo collagen is synthesized in increasing quantities in the cervix throughout gestation and that a lack of relaxin does not result in abnormally high amounts of collagen in these tissues. However, administration of human relaxin to pregnant Rln1$^{-/-}$ mice caused a significant decrease in α_1(I) collagen gene expression in the cervix on day 18.5 gestation,[35] demonstrating the ability of exogenous relaxin to reduce collagen synthesis. Relaxin may, therefore, be capable of suppressing collagen synthesis by a direct action on cervical fibroblasts.

The other common hypothesis of relaxin action involves activation of collagen degrading enzymes in the extracellular matrix. Relaxin stimulates matrix metalloproteinase (MMP)-1 activity in cultured guinea pig cervical cells,[40] and MMP-1, gelatinases and stromelysin-1 in human lower uterine segment fibroblasts[41] and normal human cervical stromal cells.[42] These results using in vitro cell culture systems demonstrated that relaxin is a positive regulator of MMP expression. But in the cervix of wild type mice, there is no correlation between increased MMP expression and changes in tissue architecture. Only the gelatinase MMP-2 mRNA levels are greater at term compared with earlier stages of gestation. There are significant decreases in MMP-13 and MMP-7 expression and no change in MMP-9 and MMP-3. The situation in pregnant Rln1$^{-/-}$ mice is reversed. Expression of all MMPs examined, except MMP-2, is significantly higher compared with Rln1$^{+/+}$ mice (Table 1). Despite this increased level of MMP expression in the cervix of Rln1$^{-/-}$ mice, there is no clear histological evidence of collagen degradation in this tissue. Interestingly, when pregnant Rln1$^{-/-}$ mice are treated exogenously with a chronic infusion of human relaxin, there are significant decreases in cervical MMP-13 gene expression and no effects on MMP-2.[35] These findings are not dissimilar from earlier work in the pig cervix which described negative effects of relaxin treatment on tissue-associated MMP-2 and MMP-9 activity and no difference in gelatinase protein expression between control and relaxin treated animals.[43] Recent data in the rhesus monkey demonstrated that relaxin negatively regulates endometrial MMP-1 and MMP-3 protein expression in vivo.[44] These data are in contrast to the relaxin-induced increases in MMP-2 reported in nonreproductive tissues.[45-47] One problem with this data is that it only demonstrates MMP activity. But this gives no measure of the interaction between relaxin and MMP production, so the interpretation is limited to an association with MMP activation. The one exception is the work of Conrad and colleagues who clearly demonstrated the positive effects of relaxin treatment in male rats on MMP-2 gene and protein expression and MMP-2 activity in small renal arteries.[48,49]

The processes by which MMPs regulate the extracellular matrix are complex and multifactorial. One aspect is the role of tissue inhibitors of metalloproteinases (TIMPs), which directly regulate MMP activity. In the pig uterine cervix, relaxin enhanced expression of TIMP-1 and TIMP-2, whereas expression of both TIMPs in the vaginal cervix did not differ between control and relaxin-treated animals.[50] Relaxin treatment also increases TIMP-1 in the endometrium of the rhesus monkey.[44] The expression of TIMPs in reproductive tissues of Rln1$^{-/-}$ mice has not been assessed, but perhaps inhibitor activity of TIMPs is enhanced. In summary, relaxin's interactions with MMPs remain an area of controversy but in the in vivo data do not support a stimulatory effect of relaxin on MMP expression in reproductive tissues. It is, therefore, unlikely that relaxin acts via MMPs to reduce the density of collagen fibers in the cervix.

Structural changes in the cervix during pregnancy are not limited to the extracellular matrix. The proliferative activity of the cervical epithelium increases at two well-differentiated time points in the pregnant rat.[51,52] The first occurs in mid-gestation and the second close to delivery. The effect of this cell proliferation is to increase the height of the luminal epithelium. In the cervical stroma, cell proliferation is generally much lower compared with the epithelium, but there is a net increase in cell number because very few cells undergo apoptosis.[52] This cervical cell proliferation is attributed, in part, to a decrease in the rate of programmed cell death. The rate of apoptosis in both cervical compartments varies between stages of pregnancy. Lee et al[53] showed that the apoptotic index in the cervical epithelium was approximately 2% in early gestation in the rat, declined to approximately 0.5% in mid-late gestation and increased dramatically to 18% by the second day after delivery. Similarly, Ramos et al[52] reported that the apoptotic activity in the cervical epithelium never exceeded 1.8%, with the highest scores for programmed cell death on day 5 and the lowest indices between days 13 and 23. A dramatic increase in epithelial apoptosis was observed on the day after parturition, reaching values of 9%.[52] Stromal compartments had increased apoptotic indices postpartum, as seen in the epithelium. But the apoptotic rate in the cervical stroma was always lower than in the epithelium and was not observed in endothelial cells. These data strengthen the current hypothesis that apoptosis plays a major role in regulating cervical epithelial and stromal cell proliferation during pregnancy.

Relaxin plays an important function is stimulating cell proliferation and reducing apoptosis in the cervical epithelium and stroma during late pregnancy.[53,54] It promotes a marked increase in the accumulation of new epithelial and stromal cells.[54] One explanation of these data is the direct effect of relaxin on programmed cell death. Immunoneutralization of endogenous relaxin in late pregnant rats with MCA1 increased the rate of apoptosis in cervical cells.[55] The effect was greatest in late pregnancy when the rates of apoptosis in cervical epithelial cells and stromal cells were up to 10-fold higher in MCA1-treated rats compared with controls.[53]

Effects of Relaxin on the Vagina

Many of the actions of relaxin in the vagina are similar to those in the cervix. Early studies demonstrated that relaxin promoted growth of the vagina in pregnant rats, mice and pigs (reviewed in ref. 2). This was shown by the increase in wet and dry weights and vaginal collagen content.[56] In both MCA1-treated rats and Rln1$^{-/-}$ mice, the collagen fibers do not disperse and there is a marked lack of epithelial proliferation.[39,55] Administration of relaxin to Rln1$^{-/-}$ mice reverses this phenotype and in particular stimulates a dramatic increase in epithelial cell number. The recent work of Sherwood and colleagues has clearly demonstrated that the increase in vaginal epithelial cell number in the late pregnant rat involves an inhibition of apoptosis.[55] A physiological role for relaxin in the vagina has not been defined as such, but the relaxin-induced morphological changes are likely to facilitate the process of parturition.

Effects of Relaxin on the Uterus

Relaxin has been dismissed as an important player in uterine physiology largely because pups of Rln1$^{-/-}$ and Lgr7$^{-/-}$ mice are born alive, with no delay and within normal birth weights.[6,21] Furthermore, women who become pregnant through ovum donation have normal pregnancies

Table 2. A summary of the putative factors involved in the different mechanisms of relaxin action in the uterus associated with angiogenesis, implantation and growth

	Stromal Cells	Epithelial Cells	Decidual Cells	Uterus
VEGF	↑[82,83]	↑ & ↓[83]		↑[100]
Interleukin-11	↑[77]			
IGF-I, IGF-II				↑ secretion only[66]
IGFBPs	↔ but ↑ with MPA[66,76,78]		↑[29,78]	↑ secretion only[66]
E-cadherin		↑[69]		↑[69]
Connexins				↑[68]
Prolactin	↑[75,77,78]		↑[78]	
ER alpha				↓[44] & ↔[24,71]
ER beta				↔[44] & ↓[24,71]
PR				↓[44]

despite having no circulating relaxin.[57] However, it is important to recognize that experiments involving administration of exogenous relaxin have yielded some important functional data. These actions of relaxin in the endometrium, myometrium and placenta have been reviewed extensively,[2,8,58] so this section will focus on the proposed mechanisms of relaxin action, particularly in the endometrium (Table 2).

Uterine Growth

The growth effects of relaxin on the uterus have been well described in many species. Relaxin causes an increase in water content, protein, collagen and glycogen concentration in the uteri of estrogen primed, nonpregnant rats and an increase in uterine weight.[59-62] In addition, relaxin promotes uterine growth in the prepubertal[63,64] and neonate pig.[65] There is evidence to suggest that the growth effects of relaxin in prepubertal pigs are mediated by insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs).[66,67] Uterine fluids collected from relaxin-treated gilts contained significantly higher amounts of IGF-I, IGF-II, IGFBP-2 and IGFBP-3 compared with controls.[66] However, relaxin administration did not alter IGF-I or-II gene expression in uterine tissue or systemic IGFs and IGFBPs. These data demonstrate a mechanism by which relaxin could contribute to uterine and conceptus growth in the early establishment of pregnancy. The work of Bagnell and colleagues in the prepubertal pig model also demonstrated relaxin-induced increases in gap junction proteins connexins and the glycoprotein E-cadherin, both thought to be important mediators of uterine growth and remodeling. Specifically, relaxin administration enhanced the expression of connexin-26, -32 and -43.[68] It was suggested by these authors that relaxin may mediate cell-cell communication between endothelial cells and the surrounding stroma and smooth muscle by increasing connexin protein expression. Relaxin-induced uterine growth in the prepubertal pig is also associated with a significant increase in epithelial cadherin (E-cadherin) protein and mRNA levels.[69] This calcium-dependent adhesion molecule is thought to mediate cell-to-cell recognition and maintain tissue integrity. The prepubertal pig is an interesting model because it lacks the local or systemic steroid hormones progesterone and estradiol. Therefore, it does not necessarily replicate the endocrine environment of pregnancy in many species. However, it has highlighted a number of novel mechanisms through which relaxin is capable of producing growth effects in the uterus.

In other animal models, relaxin's growth-promoting effects in the uterus are largely dependent on estrogen and progesterone. When administered with these steroids, relaxin stimulates growth by causing both cellular hyperplasia and hypertrophy.[70] More recent studies demonstrated that the uterotropic effects of relaxin are blocked by the specific estrogen receptor (ER)α antagonist ICI 182,780 in immature ovariectomized rats,[62] and that relaxin treatment decreases uterine ERβ

expression within 6 hours, but has no effect on ERα.[71] Treatment of $Rln1^{-/-}$ mice from day 12.5 gestation with a continuous infusion of recombinant human relaxin for 6 days has no effect on ERα gene expression but causes a significant down-regulation in ERβ expression and reverses the ERβ phenotype observed in $Rln1^{-/-}$ mice.[24] These data mirror the data of Pillai et al,[71] and support their idea that a down-regulation in ERβ expression by relaxin may be essential to allow for full activation and/or expression of ERα in the uterus. Several groups have reported that both ERβ1 and ERβ2 inhibit ERα-mediated transcriptional activity or signaling,[72,73] so a relaxin-induced down-regulation of ERβ may be a prerequisite for estrogen and other ER activators to stimulate their target tissues. However, this idea was recently challenged in ovariectomized rhesus monkeys that were primed with exogenous estradiol and progesterone in a manner that simulated a human menstrual cycle. Relaxin treatment significantly decreased uterine protein levels of ERα and both isoforms of the progesterone receptor, but had no effect on ERβ.[44] These authors concluded that relaxin may be responsible for the decline in endometrial expression of ERα and progesterone receptors that occurs during the late secretory phase of the human cycle. As with many responses to relaxin, there are large differences between species. It is possible that the variation in the response of ERs to relaxin treatment is due to the different experimental paradigms used, including stage of reproductive cycle and circulating steroid hormone levels. Furthermore, it is yet to be established how the peptide hormone activates steroid receptors in vivo.

Decidualization

Early studies using human endometrial stromal cells demonstrated that relaxin stimulates prolactin secretion after administration of relaxin.[74] Relaxin does not result in endometrial stromal cell growth, only increases prolactin production.[75] In order for relaxin to have an effect in stromal cells, a progestin (MPA) needs to be present. Relaxin also causes a transient stimulation of prolactin and IGFBP-1 mRNA within the endometrium. However, when relaxin was administered with MPA, higher mRNA levels were measured then if cells were treated with MPA alone or had MPA withdrawn.[76] More recent studies have further demonstrated relaxin's potential involvement in decidualization as treatment of human endometrial stromal cells with the hormone increases interleukin-11 mRNA expression and secretion via cAMP/protein kinase A pathways.[77] These authors proposed that relaxin acts in synergy with prostaglandin E2 to stimulate interleukin-11 production in the mid-late secretory phase of the cycle, before prolactin is detected and may therefore initiate endometrial cell differentiation. In other work, relaxin promotes induction of IGFBP-1 by binding to the cAMP regulatory element (CRE) in the IGFBP-1 promoter.[78] However, a progestin (MPA) needs to be administered together with relaxin,[78] or stromal cells need to be transfected with an LGR7 expression vector, in order to increase IGFBP-1 expression.[29] Tseng and colleagues also reported that relaxin increased the phosphorylation of CRE binding protein, indicating that relaxin activates the protein kinase A system. The complex nature of relaxin's interaction with the IGFBP-1 promoter is further demonstrated by studies using protein kinase A inhibitors. Relaxin-induced IGFBP-1 promoter activity was inhibited by the cAMP dependent protein kinase A inhibitor, H-89. Similarly, activation of prolactin by relaxin appears to be mediated through the region in the prolactin promoter containing multiple CCAAT/enhancer-binding proteins (C/EBP) binding sites.[79] Prolactin promoter activity was also inhibited by protein kinase A inhibitors. In summary, Tseng and colleagues proposed that relaxin acts via protein kinase A-dependent signaling pathways to activate two markers of decidualization, IGFBP-1 and prolactin.

Uterine Vascularization

New roles for relaxin as a vascular hormone within the uterus have also been established. Relaxin promotes endometrial and placental growth,[8,80] and may increase uterine blood flow in early pregnancy.[81] There are two explanations for these effects. The first is that relaxin causes vascular development or proliferation (angiogenesis), a view supported by in vitro cell culture studies using human endometrial stromal cells. The current hypothesis is that relaxin regulates uterine angiogenesis via vascular endothelial growth factor (VEGF). Relaxin upregulates VEGF gene expression and secretion from human endometrial stromal and glandular epithelial cells[7,82,83]

by activating the VEGF promoter region.[83] This may occur via ERα, hypoxia inducible factor 1 alpha (HIF-1α) or SP1.[83,84] Increased vascularization in prostate xenograft tumors that over-express human relaxin is also associated with elevated VEGF gene expression.[85]

This work on angiogenesis was extended to an in vivo primate model, to demonstrate that relaxin treatment stimulated new blood vessel formation in the endometrium (Fig. 4 in ref. 44). These data strengthened much of the early work in ovariectomized rats and monkeys treated with porcine relaxin in combination with estrogen. It was only when animals were pretreated with estrogen that relaxin increased arteriole number per unit area and dilated blood vessels on the endometrial luminal surface.[86] It also caused a thickening of blood vessels and the proliferation of endothelial cells in arterioles and capillaries in the endometrium.[86-88] Relaxin increases vascularization in immature rats by enlarging the diameter of arteries and veins in the area between the circular and muscular sections of the uterus, thus providing increased blood flow.[61] This data was placed in context of human physiology in a phase II/III clinical trial for the treatment of scleroderma. Women receiving human relaxin reported heavier or irregular menstrual bleeding, indicating increased endometrial vascularization.[82,89]

Relaxin has also been implicated in the regulation of uterine blood flow. A direct effect of the peptide on uterine artery relaxation has been shown in mid-pregnant rats,[90] and it increases uterine artery blood flow in conscious, ovariectomized nonpregnant rats.[91] Furthermore, in vitro analysis of uterine artery vasodilation demonstrated that treatment with relaxin increased vessel diameter in response to elevated intraluminal pressure.[91] As discussed previously, relaxin binding sites are localized to blood vessels in the pig and human uterus,[16,25,92] and on blood vessels within the human amnion and placental villi.[25] Recent work has shown Lgr7 gene and protein expression in the aorta, mesenteric and small renal arteries of nonpregnant rats and mice.[93] Therefore, relaxin could be acting directly on Lgr7 in uterine arteries to mediate vasodilation and increase uterine blood flow to the placenta.

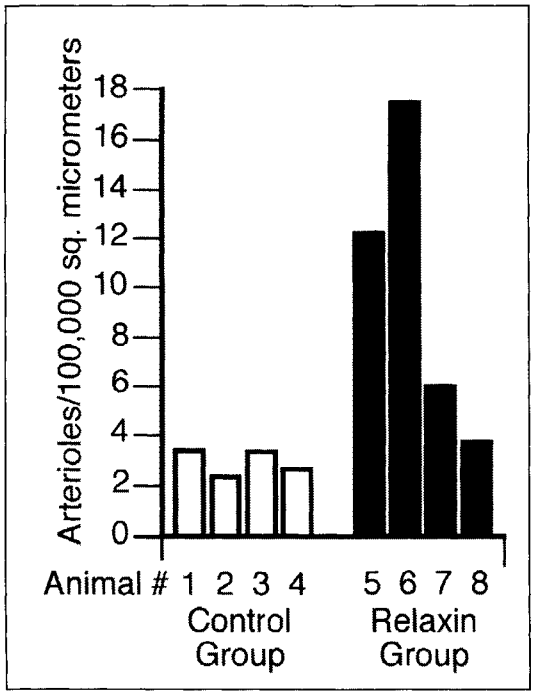

Figure 4. The effects of relaxin treatment on arteriole number in the endometrium of ovariectomized, steroid-primed rhesus monkeys. Reprinted with permission from L.T. Goldsmith et al. PNAS; 101:4685-4689. ©2004 The National Academy of Sciences (USA).

Placental Growth

In humans, relaxin is produced in low concentrations by intrauterine tissues such as the amnion, chorion, decidua, basal plate and placental trophoblast.[94] Therefore it is likely that relaxin acts as an autocrine or paracrine hormone to influence placental tissue growth.[95] Relaxin stimulates IGF-II to cause proliferation of human amniotic epithelial (WISH) cells in vitro.[80] Relaxin-treated WISH cells failed to proliferate when an antibody for IGF-II was added.[80] In addition, an in vivo component of this study identified that an increase in relaxin mRNA expression levels was correlated with a larger fetal membrane surface area and neonatal birth weight.[80] Small for gestational age infants have smaller placentas than controls, signifying that placental size is an indicator of fetal growth rate.[96] In conclusion, Millar et al suggested that relaxin could be an indicator of normal placental size and fetal growth rate. Of interest, increased risk of spontaneous abortion in horses is associated with placental insufficiency and low placental relaxin levels,[97,98] especially in mares with twin fetuses.[98,99] No study to date has examined placental growth or endometrial function in relaxin-deficient mice, nor have measurements been taken of fetal growth during pregnancy. Preliminary data from our laboratory indicate a lower conceptus weight in early gestation in $Rln1^{-/-}$ mice and a 7% reduction in fetal weight in $Rln1^{-/-}$ mice on day 18.5 gestation (1 day before expected births in mice). However, relaxin is not essential for implantation because fetuses develop to term and the average litter size is not different from wild-type mice.[6]

Summary

In order for pregnancy to be maintained until the appropriate time for parturition, many changes need to take place within the maternal reproductive tract. During early pregnancy, the endometrial stromal cells decidualize around the time of implantation and the placenta forms. In addition, uterine blood flow increases and the vascular bed proliferates to maintain a good supply of oxygen and nutrients to the fetus. The uterus also increases in size to accommodate the growing fetus and the myometrium remains quiescent to prevent premature contractions. At the end of pregnancy, the cervix softens and ripens to enable it to dilate during birth and the myometrium switches to a contractile apparatus. Although not common to all species, relaxin has been shown to play key roles in all these aspects of female reproductive physiology. In some species, a lack of relaxin can have serious implications for the maintenance of pregnancy and the birth of live young. This review has highlighted that relaxin treatment in a variety of pregnant animals has several stimulatory effects on growth factors, angiogenic factors and extracellular matrix components that could be perceived as beneficial for establishing and maintaining a successful pregnancy as well as facilitating the process of labor.

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