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Sources and biological actions of relaxin in pigs

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Although the major source of relaxin in pigs is the corpus luteum of pregnancy, there is now evidence for relaxin gene expression and translation into protein in the theca interna cells of the preovulatory follicle, the corpus luteum of the cycle and the uterus. The theca interna cells retain their ability to express the relaxin gene and protein following ovulation. During the early stages of development of the corpus luteum, the theca-derived small lutein cells are the source of the relaxin transcript. As the corpus luteum becomes fully functional, there is a switch in the site of relaxin synthesis from small theca-derived lutein cells to large granulosa-derived cells. In the absence of luteolysis, this switch is accompanied by a dramatic rise in relaxin synthesis. Relaxin has been identified in boar seminal plasma and can maintain or increase sperm motility. However, a source of relaxin in the boar has not been identified. Relaxin is an important regulator of uterine function during pregnancy acting systemically to suppress myometrial activity and promote cervical dilation at parturition. The changes in thecal relaxin production during follicle development and its ability to promote growth and changes in proteolytic enzyme activity of granulosa cells *in vitro* have led to the concept of an autocrine or paracrine role for relaxin within the follicle. Uterotrophic effects of relaxin have been reported in rodents and swine and support the hypothesis that relaxin promotes uterine growth and expansion in early pregnancy to accommodate the growing fetuses. Mammotrophic effects of relaxin in rodents have now been extended to pigs, with evidence that relaxin is necessary for normal mammary parenchymal development in late pregnancy. In most instances the mechanisms responsible for, and the physiological significance of, these diverse biological effects remain to be elucidated.

Introduction

Relaxin was first recognized as an ovarian hormone by Hisaw *et al.* (1930) as a product of the porcine corpus luteum of pregnancy, having systemic actions on uterine smooth muscle and the connective tissue of the reproductive tract. Relaxin has been recognized as a hormone in a number of species; however, pigs remain the richest source and the importance of relaxin has been well established in porcine reproductive physiology.

Although the corpus luteum of pregnancy is a major source of relaxin, tissues from nonpregnant animals have also been reported to produce relaxin (Evans *et al.*, 1983; Bagnell *et al.*, 1990a). The identification of relaxin receptors (Mercado-Simmen *et al.*, 1982) and observations of biological effects (Hall *et al.*, 1990; Zhang and Bagnell, 1993) in tissues of nonpregnant pigs extend the scope of relaxin research beyond that of a 'hormone of pregnancy'.

The present paper will focus on relaxin by reviewing the literature and presenting some of the more recent research on nontraditional sources and roles for relaxin in porcine reproductive physiology.

References to other species will be made as appropriate to provide a broader perspective and a basis for comparison. For other aspects of the physiology of relaxin in pigs (for example control of secretion, mechanism of action and receptors) the reader is referred to several comprehensive reviews (Schwabe *et al.*, 1978; Porter, 1979a; Bryant-Greenwood, 1982; Sherwood, 1988).

Chemistry of Relaxin

Studies on the chemistry of relaxin focused initially on porcine relaxin owing to its abundance in the corpus luteum of pregnancy. The isolation of porcine relaxin (Sherwood and O'Byrne, 1974) and the elucidation of its primary structure (Schwabe *et al.*, 1976; James *et al.*, 1977) were followed by development of specific and sensitive radioimmunoassays (Sherwood *et al.*, 1975a; Afele *et al.*, 1979). Recombinant DNA techniques were used to determine the nucleotide sequence of the gene coding for porcine relaxin and to produce the relaxin cDNA from mRNA of ovaries from pigs in late pregnancy (Haley *et al.*, 1982).

Porcine relaxin is a 6.3 kDa peptide consisting of an A chain of 22 amino acids and a B chain of 31 amino acids covalently linked by two disulfide bonds; the A chain contains an intradisulfide linkage (Schwabe *et al.*, 1976; James *et al.*, 1977). Relaxin is synthesized as a large, single-chain precursor, preprorelaxin, from which prorelaxin is formed by removal of a signal peptide (24 amino acids). Prorelaxin is then converted to a double chain structure after cleavage of a 104 amino acid connecting peptide (Gast, 1982, 1983). Plasma immunoreactive relaxin in pigs is secreted in a biologically active form, circulates unbound and has an amino acid composition similar to that of the form stored in the corpus luteum (O'Byrne *et al.*, 1989). The structural similarity of relaxin to insulin and insulin-like growth factors (IGF), with respect to A and B chain lengths and the position of the disulfide bridges (Schwabe and McDonald, 1977), led to the postulate that these hormones constitute a family of peptides (Blundell and Humbel, 1980). However, there is only about 25% amino acid sequence similarity between relaxin and insulin (Issacs and Dodson, 1981). Relaxin does not compete for insulin (Olefsky *et al.*, 1982) or IGF-I receptors (Hernandez *et al.*, 1988) nor does porcine insulin displace porcine relaxin from its receptor in pig myometrium (Mercado-Simmen *et al.*, 1982). Furthermore, there is no crossreactivity between relaxin and insulin in their respective radioimmunoassays (Rawitch *et al.*, 1980; Greenwood *et al.*, 1981).

Sources of Relaxin in Female Pigs

Ovarian follicle

Immunoreactive relaxin was first detected in porcine follicular fluid from cyclic and pregnant pigs, and segments of follicle wall were shown to produce relaxin *in vitro* (Bryant-Greenwood *et al.*, 1980; Matsumoto and Chamley, 1980). Using isolated granulosa and theca cells from follicles of pregnant mares' serum gonadotrophin/human chorionic gonadotrophin (PMSG/hCG)-treated prepubertal gilts, Evans *et al.* (1983) found that the theca cell layer was the principal source of follicular relaxin. Using the same model, we have confirmed the production of relaxin by theca cells *in vitro* (Bagnell, 1991) and the presence of relaxin in the theca interna layer of the preovulatory pig follicle by immunohistochemical studies (Bagnell *et al.*, 1987). More recently, using northern analysis and *in situ* hybridization, we demonstrated that relaxin gene expression is restricted to theca interna cells, and there was no evidence for relaxin mRNA in the granulosa cell layer (Bagnell *et al.*, 1990b). Moreover, the distribution and relative concentration of relaxin mRNA parallels the production *in vitro* and immunohistochemical staining reported in the developing preovulatory follicle (Evans *et al.*, 1983; Bagnell *et al.*, 1987; Bagnell, 1991).

In contrast to the studies that have identified the theca interna as the principal source of follicular relaxin, other investigators have reported production of relaxin by granulosa cells. Loeken *et al.* (1983) found that granulosa cells from large porcine follicles released relaxin in response to LH *in vitro* (Loeken *et al.*, 1983) and low amounts of relaxin mRNA were detected in extracts of porcine granulosa cells after culture with FSH and LH (Einspanier *et al.*, 1986). In both of these studies, the granulosa cells were cultured in the presence

of gonadotrophins, which are known to promote luteinization (Channing and Ledwitz-Rigby, 1975). In addition, relaxin has been found in luteinized granulosa cells of the human preovulatory follicle after ovarian hyperstimulation (Yki-Jarvinen *et al.*, 1984; Gagliardi *et al.*, 1992) and in luteinized granulosa cells of the porcine corpus luteum (Bagnell *et al.*, 1989). Collectively, these results suggest that luteinization of the granulosa cells may be required to activate expression of the relaxin gene and production of relaxin. Such a concept could provide a reasonable explanation for the apparent conflicting data on the source of follicular relaxin.

Corpus luteum

Low amounts of immunoreactive relaxin were detected in corpora lutea from cyclic pigs and maximum values were reached at about day 14 of the oestrous cycle (Sherwood and Rutherford, 1981; Denning-Kendall *et al.*, 1989). This pattern of relaxin activity in the corpus luteum of the cycle is consistent with that found in peripheral blood (Messine *et al.*, 1989) and with relaxin immunostaining of corpora lutea at different stages of the cycle (Ali *et al.*, 1986; Bagnell *et al.*, 1989; Denning-Kendall *et al.*, 1989). There is also a suggestion that the pattern of relaxin secretion by the corpus luteum parallels that of progesterone (Kotwica *et al.*, 1991).

During pregnancy, the corpus luteum is the principal source of relaxin in pigs. Bioactive relaxin in the corpus luteum increases steadily from about day 20 of pregnancy reaching maximum values at about day 110 and declining rapidly within 16 h of birth (Anderson *et al.*, 1973). Concentrations of relaxin in plasma remain below 2 ng ml^{-1} until about day 100, then increase gradually to about 10 ng ml^{-1} three days before parturition. During the 48 h before farrowing the concentrations of relaxin increase markedly to 50–250 ng ml^{-1} about 24–14 h before farrowing. Thereafter, the concentrations decline rapidly to about 10 ng ml^{-1} (Sherwood *et al.*, 1975b, 1981). The pattern of concentrations of relaxin in peripheral blood during pregnancy is consistent with the view that relaxin accumulates in electron-dense cytoplasmic granules in pregnancy and is released during their rapid degranulation and disappearance during the last two days of gestation (Belt *et al.*, 1971; Kendall *et al.*, 1978; Anderson *et al.*, 1983).

Anderson *et al.* (1983) showed that in both pregnant and unmated gilts hysterectomized on day 6 of the cycle relaxin concentrations increased steadily until day 114 after oestrus. Thereafter, the relaxin concentrations in the pregnant gilts declined rapidly just before farrowing. In the hysterectomized group, relaxin concentrations declined steadily until day 120; there was then a gradual decrease to day 150 after oestrus. Anderson *et al.* (1983) postulate that relaxin release in ageing corpora lutea may be a precisely timed, genetically controlled event independent of endocrine control by the conceptuses or uterus. Although genetic control may contribute to the timing of relaxin release, there is evidence that other factors including the hypothalamic–pituitary axis (Felder *et al.*, 1986) and luteal cells themselves (Huang *et al.*, 1991) are involved.

A number of studies have demonstrated the presence of relaxin in porcine corpora lutea by immunohistochemistry. It was found that relaxin immunostaining increased to a peak by day 14 of the cycle (Fields and Fields, 1985; Ali *et al.*, 1986; Bagnell *et al.*, 1989). Thereafter, the immunostaining decreased as the corpora lutea regressed. In a more detailed study, with corpora lutea from nonpregnant and pregnant pigs, Denning-Kendall *et al.* (1989) showed that relaxin immunostaining in both types of corpora lutea was indistinguishable up to day 14 of the cycle. In the corpus luteum from nonpregnant pigs, the staining intensity decreased after day 14. In contrast, the intensity of relaxin immunostaining increased markedly in the corpora lutea of pregnant pigs between days 11 and 14 and continued to increase in intensity up to day 31 of pregnancy. In addition, this study showed that histochemical staining for alkaline phosphatase, which is associated only with the small, theca interna-derived luteal cells (Corner, 1944) was restricted to the relaxin immunoreactive cells in the early luteal phase. As the corpus luteum developed there appeared to be a switch in the site of relaxin immunostaining to the large luteal cells which were not associated with alkaline phosphatase staining and may be derived from granulosa cells. This apparent switch in the site of relaxin immunostaining is most pronounced in the corpora lutea from pregnant pigs and was accompanied by a dramatic increase in relaxin content of the corpus luteum.

We have confirmed and extended those studies on the site of luteal relaxin production using a combination of northern analysis and *in situ* hybridization to follow the development of relaxin gene

expression during corpus luteum development (Bagnell *et al.*, 1993). Akaline phosphatase (AP) was used as a marker for theca interna-derived lutein cells and the relationship between AP-positive and relaxin mRNA-containing cells was assessed. Corpora lutea obtained at various stages of development up to 19 days after ovulation were obtained from prepubertal gilts treated with PMSG/hCG to induce follicular development and ovulation. Northern analysis revealed that relaxin gene expression increased with development of the corpus luteum and reached maximum values at about day 14 after ovulation. Thereafter, as the corpus luteum regressed, the amount of relaxin mRNA declined. The question of the cellular source of luteal relaxin was studied using small and large cell populations isolated from corpora lutea of pigs at day 10 and 16 of the oestrous cycle. We showed that only small cells expressed relaxin mRNA at day 10, whereas at day 16 the large cells were the source of relaxin gene expression. *In situ* hybridization showed that the relaxin gene transcript was associated with cells corresponding to AP-positive small, luteinized theca cells until about day 9 after ovulation. Thereafter, the first AP-negative, granulosa-derived lutein cells expressing relaxin mRNA were observed. At day 14, relaxin hybridization and AP staining were distributed throughout the luteal tissue. With regression of the corpus luteum, both AP staining and relaxin hybridization declined.

Gast (1982) reported that RNA isolated from the corpus luteum of nonpregnant sows directed the synthesis of a protein with immunological and sequence identity to authentic relaxin. We confirmed and extended those studies by showing that relaxin mRNA is present in the corpora lutea of sows at early and midluteal phases of the cycle and increases during pregnancy (Bagnell *et al.*, 1990b). By day 40 of pregnancy, luteal relaxin mRNA increased 50-fold over amounts observed at day 13 of the cycle (Bagnell *et al.*, 1990a). Lobb and Porter (1992) extended these findings by pin-pointing the first indication of amplified relaxin gene expression at day 16 of pregnancy. A further increase in relaxin mRNA expression occurred at midpregnancy with a maximum at day 65 (Lobb and Porter, 1992). Evidence that this increase in relaxin gene expression in early pregnancy is translated into protein is reflected in a dramatic rise in content of immunoreactive relaxin in the corpus luteum between days 11 and 14 of pregnancy (Denning-Kendall *et al.*, 1989).

Collectively, these studies on the source of luteal relaxin demonstrate the important contribution of the theca interna to formation of corpus luteum and development in pigs. The parallel fluctuations in relaxin transcript and relaxin protein imply active synthesis rather than sequestration of the hormone by luteal tissue. Although the significance of the switch in cell type producing relaxin during development of the corpus luteum is unknown, it is consistent with the observation that relaxin is secreted by large luteal cells of the fully developed corpus luteum (Taylor *et al.*, 1987). The mechanisms that subserve the regulation of luteal relaxin synthesis and secretion are poorly understood. However, it has been shown that calcium mobilization, cyclic nucleotides, protein kinase activation and products of the cyclooxygenase pathway of arachidonic acid metabolism are implicated in the control of relaxin secreted by cultured porcine luteal cells *in vitro* (Taylor and Clark, 1988a, b, 1989; Taylor *et al.*, 1987).

Uterus

The uterus produces relaxin in pregnant guinea-pigs (Pardo and Larkin, 1982), rabbits (Lee and Fields, 1990) and rats (Fields *et al.*, 1992). The porcine uterus was studied as a possible source of the relaxin found in the plasma of lactating sows during suckling and nuzzling (Afele *et al.*, 1979). Immunoreactive relaxin was isolated from uteri in pregnancy and lactation, but the low quantities detected were thought to represent receptor-bound hormone (Setliff and Greenwood, 1981). Immunoreactive relaxin was detected in the uterine epithelium of day 16 pregnant gilts, but not at day 13 of pregnancy nor on these days during the oestrous cycle (Zhang *et al.*, 1992). Evidence that this represented relaxin production and not receptor-bound hormone was shown by reverse transcriptase–polymerase chain reaction studies which detected relaxin mRNA in endometrial tissue of day 16 pregnant gilts (Zhang *et al.*, 1992).

Sources of Relaxin in Male Pigs

In boars, relaxin immunoactivity was reported to be associated with both interstitial and Sertoli cells of the testis (Dubois and Dacheux, 1978), but when antiserum against highly purified porcine relaxin was used, these results could not be confirmed (Arakaki *et al.*, 1980; Yamamoto and Bryant-Greenwood,

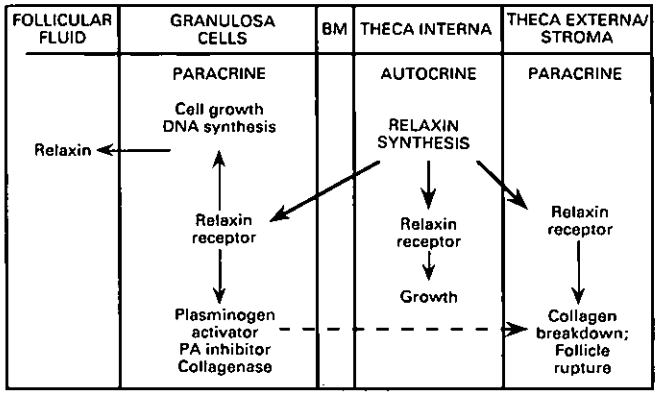

Fig. 1. Hypothesis for an autocrine or paracrine role of relaxin in the ovarian follicle. This hypothesis includes production of relaxin by theca interna cells which is detectable in follicular fluid, biological actions on cell growth and connective tissue protease activity via receptors in follicle compartments for locally produced relaxin. BM: basement membrane.

1981). Other studies have demonstrated the presence of immunoreactive relaxin in boar seminal plasma (Juang *et al.*, 1990), but did not identify the site of production. The source of relaxin in boars requires further investigation.

Biological Actions of Relaxin in Female Pigs

Follicular remodelling and growth

The growing evidence for production of relaxin by theca interna cells of the developing preovulatory follicle, in the face of undetectable relaxin concentrations in the peripheral circulation at this time, has led to the hypothesis that relaxin may play an autocrine or paracrine role in the follicle (Bryant-Greenwood, 1982; Bagnell *et al.*, 1984). Our current view of the way relaxin functions within the follicle is shown (Fig. 1). This working hypothesis meets in part the criteria established for an autocrine or paracrine system. These criteria include (1) local production: relaxin is a product of theca interna cells, (2) local action: relaxin acts on thecal cells (autocrine) or adjacent granulosa and theca externa/stromal cells (paracrine), (3) receptors on nearby target tissues to produce a biological response and (4) developmental alterations with changing physiological state, that is changes in production, action, receptor concentration and biological sensitivity.

An important biological action of relaxin is its ability to remodel collagen in its target tissues, the interpubic ligament and uterine cervix during pregnancy (Schwabe *et al.*, 1978). The process of collagen remodelling in the last two tissues can be compared with that occurring in the connective tissue of the follicle wall before ovulation (Espey, 1974). Proteolytic enzymes have been implicated in the process of follicle rupture, and plasminogen activator (PA), a protease produced by follicular cells, is considered to be one of the key enzymes involved (Hsueh *et al.*, 1988). Plasminogen activator converts plasminogen to plasmin, which activates collagenase and degrades proteoglycan.

It was shown, using porcine granulosa cells from large and small follicles, that PA activity was three times greater in granulosa cells from large follicles after culture for 24 h (Bagnell, 1991). Addition of relaxin had no effect on PA activity, whereas addition of FSH resulted in a twofold increase in PA activity by granulosa cells from both small and large follicles. However, relaxin enhanced the effects of FSH by increasing PA activity of granulosa cells when compared with cells cultured with FSH alone. These results are similar to those in rats in which gonadotrophin priming of granulosa cells was required to demonstrate an effect of relaxin on PA activity (Too *et al.*, 1984). In addition, the increased PA activity of granulosa cells from large follicles is consistent with data showing that PA activity in the porcine preovulatory follicle increased with approaching ovulation (Politis *et al.*, 1990).

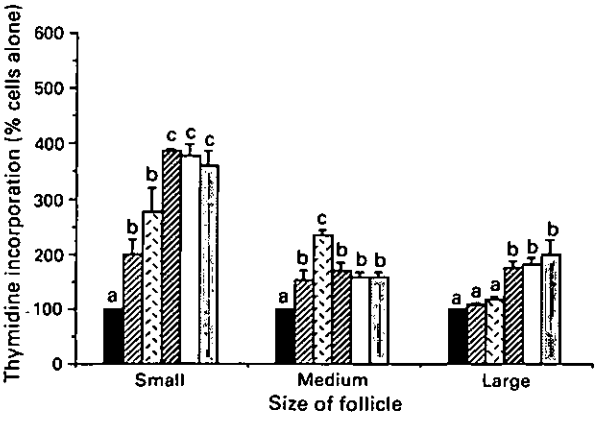

Fig. 2. Effect of relaxin on [^{3}H]thymidine incorporation by granulosa cells from developing pig follicles. Cells from small (1–2 mm), medium (3–5 mm) and large (≥6 mm) follicles were plated in serum-containing media for 48 h. After attachment, cells were incubated in serum-free media with relaxin (▨) 1, (▨) 10, (▨) 30, (□) 100 and (■) 1000 ng ml^{-1} or (■) alone for 24 h. [^{3}H]thymidine incorporation into DNA was then determined. Data are means ± SEM of four experiments. Bars with different superscripts within each follicle size were significantly different ($P < 0.05$) (Reproduced with permission from Zhang and Bagnell, 1993).

The anabolic effects of relaxin in other reproductive tissues (Hall *et al.*, 1990; Hurley *et al.*, 1991) and of members of the insulin-like family of hormones on ovarian cells (Baranao and Hammond, 1984; May *et al.*, 1988) prompted us to investigate the effects of relaxin alone and in combination with insulin or IGF-I on porcine follicular cells (Zhang and Bagnell, 1993). Relaxin alone stimulated [^{3}H]thymidine incorporation into DNA of granulosa cells from small, medium and large porcine follicles (Fig. 2). After 6 days of exposure to relaxin *in vitro*, there was a dose-dependent response in the proliferation of granulosa cells from small follicles (Fig. 3). The increased numbers of cells were correlated with the relaxin-induced increase in [^{3}H]thymidine incorporation into DNA in these cells. In addition, relaxin increased insulin- and IGF-I-induced DNA synthesis in granulosa cells at all stages of follicular development, suggesting an interaction of relaxin with these factors during follicular growth. These observations indicate that relaxin acts as a growth factor in developing pig follicles.

The changes in relaxin production with development of the preovulatory follicle, the increase in granulosa cell proteolytic enzyme activity *in vitro* in response to relaxin and the growth-promoting action of relaxin on granulosa cells support the concept of an autocrine or paracrine role for relaxin in preovulatory follicular development in pigs. Whether relaxin acts on other cells in the ovary or plays a role in the remodelling of connective tissue and rupture of the follicle *in vivo* is unknown. Relaxin has been shown to influence aromatase activity of human endometrial stromal cells (Tseng *et al.*, 1987), but a similar action on porcine granulosa cells was not observed (Bagnell, 1991). Other possible actions of relaxin within the ovary, such as altering collagen content of the follicle wall or influencing smooth muscle activity have not been investigated. Evidence for ovarian relaxin receptors is based on the biological response of granulosa cells to relaxin *in vitro*; however, an ovarian relaxin receptor remains to be characterized.

Uterine accommodation

The establishment and maintenance of pregnancy involves metabolic and physical changes in the uterus to accommodate the growing fetuses. Relaxin is an important regulator of myometrial activity during pregnancy; however, a lesser understood action is the ability of relaxin to stimulate uterine growth. In rats, uterotrophic actions of relaxin are suggested from studies showing imbibition of water by uterine

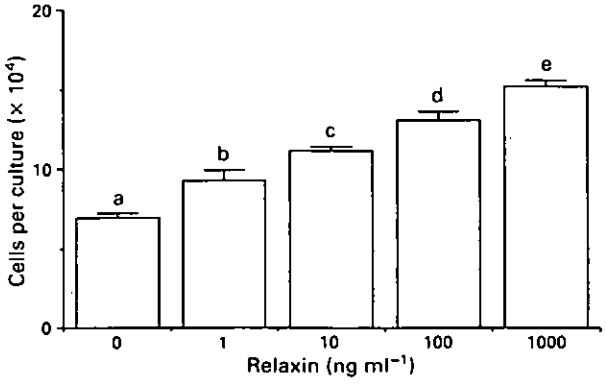

Fig. 3. Effects of relaxin on proliferation of granulosa cells from small pig follicles. Granulosa cells from small follicles were plated as described in Fig. 2. After attachment, cells were incubated in serum-free media with relaxin (1–1000 ng ml^{-1}) for 6 days. Media were changed at 72 h; fresh hormone was added for an additional 72 h and number of cells was counted. Data are means ± SEM for four experiments. Bars with different superscripts were significantly different ($P < 0.05$) (Reproduced with permission from Zhang and Bagnell, 1993).

tissues and increased amounts of soluble protein, collagen, glycosaminoglycans and glycogen in the uterus (Steinetz *et al.*, 1957; Vasilenko *et al.*, 1980, 1981; Vasilenko and Mead, 1987). The increase in uterine weight after administration of relaxin can be attributed largely to the accumulation of fluid in uterine tissues, which may be due to increased vascularity or enhanced blood flow (Vasilenko *et al.*, 1986). However, on the basis of these observations, Vasilenko and Mead (1987) postulated a uterotrophic role for relaxin in the rat uterus to accommodate the rapidly growing fetuses during early pregnancy. Hall *et al.* (1990) demonstrated uterotrophic effects of relaxin based on increased uterine dry weight, protein and DNA content, following administration of relaxin to prepubertal gilts. These latter changes were accompanied by cervical softening and imbibition of water by the uterus. In a recent study, Hall *et al.* (1992) showed that relaxin can promote hypertrophic and hyperplastic growth of the pig uterus in the presence or absence of oestrogen or progesterone. In addition, oestrogen appears to enhance the effects of relaxin stimulated uterine growth, which is consistent with results from studies in rats (Kroc *et al.*, 1959; Vasilenko and Mead, 1987). Although the mechanisms by which relaxin stimulates uterine growth have not been defined, it is possible that relaxin may act directly through a uterine receptor which in turn could be regulated by oestrogen (Mercado-Simmen *et al.*, 1982).

Myometrial relaxation

Relaxin-induced inhibition of uterine smooth muscle activity *in vivo* and *in vitro* has been well documented in pregnant and nonpregnant pigs (Porter, 1979b). Relaxin is thought to prevent premature labour, but, paradoxically, it facilitates delivery by inducing cervical ripening before parturition (Bryant-Greenwood, 1982). To explain this apparent paradox, Porter (1979b) suggested that relaxin inhibits uterine contraction during the antepartum period of progesterone withdrawal while priming the myometrium to respond to oxytocic signals that generate highly coordinated labour contractions and expulsion of the fetuses. This explanation is supported by the results of studies using the oestrogen-primed, ovariectomized nonpregnant mini-pig, showing that relaxin lowers the frequency of contractions *in vitro*, without affecting the amplitude (Porter and Watts, 1986). In addition, the myometrium remained responsive to oxytocin despite the infusion of relaxin. These studies were supported and extended by showing that in myometrial tissues from pregnant and nonpregnant pigs, relaxin inhibited spontaneous contractions *in vitro*, but had no effect on oxytocin-, PGE_2- or $PGF_{2\alpha}$-induced contractions (MacLennan *et al.*, 1986; Pupula and MacLennan, 1989).

Cervical softening

During most of mammalian pregnancy, the cervix is a firm, inextensible barrier protecting the fetus from the external environment and premature delivery. However, in late pregnancy, the tensile properties of the cervix change promoting increased distensibility or 'softening' and dilation to allow passage of the fetuses at parturition. In the firm cervix, collagen fibrils are densely arranged with little proteoglycan-rich matrix, whereas in the softened cervix, the collagen fibrils are dispersed and randomly oriented with an increase in matrix separating the fibrils (Sherwood, 1988). There is good evidence that relaxin plays a major role in the remodelling of the cervix in late pregnancy in a number of species and this has been the subject of several reviews (Steinetz *et al.*, 1980; Porter, 1981).

Zarrow *et al.* (1956) were the first to report that porcine relaxin following oestrogen priming produced maximum dilation of the cervix by the third day of treatment in pigs. Oestrogen alone has been shown to promote cervical softening in pregnant sheep and women (Fitzpatrick and Liggins, 1980); however, cervical remodelling in pigs cannot be attributed to oestrogen alone. During oestrus, when oestrogen concentrations are high, the porcine cervix is relatively firm in comparison with other stages of the cycle (Rigby, 1967; Meredith, 1977). In addition, treatment of ovariectomized, nonpregnant pigs with oestrogen caused constriction of the cervix (Smith and Nalbandov, 1958), whereas cervical softening was reported in prepubertal gilts treated with relaxin *in vivo* (Hall *et al.*, 1990). Relaxin-induced growth and softening of the cervix in rats and mice appears to be oestrogen dependent (Kroc *et al.*, 1959). Likewise in pregnant pigs, marked changes in the physical properties of the cervix have been reported which were temporally correlated with high serum oestrogen or oestrogen and relaxin (Eldridge-White *et al.*, 1989). In ovariectomized pregnant pigs, increases in the physical properties (extensibility, lumen diameter and wet weight) of both the uterine and vaginal portions of the cervix occur only after treatment with relaxin (O'Day *et al.*, 1989). These relaxin-induced physical changes in the cervix are correlated with changes in biochemical composition of the tissue. For example, relaxin treatment decreased collagen concentrations while increasing water content, dry weight and glycosaminoglycan:collagen ratio in the cervix of ovariectomized gilts during the last third of gestation (O'Day-Bowman *et al.*, 1991). These data provide an explanation for the observations that birth is prolonged and the incidence of live births is low in ovariectomized pigs, unless relaxin replacement therapy is provided (Nara *et al.*, 1982). Taken together, these data support the concept that relaxin plays an important role in the preparation of the cervix for delivery.

Mammary gland growth

Mammotrophic effects of relaxin have been described in guinea-pigs (Garrett and Talmadge, 1952), mice (Bani and Bigazzi, 1984), rabbits (Garrett and Talmadge, 1952) and rats (Wright and Anderson, 1982). In addition, relaxin has been reported to stimulate growth of human MCF-7 breast cancer cells (Bigazzi *et al.*, 1992). Indirect evidence for mammotrophic effects of ovarian relaxin in pregnant pigs came from studies in which ovariectomy at midgestation resulted in a delay or inhibition of mammary growth (Elliott and Dziuk, 1973; Buttle, 1988). Hurley *et al.* (1991) examined the effects of relaxin on the development of mammary tissue during the last third of pregnancy in gilts. Ovariectomy on day 80 or 100, followed by progesterone replacement, resulted in significant reduction in mammary parenchymal tissue development supporting the concept that the ovary is important for mammary growth. Relaxin therapy restored mammary gland growth to control values indicating that relaxin plays a role in porcine mammary development in late gestation.

Biological Actions of Relaxin in Male Pigs

It has been shown that purified porcine relaxin can maintain or increase sperm motility; in contrast, antiserum to porcine relaxin inhibits sperm motility (Juang *et al.*, 1989). In addition, the presence of immunoreactive relaxin in boar seminal plasma was highly correlated with increased sperm motility (Juang *et al.*, 1990). These studies indicate a potential role for relaxin in boars, but it is clear that much work is needed to determine the source and biological actions of relaxin in males.

Conclusion

Although multiple sources and biological actions for relaxin have been identified in pigs, important fundamental questions remain concerning the control of relaxin synthesis and its biological role as both a systemic and locally acting hormone during various reproductive states. The contribution of relaxin to the process of follicular growth, uterine accommodation and mammary development support a growth-promoting effect for the hormone in target tissues. Furthermore, regulation of porcine relaxin secretion, its mechanism of action, isolation and characterization of relaxin receptors from target tissues are areas of investigation that will provide important fields of study in the future.

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