Relaxin’s Physiological Roles and Other Diverse Actions

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Relaxin has vital physiological roles in pregnant rats, mice, and pigs. Relaxin promotes growth and softening of the cervix, thus facilitating rapid delivery of live young. Relaxin also promotes development of the mammary apparatus, thus enabling normal lactational performance. The actions of relaxin on the mammary apparatus vary among species. Whereas relaxin is required for development of the mammary nipples in rats and mice, it is essential for prepartum development of glandular parenchyma in pregnant pigs. During pregnancy relaxin also inhibits uterine contractility and promotes the osmoregulatory changes of pregnancy in rats. Recent studies with male and nonpregnant female rodents revealed diverse therapeutic actions of relaxin on nonreproductive tissues that have clinical implications. Relaxin has been reported to reduce fibrosis in the kidney, heart, lung, and liver and to promote wound healing. Also, probably through its vasodilatory actions, relaxin protects the heart from ischemia-induced injury. Finally, relaxin counteracts allergic reactions. Knowledge of the diverse physiological and therapeutic actions of relaxin, coupled with the recent identification of relaxin receptors, opens numerous avenues of investigation that will likely sustain a high level of research interest in relaxin for the foreseeable future. (Endocrine Reviews 25: 205–234, 2004)

I. Introduction

A. Historical and scientific perspective

FRÉDERICK L. HISAW (1) discovered relaxin in 1926 when he found that the injection of serum from pregnant guinea pigs or rabbits into virgin guinea pigs shortly after estrus induced a relaxation of the pubic ligament. Relaxin is produced in the reproductive tract of many mammals during pregnancy. The source of relaxin varies among species. Whereas circulating relaxin is produced in the corpora lutea in rats, mice, and pigs, it is produced in the placenta in rabbits and hamsters and in the uterus in guinea pigs (2).

Research on relaxin has waxed and waned (Fig. 1). After the economic depression and world conflict in the 1930s and 1940s, there was a surge of research interest on relaxin. From the late 1940s through the early 1960s, impure preparations of relaxin were reported to have numerous effects on the reproductive tract in nonpregnant mammals. The pioneering discoveries that relaxin inhibits spontaneous uterine contractility in estrogen-primed guinea pigs (3), promotes cervical softening in estrogen-primed cattle (4), and promotes elongation of the interpubic ligament in estrogen-primed mice (5) predicted physiological roles that were later established for endogenous relaxin during pregnancy. During the late 1950s and early 1960s, the Warner-Chilcott Laboratories provided an impure preparation of porcine relaxin (Releasin) and supported studies in humans that examined the use of relaxin as a therapeutic agent for three clinical problems (2). It was postulated that, by increasing skin elasticity, relaxin would have beneficial effects in patients with progressive systemic sclerosis. It was also postulated that, by inhibiting uterine contractility, relaxin would prevent premature labor, and, by softening the cervix, the hormone would reduce the duration of labor. Clinical efforts with impure porcine relaxin were not sustained beyond the mid-1960s for several reasons, including lack of consistent effectiveness, safety problems,

B. Future directions

Abbreviations: GPCR, G protein-coupled receptor; H2 relaxin, human 2 relaxin; icv, intracerebroventricular; INSL, Leydig cell insulin-like peptide; IP3, inositol triphosphate; IR, ischemia and reperfusion; LGR7KO, LGR7 knockout; M1 and M3 relaxin, mouse 1 and mouse 3 relaxin; M1RKO, M1 relaxin knockout; MLCK, myosin light chain kinase; MMP, matrix metalloproteinase; OVLT, organum vasculosum of the lamina terminals; P1 and P3 relaxin, pig 1 and pig 3 relaxin; PKA, protein kinase A; PLC, phospholipase C; PVN, paraventricular nuclei; R1 and R3 relaxin, rat 1 and rat 3 relaxin; R2 relaxin, recombinant H2 relaxin; RLF, relaxin-like factor; SFO, subfornical organ; SON, supraoptic nuclei; TIMP, tissue inhibitor of matrix metalloproteinase; VEGF, vascular endothelial growth factor.

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endocrine processes. Several novel therapeutic actions of relaxin are those that occur after implantation in pregnant rats, mice, and pigs. Roles of relaxin during pregnancy in the rat, mouse, and pig (2, 10). Findings during pregnancy in the rat, mouse, and pig (2, 10). Findings in these three species encourage the view that relaxin has important effects in other species. Second, the recent discovery of an additional form of relaxin that is expressed in highest levels in the brain (11) provides inferences that relaxin may act locally as a neurotransmitter within the brain. Third, the recent discovery of the relaxin receptors (12–14) enables rigorous molecular approaches toward the identification of not only the target tissues for relaxin but also the molecular mechanism(s) whereby relaxin brings about its effects. Finally, the discovery of relaxin’s actions on nonreproductive tissues such as the brain (15–17), kidney (18, 19), and heart (20, 21) in both males and females have opened new avenues of investigation not only into relaxin’s physiological roles but also its therapeutic potential.

B. Scope of review

Before describing the actions of relaxin, the Introduction provides brief descriptions of the structures of relaxin and its receptor, as well as the source and secretion of relaxin in rats, mice, pigs, and humans. Section II describes in physiologically sequential order the actions of relaxin during female reproductive processes in rats, mice, and pigs. These species offer experimental advantages. In rats and pigs, the corpora lutea contain sufficient relaxin in late pregnancy to enable its isolation in large enough quantities to use for physiological studies. Moreover, the ovaries can be removed to examine the physiological roles of circulating relaxin during pregnancy in both species. Mice have the advantage of using gene targeting to create relaxin-deficient and/or relaxin receptor-deficient animals. Whereas roles for relaxin during follicular growth, ovulation, and implantation have been postulated, the reader is told at the outset that the only well-documented and, in some cases, physiological roles of relaxin are those that occur after implantation in pregnant rats, mice, and pigs. Section III describes recently discovered and surprising actions of relaxin on male reproductive processes. Several novel therapeutic actions of relaxin on nonreproductive tissues in male and female rodents have been reported recently. These poorly understood actions, which are described in Section IV, may have clinical potential in humans and other species. Section V contains the conclusions and a view of future directions for relaxin research. For additional information on the history, chemistry, and physiology of relaxin, the reader is referred to other reviews (2, 15, 16, 22–25) and conference proceedings (26, 27).

C. Structure of rat, mouse, pig, and human relaxin

Relaxin belongs to the insulin/relaxin superfamily of structurally related hormones that in the human includes insulin; IGF-I (28); IGF-II (29); relaxin 1 (30); relaxin 2 (31); relaxin 3 (11); Leydig cell insulin-like peptide (INSL3), which is also designated relaxin-like factor (RLF) (32); early placenta insulin-like peptide (INSL4) (33); INSL5 (34); and INSL6, which is also designated relaxin/insulin factor 1 (35, 36). Whereas the overall sequence identity among family members is low, they are all first synthesized as a prohor-
mone that is comprised of a signal sequence and a B-C-A domain configuration. Within the B and A domains, there are highly conserved cysteine residues that link the A and B domains by two interdomain disulfide bonds and form an A chain intradomain disulfide bond (see the structure of rat relaxin in Fig. 2A). In several members of the family, including relaxin, INSL3/RLF, and insulin, the C domain peptide is removed during processing of the mature peptide.

The amino acid sequences of relaxin in the four species emphasized in this review are shown in Fig. 2B. Rat 1 (R1) relaxin, mouse 1 (M1) relaxin, pig 1 (P1) relaxin, and human 2 (H2) relaxin are the only forms of relaxin known to be secreted into the blood. All that is known concerning the actions of relaxin was obtained in studies involving these four forms of relaxin. The gene for human 1 (H1) relaxin is located on chromosome 9 in close proximity to that of H2 relaxin at 9p24 (44). Genes homologous to H1 relaxin have been identified in only four great ape species (44–46), and the occurrence of H1 relaxin is thought to be a consequence of gene duplication during primate evolution about 25 million years ago (47). Whereas H1 relaxin has bioactivity comparable to that of H2 relaxin (48), its gene expression as determined by RT-PCR has been reported in only a few tissues including the decidua, placenta, and prostate (49); and it remains to be demonstrated that H1 relaxin protein is either produced or released in quantities sufficient to be detected in the blood or prostatic fluid.

Recently, human 3 (H3) relaxin and its nearly identical orthologs mouse 3 (M3) relaxin, rat 3 (R3) relaxin, and pig 3 (P3) relaxin were discovered (11, 42, 43). The H3 gene is localized on chromosome 19 at 19p13.3 in close proximity to the RLF gene (11). Relaxin 3 gene is expressed in greatest levels in the ventromedial dorsal tegmental nucleus in the rat and mouse brain, where it has been postulated to act locally as a neuropeptide (13, 42). Synthetic H3 relaxin displays relaxin bioactivity that is about two orders of magnitude lower than that of recombinant H2 (rH2) relaxin in both the human monocyte cell line THP-1 (11) and human 293T cells.
transfected with H2 relaxin receptors (50). There is evidence that relaxin 3 protein is produced by the brain. Small quantities of P3 relaxin (10–15 ng/kg) were isolated from pig brains (13). However, it remains to be determined whether relaxin 3 is of physiological significance in any species. With the exception of the four recently discovered relaxin 3 gene orthologs, which share 90–100% amino acid sequence homology (Fig. 2), there has been limited evolutionary conservation of relaxin among the more than 25 species in which the sequence is known (2, 11, 23). Although rats and mice are in the same taxonomic order (Rodentia), the extent of amino acid sequence identity between R1 relaxin and M1 relaxin is only 75%, and other comparisons of relaxin’s structure among the four species shown in Fig. 2B range from 37–48%.

Twelve of the amino acids in relaxin are invariant or highly conserved among species. The six cysteine residues involved in disulfide bond formation are invariant (2, 11, 13, 23, 42, 43, 47) with the exception of M1 relaxin, in which an additional tyrosine residue is found penultimate to the C terminus of the A chain (38). The amino acid motif Arg-X-X-Arg, located in the middle of the B chain is required for relaxin bioactivity (2, 51). The arginines in positions B16 and B20 are located in the middle of the B chain is required for relaxin activation of relaxin among the more than 25 species in which the sequence is known (2, 11, 23). Although rats and mice are in the same taxonomic order (Rodentia), the extent of amino acid sequence identity between R1 relaxin and M1 relaxin is only 75%, and other comparisons of relaxin’s structure among the four species shown in Fig. 2B range from 37–48%.

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Because of the structural similarities not only of INSL3/RLF and relaxin but also their receptors, a brief description of the structure of INSL3/RLF and its major physiological role is warranted. INSL3 is also designated RLF because it contains a portion of the putative relaxin receptor-binding region (Arg-X-X-Arg), which is offset four amino acid residues toward the C terminus relative to its position in relaxin (52). However, the binding of INSL3/RLF to its receptor appears to require the five-amino acid motif, Gly-Gly-Pro-Arg-Trp, located on the C-terminal end of the B chain (53). INSL3/RLF is produced by testicular Leydig cells and causes descent of the testis during embryonic development (54).

D. Structure of relaxin receptors and signal transduction pathways

For many years, discovery of relaxin receptors proved to be elusive. In 2001 a major breakthrough occurred. This breakthrough was a consequence of an ongoing investigation of a family of leucine-rich guanine nucleotide-binding (G protein)-coupled receptors designated LGRs, which include FSH, LH, and TSH receptors. By using genomics and astute observations of phenotypic expression reported in knockout mice, Hsueh and co-workers and others discovered that two orphan LGR receptors, designated LGR7 and LGR8, are receptors for relaxin (12) and INSL3/RLF (12, 55, 56), respectively (Fig. 3). LGR7 and LGR8, which are 757 (12) and 737 (55) amino acids in length, respectively, share about 60% amino acid sequence identity and contain 10 leucine-rich repeats in their extracellular domain. Through their activation of LGR7 and LGR8, both relaxin and INSL3/RLF elicit bioactivity, at least in part, through the stimulation of the Gc-cAMP-protein kinase A (PKA)-dependent signaling pathway (12, 56). Available evidence indicates that INSL3/RLF binds to and activates the LGR8 receptor but not the LGR7 receptor (50, 56). Relaxin binds with high affinity and activates the LGR7 receptor (12, 50, 56). Relaxin, however, may not act solely through LGR7. Whereas relaxin binds with greatest affinity to cells containing the LGR7 receptor, it also binds with low affinity to cells that contain only LGR8 receptors (50). Moreover, rH2 relaxin and P1 relaxin induce cAMP production in cells that contain only LGR8 receptors (12, 50, 56). It remains to be determined whether circulating relaxin elicits any biological actions in vivo through LGR8. LGR7 transcripts have been identified in reproductive tissues, as well as nonreproductive tissues such as the brain, kidney, heart, and lung, where actions of relaxin have been reported (Table 1). A splice variant of LGR7 that is attributable to deletion of an exon located C-terminal to the initial leucine-rich repeat in the extracellular domain of the receptor has been identified (12). Neither the function, if any, nor the tissue distribution of the truncated LGR7 has been reported.

Recently, two orphan G protein-coupled receptors designated GPRC135 and GPRC142 that bind relaxin 3 but not
other members of the insulin/relaxin peptides were identified (13, 14). These putative relaxin 3 receptors differ structurally and functionally from LGR7 and LGR8. They have relatively short N-terminal extracellular domains, and they are coupled to cAMP inhibition. Whereas the predominant expression of GPCR135 mRNA is in the brain (13), the expression of GPCR142 occurs in numerous peripheral tissues as well as the brain (14). The dominant brain expression of relaxin 3 and GPCR35 led to the postulation that this ligand-receptor pair plays a role in the nervous system (13). In view of the present lack of information concerning the function of recently discovered relaxin 3, the remainder of this review will be confined to a description of the far better understood circulating relaxin in rats (R1), mice (M1), pigs (P1), and humans (H2).

The intracellular signaling pathways initiated by relaxin are not well understood for any target cell. There is accumulating evidence that relaxin initiates its effects through multiple pathways. Consistent with activation through GPCR activation of Gs, relaxin was reported to increase cAMP and activate PKA in several cells and tissues (2, 22, 63–67). A recent study with a human monocyte cell line THP-1 provided evidence that relaxin-stimulated cAMP accumulation is biphasic and that activation of phosphoinositide 3-kinase is required for the second wave of cAMP (68). There are also reports supporting the view that relaxin uses a receptor tyrosine kinase-signaling pathway in primary human uterine cells and THP-1 cells (65, 69, 70); the MAPK pathway in human endometrial stromal cells, THP-1 cells, and human coronary artery smooth muscle cells (71); and ERK-1/2 in human umbilical vein endothelial cells and epithelial (HeLa) cells (72). Bani and co-workers obtained evidence that relaxin also signals through increased nitric oxide generation and subsequent production of cGMP with studies that used bovine vascular smooth muscle cells (73), rat coronary endothelial cells (74, 75), human basophils (76), human neutrophils (77), human breast cancer cells (78), guinea pig heart (79), mouse uterus (80), and mouse small intestine (81). The significance of distinct signal pathways is presently unknown. It was postulated that they may enable relaxin to be a pleiotropic hormone with a broad range of biological effects on numerous organs and tissues (68).

E. Source and secretion of rat, mouse, pig, and human relaxin

The corpora lutea are the source of circulating relaxin and progesterone throughout pregnancy in the rat, mouse, and pig. Whereas the corpora lutea are also the source of circulating relaxin throughout pregnancy, the placenta becomes the primary source of progesterone during the last two thirds of human pregnancy (2). In rats, mice, and pigs, a portion of the relaxin accumulates in storage granules within luteal cells until 2–3 d before delivery (2). The profiles of circulating relaxin, progesterone, and estrogen during pregnancy in rats, pigs, and humans are shown in Fig. 4. In rats, relaxin is detectable by d 10 of a 23-d pregnancy, and levels rise rapidly to about 100 ng/ml by d 20 (82). Then, during the 2–3 d before birth, the luteal cells degranulate, and serum relaxin levels surge to max-

inal levels that frequently exceed 150 ng/ml. This surge in relaxin levels is temporally coincident with the precipitous antepartum decline in progesterone levels that occurs at functional luteolysis and is required for delivery in rats. The general profiles of serum levels of relaxin, progesterone, and estrogen during the 19-d mouse pregnancy are similar to those in the rat (85, 86). The effects of relaxin on reproductive tissues are either dependent upon or augmented by estrogen in rats, mice, and pigs (2). Developing ovarian follicles are the source of rising estrogen levels during late pregnancy in rats and mice (84, 86).

The profile of serum relaxin in the pig differs from that in the rat throughout most of the 114-d gestation period (Fig. 4B). Relaxin rises gradually from about 0.15 ng/ml on d 6 to about 10 ng/ml on d 110 of pregnancy (87, 88). Then, as with rats, a surge in serum relaxin to levels that generally range from 50–150 ng/ml occurs coincident with functional luteolysis. The placenta is the source of the rapidly rising levels of estrogen during late pregnancy in pigs.

The profile of serum relaxin levels during human pregnancy (89, 90) differs markedly from those in the rat, mouse, and pig in three ways (Fig. 3C). First, the profile of serum relaxin levels follows that of human chorionic gonadotropin: it is highest during the first trimester when the corpus luteum is most active, and it declines throughout the remainder of pregnancy. Second, maximal serum levels of relaxin in humans are only about 1 ng/ml, which is about two orders of magnitude lower than maximal levels in rats and pigs. Finally, because relaxin does not accumulate in human luteal cells as pregnancy progresses (92) and functional luteolysis does not occur, there is no antepartum surge in serum relaxin levels. Whereas the corpus luteum remains the source of circulating relaxin (H2 relaxin) throughout the approximately 40-wk pregnancy (2), the placenta becomes the dominant source of progesterone and estrogen during the second and third trimesters of human pregnancy (2).

Secondary sources of R1, M1, P1, and H2 relaxin that may produce sufficient hormone to act locally in a paracrine manner have been identified in reproductive and nonreproductive tissues (2, 11, 42, 93). Because the well-documented effects of relaxin are attributable to circulating luteal relaxin, potential actions of relaxin in other sources are given limited attention in this review.

With the exception of a few reports of historical importance, all studies of relaxin’s actions described in this review were conducted by either removing endogenous relaxin in vivo or by administering pure relaxin either in vivo or in vitro. Two pure hormone preparations were administered for essentially all studies. Purified porcine (P1) relaxin (94) was used for in vivo studies in pigs, and rH2 relaxin was used for clinical investigations in humans. Despite the marked differences in their primary structures, both P1 relaxin and rH2 relaxin have high and similar bioactivity in vivo in rodents (2, 18). Moreover, both hormones appear to be equally effective in in vitro systems using either rodent or human cells, tissues, and organs (2, 50, 65, 71, 72, 74, 78, 80, 81).

II. Effects of Relaxin during Female Reproductive Processes

Section II describes the effects of relaxin on five female reproductive processes. Although nearly all studies were done in rats, mice, and pigs, the limited available information relevant to these processes in the human and/or other primates is included.

A. Follicular growth and ovulation

Whereas relaxin immunoactivity has not been detected in the peripheral blood during the follicular phase of the estrous cycle in any species, relaxin mRNA and/or immunoactivity has been detected in preovulatory follicles in pigs and a variety of other mammalian species including primates (2, 95–98). Findings in immature pigs led to the hypothesis that relaxin may promote follicular growth through intraovarian autocrine and/or paracrine mechanisms (95, 99). Bagnell et al. (95) reported that the theca interna in prepubertal piglets produces relaxin, and the levels of relaxin in follicular fluid increase with follicular size. Moreover, they found that low doses of P1 relaxin promote growth of both granulosa and theca cells in vitro (100–102). Consistent with these findings, specific relaxin-binding sites were localized in both the theca and granulosa cells of developing follicles (103).

There is also limited evidence that relaxin may act locally to promote follicular development and ovulation in rats. Proteinase enzymes, including plasminogen activator and collagenase, play an essential role in bringing about the extracellular matrix remodeling required for follicular development and ovulation, and it was demonstrated that P1 relaxin promotes the secretion of these and possibly other proteinases from primary cultures of rat granulosa cells and theca-interstitial cells (104, 105). Additionally, it was reported that rH2 relaxin induces ovulation in the in vitro perfused rat ovary (106), and that passive immunization of circulating R1 relaxin with a monoclonal antibody for R1 relaxin (designated hereafter as MCA1) reduces the number of ovulated oocytes when immature rats are induced to superovulate with gonadotropins (105).

Studies in mice do not support a role for relaxin in either follicular development or ovulation. P1 relaxin did not increase follicular growth or antrum formation in cultures of mouse preantral follicles (107). Moreover, recent studies in M1 relaxin knockout (M1RKO) mice and relaxin receptor LGR7 knockout (LGR7KO) mice provide definitive evidence that M1 relaxin is not required for either follicular development or ovulation in mice. Female mice without either a functional M1 relaxin gene or a functional LGR7 relaxin receptor gene are fertile, and the average litter size does not differ from that of controls (10, 59). A role for M3 relaxin cannot be ruled out because its gene is expressed in ovaries of nonpregnant mice (11). Additionally, one must be mindful that differences exist in the actions of relaxin among species. It remains to be demonstrated that endogenous relaxin plays a role in either follicular growth or ovulation during normal estrous cycles in any species.
B. Implantation

In the 1960s Hisaw and co-workers (108) examined the influence of prolonged administration of progesterone, estrogen, and partially purified P1 relaxin on the histological characteristics of the endometrium in juvenile or ovariectomized rhesus monkeys. The finding that relaxin promoted growth of the endometrium, which included proliferation of the endothelial cells located in the distal portions of the spiral arteries, led these workers to postulate that one of the functions of relaxin is to assist in the preparation of the endometrium for implantation. A recent clinical trial in women with diffuse scleroderma provided additional evidence that relaxin may promote angiogenesis in the human endometrium. During the course of 24 wk of continuous sc infusion, women receiving rH2 relaxin reported heavy, irregular, or prolonged menstrual bleeding more often than women receiving placebo (109). Because of the interest in the clinical potential of relaxin and the availability of tissue, most of relaxin’s actions on the endometrium have been determined with in vitro studies using human endometrial cells. Studies that used either P1 relaxin or rH2 relaxin with primary cultures of normal human endometrial cells demonstrated that relaxin binds with high affinity and specificity (110) and increases the expression of hormones, growth factors, and other molecules associated with either decidualization, angiogenesis, or other processes at implantation in humans and other species. Employing cultures of stromal cells, Tseng and co-workers (2, 111) demonstrated relaxin exerts a synergistic effect with progesterin on the expression of prolactin, aromatase activity, estrone sulfate sulfatase, and IGF-binding protein-1, which is a major protein synthesized and secreted by decidualized cells. Vascular endothelial growth factor (VEGF), which has been implicated in the new vessel growth and vasodilation that occur in the endometrium at implantation (112), increases with the addition of relaxin to cultures of human endometrium stromal and epithelial cells (109, 113, 114). Glycodelin, a glycoprotein produced and secreted by the secretory endometrium, has been postulated to contribute locally to immunosuppression at implantation (115). Stewart et al. (116) demonstrated that there is a close temporal and quantitative relationship between circulating relaxin and glycodelin profiles during the luteal phase of the menstrual cycle and early pregnancy and that sc administration of rH2 relaxin for 28 d increases the secretion of glycodelin in women demonstrating ovarian cyclicity. It was also reported that P1 relaxin promotes the expression of glycodelin mRNA and protein in primary cultures of human endometrial epithelial cells (117). There is also limited evidence that relaxin increases immunostaining for cyclooxygenase-2 (113) and inhibits the expression of collagenase (114) in primary cultures of human endometrial cells. Relaxin appears to mediate its effects on human endometrial stromal cells, at least in part, through the generation of cAMP (118–120). There is limited evidence that relaxin acts in concert with estrogen and progesterone to enhance the expression of Hoxa-10, an endometrial transcription factor that is required at implantation in mice (121).

There have been a few in vivo studies of the effects of relaxin on the endometrium in estrogen or estrogen plus progesterone-primed nonpregnant primates and rodents. Porcine relaxin in concert with estrogen was reported to increase endometrial thickness, number of glands, blood vessel content, and VEGF expression in ovariectomized nonpregnant marmosets (122). Similarly, P1 relaxin was reported to promote increased endometrial thickness, loosening of collagen framework, and dilation of blood vessels in rats and mice (123, 124). Finn et al. (125), however, reported that the sc administration of P1 relaxin to estrogen plus progesterone-treated mice does not promote decidualization of the endometrium.

If relaxin plays a role at implantation, it must be available to the uterine endometrium in quantities sufficient to be effective. In humans and macaque monkeys, circulating relaxin is detectable but less than 200 pg/ml at implantation (126, 127). Relaxin levels then rise in close association with chorionic gonadotropin in conceptive cycles (126, 127). A clinical observation makes it seem unlikely that circulating relaxin is important at implantation in humans. Women who experience premature ovarian failure can become pregnant through ovum donation. Implantation occurs in these women despite the fact that they have neither a corpus luteum nor detectable serum relaxin levels (128). The possibility that luteal relaxin contributes to implantation cannot be ruled out for primates. In the marmoset, a New World monkey, serum relaxin levels rise a few days before implantation, and they are higher than in humans and Old World monkeys (129).

It is conceivable that small amounts of relaxin produced locally support implantation through autocrine/paracrine mechanisms. Relaxin mRNA and/or immunoreactivity was localized in the endometrium during the luteal phase of the cycle or early pregnancy in humans (114, 130, 131), marmosets (98), pigs (132), rabbits (133), and guinea pigs (134). At present, little is known concerning the amounts, regulation, and function of endometrial relaxin in any species.

As with ovulation, definitive information concerning a putative role for relaxin at implantation comes from findings with both M1RKO and LGR7KO mice. The fact that these animals are fertile, and the average litter size does not differ from that of wild-type controls (10, 59), indicates that M1 relaxin is not essential at implantation in mice. As with ovulation, it remains to be demonstrated that relaxin plays a role at implantation in any species.

C. Effects between implantation and parturition

Studies conducted in pregnant rats and pigs provide convincing evidence that endogenous relaxin produces physiological effects between implantation and parturition. Relaxin affects 1) uterine growth and development, 2) myometrial contractility, 3) central regulation of plasma osmolality, 4) cardiovascular adaptations, and 5) the fetus. With some effects, differences have been demonstrated among species. There is presently no evidence that relaxin is required to maintain pregnancy in any species. The average litter size in rats, mice, and pigs that are devoid of either circulating bioactive relaxin (10, 135–137) or relaxin receptors (59) during the second half of pregnancy does not differ from that of controls. Also, women who become pregnant with
ovum donation maintain their pregnancies (138) despite the fact that relaxin is not detectable in the circulating blood (128). The physiological importance of the effects of relaxin between implantation and parturition remains to be demonstrated.

1. Uterine growth and development. The administration of P1 relaxin promotes growth of the uterine myometrium and endometrium in nonpregnant rodents and pigs (2, 139–142). Uterotropic effects of relaxin in nonpregnant rats and/or pigs were reported to be associated with dilation of small arteries and/or veins (123) and increased content of water, protein, collagen, glycogen, DNA, IGFs, IGF-binding proteins, connexins, E-cadherin, VEGF, and tissue inhibitor of matrix metalloproteinases (TIMPs) (2, 142–146). The uterotropic effects of relaxin are markedly influenced by estrogen. Whereas the administration of P1 relaxin alone over relatively short time periods ranging from 6–54 h (acute period) promotes growth of the uterus in immature and/or ovariectomized rats and pigs (2, 139, 142, 147, 148), greater growth is obtained when rats are primed with estrogen before relaxin treatment (147). Moreover, when ovariectomized pigs are treated with P1 relaxin for prolonged periods of 10 and 14 d (chronic period), the hormone fails to have a significant effect on uterine growth in the absence of estrogen priming (140, 141). The mechanism(s) whereby relaxin and estrogen act in combination to promote uterine growth remains poorly understood. Estrogen may up-regulate relaxin receptors (2, 148, 149). There is also evidence that the acute effects of relaxin on the rat uterus are mediated through ligand-independent activation of the estrogen receptor (150). It was recently reported that estrogen and relaxin inhibit the expression of estrogen receptor-β in the rat uterus, and it was postulated that down-regulation of estrogen receptor-β might be necessary for estrogen or other estrogen receptor activators to exert their full trophic effects on the uterus (151). Progesterone also influences the effects of relaxin on uterine growth in rats and pigs, but the influence of progesterone may differ in the two species. Whereas progesterone inhibited acute relaxin-induced increases in uterine wet weight and collagen content in ovariectomized prepuberal rats (147), progesterone augmented both the acute (139) and chronic (141) uterotrophic effects of relaxin in ovariectomized gilts.

The discovery that relaxin has marked effects on growth and development of the uterus in nonpregnant rats and pigs led to the hypothesis that endogenous relaxin plays a role in accommodating the developing fetuses during pregnancy (2). Experimentation did not support the hypothesis in pregnant rats and mice, but did in pigs (Fig. 5). When circulating R1 relaxin was neutralized with monoclonal antibody MCA1 throughout the second half of rat pregnancy, the uterus was as large at term in relaxin-deficient animals as in controls (152). Consistent with this finding, uterine wet and dry weights increased as dramatically during pregnancy in MIRKO mice as they did in wild-type controls (157). In contrast, when gilts were ovariectomized on d 40 and given hormone replacement therapy with only progesterone, the wet weight of the uterus at term was approximately 30% lower than that in intact controls (135).

2. Myometrial contractile activity. Numerous in vivo or in vitro pharmacological studies employed nonpregnant animals to demonstrate that either P1 relaxin or R2 relaxin reduces the frequency of myometrial contractions in several species, including rats, mice, and pigs (2). In rats and pigs, myometrial activity is low when serum relaxin levels are elevated, and then it increases markedly at delivery (158–160). When rats were ovariectomized on d 9 of pregnancy and given hormone replacement therapy with physiological levels of progesterone and estrogen throughout the remainder of pregnancy, the frequency of intrauterine pressure cycles remained well above that in intact controls. In contrast, when hormone replacement therapy included both steroids and P1 relaxin, the frequency of intrauterine pressure cycles declined to levels that did not differ from those in controls (158) (Fig. 6).

Other hormones, including progesterone, estrogen, oxytocin, and prostaglandins, have direct actions on the myometrium during pregnancy, and the interaction of relaxin with these hormones is only beginning to be understood. Progesterone plays an essential role in maintaining pregnancy by providing hormonal support for the endometrium and preventing strong, highly coordinated uterine contractions. There is in vitro evidence that progesterone increases the sensitivity of the myometrium to the quiescent effect of relaxin in rats and pigs (161–163). Studies in ovariectomized nonpregnant rats demonstrated that estrogen also markedly increases the sensitivity of the myometrium to the quiescent effect of relaxin (2, 22, 164), and it may do so by inducing the formation of relaxin receptors (148, 149, 164–166). With the increase in serum estrogen levels and decline in progesterone

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levels that occurs during late pregnancy in rats, mice, and pigs, there is an increase in the production of uterine components, such as gap junctions, oxytocin, oxytocin receptors, and prostaglandins, that increase the myometrium’s capacity for highly coordinated contractions (2). Numerous studies in rats and pigs provide evidence that pharmacological doses of relaxin inhibit myometrial contractions induced by either oxytocin or prostaglandin (2). However, it is not presently known whether endogenous relaxin prevents or diminishes contractions induced by oxytocin or prostaglandins before delivery. It is known that under normal physiological conditions at term pregnancy, potent and highly coordinated contractions of the myometrium occur in rats and pigs despite moderate levels of relaxin in the serum.

The mechanisms regulating uterine contractility and their hormonal control by relaxin, oxytocin, and other hormones are complex and partially understood (2, 22, 167–170) (Fig. 7). It is well known that the signaling cascades that control the concentration of intracellular free calcium (Ca^{2+}) regulate the contractile state of the myometrium. An increase in myometrial cell Ca^{2+} increases the formation of the Ca^{2+}-calmodulin complex, which then binds to and activates myosin light chain kinase (MLCK). The activated MLCK phosphorylates the 20-kDa regulatory chain of myosin and thereby enhances the interaction of myosin with actin and actin-activated myosin ATPase to bring about contraction. Phosphorylation of MLCK reduces the capacity of MLCK to combine with Ca^{2+}-calmodulin to form the active Ca^{2+}-calmodulin-MLCK complex (2, 167, 168). Relaxin treatment blocks the above cascade of events. After relaxin treatment, rat myometrial cAMP increases, PKA activity increases, affinity of MLCK for the Ca^{2+}-calmodulin decreases, MLCK activity decreases, myosin light chain phosphorylation decreases, actomysin ATPase activity decreases, and...

**FIG. 6.** Influence of endogenous relaxin on uterine contractility in pregnant rats. Mean frequency (±SEM, n ≥ 25) of intramural pressure cycles from d 9 until d 23 in intact pregnant rats (control), ovariectomized pregnant rats treated with progesterone and estrogen (OPE), and ovariectomized pregnant rats treated with progesterone, estrogen, and porcine relaxin (OPER). [Reprinted with permission from S. J. Downing and O. D. Sherwood: *Endocrinology* 116:1206–1214, 1985 (158). © The Endocrine Society.]

**FIG. 7.** Intracellular mechanisms whereby relaxin and oxytocin regulate contractions of uterine myometrial cells. Effects of relaxin and oxytocin that are supported by experimental data in rat and/or human myometrial cells are indicated by solid arrows. Possible effects of relaxin that have not been demonstrated are shown with dashed arrows. AC, Adenylyl cyclase; Gs, stimulatory G protein; R-PKA, regulatory subunit of cAMP-dependent protein kinase A; CaM, calmodulin; G, G protein; PLC, phospholipase C; PIP2, phosphoinositl 4,5-bisphosphate; DAG, diacylglycerol; AKAP, A-kinase associated protein. Ion channels that control influx of calcium at the level of the plasma membrane are capacitated calcium entry (CCE) and voltage-operated (VOC). [Modified with permission from B. M. Sanborn et al.: *Progress in Relaxin Research*, World Scientific Publishing Co, Singapore, 1995 (167)].
uterine contractility diminishes (2, 22, 167, 168, 170). Control of phosphorylated light chain/light chain and phosphorylated MLCK/MLCK ratios is complex, and it has not been definitively established how relaxin achieves its effects. The actions of relaxin on myometrial cells are only partially mediated through activation of PKA (2, 22, 167, 170). There is limited evidence that relaxin also acts by reducing intracellular Ca\(^{2+}\) levels by promoting increased Ca\(^{2+}\) efflux and inhibiting mobilization of Ca\(^{2+}\) from intracellular microsomal stores (2, 22, 167, 168). A potential mechanism for promoting hyperpolarization and repolarization that can influence the Ca\(^{2+}\) transient and affect uterine contractility is the opening of K\(^+\) channels (22, 168). Sanborn and co-workers (63) demonstrated that relaxin stimulates myometrial Ca\(^{2+}\)-activated K\(^+\) channel activity and does so through PKA in a human myometrial cell line. There is also limited, but not consistent, evidence that relaxin may stimulate the opening of ATP-dependent K\(^+\) channels in isolated rat uterus and myometrium (22, 170). Also inconsistent are reports that relaxin may up-regulate the \(\tau\)-arginine-nitric oxide pathway to increase the second messenger cGMP, thereby inhibiting uterine smooth muscle contractility (2, 80, 170).

Relaxin also attenuates the contractile effects of oxytocin on human myometrial cells. Oxytocin binds to a G protein (Goq)-coupled receptor, thereby activating phospholipase C (PLC). The PLC stimulates phosphoinositol 4,5-bisphosphate turnover and production of inositol triphosphate (IP\(_3\)) and diacylglycerol. IP\(_3\) and diacylglycerol increase intracellular Ca\(^{2+}\) by promoting both Ca\(^{2+}\) release from intracellular endoplasmic reticulum and Ca\(^{2+}\) entry into the cell. Studies that employed P1 relaxin with immortalized pregnant human myometrial cells provide evidence that relaxin inhibits the effects of oxytocin through activation of PKA. After relaxin stimulation, the regulatory subunit of PKA is anchored to the myometrial plasma membrane through association with a kinase anchoring proteins (AKAP), and this obligatory anchoring is required for inactivation of Goq/PLC coupling (171). There is evidence that Goq/PLC inactivation is attributable to phosphorylation of PLC\(\beta_1\) and not Goq (172).

The recently identified relaxin receptor LGR7 (12) is shown to couple directly to a Go protein to bring about all of relaxin’s effects through the PKA pathway in Fig. 7. This is probably an oversimplification. There is evidence that relaxin’s activation of cAMP in myometrial cells is, at least in part, indirect and through a tyrosine kinase-linked receptor. Kuznetsova et al. (173) reported that the tyrosine kinase inhibitor tyrphostin 47 reduced the increase in cAMP content of human myometrium homogenates produced by synthetic H2 relaxin, and Dodge and Sanborn (64) found that the tyrosine kinase inhibitor genistein reversed relaxin’s effect on oxytocin-stimulated phosphoinositol 4,5-bisphosphate turnover in human myometrial cells. A physiological role of relaxin on human myometrial contractility remains to be demonstrated. Whereas relaxin induces responses in human myometrial cells, there is limited evidence that relaxin does not inhibit contractions of human uterus in vitro (2, 22, 170). The physiological significance of relaxin’s effects on uterine contractility remains to be demonstrated.

3. Central regulation of plasma osmolality. Plasma osmolality is maintained within a narrow range by a complex interaction between thirst mechanisms, neurohypophyseal release of vasopressin, and the actions of this antidiuretic hormone on the kidney (174). Studies in humans and rats demonstrated that during pregnancy there is an approximately 10 mosmol decline in plasma osmolality that occurs without a change in plasma vasopressin concentrations, and this adaptation of pregnancy is attributable to a reduced osmotic threshold for both thirst and vasopressin secretion (174). There is evidence that relaxin contributes to the decline in plasma osmolality that occurs during pregnancy in rats and mice. Plasma osmolality in nonpregnant rats declined about 10 mosmol after the infusion of either rH2 relaxin or P1 relaxin for 5 or 6 d (19, 175, 176). In pregnant rats, a reduction in plasma osmolality coincides with the elevation in serum relaxin levels during the second half of pregnancy (82, 174). When pregnant rats were made R1 relaxin deficient by either passive immunization with monoclonal antibody MCA1 or ovarectomy, a reduction in plasma osmolality failed to occur (19). Consistent with findings in rats, plasma osmolality during late pregnancy in M1RKO mice is about 10 mosmol higher than in wild-type controls (10). Relaxin may not contribute to the reduction of plasma osmolality that occurs during pregnancy in humans. Plasma osmolality was reported to fall and not differ from that in normal controls in women with singleton pregnancies after ovum donation despite the fact that they have undetectable serum relaxin (128, 177).

a. Drinking. The effects of relaxin on the osmotic threshold during pregnancy are probably mediated, at least in part, through its central effects on drinking and vasopressin secretion from the posterior pituitary. This hypothesis has been examined only in rats. Water consumption increases markedly during the second half of rat pregnancy (178–180), and two lines of evidence indicate that relaxin stimulates drinking during this period through actions on the brain. Either intracerebroventricular (icv) or iv administration of relaxin promotes drinking within minutes in nonpregnant rats (180–185). Second, when passive immunization with monoclonal antibodies for R1 relaxin was used to remove relaxin from either the peripheral circulation (154) or icv fluid (180) throughout the second half of rat pregnancy, water consumption was reduced. Moreover, the reduction in drinking was most profound when relaxin was neutralized in the icv fluid (180).

b. Vasopressin secretion. Whereas plasma osmolality declines during pregnancy in rats, blood levels of vasopressin do not change significantly (174). Perhaps relaxin acts centrally to contribute to the phenomenon. Either icv or iv administration of P1 relaxin causes secretion of vasopressin in anesthetized nonpregnant rats (15, 186–191). One can speculate that by acting centrally, endogenous relaxin contributes to the reduced osmotic threshold for vasopressin secretion that occurs during rat pregnancy. By maintaining serum concentrations of vasopressin that are similar to those of nonpregnant rats, water retention is promoted in the renal collecting ducts in pregnant rats despite plasma osmolality that would markedly reduce vasopressin secretion in non-
pregnant rats. At this time, however, the central effects of endogenous relaxin on vasopressin secretion in pregnant rats have not been demonstrated.

c. Central control mechanisms. Relaxin appears to initiate its effects on drinking and vasopressin secretion in rats through actions on the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), two circumventricular organs located on the anterior wall of the third cerebral ventricle that lack a blood-brain barrier and are accessible to circulating relaxin (Fig. 8). High-affinity binding sites for rh2 relaxin were reported in both the SFO and OVLT (192). Additionally, after iv administration of either P1 relaxin or rH2 relaxin, the expression of c-fos increased in group of neurons located in the more peripheral and dorsal parts of the SFO and in the dorsal cap region of the OVLT (184, 185, 193–195).

The SFO and OVLT probably mediate the central actions of relaxin, at least in part, by activating efferent pathways projecting to the paraventricular (PVN) and supraoptic (SON) nuclei, the magnocellular hypothalamic sites that produce vasopressin and oxytocin. After iv administration of relaxin, the firing rate of vasopressin neurons increased in the SON (189), and c-fos expression increased not only in the SFO and OVLT but also in the PVN and SON (190, 191, 193, 194). There are both direct and indirect neural pathways from the circumventricular organs to the hypothalamic nuclei that produce vasopressin and oxytocin. Retrograde neuronal tracing identified subsets of c-fos-positive SFO and OVLT cells that project to the PVN and SON (184). Efforts have been made to differentiate the functions of the SFO and OVLT. Summerlee and Wilson (196) reported that radiofrequency lesions of the SFO on d 12 of pregnancy reduced the rate of increase in water consumption during the second half of pregnancy. Consistent with this finding, McKinley and co-workers (185) recently found that electrolytic ablation of the SFO blocked relaxin’s actions on drinking, but ablation of the OVLT did not. These workers postulated the OVLT may stimulate vasopressin secretion (185). Osmosensitive neurons reside in the dorsal cap of the OVLT, where enhanced c-fos expression is observed after relaxin administration. Because these OVLT neurons have efficient projections to the SON, they could be the neuroanatomical site where relaxin resets the osmostat during pregnancy (175, 197). There is evidence that the neuronal pathways originating in the SFO and OVLT that mediate the effects of relaxin on drinking and vasopressin secretion do so by utilizing angiotensin produced within the brain as a neurotransmitter that acts through angiotensin 1 receptors (182, 183, 188, 190, 198).

4. Cardiovascular adaptations. During human pregnancy, cardiovascular adaptations that are observed by 5–8 wk include not only increased plasma volume, cardiac output, and heart rate but also decreased blood pressure and vascular resistance (199, 200). Similar cardiovascular changes occur relatively later in rat pregnancy (178, 201, 202). Recent studies provide evidence that relaxin may contribute to cardiovascular adaptations during pregnancy through effects on the kidney, vasculature, and heart.

a. Glomerular filtration and effective renal plasma flow. Conrad and co-workers (19) and Conrad and Lindheimer (203) postulated that the circulations of nonreproductive organs such as the kidney serve as arteriovascular shunts that bring about a fall in ventricular afterload during pregnancy. The decrease in ventricular afterload initiates the increase in cardiac output and expansion of plasma volume that occurs during rat and human pregnancy. In pregnant rats, glomerular filtration rate and effective renal plasma flow increase whereas effective renal vascular resistance declines (19), and these renal adaptations are maximal during the second half of pregnancy (204) when serum relaxin levels are elevated. The above renal adaptations of pregnancy failed to occur when circulating R1 relaxin was neutralized with monoclonal antibody MCA1 (19). Relaxin may play a role in these adaptations of pregnancy through direct effects on the vasculature. Conrad and co-workers (18, 19, 205, 206) demonstrated that relaxin is a potent renal vasodilator in rats, promoting both a reduction in myogenic reactivity of small arteries and attenuation of the vasoconstrictive response of the vasculature to angiotensin II. There is evidence that relaxin up-regulates endothelin type B receptors on the vascular endothelium and also the release of nitric oxide, a potent vasodilator (18, 72, 176, 205). Recently, Conrad and co-workers (206) obtained novel evidence that relaxin’s effects on the renal vasculature are dependent upon increased activity of vascular matrix metalloproteinase (MMP) 2. Findings indicate that this vascular gelatinase acts upstream of, and in series with, the endothelial endothelin type B receptor-NO signaling pathway. It appears to do so by processing big endothelin 1 to endothelin-1-32.

b. Vasodilation and blood pressure. In rats, there is a small reduction in blood pressure during midpregnancy and a larger decline during the 2 or 3 d that precede delivery (204, 207, 208). It is conceivable that endogenous circulating relaxin contributes to this decline in blood pressure. Relaxin has been reported to dilate not only microvessels such as arterioles that are surrounded by a smooth muscle coat but also capillaries and postcapillary venules in numerous sites throughout the body. In rodents, relaxin promotes dilation of microvessels in not only reproductive organs (123, 124, 209,
all blood vessels. Whereas elucidated.

signal transduction, if any, remains to be

79, 205, 211, 216, 217). The connection between these two

thase II, thereby increasing nitric oxide production (18, 72, 74,

also to stimulate the activity of inducible nitric oxide syn-

atheries and other sites, at least in part, by acting on endo-

dler cells to up-regulate endothelin type B receptors and also to stimulate the activity of inducible nitric oxide synthase II, thereby increasing nitric oxide production (18, 72, 74, 79, 205, 211, 216, 217). The connection between these two pathways in relaxin signal transduction, if any, remains to be elucidated.

It is possible that relaxin does not promote vasodilation in all blood vessels. Whereas in vitro treatment with rH2 relaxin was reported to cause a rapid relaxation of human preconstricted gluteal arteries in an endothelium-dependent manner, it did not influence small pulmonary resistance arteries, uterine myometrial arteries, or placental stem villus arteries (217, 218). Moreover, the hormone does not appear to play a role in the antepartum decline in blood pressure in rats. Acute iv administration of relaxin during late pregnancy did not influence blood pressure in conscious rats (219) or anesthetized rats (191). Moreover, removal of endogenous relaxin by either bilateral ovariectomy (208) or by passive immunization of endogenous relaxin (19) failed to influence mean arterial pressure in pregnant rats. Consistent with this finding, short-term infusion of rH2 relaxin had no effect on blood pressure in pregnant rhesus monkeys (220).

c. Heart contractile activity. The heart is a target organ for relaxin. Sites that bind radiolabeled rH2 relaxin with high affinity and specificity were reported in rat atria (110, 149, 221, 222), and mRNA for the relaxin receptor LGR7 was also identified in the rat (57, 58), mouse (59), and human (12) heart. Numerous in vitro studies have shown that relaxin has potent, direct and concentration-dependent chronotropic and inotropic effects on the isolated rat heart. Relaxin increased not only the rate of spontaneous contractions in perfused intact hearts (211, 223–225) and isolated right atria (20, 21, 48, 226, 227) but also the force of electrically stimulated contractions in isolated left atria (20, 21, 48, 226, 227). Relaxin may act on both atrial and ventricular pacemakers because relaxin increased heart rate in heart preparations in which the atria had been removed (223). The signal transduction pathways whereby relaxin enhances contractility of the heart have received limited experimental attention. Relaxin’s chronotropic effects on isolated perfused rat hearts were accompanied by the secretion of atrial natriuretic peptide, and both effects of relaxin appeared to involve cellular signal transduction pathways involving PKA, protein kinase C, and calcium/calmodulin-dependent protein kinases (225). When individual cells were examined with whole-cell patch clamp, relaxin was found to inhibit outward potassium currents, increase action potential duration, and enhance calcium entrance into rat atrial myocytes (228, 229). In similar experiments, relaxin increased the rate of action potentials and L-type calcium current in rabbit sinoatrial node cells (230). These effects involved the activation of PKA (228–230).

Does endogenous relaxin influence heart contractility in intact pregnant rats? Acute iv or icv administration of P1 relaxin increased heart rate in urethane-aneurinitized nonpregnant rats (186, 187, 231), and infusion of rH2 increased heart rate in unanesthetized nonpregnant rats (21). However, acute arterial administration of rH2 relaxin on d 19 did not influence heart rate in conscious pregnant rats (219). The effect, if any, of endogenous circulating relaxin on heart rate in pregnant rats has not been reported.

Interestingly, the central and cardiovascular effects of relaxin appear to be as profound in male as in female rodents. In males, relaxin was reported to decrease plasma osmolality (176) and increase drinking (181, 183, 185, 194), vasopressin secretion (232), glomerular filtration rate, effective renal plasma flow (176), vasodilation (212–214), and heart rate (20, 21, 223). Thus, unlike the female rodent reproductive tract, relaxin’s effects on the brain, kidney, general vasculature, and heart do not require elevated circulating estrogen.

5. Fetus. There is evidence that circulating maternal relaxin influences fetal development during rat pregnancy. When rats were deprived of circulating relaxin throughout the second half of pregnancy by either immunoneutralization of R1 relaxin with monoclonal antibody MCA1 or ovariectomy, fetal weights were significantly greater than in controls (137, 152, 154–156). It is not known whether this enlargement of fetuses is attributable to the effects of relaxin deprivation on the mother or on the fetus. It does appear likely that only small amounts of maternal relaxin pass to the fetal serum. Whereas serum levels of relaxin in rat fetuses are not known, those in hamster and human fetuses were reported to be low (233) and nondetectable (234, 235), respectively. Consistent with this view are reports of transplacental passage of small amounts of rH2 relaxin in pregnant rhesus monkeys (236, 237). Again, one must be mindful of possible differences among species. An influence of circulating maternal relaxin on fetal weights was not observed with either M1RKO mice (238) or ovariectomized pigs (135). There is also limited evidence that relaxin contributes to testicular descent during late rat pregnancy (239), and that will be described in more detail in Section III.

D. Parturition

1. Time of onset of delivery. The preponderance of data indicates that circulating endogenous relaxin does not influence the duration of pregnancy. The time of onset of delivery in rats in which circulating R1 relaxin was immunoneutralized with monoclonal antibody MCA1 (240, 241) and in mice that lacked either a functional relaxin gene (10) or relaxin receptor LGR7 gene (59) did not differ from that of controls. There is one report that is not consistent with these findings. When the soluble ligand-binding portion of the human relaxin receptor LGR7 was administered sc to antagonize endogenous circulating relaxin the last 4 d of mouse pregnancy, delivery was delayed by 27 h (12).
Relaxin 1 mRNA and/or immunoreactivity have been reported to be produced within the rat and mouse brain (11, 93, 221, 242), and there is limited evidence that there may be a central R1 relaxin system involving the SFO that influences the time of onset of birth in rats (196, 243). The onset of both luteolysis and delivery was advanced approximately 24 h after central immunoneutralization of relaxin by daily injection of monoclonal antibody for R1 relaxin into the right lateral ventricle throughout the second half of pregnancy (180). It was postulated that central R1 relaxin may influence the time of delivery by acting on the SFO to inhibit oxytocin secretion (180, 196). There are observations that are not consistent with this hypothesis. Intravenous administration of P1 relaxin increased the secretion of oxytocin in unanesthetized nonpregnant and pregnant rats (189, 244). Moreover, neither the time of onset nor the duration of active labor in oxytocin knockout mice differed from those of wild-type controls (245).

2. Duration of delivery and incidence of live young. Two experimental approaches demonstrated that circulating relaxin has vital effects at parturition in rats and pigs. When primiparous rats and pigs were bilaterally ovarioctomized during the second half of pregnancy and given hormone replacement with physiological amounts of progesterone plus estrogen (rats) or progesterone only (pigs), the duration of delivery was several times longer, and the incidence of live births was far lower than in intact controls (136, 244, 246, 247).

However, when hormone replacement included physiological levels of P1 relaxin, birth parameters were similar to those of controls (136, 246, 247). Comparable findings were obtained in both rats (240, 241, 248) and pigs (249) when circulating relaxin was immunoneutralized (Fig. 9).

Recent studies with pregnant M1 relaxin knockout and relaxin receptor LGR7 knockout mice indicate that relaxin is also required for normal delivery in mice. Two of eight M1RKO mice were unable to deliver their pups normally. Moreover, in one of the mice, all pups were either stillborn or died in utero during parturition (10). Similarly, 25 of 162 pups (distributed among nine of 21 litters) born of LGR7KO mice were found dead on the morning of delivery (59). Relaxin deficiency does not disrupt delivery as dramatically in mice as it does in rats and pigs. Six of eight M1RKO mice and 12 of 21 LGR7KO mice appeared to have delivered their young as rapidly as did the wild-type controls (10, 59).

At present, there is no solid evidence that endogenous relaxin facilitates delivery in humans. Circulating levels of relaxin are so low (Fig. 4C) that they may not be sufficient to do so. The extremely high cesarean section rate of more than 50% in women who become pregnant after oocyte donation and have no functional corpus luteum (128, 250) may be largely attributable to proactive clinical management rather than to an absence of systemic relaxin. Also complicating our understanding of the role of relaxin at term in humans is the possibility that the small amounts of H1 relaxin and H2 relaxin that are produced by the decidua and placenta act through local autocrine/paracrine signaling to increase the expression of MMPs in fetal membranes and thereby bring about their rupture and the induction of delivery (2, 23, 251).

Available evidence supports the view that the primary means whereby relaxin facilitates birth in rats, mice, and pigs is by promoting dramatic growth and remodeling of the cervix. The effects of relaxin on two other portions of the birth canal—the vagina and the interpubic ligament—may also play roles at the time of delivery in some species.

a. Cervix. During pregnancy relaxin plays a major role in bringing about the dramatic growth of the cervix that occurs in rats (136, 137, 153–156, 241, 248, 252), mice (157), and pigs (135, 253) (Fig. 5). Both the wet weight of the cervix and the circumference of the cervical lumen at term in control rats and pigs are about 2-fold greater than those in relaxin-deficient animals (137, 253) (Fig. 10, A and B). Numerous and relatively large cervical lumen involutions in relaxin-replete control rats enable far greater expansion of cervical lumens than is possible in relaxin-deficient monoclonal antibody MCA1-treated rats (210). Recently, progress has been made toward an understanding of the mechanisms whereby endogenous relaxin contributes to growth of the rat cervix. Relaxin promotes the accumulation of both epithelial cells and stromal cells (136, 137) not only by stimulating cell proliferation (254) but also by inhibiting apoptosis (255). Within the stroma, relaxin’s effects on cell proliferation and apoptosis are primarily on fibroblasts and not on smooth muscle cells (254, 255).

Endogenous relaxin has also been demonstrated to promote a dramatic increase in the extensibility (softening) of the cervix in pregnant rats and pigs (2, 152, 252, 253, 256) (Fig. 11). Studies have provided inferences concerning the mechanisms whereby relaxin brings about softening of the cervix. The cervix contains smooth muscle. The proportion of smooth muscle to other cervical components diminishes as
pregnancy progresses and varies among species. However, it is the extracellular matrix components, including type I collagen, type III collagen, type IV collagen, elastin, proteoglycans, and glycosaminoglycans, that are thought largely responsible for the tensile properties of the cervix. Light microscopic analysis of cervices removed during late pregnancy from relaxin-deficient rats, pigs, and mice revealed that the collagen fiber bundles fail to disperse or to become as disorganized as do those in cervices removed from controls (2, 157, 210, 257, 258). The relaxin-induced dispersion of collagen fiber bundles may allow the fibers to be readily pulled past one another as the fetus enters the cervix. Elastin fiber-like structures are more prevalent and longer in relaxin-deficient pregnant rats than in controls (210). Perhaps the organization of elastin influences cervical extensibility in the rat and other species in which cervical elastin is prevalent. Arterial cross-sectional area is also smaller in the cervices of relaxin-deficient pregnant rats than in controls (210). Endogenous relaxin plays a major role in promoting changes in the biochemical composition of the cervix that occur during late pregnancy in rats and pigs (252, 259). Relaxin increases cervical hydration, dry weight, and glycosaminoglycans (dermatan sulfate, heparan sulfate, hyaluronic acid) content. Because relaxin has little effect on collagen content during pregnancy, the ratio of glycosaminoglycans to collagen increases in relaxin-replete rats and pigs during late pregnancy (252, 259). The predominant cervical proteoglycan in rats and pigs is a relatively small dermatan sulfate proteoglycan that consists of a core protein and a single glycosaminoglycan chain (259, 260). Small dermatan sulfate proteoglycans such as decorin and biglycan associate specifically with type I collagen and inhibit fibrillogenesis (261). Thus, dermatan sulfate may accumulate in the interstites among cervical collagen fibrils, thereby either dispersing them or preventing their aggregation (260). The mechanism(s) associated with relaxin-induced hydration of the cervix is not well understood, but enlargement of the cervical arteries (210) may be a factor. The cervix is a target for relaxin (2), but certain identification of the cervical cells that contain relaxin receptors is needed. An immunohistochemical procedure was used to
identify binding sites for relaxin in epithelial cells, circular and longitudinal smooth muscle cells, and cells associated with blood vessels in rat (262), pig (263), and human (264) cervices. There is also evidence that cervical fibroblasts respond to relaxin in vitro (70, 265). A single study to date has localized relaxin receptor LGR7 immunoreactivity in rat cervical smooth muscle cells but not in epithelial cells (12).

The molecular mediators of relaxin’s effects on softening of the cervix are complex and remain poorly understood. Because it is the extracellular matrix that is largely responsible for the tensile properties of the cervix, a widely held view is that degradation of collagen and perhaps other extracellular components is key to cervical softening (2). The possible role of relaxin in collagen degradation has been explored in a few laboratories. It was reported that rH2 relaxin up-regulates the activity of MMP-1 (collagenase) in primary cultures of cervical cells from pregnant guinea pigs (265) and increases levels of both mRNA and protein for pro-MMP-1, pro-MMP-2 (gelatinase), and pro-MMP-3 (stromelysin) in human lower uterine fibroblasts through a tyrosine kinase signaling pathway (70). P1 relaxin was reported to stimulate the secretion of several metalloproteinases in primary cultures of human cervix fibroblast-like cells (266). However, when immature pigs were administered P1 relaxin, there was a decrease in MMP-2 (267) and an increase in TIMP-1 and TIMP-2 (145). In view of the limited studies and inconsistency of findings, it is premature to conclude that degradation of collagen plays a central role in relaxin-induced cervical softening.

Prostaglandins have also been implicated as potential mediators of cervical softening. Whereas prostaglandin E2 administration promotes cervical softening in rats, humans, and other species (2, 268, 269) and is used frequently to promote ripening of the human cervix before delivery (270), it remains to be demonstrated that prostaglandins mediate relaxin’s effects on the cervix. When the cyclooxygenase inhibitor indomethacin was used to block prostaglandin synthesis, P1 relaxin’s effects on neither cervical wet weight nor softening were inhibited in ovariectomized nonpregnant rats (271). There is limited evidence that nitric oxide contributes to softening of the cervix. Blocking the synthesis of nitric oxide with the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester restrained cervical softening, and local application of nitric oxide donors promoted cervical softening in pregnant rats (268, 272). Two studies aimed at determining whether nitric oxide mediates relaxin’s effects on cervical softening in rats are not consistent (268, 273). There is limited evidence that nitric oxide may contribute to relaxin’s effects on cervical wet weight (273).

An understanding of relaxin’s effects on the cervix requires knowledge of the influence of the individual and combined effects of the steroids estrogen and progesterone, which are also elevated during late pregnancy. Limited studies have been conducted in rats and pigs, and findings are not in total agreement. First, let us consider cervical growth. In both species, estrogen alone promotes growth of the cervix, but progesterone does not (274–276). Relaxin’s effects on growth of the cervix are estrogen dependent in rats (274, 275), but not in pigs (276, 277). Concurrent administration of progesterone does not have much effect on the growth of the cervix that is induced by the combination of estrogen plus relaxin in either the rat or the pig (274–276).

The administration of neither estrogen nor progesterone independently promotes marked cervical softening in non-pregnant rats and pigs (274–277). Moreover, in the presence of physiological levels of both estrogen and progesterone during late pregnancy in relaxin-deficient rats and pigs, the cervix fails to soften (152, 253, 256). Pharmacological levels of estrogen actually decrease the extensibility of the cervix in pregnant rats (278). Whereas estrogen and progesterone alone and in combination do not promote extensibility of the cervix, relaxin’s effects on softening of the cervix are influenced by these steroids. Again, differences exist between rats and pigs. The cervical softening effects of relaxin are estrogen dependent in rats (274, 275), but not in pigs (276, 277). It remains unclear whether estrogen augments relaxin’s effects on softening of the cervix in pigs (276, 277) as it does in rats (275). There is evidence that progesterone inhibits cervical softening during the second half of rat pregnancy. Either the administration of the antiprogesterone RU 486 to intact pregnant rats (279) or the withdrawal of progesterone implants from ovariectomized pregnant rats (248) promotes marked softening of the cervix. In the pregnant rat, softening of the cervix increases gradually from midpregnancy until a few hours before delivery when it increases markedly (2). This increase in cervical extensibility may be attributable not only to the surge in serum relaxin but also to the precipitous decline in serum progesterone that occurs at luteolysis (Fig. 4A).

Relaxin has potential advantages over the presently used prostaglandin E2 (dinoprostone) as an agent to prepare the cervix for delivery because it promotes both growth and softening of the cervix within 8 h (271), and it inhibits rather than stimulates uterine contractility (2). Because two clinical trials failed to demonstrate an effect of rH2 relaxin on birth in pregnant women, it is important to understand the shortcomings of those trials and the conclusions of the investigators. The clinical trials (8, 9) were conducted with the hypothesis that when rH2 relaxin is placed in methyl cellulose gel and then deposited in the vagina, the hormone effectively passes through the squamous epithelium, enters the blood, and thereby reaches relaxin receptors within the cervix. That does not appear to happen. Both groups of investigators found that circulating blood levels of relaxin were not influenced by treatment with up to 4 mg of rH2 relaxin (8, 9), and they both postulated that the lack of adsorption of relaxin may be the reason that treatment failed to influence birth.

b. Vagina. Relaxin also brings about marked growth of the vagina in rats (136, 155, 156, 255), pigs (135), and mice (157) during pregnancy (Fig. 5 and Fig. 10, C and D). Studies of the vagina in pregnant rats and mice indicate that relaxin promotes increased wet weight, dry weight, arterial cross-sectional area, epithelial cells, and stromal cells (136, 156, 157). Relaxin acts to increase vaginal epithelial cells in the rat, at least in part, by reducing the rate of apoptosis (255). Relaxin also increases extensibility of the vagina in rats (156). Consistent with this finding, collagen fiber bundles in vaginas of relaxin-deficient rats and mice fail to disperse or to become as disorganized as do those in vaginas from controls (156,
157) (Fig. 10, C and D). The vaginal cells that contain relaxin receptors remain to be established. In the vagina, as in the cervix, specific and saturable relaxin binding sites were reported associated with luminal epithelial cells as well as circular and longitudinal smooth muscle cells in the rat (155) and human (264). Relaxin receptor LGR7 immunoactivity, however, was reported in rat vaginal smooth muscle cells and not in epithelial cells (12). The physiological significance of relaxin’s actions on the vagina in rats, pigs, and mice cannot be readily separated experimentally from that of relaxin’s effects on the cervix. It does seem likely that relaxin–induced growth and softening of the vagina facilitate delivery.

c. Pubic joint. Transformation of a pubic symphysis that consists of fibrocartilage to a flexible and elastic interpubic ligament is a pelvic adaptation that occurs during pregnancy in several mammalian species, including mice, guinea pigs, bats, and humans (2, 280). The extent to which the pubic joint undergoes a transformation during pregnancy varies among species. The rat interpubic joint contains a central core of hyaline cartilage that is surrounded by fibrocartilage areas (281), and it is not transformed to an elongated and elastic interpubic ligament during pregnancy (2, 280–282). It is relaxin’s capacity to promote transformation of the interpubic ligament that led to the hormone’s discovery in guinea pigs (1) and subsequently to the development of the widely used mouse interpubic ligament bioassay (283). As with the rodent reproductive tract, the effects of relaxin on the interpubic ligament in mice and guinea pigs are estrogen dependent (2).

The advent of the M1RKO mouse provided strong evidence that endogenous relaxin plays a major role in promoting transformation of the interpubic ligament during pregnancy in the mouse. In M1RKO mice the interpubic ligament length, wet weight, dry weight, and water content are significantly lower than in wild-type mice at term pregnancy (10, 157). The mechanisms whereby relaxin brings about its effects on the interpubic ligament in mice, guinea pigs, and other species are not well understood (2, 280). However, as with the cervix and vagina, endogenous relaxin reduces the density of collagen fiber bundles in the interpubic ligament (157). The physiological significance of relaxin-induced transformation of the interpubic ligament during pregnancy is likely to vary from species to species. Surprisingly, the more than 4-fold increase in length of the interpubic ligament that occurs in pregnant mice is not essential at delivery. Mice that had their pubic bones tied together (284), as well as most M1RKO mice (10), delivered their young as rapidly as did wild-type controls. It is presently not known whether endogenous relaxin contributes to the modest separation of the pubic symphysis that occurs during pregnancy in humans or other primates (2).

E. Lactation

Endogenous relaxin has dramatic effects on development of the mammary apparatus in pregnant pigs, rats, and mice. However, relaxin’s effects in pigs differ from those in rodents. Whereas relaxin has profound effects on the development of the mammary glands in pigs, it is the hormone’s effects on the development of the mammary nipples that are most dramatic and vital in rodents.

1. Mammary glands. In primiparous pigs, mammary lobuloalveolar development begins on about d 80, and it continues until term (285), a period that coincides not only with rising levels of estrogen and relaxin (Fig. 4B) but also relaxin-dependent growth and softening of the cervix (88, 253). Studies that employed ovariectomized primiparous pigs and hormone replacement demonstrated that endogenous relaxin plays a major role in promoting the development of the mammary gland parenchyma (285, 286). A subsequent study in ovariectomized nonpregnant gilts provided evidence that relaxin’s effects on mammary parenchymal development are estrogen dependent in pigs, and that they are accompanied by a reduction in the organization of collagen fiber bundles in the stroma (287). Endogenous relaxin has no apparent effect on the weight of the mammary glands during the second half of pregnancy in rats (288, 289) and mice (157). Nevertheless, as with the cervix and vagina, relaxin influences mammary gland differentiation. In rats, relaxin reduces the density and organization of collagen fiber bundles, reduces the length of elastin fibers, and increases the cross-sectional area of arterioles (288, 289). Moreover, in both rats and mice, relaxin increases alveolar development (10, 288).

Relaxin likely mediates mammary development through direct effects on the mammary glands. Relaxin-binding sites have been reported in epithelial cells associated with lactiferous ducts and lobulo-alveolar structures in rats (262), pigs (263), and humans (264).

Available evidence indicates that relaxin plays little, if any, role in lactogenesis (2, 10, 289). There is limited evidence that relaxin may suppress the milk ejection reflex by acting either on the rat brain to inhibit oxytocin secretion (290) or directly on the myoepithelial cells of the goat mammary glands to inhibit milk expression (291). Because serum levels of relaxin are extremely low to nondetectable during lactation, it was postulated that relaxin produced in the brain of the rat (290) and in the mammary glands in the goat (291) bring about local effects on milk release. The physiological significance, if any, of the putative effect of relaxin on the milk ejection reflex remains to be demonstrated.

2. Mammary nipples. Immunoneutralization of circulating R relaxin throughout the second half of pregnancy with monoclonal antibody MCA1 led to the surprising discovery that endogenous relaxin is required for the development of the mammary nipples that occurs during the second half of pregnancy in rats (288) (Fig. 12). This effect of relaxin is absolutely vital. The nipples in relaxin-deficient rats are so small at term that the pups cannot grasp them to obtain milk (153, 288). More recent studies that examined the phenotypes of postpartum M1RKO mice (10) and LGR7KO mice (59), as well as the physiological consequences of the antepartum administration of the ligand-bound portion of the relaxin receptor LGR7 (12), demonstrated that relaxin also has a vital role on nipple development in pregnant mice. Analysis of rat and mouse nipple histology demonstrated that relaxin promotes a reduction in the density of collagen fiber bundles that is similar to that which occurs in the cervix, vagina, and
mammary glands (10, 153). Moreover, morphometric analysis of rat nipples indicated that relaxin reduces the length of elastin fibers and enlarges the cross-sectional area of arteries (153) (Fig. 12, C and D). The nipples of relaxin-deficient primiparous pigs are moderately well developed at term (285, 286), and piglets can grasp them and obtain milk (286). There is evidence that relaxin acts directly on the nipples to bring about its effects. Specific relaxin binding sites were reported in nipples from rats (262, 292), pigs (263), and humans (264). Recently, LGR7 mRNA expression was reported in the connective tissue at the base of the nipple and just beneath the epithelium in mice (59). Additionally, sc injection of P1 relaxin at the base of a left abdominal rat nipple promoted significant growth of that nipple relative to its right counterpart (292). Little is known concerning the roles of ovarian steroids on nipple development. A single study indicated that, unlike the rat cervix, relaxin-induced growth of the rat nipple occurs in the absence of estrogen (292).

III. Effects of Relaxin in the Male

There is evidence that relaxin is produced in small amounts within the male reproductive tract in several species (2). Both H1 and H2 relaxin gene expression were reported in the human prostate (293, 294), and relaxin immunostaining was reported in pig seminal vesicles (295). Available evidence in pigs, humans, cattle, and goats indicates that the relaxin produced in the reproductive tract is released primarily, if not exclusively, into the seminal fluid (2, 296, 297), where it may act as an exocrine factor to improve the motility of spermatozoa and thereby enhance fertility (2, 297, 298).

Recently, the physiology of relaxin in male rodents has been given experimental attention, and the results have made it clear that relaxin can no longer be considered strictly a hormone of pregnancy. Both the testis and the prostate express R1 and M1 relaxin (93, 299), and both tissues have been reported to express the relaxin receptor LGR7 (57, 299). The phenotypes of the M1RKO and LGR7KO mice provide strong evidence that endogenous relaxin plays a role in growth and development of the male reproductive system in mice. By the age of sexual maturity, male M1RKO and LGR7KO mice show retarded growth of the reproductive tract (59, 299). Histological examination indicated that sperm maturation was markedly decreased in the testis, and that this may be attributable, at least in part, to an increase in the rate of apoptosis in early stages of spermatogenesis (59, 299). The epididymis, seminal vesicles, and prostate were also reported to be smaller in the M1RKO and/or LGR7KO mice than in wild-type controls. Histological analysis revealed developmental anomalies in the epididymis and prostate of M1RKO and/or LGR7 mice. In the epididymis, tubule compactness, collagen staining, and apoptosis of epithelial cells

*FIG. 12. Influence of immune neutralization of circulating R1 relaxin throughout the second half of pregnancy on nipple development in rats. Photographs of representative abdominal and inguinal mammary nipples on postpartum d 1 in rats treated with control antibody MCAF for fluorescein (A) and monoclonal antibody MCA1 for rat relaxin (B). Photomicrographs of representative cross-sections of abdominal nipples obtained on d 22 of pregnancy from rats treated with MCAF (C) and MCA1 (D). LD, Lactiferous duct. Bar in panel C, 270 μm. C and D are the same magnification. MCAF, Monoclonal antibody for fluorescein. [Panels C and D adapted from M. J. Kuenzi and O. D. Sherwood: Endocrinology 131:1841–1847, 1992 (153). © The Endocrine Society.]*
were greater than in wild-type controls (59, 299). The reduced growth of the prostate was accompanied by increased collagen, increased apoptosis of epithelial cells, and a decrease in glandular epithelium relative to wild-type controls. These developmental deficiencies are of considerable importance. Whereas both M1RKO and LGR7KO mice are fertile, fertility is markedly lower than in wild-type controls (59, 299).

Does relaxin influence testicular descent in rodents? Testicular descent occurs in male fetuses during the second half of pregnancy. The first or transabdominal phase of testicular descent involves enlargement of the gubernaculum and regression of the cranial suspensory ligament (300). Several lines of evidence support the view that INSL3/RLF plays a major role in testicular descent. The testes fail to descend and have undeveloped gubernaculum in mice devoid of either INSL3/RLF (301) or its receptor LGR8 (55). Rat gubernaculums cells contain the INSL3/RLF receptor LGR8 (56) and proliferate in response to INSL3/RLF (60). Finally, the fetal testes produce (60, 302) and secrete (303) INSL3/RLF during late pregnancy in rats when testicular descent occurs. Nevertheless, it is possible that relaxin contributes to testicular descent in the rat. Whereas the gubernaculum does not contain the relaxin receptor LGR7 (60), relaxin binds to the INSL3/RLF receptor LGR8 and induces modest activity in cultures of gubernacular cells obtained from rat fetuses (56, 60). At present, the evidence that endogenous relaxin may contribute to testicular descent during rat pregnancy is limited to a brief report that gubernaculum growth and testicular descent were delayed in male fetuses when their mothers were passively immunized with a monoclonal antibody for R1 relaxin from d 15–21 of pregnancy (239). The apparent normal descent of the testis in M1RKO and LGR7KO mice (59, 299), in conjunction with the failure of testicular descent in mice devoid of either INSL3/RLF (301) or its receptor LGR8 (55), does not support a role of relaxin in testicular descent in mice. Moreover, it has been suggested that these data provide in vivo evidence that relaxin and INSL3/RLF are the cognate receptors for LGR7 and LGR8, respectively (59).

There is also recent evidence of puzzling gender differences in phenotypic expression of M1 relaxin deficiency. In male M1RKO mice there was an increase in heart and lung collagen content, and the function of both organs was altered (61, 304). These phenotypes may be attributable to the absence of local relaxin acting in an autocrine/paracrine manner on cardiac LGR7 receptors (59). Expression of M1, as well as M3 relaxin, was reported in mouse heart and lung (11).

IV. Effects of Relaxin on Nonreproductive Processes

Studies that were largely conducted with animal models of pathologies demonstrated that relaxin has actions beyond the physiological actions identified to date for endogenous relaxin in rodents and pigs. The therapeutic actions of exogenous relaxin on nonreproductive processes do not appear to be estrogen dependent, and they occur in males as well as in females.

A. Fibrosis

Fibrosis is the excessive accumulation of extracellular matrix components that include collagens, glycoproteins such as fibronectin, and proteoglycans. A report in 1956 that prolonged administration of a preparation of P1 relaxin increased skin elasticity in rats led clinical investigators to explore the use of impure P1 relaxin in patients with scleroderma (2). Scleroderma (systemic sclerosis) is a connective tissue disease of unknown etiology in which tissue fibrosis is the predominant clinical feature and largely determines morbidity and mortality (305). The disease is characterized by fibrotic intimal hyperplasia of small arteries and arterioles (Raynaud’s phenomenon, renal crisis, pulmonary hypertension), as well as extravascular fibrosis of the skin and internal organs, including the lung, kidneys, and heart (305). In 1958, Casten and Boucek (306) reported that daily im injection of partially purified P1 relaxin over a period from 6–30 months influenced favorably three features of scleroderma—skin tightness, Raynaud’s phenomenon, and trophic ulcers. Although reports were not entirely consistent, other workers in the late 1950s and early 1960s generally reported that the administration of impure P1 relaxin brought about beneficial effects in scleroderma patients (2). Nevertheless, further efforts were not undertaken during the 1960s to examine the effects of relaxin on scleroderma for several reasons, including the uncertainties and risks associated with the use of impure hormone preparations (2, 7).

The availability of highly purified P1 relaxin in the late 1970s and rh2 relaxin in the late 1980s made possible rigorous examination of relaxin’s effects on fibrosis in tissues other than the reproductive tract. Experiments conducted in vitro provided evidence that relaxin has the capacity to reduce the synthesis and/or increase the rate of degradation of extracellular matrix components in the skin, lung, liver, and kidney. In the early 1990s, Unemori and co-workers reported that rh2 relaxin reduced the overexpression of interstitial collagen not only in cultures of normal human dermal fibroblasts that were stimulated to overproduce collagen by the cytokines TGFβ and IL-1 (307) but also in human scleroderma fibroblast lines (308). Also consistent with an antifibrotic effect, relaxin increased the synthesis of collagenase and decreased the synthesis of TIMP-1 in cultures of normal human dermal fibroblasts (307). More recently, Unemori et al. (309) reported that rh2 relaxin inhibits not only TGFβ-induced overexpression of interstitial collagen and fibronectin, but also stimulates collagenase expression in cultures of normal human lung fibroblasts. The addition of either rh2 relaxin (310) or P1 relaxin (311) was reported to both inhibit collagen deposition and reduce TIMP-1 and TIMP-2 secretion in cultures of activated rat hepatic stellate cells, the effector cells in liver fibrogenesis. Additionally, rh2 relaxin decreased TGFβ-induced fibronectin protein and did so by increasing ubiquitin-dependent degradation of fibronectin in cultures of three types of mouse renal cells implicated in fibrosis (312).

Relaxin has been administered to pigs and rodents to examine its antifibrotic effects on the skin in vivo. Pigs were chosen for two studies (313, 314) of relaxin’s effects on the dermis because pig skin is similar to that of the human (313). Tissue expansion was facilitated when P1 relaxin was administered intradermally over tissue expanders for 7 d (314) and when rh2 relaxin was administered iv into male piglets that were implanted with tissue expanders (313). Consistent
with these findings in pigs, the sc infusion of rH2 relaxin for 14 d decreased the deposition of collagen in two rodent models of sc fibrosis (315).

Relaxin has also been reported to have antifibrotic effects on the lung, liver, and kidney in vivo in rodent models of fibrosis. When rH2 relaxin was infused sc into female mice for 14 d, it inhibited bleomycin-induced pulmonary fibrosis by reducing both alveolar thickening and collagen deposition (309). Similarly, continuous infusion of rH2 relaxin inhibited the accumulation of collagen in the airways of sensitized mice exposed to ovalbumin aerosol (316). Consistent with these findings, the sc infusion of rH2 relaxin into M1RKO mice for 14 d inhibited the progressive pulmonary fibrosis that occurs in these animals (61). The continuous sc infusion of rH2 relaxin into male rats for 28 d reduced liver weight and collagen levels when hepatic fibrosis was induced with carbon tetrachloride (310). Male rats were used in two studies to examine relaxin’s effects after experimental induction of fibrosis in the kidney. In one study, continuous sc infusion of rH2 relaxin inhibited bromoethylamine-induced interstitial fibrosis and the infiltration of macrophages, a cell type frequently associated with interstitial fibrosis (317). In the second study, daily ip administration of rH2 relaxin decreased proteinuria, serum creatinine, and interstitial fibrosis in an antiglomerular membrane model of renal fibrosis that was induced by immunization with the α3-chains of type IV collagen (312). Consistent with these findings, relaxin diminished the severity of sclerotic changes in glomeruli in two models of renal mass reduction (318).

The generally positive reports of early clinical studies that employed impure P1 relaxin in subjects with scleroderma, in addition to encouraging findings with in vitro and in vivo animal studies of the effects of pure relaxin on fibrosis in the 1980s and early 1990s, led Connective Therapeutics to initiate clinical studies in 1994 to assess rH2 relaxin for safety and efficacy in preventing the progression of stable diffuse scleroderma in human subjects. A phase II trial that employed about 20 patients per group examined the continuous sc infusion of 25 μg/kg or 100 μg/kg of body weight rH2 relaxin per day for a 24-wk period (319). The modified Rodnan skin score was employed as the primary measure of efficacy. Portions of the results of this study were favorable. The administration of rH2 relaxin was found to be safe. Moreover, 24 wk of treatment with the 25 μg/kg dose of rH2 relaxin, but not the 100 μg/kg dose, had statistically significant beneficial effects on skin thickness and mobility. In response to these encouraging results, a phase II/III study that employed more than 40 patients per group was conducted at doses of 25 μg/kg and 10 μg/kg rH2 relaxin (7). Unfortunately, skin elasticity, hand extension, oral aperture, cutaneous ulcers, and pulmonary function in rH2-treated patients did not differ from the placebo controls (7). It is unlikely that the failure of the clinical trials was attributable to insufficient rH2 relaxin. Heavy and irregular menstrual bleeding, which was postulated to be attributable to relaxin’s vasodilatory and/or angiogenic effects on the endometrium, was significantly more frequent in relaxin-treated subjects than in placebo controls (7). These recent failed clinical trials have raised serious doubts concerning relaxin’s potential for the treatment of scleroderma.

The recent development of the M1RKO mouse provided the first evidence that endogenous relaxin may act as a protective agent against fibrosis, particularly in males (61, 304). In the heart of M1RKO mice, there was an increase in left ventricular collagen and chamber stiffness that was accompanied by atrial hypertrophy and impeded left ventricular diastolic filling and venous return (304). In the lungs, there was a progressive increase in wet weight, collagen content, alveolar congestion, and bronchiolar epithelium thickening that was associated with altered lung function (61). Treatment of the M1RKO mice with rH2 relaxin reversed collagen deposition in both organs (61, 304).

B. Wound healing

The effective healing of wounds is dependent upon interactions that require ample local blood vessels (platelets, macrophages, neutrophils, endothelial cells, and smooth muscle cells) (320). Clinical studies in the late 1950s in which impure P1 relaxin brought about the healing of ischemic ulcers on fingers and toes provided the initial encouragement that relaxin might enhance wound healing (306, 321). More recent studies in which pure P1 relaxin or rH2 relaxin was administered to rodents also provided evidence of actions of relaxin on the vasculature and platelet aggregation that are supportive of a possible role of relaxin in wound healing. Relaxin was reported to induce vasodilatation not only in reproductive organs (123, 124, 209) but also the heart (79, 211), kidney (18), mesocecum (214), and liver (213) through a nitric oxide-mediated mechanism. Also consistent with the possibility that relaxin fosters the availability of blood at wound sites is the limited evidence that relaxin counteracts hypercoagulation. Porcine relaxin inhibited not only collagen- or thrombin-induced aggregation of human and rabbit platelets in vitro (322) but also reduced circulating platelets after 4 d of ip administration to male rats (323), and these effects were mediated, at least in part, by nitric oxide.

Recently, Unemori et al. (324) and Huang et al. (325) reported that continuous infusion of rH2 relaxin increased blood vessel content relative to controls in three rodent models of ischemic wound sites. Moreover, these workers provided evidence that relaxin increased the expression of the angiogenic cytokines VEGF and basic fibroblast growth factor (326) not only in cells removed from wound chambers in a rat model of sc wound healing but also cultures of human monocyte/macrophages (THP-1 cells) (324). Supportive of the view that relaxin contributes to wound healing is the observation that full thickness wound size was smaller in diabetic mice that received relaxin than in controls after 14 d of hormone treatment (325).

C. Cardiac protection

Bani Sacchi and co-workers demonstrated that P1 relaxin protects against myocardial injury caused by ischemia and reperfusion (IR) in the hearts of rats (212) and guinea pigs (79). When relaxin was present during IR, it reduced areas of damage, ventricular arrhythmias, mortality, neutrophil accumulation, and morphological indications of myocardial cell injury in male rats (212). Relaxin’s cardioprotective ef-
effects after IR in isolated male guinea pig hearts included increased coronary flow, improved cardiac contractility, and reduced morphological indications of myocardial cell injury (79). The mechanisms whereby relaxin protects the heart during IR are not known with certainty. Masini et al. (79) postulated that relaxin’s beneficial actions are partially attributable to the hormone’s vasodilatory effects. Bani Sacchi and co-workers demonstrated that relaxin increases dilation of small vessels in the rat heart (212) and increases coronary blood flow in isolated and perfused male rat and guinea pig hearts (211). Unemori and co-workers (327) examined the influence of relaxin in a rat model of myocardial infarction. They found that relaxin may influence the perfusion of ischemic sites by inducing VEGF and basic fibroblast growth factor, thus augmenting collateral vessel formation. Relaxin may also mediate its cardioprotective effects through mast cells. Mast cells undergo degranulation and histamine release during IR in both rat and guinea pig hearts. Recently, Bani and co-workers (75, 77) provided evidence that it may be through relaxin’s effects on neutrophils and endothelial cells that the hormone exerts its cardioprotective effects. In response to tissue injury such as IR, neutrophils adhere to and pass through the vascular endothelium to reach the interstitium where they become activated and release high amounts of harmful reactive oxygen species and lysozymes. Inflammatory mediators generated during tissue injury stimulate the expression of endothelial cell adhesion molecule-1 (75). Bani and co-workers reported that P1 relaxin inhibited both the pharmacological activation of human neutrophils (77) and the capacity of lipopolysaccharide-primed rat coronary endothelial cells to adhere to neutrophils (75). The cardioprotective effect of relaxin in IR appears to be attributable to the stimulation of endogenous nitric oxide production (79). Available evidence indicates that relaxin brings about its vasodilatory effects, at least in part, by acting directly on coronary endothelial cells and neutrophils to up-regulate inducible nitric oxide synthase II, thereby stimulating production of the potent vasodilator nitric oxide (74, 75, 77, 79, 211). The physiological significance of relaxin’s apparent cardioprotective effects is not known. It was postulated that circulating relaxin emanating from the corpus luteum could prevent ischemic heart disease in women during nonconceptive cycles and pregnancy (79).

There is limited evidence that relaxin produced locally may provide cardioprotective actions and/or predict the severity of chronic heart failure. It has been reported that R1 relaxin, R3 relaxin, and relaxin receptor LGR7 are expressed in the rat heart (57, 58, 328) and that both M1 and M3 are expressed in the mouse heart (11). The possibility that relaxin produced locally has a cardioprotective effect is supported by the recent report that male M1RKO mice have increased ventricular collagen and chamber stiffness (304). The human heart has been reported to secrete relaxin. Dschietzig, et al. (329) reported that gene expression of both H1 and H2 relaxin increased in failing atrial and ventricular tissue, and plasma concentrations of myocardial expression of the genes for H1 and H2 relaxin correlated positively with the severity of congestive heart failure. Fisher et al. (330) also found that H2 relaxin is secreted by the heart in increased amounts in patients with chronic heart failure but that plasma H2 relaxin concentrations are not a predictor of clinical outcome. In vitro studies with pulmonary artery endothelial cells in a flow chamber model that mimicked the hemodynamic changes associated with coronary heart failure provided evidence that relaxin suppressed the hemodynamically induced increase in endothelin 1 secretion through the induction of endothelin type B receptors (329).

D. Allergic responses

Resident mast cells and circulating basophils share IgE receptors and, through the release of granular agents such as histamine and other vasoactive agents, trigger pathogenic changes that mediate allergic reactions and inflammation (75, 76, 331). Relaxin inhibits granule exocytosis and histamine release by both mast cells and basophils through a nitric oxide-mediated process that prevents the increase in intracellular Ca2+ that triggers granule release from these cells (76, 77, 332). There is evidence that relaxin protects against the pathogenetic events underlying allergic reactions and may do so through effects on resident mast cells (333), neutrophils (77), and/or endothelial cells (75). Porcine relaxin counteracted both the asthma-like reactions (coughing and dyspnea) induced by inhaled antigen in sensitized adult male guinea pigs (333) and the cardiac anaphylaxis-like reactions (reduced coronary flow and heart rate) induced by antigen in perfused hearts from sensitized male guinea pigs (331). Relaxin’s protective effects in these male guinea pig models of allergy were associated with prevention of mast cell degranulation (333), decreased histamine release (331), and reduced interstitial neutrophil content (333). Additional biochemical findings with the cardiac anaphylaxis model led to the postulation that relaxin’s protective effects may be mediated by readily diffusible nitric oxide that increases cGMP and decreases intracellular Ca2+, thereby not only reducing the release of histamine from cardiac mast cells but also decreasing vascular tone of coronary vessels (331).

V. Conclusions and Future Directions

A. Conclusions

Research conducted since the early 1980s has elevated the status of relaxin from a poorly understood and frequently ignored hormone to one that requires attention when the hormonal regulation of pregnancy and parturition is considered. It is now established that circulating relaxin is essential during pregnancy in at least three species—rats, mice, and pigs. Two vital roles have been identified. Relaxin promotes growth and softening of the cervix and vagina and thereby enables rapid and safe delivery of the fetuses. Relaxin also promotes growth and development of the mammary apparatus in these three species. Relaxin’s effects on the nipples are required for normal lactational performance in rats and mice. Relaxin has far more striking effects on mam-
mary parenchymal tissue development during late pregnancy in pigs than in rats and mice. The fact that relaxin has remarkable effects on development of the cervix and mammary apparatus (glands and/or nipples) in rats, mice, and pigs encourages the view that relaxin may have similar effects in other species. One must keep in mind, however, that there is great diversity in the physiology of relaxin among species. An extreme example is the sheep. Available evidence indicates that relaxin is neither produced nor secreted in sheep (334).

Studies in rats demonstrated that endogenous circulating relaxin has additional actions during pregnancy (Fig. 13). Relaxin inhibits uterine contractile activity, increases both glomerular filtration rate and effective renal plasma flow, and promotes drinking. The physiological importance of these actions during pregnancy remains to be demonstrated. Pregnancy is maintained, and both litter size and viability do not differ from controls in relaxin-deficient rats, mice, and pigs. It has also been postulated that relaxin may play a role in follicular development, ovulation, and/or implantation. Whereas both in vitro and in vivo studies provide evidence that the administration of exogenous relaxin has effects on ovarian and endometrial cells, there is presently no evidence that endogenous relaxin contributes to these three reproductive processes in any species. Reports to date with both the M1RKO mouse and the LGR7KO mouse support the view that M1 relaxin is not required for normal follicular development, ovulation, or implantation in mice. The possibility that small amounts of locally produced relaxin act through a paracrine/autocrine mechanism(s) to contribute to these processes cannot be ruled out for any species.

Far less progress has been made toward an understanding of the actions of endogenous relaxin in the male. Available evidence largely supports the view that relaxin is not a circulating hormone in males. However, recent reports of underdeveloped testis, underdeveloped reproductive tract, and reduced fertility in both M1RKO and LGR7KO male mice provide compelling evidence that low levels of circulating and/or locally produced M1 relaxin play a role in the development of the male reproductive system. Also, the observations of cardiac and pulmonary fibrosis in male M1RKO mice infer that endogenous relaxin has antifibrotic protective effects in some organs.

Since the mid-1990s, numerous and diverse reports of therapeutic actions of exogenous relaxin on nonreproductive processes such as fibrosis, wound healing, cardiac protection, and allergic response have emerged. These actions are fascinating because they occur in males and females, are remarkably diverse, open unanticipated avenues of investigation of relaxin’s actions, and have clinical potential.

B. Future directions

With the recent discovery of relaxin form 3 and the identification of the cognate relaxin receptors for both relaxin forms 1 (LGR7) and 3 (GPCR135), it appears that all existing forms of relaxin in the mouse, rat, pig, and human and the receptors that trigger relaxin’s actions are known. These findings make it possible to examine the physiological actions of relaxin in a more comprehensive and precise manner than was possible in the past. Several examples of important future objectives come to mind. It is now possible to determine whether M3 relaxin is produced and has important autocrine/paracrine actions by examining the phenotype of male and female M3 relaxin knockout mice. Knowledge of the structures of the relaxin receptors makes possible the identification of potential target tissues for both forms of the hormone, the identification of factors that regulate relaxin receptor expression, and rigorous experimentation to determine whether other members of the family of relaxin-related genes, such as INSL4–6, have the capacity to induce relaxin bioactivity. The phenotype of the M1 relaxin receptor LGR7KO mouse was recently published (59), and the phenotype of the cognate M3 relaxin receptor GPCR135 knockout mouse will most surely soon follow. The totality of the physiological effects of M1 relaxin plus M3 relaxin in mice should be revealed by examining the phenotype of LGR7 and GPCR135 double-knockout male and female animals.

**Fig. 13.** Relaxin’s diverse actions in rats. Those seven targets where effects of circulating relaxin have been demonstrated during pregnancy are indicated with solid lines. There is also evidence of action of relaxin in the skin, ovary, liver, heart, and lung. Relaxin’s actions in the nonreproductive targets occur in both male and female rats, and their physiological significance is not understood.
Because the physiological roles of relaxin vary among species, the reality is that we have some understanding of the endogenous hormone’s actions in only rats, mice, and pigs. Accordingly, there is need to investigate the role of relaxin in additional species. There are no known relaxin antagonists. Currently, neutralization of endogenous relaxin affords the most rigorous experimental approach to examine the role of circulating relaxin in nearly all species. Advantages to this approach are its potential for application to a variety of species at specific time frames during physiological processes of interest. It is presently uncertain whether relaxin produced at secondary sources that may act locally within tissues can be effectively neutralized by systemic administration of either relaxin antibodies or the ligand-binding ectodomain of the relaxin receptor. It seems particularly important to examine this approach toward gaining a better understanding of the possible physiological significance of endogenous relaxin in a primate. The advent of the M1RKO mouse and the LGR7KO mouse has not only confirmed findings concerning the actions of circulating R1 relaxin during pregnancy in rats, but also identified possible unsuspected actions of relaxin in the reproductive tract, heart, and lungs of male mice. M1RKO and LGR7KO mice, in combination with the likely future creation of M3 relaxin and M3 receptor knockout mice, have strong potential for identifying the local roles of relaxin in secondary sources such as the mouse brain and heart. There are considerations that should be kept in mind, however, as findings with these animals occur. Although LGR7 is more highly responsive to relaxin than is LGR8, it is possible that LGR8 acts as a paracrine receptor for locally produced relaxin. Accordingly, the production of a knockout mouse that is deficient in both LGR7 and LGR8 genes may be required to identify the totality of relaxin’s physiological roles. Moreover, limitations of the gene knockout approach include the present restriction to only the mouse, the inability to examine the role of relaxin at specific times during the physiological process of interest, and the potential for recruitment of compensating mechanisms that can mask the physiological role of relaxin.

It is well established that relaxin’s actions on reproductive tissues are dependent upon and/or are augmented by estrogen. However, essentially nothing is known concerning the mechanism(s) whereby relaxin works in concert with estrogen to bring about its effects. To not only understand the basic mechanisms associated with relaxin’s actions but also to make possible a well-informed experimental approaches toward realizing relaxin’s clinical potential, the role that estrogen plays in enabling relaxin’s actions in the target of interest must be understood.

What can be concluded concerning the actions of relaxin in the human and its potential for therapeutic application? It is well established that H2 relaxin is produced and secreted in relatively small amounts during pregnancy, but that no physiological role for circulating relaxin has been identified. There is evidence that small amounts of H1 and H2 relaxin are produced in placental and decidual tissues during pregnancy, and they may act locally through autocrine/paracrine mechanisms. Regardless of whether the relatively small amounts of endogenous ovarian and extracellular human relaxin are sufficient to have important physiological roles, there are reasons to believe that the hormone has clinical potential. Although recent clinical trials failed to demonstrate that rH2 relaxin has therapeutic effectiveness in the treatment of scleroderma, they did demonstrate that the hormone is active in the human and that prolonged treatment is well tolerated.

Whereas discovering and gaining a better understanding of the physiological effects of relaxin are sufficient reward for many “relaxinologists,” it is the potential clinical applications of relaxin that both capture the interest of the public at large and dictate the level of funding for relaxin research. The amazingly diverse actions of relaxin on reproductive tissues in the female and nonreproductive tissues in both the female and the male provide numerous avenues of investigation that have clinical implications. For example, in the female, relaxin holds promise of providing a better understanding of cervical and vaginal growth and remodeling, mammary apparatus development, and uterine contractility. In both females and males, the use of relaxin will lead to a better understanding not only of brain, renal, and heart function, but also vasodilation, fibrosis, and wound healing. With all of the promising unexplored opportunities for important discoveries of the actions of relaxin, it seems nearly certain that there will be a high level of research interest in this fascinating hormone for the foreseeable future.

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