Action of relaxin on uterine contractions – a review

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Action of Relaxin on the Uterus

Relaxin is a potent, reversible inhibitor of spontaneous uterine contractions in a number of species, including rats, mice, guinea-pigs, rabbits, sheep and pigs, *in vivo* and *in vitro*. The potency of relaxin on the isolated uterus is about $1\text{--}5\text{ nmol l}^{-1}$ (assuming a molecular mass of 6 kDa; Table 1); relaxin is therefore one of the most potent uterine relaxants known. The isolated human uterus, however, has been reported to be relatively insensitive to relaxin. Human recombinant relaxin has only recently become available and, therefore, in this review it may be assumed that the studies described were conducted using porcine relaxin unless stated otherwise. Low doses of relaxin selectively reduce the frequency of contractions in rats *in vivo* and at high doses complete inhibition of myometrial contractions is achieved (Downing and Hollingsworth, 1991, 1992a). The duration of inhibition is related to the dose of relaxin administered. Relaxin has a rapid onset of action *in vivo* (within 1–2 min of administration of an intravenous dose) and recovery of uterine contractions to control values occurs rapidly after clearance of relaxin from the circulation. The half-life of endogenous relaxin in the circulation of the mid- and late-pregnant rat is 80 and 50 min, respectively (Sherwood *et al.*, 1984) and human relaxin shows half-lives of 15 and 60 min for the second and third decay phases in the rat, respectively (Cossum *et al.*, 1992). Similarly, *in vitro*, uterine contractions are inhibited rapidly upon addition of relaxin to the tissue bath (Wiqvist and Paul, 1958; Bradshaw *et al.*, 1981; Anderson, 1984), and recovery of uterine contractions occurs after relaxin is washed from the bathing medium. Collectively these observations suggest that relaxin interacts with a plasma membrane receptor in the myometrium. Radiolabelled relaxin has been shown to exhibit specific binding to uterine tissue (McMurtry *et al.*, 1978; Cheah and Sherwood, 1980; Mercado-Simmen *et al.*, 1980, 1982a,b; Osheroff *et al.*, 1990, 1992; Yang *et al.*, 1992). Displaceable relaxin binding is localized to the lateral aspect of the uterine horn including the area of the basalis and myometrium (Osheroff *et al.*, 1992). It appears, however, that receptor density is naturally low in relaxin target tissues, rendering isolation and characterization of receptors for relaxin difficult (Yang *et al.*, 1992).

Uterine sensitivity to relaxin as an inhibitor of spontaneous contractions *in vivo* and *in vitro* is modulated by oestrogen and progesterone, but the extent of the modulation has varied between studies. After treatment for 48 h with a low, physiological dose of oestradiol benzoate ($0.4\text{ }\mu\text{g kg}^{-1}\text{ day}^{-1}$), a two-fold increase in uterine sensitivity to relaxin was observed (Downing and Hollingsworth, 1992a). Progesterone treatment

alone had little effect on uterine sensitivity to relaxin, but combined oestrogen and progesterone treatment produced a tenfold increase in myometrial sensitivity to relaxin. Brenner *et al.* (1984) reported that progesterone pretreatment of rats *in vivo* increased the sensitivity of their uteri to relaxin *in vitro*, although the change in sensitivity was less than twofold. Other studies have indicated that progesterone can synergize with relaxin *in vitro* as an inhibitor of uterine contractions (Sarosi *et al.*, 1983; MacLennan *et al.*, 1986). The concentrations of progesterone used ($1\text{ }\mu\text{g ml}^{-1}$), however, also inhibited uterine contractions, thus the resultant effect could simply have been one of addition.

It has been suggested that oestrogen increases the concentration of myometrial relaxin receptors (Mercado-Simmen *et al.*, 1980, 1982a). More recently, Osheroff *et al.* (1992) reported that the number of relaxin binding sites in uterine tissue was reduced by 53% in ovariectomized rats and was restored to 90% of control values after oestrogen treatment of ovariectomized animals. Uptake of radioiodinated relaxin in uterine tissue *in vivo* was increased twofold over that in ovariectomized control rats by treatment with $0.4\text{ }\mu\text{g}$ oestradiol benzoate $\text{kg}^{-1}\text{ day}^{-1}$ for 2 days (Downing and Hollingsworth, 1993). In contrast, oestradiol benzoate plus progesterone treatment did not increase further uterine uptake of radioiodinated relaxin. This result suggests that the enhanced uterine sensitivity to relaxin as an inhibitor of spontaneous contractions observed after combined oestrogen and progesterone treatment may be due to both more receptors and additional factors such as enhanced signal transduction processes.

Whereas the potency of relaxin as an inhibitor of spontaneous uterine contractions is only moderately enhanced by oestrogen pretreatment, the ability of relaxin to inhibit spasmogen-stimulated contractions *in vivo* is markedly influenced by oestrogen (Porter *et al.*, 1979, 1981a; Downing and Hollingsworth, 1992b; Table 1). In ovariectomized rats, the potency of relaxin as an inhibitor of uterine contractions driven by oxytocin or prostaglandin $F_{2\alpha}$ was markedly reduced compared with its potency as an inhibitor of spontaneous contractions (Fig. 1). After treatment of ovariectomized rats with a low dose of oestradiol benzoate ($0.4\text{ }\mu\text{g kg}^{-1}$) for 2 days, the potency of relaxin as an inhibitor of oxytocin-stimulated contractions was similar to its potency for inhibition of spontaneous contractions (Downing and Hollingsworth, 1992b). *In vitro*, relaxin can reduce contractions stimulated electrically or by oxytocin or prostaglandins *in uteri* from oestrogen or oestrogen plus progesterone-treated rats (Chamley *et al.*, 1977; Bradshaw *et al.*, 1981; Goldsmith *et al.*, 1989; McGovern *et al.*, 1992). In the absence of oestrogen pretreatment, however, relaxin is ineffective in inhibiting stimulated uterine contractions (Steinetz *et al.*, 1982; Osa *et al.*, 1991). The marked contrast in the influence of oestrogen on the potency of relaxin

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Table 1. Potencies of relaxins from different species against spontaneous and stimulated uterine contractions

Source of relaxin	Species tested	Spontaneous/ stimulated	Approximate ID ₅₀ /IC ₅₀ value or effective concentration range	Reference
<i>In vitro</i>				
Pig	Pig	Spontaneous	> 50 ng ml ⁻¹	MacLennan <i>et al.</i> , 1986
Pig	Rat	Spontaneous	5 ng ml ⁻¹	Downing and Hollingsworth, 1991
Pig	Rat, O-treated	KCl (28 mmol l ⁻¹)	about 160 ng ml ⁻¹	MacLennan <i>et al.</i> , 1986
Pig	Rat, pregnant	KCl (28 mmol l ⁻¹)	> 500 ng ml ⁻¹	MacLennan <i>et al.</i> , 1986
Pig	Mouse O-treated	Spontaneous	4–16 ng ml ⁻¹	Reinig <i>et al.</i> , 1981
Pig	Guinea-pig, O-treated	Spontaneous	40 ng ml ⁻¹	Reinig <i>et al.</i> , 1981
Pig	Human, nonpregnant, pregnant	Spontaneous	> 10 µg ml ⁻¹	MacLennan <i>et al.</i> , 1986
Human	Human, nonpregnant, pregnant	Spontaneous	Minimal effect up to 1.3 µg ml ⁻¹	MacLennan and Grant, 1991
Human	Human, pregnant	KCl, oxytocin, PGF _{2α}	> 10 ⁻⁷ mol l ⁻¹	Petersen <i>et al.</i> , 1991
Human	Rat	Vasopressin	10 ⁻¹⁰ –10 ⁻⁷ mol l ⁻¹	Petersen <i>et al.</i> , 1991
Human	Pig	Spontaneous	190–600 ng ml ⁻¹	MacLennan and Grant, 1991
Shark	Mouse, O-treated	Spontaneous	Ineffective, > 160 ng ml ⁻¹	Reinig <i>et al.</i> , 1981
Shark	Guinea-pig, O-treated	Spontaneous	25–100 ng ml ⁻¹	Reinig <i>et al.</i> , 1981
<i>In vivo</i>				
Pig	Rat, ovariectomized	Spontaneous	10 µg kg ⁻¹	Downing and Hollingsworth, 1992a,b
		Oxytocin-stimulated	> 200 µg kg ⁻¹	
Pig	Rat, O-treated	Spontaneous	5 µg kg ⁻¹	Downing and Hollingsworth, 1992a,b
		Oxytocin-stimulated	10 µg kg ⁻¹	
Pig	Rat, O + P-treated	Spontaneous	1 µg kg ⁻¹	Downing and Hollingsworth, 1992a,b
Pig	Sheep, O-treated	Spontaneous	about 20 µg kg ⁻¹	

O: oestrogen; P: progesterone; IC₅₀ value: concentration that produces 50% inhibition *in vitro*; ID₅₀ value: dose that produces 50% inhibition *in vivo*.

against spasmogen-induced contractions compared with spontaneous contractions suggests that the mechanism of action of relaxin in the two circumstances is different.

There appears to be variation in the potencies of relaxin from different species as inhibitors of uterine contractions (Table 1). Porcine relaxin is effective in inhibiting contractions in uterine tissue from most species so far examined, including rats, mice, sheep, pigs, guinea-pigs and rabbits. Shark relaxin inhibits uterine contractions in guinea-pig uterus but is ineffective in uterine tissue from mice (Reinig *et al.*, 1981). Human recombinant relaxin has a lower potency against spontaneous contractions of porcine uterus than has porcine relaxin (MacLennan and Grant, 1991). Human relaxin has been reported to be ineffective in inhibiting contractions of term pregnant human uterus *in vitro* (MacLennan and Grant, 1991; Petersen *et al.*, 1991). Such variation in potency between species may be due to variation in the amino acid sequence between relaxins (Schwabe and Bullesbach, 1990, for review). A second contributing factor may be the variation in relative importance of relaxin as an inhibitor of uterine contractions during pregnancy. Low sensitivity to relaxin of uteri isolated from pregnant animals may reflect tolerance developed to endogenous relaxin *in vivo* (see later).

Mechanism of Action of Relaxin

Relaxin interacts with a uterine plasma membrane receptor. Several possible post-receptor signal-transduction mechanisms

for relaxin have been proposed, including generation of cyclic adenosine 3'5' monophosphate (cAMP), opening of plasma membrane potassium (K⁺) channels and changes in intracellular calcium ion (Ca²⁺) concentration (Fig. 2). The last process may be subsequent to earlier events.

Evidence for a role for cAMP in the mediation of the action of relaxin is provided largely from *in vitro* studies, using uteri from oestrogen-treated rats or myometrial cells in culture. Porcine relaxin increased cAMP concentration in the rat myometrium in a concentration-dependent manner in the presence of phosphodiesterase inhibitors (Judson *et al.*, 1980; Sanborn *et al.*, 1980) and in rat myometrial cells in culture (Hsu *et al.*, 1985). Human recombinant relaxin and relaxin isolated from human corpora lutea increase cAMP concentrations in a concentration-dependent fashion in cultured human endometrial cells (Fei *et al.*, 1990) and in cultured uterine cells from newborn rhesus monkeys (Kramer *et al.*, 1990), again under conditions in which phosphodiesterases are inactivated. Propranolol did not antagonize relaxin-induced generation of cAMP, suggesting that relaxin-stimulable adenylyl cyclase is not linked to myometrial β₂-adrenoceptors (Hsu *et al.*, 1985). The time course of generation of cAMP by relaxin, however, appears to follow mechanical inhibition (Cheah and Sherwood, 1980; Judson *et al.*, 1980; Sanborn *et al.*, 1980; Osa *et al.*, 1991). Activation of cAMP-dependent protein kinase by relaxin was apparent by 30 s after administration of the hormone and was maximal by 5 min (Kemp and Niall, 1981), again a time course slower than that of mechanical inhibition.

Fig. 1. Dose-response curves for inhibition by relaxin of uterine contractions in (a) vehicle-treated ovariectomized rats (spontaneous contractions (○); oxytocin stimulated contractions (●); 12 rats) and (b) oestradiol-treated ovariectomized rats (spontaneous contractions (○); oxytocin stimulated contractions (●); 10 rats). Values are means $\pm$ SEM. Ordinate scales: inhibition of integral of uterine contractions over 60 min as percentage of integral of 60 min preceding i.v. bolus dose. Abscissa: dose of relaxin on a log scale. (Reprinted with permission from Downing and Hollingsworth, 1992b.)

Evidence for a role for cAMP changes in the action of relaxin on the uterus *in vivo* is even less convincing. This is particularly apparent when compared with cAMP changes observed after administration of β_2 -adrenoceptor agonists, the actions of which are more clearly linked with generation of cAMP (Johansson and Andersson, 1980, 1981). Administration of an i.v. bolus of salbutamol produced a marked (19-fold) increase in uterine cAMP concentrations in the absence of phosphodiesterase inhibitors, in the ovariectomized rat *in vivo*, for a period of 10 min, followed by a decline in cAMP concentrations (Downing *et al.*, 1992). The time course of inhibition of uterine contractions was correlated with the time course of changes in cAMP concentrations. In contrast, administration of an i.v. bolus of relaxin, which produced a similar marked and prolonged inhibition of uterine contractions, induced only a small increase (threefold) in uterine cAMP concentrations which was of short duration. It is possible that relaxin-induced changes in myometrial cAMP were masked by the presence of the endometrium, or that for relaxin, only small changes in cAMP concentrations are required to bring about the cascade of intracellular events leading to smooth muscle relaxation.

For those agonists for which cAMP is a second messenger, the increased cAMP concentrations are considered to result in activation of cAMP-dependent protein kinase A. This enzyme catalyses phosphorylation of the catalytic subunit of the enzyme myosin light chain kinase (MLCK) (Conti and Adelstein, 1981). Phosphorylation of MLCK reduces its capacity to bind to the calcium-calmodulin complex and, therefore, inhibits formation

of the active MLCK-calcium-calmodulin complex required for the phosphorylation (activation) of the 20 kDa light chain of myosin, activation of actin and myosin crossbridges and smooth muscle contraction (Adelstein *et al.*, 1981). Relaxin has been shown to decrease myosin light chain kinase activity and decrease the phosphorylation of myosin light chains in rat uterus (Nishikori *et al.*, 1983; Cole and Garfield, 1986; Hsu and Sanborn, 1986). In addition, the purportedly selective protein kinase A inhibitor, H-8, reduced the ability of relaxin to inhibit the oxytocin-stimulated rise in intracellular calcium ion concentrations and [^3H]inositol phosphate formation in rat myometrial cells in culture (Anwer *et al.*, 1990).

A second mechanism by which smooth muscle relaxation can be produced is that of K^+ channel opening. Drugs known to open potassium channels (e.g. cromakalim) have been shown to cause hyperpolarization of the plasma cell membrane of smooth muscle by opening K^+ channels (possibly the ATP-sensitive K^+ channel), such that plasma membrane Ca^{2+} channels are induced to close (Hamilton *et al.*, 1986; Noack *et al.*, 1992). The influx of Ca^{2+} via voltage-operated channels is prevented. The reduction in influx of Ca^{2+} results in low intracellular Ca^{2+} concentration which, in turn, favours phosphorylation of MLCK. Hyperpolarization of uterine smooth muscle has not been observed for cromakalim (Hollingsworth *et al.*, 1987) or relaxin (Chamley and Parkington, 1984; Osa *et al.*, 1991). It has been suggested, however, that the actions of cromakalim may be limited to opening K^+ channels in the plasma membrane of pacemaker cells in the myometrium; the hyperpolarization produced would therefore be too localized to be detected by conventional means (Hollingsworth *et al.*, 1987). A characteristic feature of the action of drugs that open K^+ channels *in vitro* is that, while they are effective in relaxing a spasm of smooth muscle induced by a low concentration of KCl ($< 20 \text{ mmol l}^{-1}$), they are ineffective at relaxing spasm produced by high KCl concentrations ($> 40 \text{ mmol l}^{-1}$). This is because, at high KCl concentrations the plasma membrane is depolarized to such an extent that Ca^{2+} channels are held open. The hyperpolarization produced by opening K^+ channels is then insufficient to offset the depolarization induced by the high concentration of KCl. A greater reduction in uterine spasm evoked by low KCl concentrations than by high KCl concentrations has been demonstrated in uteri for cromakalim (Piper *et al.*, 1990) and to some extent for relaxin (St-Louis, 1981). Other workers have observed that high concentrations of relaxin are partially effective in reducing uterine spasm induced by high KCl concentrations (MacLennan *et al.*, 1986; Hughes *et al.*, 1992; Table 1).

It has been reported that the oral hypoglycaemic agent, glibenclamide, is a relatively selective blocker of ATP-sensitive K^+ channels (Quast and Cook, 1988). *In vivo*, glibenclamide produced a parallel rightward shift in the dose-response curve for cromakalim as an inhibitor of uterine contractions in ovariectomized rats (Downing and Hollingsworth, 1991). As might be expected, glibenclamide had no effect on uterine sensitivity to the β_2 -adrenoceptor agonist, salbutamol. Glibenclamide produced a rightward shift in the dose-response curve for relaxin inhibition of uterine contractions, but in this case, in a non-parallel fashion (Fig. 3). Glibenclamide also reversed an established inhibition of uterine contractions by cromakalim or relaxin but not inhibition induced by salbutamol.

Fig. 2. Schematic representation of the mechanisms of action of the uterine relaxants relaxin, salbutamol (a β_2 adrenoceptor agonist) and cromakalim (a K^+ channel opener) in the myometrial cell. AC: adenylyl cyclase; $G_{\beta\gamma}$ and $G_{\alpha s}$: subunits of G protein; PPi: inorganic phosphate; MLCK-P: phosphorylated myosin light chain kinase; RLX: relaxin; β_2 receptor: β_2 adrenoceptor.

Fig. 3. (a) Effect of relaxin i.v. on uterine contractions in ovariectomized rats before (○) and after (●) infusion of vehicle (0.02 mol sodium hydroxide l^{-1} in 4% glucose, 5 ml kg^{-1} , i.v.), 40 rats before infusion, 10 rats per point after infusion. (b) Effect of relaxin i.v. on uterine contractions in ovariectomized rats before (○) and after (●) infusion of glibenclamide (20 mg kg^{-1} , i.v.), 40 rats before infusion, 10 rats per point after infusion. Values are means $\pm$ SEM. Ordinate scales: inhibition of integral of uterine contraction over 60 min as a percentage of integral for 60 min preceding i.v. relaxin dose. Abscissa scales: dose of relaxin on a log scale. (Reprinted with permission from Downing and Hollingsworth, 1991.)

The results suggest that ATP-sensitive opening of K^+ channels may be a significant mechanism in the action of relaxin *in vivo*. *In vitro*, however, cromakalim was antagonized by glibenclamide as an inhibitor of uterine contractions (Piper *et al.*, 1990), but relaxin was not antagonized (Hughes *et al.*, 1992). This conflict in observations between experiments *in vivo* and *in vitro* suggests either that there is an alternative mechanism of action of relaxin *in vitro* or possibly an additional action of glibenclamide *in vivo*.

Relaxin increases Ca^{2+} efflux from rat uterine tissue *in vitro* (Ginsburg *et al.*, 1988) and in cultured rat myometrial cells (Rao and Sanborn, 1986). Relaxin also inhibits the oxytocin-induced increase of intracellular free Ca^{2+} concentrations and accumulation of inositol monophosphate, inositol bisphosphate and inositol trisphosphate (Anwer *et al.*, 1989) in cultured rat myometrial cells. These events may be secondary to earlier biochemical or ion channel changes.

In addition to modulation of intracellular events, relaxin may also reduce intercellular communication by reduction of myometrial gap junction permeability (Cole and Garfield, 1986), possibly by a cAMP-dependent mechanism. Such an effect would reduce electrical coupling between myometrial cells and prevent development of strong coordinated contractions.

The involvement of catecholamines as mediators of relaxin action has also been investigated, although with conflicting results. It has been suggested that release of intramural catecholamines contributes to the action of relaxin (St-Louis, 1982) and also that relaxin acts via the myometrial β -adrenoceptor (Rudzick and Miller, 1962a, b). The lack of antagonism of relaxin by propranolol *in vivo* (Porter *et al.*, 1979) or *in vitro* (Sanborn *et al.*, 1981; Chamley and Parkington, 1984) does not substantiate an action of relaxin via β -adrenoceptors.

In summary, although increases in cAMP concentrations and K^+ channel opening may play a part in mediation of inhibition of uterine contractions by relaxin, neither event has been conclusively shown to be solely responsible for the actions of relaxin.

Tolerance

Relaxin, given as a single i.v. bolus dose, produces rapid inhibition of uterine contractions. It was observed, however, that during continuous, prolonged infusion of relaxin, inhibition of uterine contractions was not maintained and that some return of contractions occurred (Cheah and Sherwood, 1981). Development of tolerance to relaxin as an inhibitor of uterine contractions *in vitro* has been shown to occur rapidly (Wiqvist, 1959). Tolerance was most pronounced in the longitudinal muscle layer of the myometrium (Chamley and Parkington, 1984), where complete desensitization to relaxin was observed after a single administration. In contrast, the circular muscle layer remained responsive to relaxin upon repeated administration. Administration of porcine relaxin to uterine tissue from nonpregnant rats *in vitro* resulted in inhibition of spontaneous or electrically driven contractions. Relaxin administered to uterine tissue from late pregnant rats, the endogenous relaxin concentrations of which were high, was, however, ineffective (Porter, 1982) or less potent (Chamley and Parkington, 1984; MacLennan *et al.*, 1986). Injections of porcine relaxin administered twice daily to ovariectomized pregnant rats resulted in reproducible inhibition of uterine contractions throughout the second half of pregnancy (Downing and Sherwood, 1985b), which might suggest that tolerance is not a physiologically relevant phenomenon. The dosage regimen used, however, would have resulted in fluctuating plasma concentrations of relaxin, circumstances under which tolerance would be minimized. The above observations suggest that tolerance to relaxin develops in the uterus upon continued, prolonged exposure to the hormone. More quantitative information on development of tolerance to relaxin has been obtained by Downing and Hollingsworth (1992a). Continuous i.v. infusion of relaxin to ovariectomized rats over 40 h ($20 \mu\text{g kg}^{-1} \text{h}^{-1}$) produced a tenfold reduction in uterine sensitivity to relaxin. Partial recovery of uterine sensitivity occurred during the 24 h following cessation of infusion. The extent of tolerance development is dose dependent since infusion of relaxin at a higher rate ($50 \mu\text{g kg}^{-1} \text{h}^{-1}$) produced a 130-fold reduction in uterine sensitivity (Fig. 4). Treatment of ovariectomized rats with oestrogen, progesterone or oestrogen plus progesterone did not influence the extent of tolerance developed; however, a more rapid recovery of uterine sensitivity to relaxin after cessation of infusion was observed in oestrogen plus progesterone-treated rats.

It has been suggested that tolerance to other hormones is due to uncoupling of receptors from generation of intracellular second messengers, internalization of membrane receptors or degradation of these receptors. Uterine tolerance to other relaxants, including drugs that open K^+ channels and β -adrenoceptor agonists has also been observed *in vivo* (Abel and Hollingsworth, 1986; Downing and Hollingsworth, 1992a). The mechanism of tolerance to drugs that open K^+ channels

Fig. 4. Dose-response curves for inhibition of uterine contractions by relaxin (a) before (○) and after (●) infusion of saline or (b) before (○) and after (●) infusion of relaxin ($50 \mu\text{g kg}^{-1} \text{h}^{-1}$ for 40 h) in ovariectomized rats (four rats per dose). Values are means $\pm$ SEM. Ordinate scales: inhibition of integral of uterine contraction over 60 min as a percentage of integral for 60 min preceding bolus dose. Abscissa scales: dose of relaxin on a log scale. (Reprinted with permission from Downing and Hollingsworth, 1992a.)

is not known, but tolerance to β -adrenoceptor agonists has been shown initially to involve phosphorylation of the β -adrenoceptor with consequent impaired ability of the receptor to couple with adenylyl cyclase (Levitzki, 1986; Lefkowitz *et al.*, 1990). In addition, concomitant with uncoupling of receptors from adenylyl cyclase, β -adrenoceptors are irreversibly lost from the plasma membrane (downregulation). The lack of cross-tolerance among relaxin, cromakalim and salbutamol (Downing and Hollingsworth, 1992a) suggests that the site of tolerance development to relaxin occurs before any common mechanism in the intracellular pathway leading to smooth muscle relaxation. Tolerance to relaxin, therefore, presumably occurs at a relatively early biochemical stage, such as receptor concentration or coupling to second messenger systems. Uterine uptake of radioiodinated relaxin showed little reduction in rats that were tolerant to relaxin compared with relaxin-naïve rats, suggesting that for relaxin, tolerance may occur subsequent to its binding (Downing and Hollingsworth, 1993). Studies on the precise mechanism of tolerance to relaxin are still awaited. Tolerance to non-uterine actions of relaxin (e.g. cervical softening) has not been reported suggesting that the biochemical mechanisms underlying uterine relaxation and other effects of relaxin on the reproductive tract differ.

Physiological Functions of Relaxin Action on the Uterus During Pregnancy and Parturition

The physiological effects of relaxin on the connective tissue of the reproductive tract to promote cervical softening, growth of the interpubic ligament and uterus and development of the mammary gland are important for normal pregnancy and

parturition in several species (Sherwood, 1988, for review). It has been demonstrated that these effects are essential for normal parturition and live births (Nara *et al.*, 1982; Downing and Sherwood, 1985a; Lao-Guico-Lamm and Sherwood, 1988). The relative importance of relaxin as an inhibitor of uterine contractions varies among species. In some species, such as pigs and rabbits, progesterone is of principal importance for the major part of pregnancy for preventing spontaneous contractions and responses to stimulants such as oxytocin, prostaglandins and stretch imposed on the myometrium owing to the growing fetus (the 'progesterone block', Csapo, 1956; Porter and Watts, 1985). In other species (rats, guinea-pigs) progesterone has little influence on spontaneous uterine contractions (Porter and Challis, 1974). The role of progesterone, if any, as an inhibitor of uterine contractions in humans has not been established.

In species in which progesterone is important in restraining uterine contractions, relaxin may function to prevent the development of uterine contractions during the period of progesterone withdrawal, just before parturition. In pigs, serum relaxin concentrations are low until 48 h before birth. During this antepartum period, plasma concentrations of relaxin increase rapidly to reach a peak 14 h before parturition and then decline rapidly to the time of delivery (Sherwood *et al.*, 1975; Watts *et al.*, 1988). The increased relaxin concentrations observed just before parturition may synergize with oestrogen to prevent premature stimulation of uterine contractions by oxytocin and prostaglandins.

In species, such as rats, in which progesterone does not inhibit uterine contractions, relaxin may be important in preventing uterine contractions during a major part of pregnancy (Downing and Sherwood, 1985b). In rats, serum relaxin concentrations increase from day 10 of pregnancy, with a surge to maximum concentrations on day 21, followed by a decline before birth, although serum relaxin concentrations remain high during birth (Sherwood *et al.*, 1980, 1983). During pregnancy, relaxin occurs in serum as three forms (60 kDa, 13 kDa and 6 kDa), the proportions of which change in a consistent manner as pregnancy progresses (Sherwood *et al.*, 1984). Tolerance may occur to varied extents to the different forms of relaxin. The more rapid recovery of uterine sensitivity to relaxin observed after cessation of prolonged infusion of relaxin to oestradiol plus progesterone-treated rats (Downing and Hollingsworth, 1992a) may indicate that combined actions of oestrogen and progesterone also serve to limit the extent of development of tolerance during pregnancy. As oestrogen concentrations increase during late pregnancy (Shaikh, 1971), synergism between relaxin and oestrogen may serve to restrain myometrial activity in the face of increasing contractile stimuli. At term, high oestrogen and relaxin concentrations might be expected to prevent, or limit, myometrial responses to oxytocin and prostaglandins and thus attenuate the strong contractions required to dilate the cervix and expel the fetuses. Development of tolerance to relaxin at term may effectively withdraw relaxin inhibition of uterine contractions, allowing oxytocin and prostaglandins to stimulate parturient activity. In species such as pigs, in which relaxin action on the uterus may be confined to the immediate antepartum period, tolerance to relaxin may not have a significant role. In accordance with this suggestion is the observation that only one form of relaxin is found in serum in pigs during pregnancy (O'Byrne *et al.*, 1989).

It is not known whether development of tolerance to relaxin occurs in other target tissues, or whether it is confined to the uterus. If the latter is the case, development of uterine tolerance may permit parturient myometrial activity and continued relaxin action on the connective tissue of the cervix, pubic ligament and development of mammary tissue.

Conclusion

Relaxin is a potent inhibitor of uterine contractions, with a rapid onset of action and, once the hormone is cleared from the circulation, the uterus quickly returns to full contractile activity. Although it is yet to be established that the effects of relaxin on uterine contractions are physiologically important in any species, the nature of the inhibition produced by relaxin suggests that the hormone may be necessary for the fine control of uterine contractions, particularly during the immediate antenatal period and parturition. Relaxin may be important in restraining uterine contractions during the period of declining progesterone influence and increasing sensitivity of the myometrium to oxytocin. If, however, further studies support the current findings of an absence of a relaxant action of human relaxin on human uterus (MacLennan and Grant, 1991; Petersen *et al.*, 1991), then its use for prevention of preterm birth may be precluded. As studies on relaxin progress, its potential for therapeutic use clinically and in animal husbandry, both as a uterine relaxant and as a cervical softening agent, will become more apparent.

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