The effect of oestrogen and relaxin on uterine and cervical enzymes: collagenase, proteoglycanase and β-glycuronidase

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Abstract. Relaxin (Rlx) classically causes uterine quiescence during pregnancy and cervical dilatation prior to parturition. Its actions involve major changes in the components of the extracellular matrix of these tissues. The activities of three enzymes, collagenase, proteoglycanase and β-glucuronidase, major determinants of the integrity of the extracellular matrix have been measured in the rat uterus and cervix in different reproductive states. The results show that there are marked differences in the changes of these enzymes occurring in the uterus and cervix during the course of pregnancy and the puerperium. It was not possible to directly relate these changes to a single hormonal event over this period of major endocrine fluctuations. Two models have therefore been used in an attempt to delineate the effects of oestrogen and Rlx on the tissue enzyme levels or their secretion into culture medium. In the first model cyclic animals were treated with oestrogen alone or oestrogen followed by Rlx and tissue enzyme levels measured. The addition of Rlx treatment reversed an inhibiting effect of oestrogen alone on both uterine and cervical collagenase and proteoglycanase activities, at the same time as completely obliterating the stimulating effect of oestrogen on uterine and cervical β-glucuronidase activity. A second model used in vivo oestrogen priming of cyclic rats followed by in vitro Rlx treatment and measurement of the enzymes secreted into the culture medium over 7 days. The results showed as in the first model that Rlx treatment could in particular overcome the inhibiting effect of oestrogen on uterine proteoglycanase secretion without affecting β-glucuronidase levels. In contrast, the effect of Rlx on the cervix was to decrease collagenase and proteoglycanase secretion whilst not affecting the β-glucuronidase levels.

The uterus undergoes major changes in shape and structure during the course of pregnancy to accommodate the developing foetuses. The cervix, on the other hand, forms a fibrous barrier throughout gestation to withhold the uterine contents but then undergoes extensive structural and functional changes prior to parturition to allow the passage of the foetuses at birth. Much evidence has now been accumulated demonstrating that Rlx is involved in the structural changes occurring in the uterus and cervix (Porter 1979). The actions ascribed to Rlx on these tissues occur at different times of pregnancy; those on the uterus during the major portion of pregnancy whilst those on the cervix only a few days before parturition. After parturition the events of involution in both tissues occur simultaneously.

Uterine growth during pregnancy, cervical dilatation prior to birth as well as post-partum involution are thus precisely timed events involving major changes in the components of the extracellular matrix of these tissues. The distribution of

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uterine collagen (Harkness & Harkness 1954), cervical collagen and glycosaminoglycans (Danforth et al. 1960, 1974), and cervical elastin (Leppert et al. 1982) have been studied during the course of pregnancy. The structural changes in both the uterus and cervix are controlled to some extent by progesterone (Jeffrey et al. 1971) as well as oestrogen (Hollingsworth et al. 1979) and the effects of these steroids on collagenolytic enzymes, e.g., collagenase (Woessner & Ryan 1973) have been studied.

Rx is known to alter uterine collagen content as well as to bring about cervical softening in several different species (Porter 1979). Accordingly we have studied the effect of oestrogen with and without Rx on the levels of uterine and cervical collagenase, proteoglycanase and β-glucuronidase, enzymes involved in the degradation of collagen, proteoglycan and hyaluronic acid, respectively. Oestrogen priming has been used extensively in this study because of its known actions on these enzymes as well as its action on the control of the Rx receptor (Mercado-Simmen et al. 1982).

Material and Methods

Porcine Rx was prepared routinely in our laboratory by the method of Sherwood & O’Byrne (1974); the fraction CM-a (3000 GPU/mg) was used in this study. The materials used and their sources were: oestradiol-17β-cyclopentanepropionate (E2-17β), 4 aminophenyl mercuric acetate (APMA), phenolphthalein mono-β-glucosiduronic acid sodium salt, phenolphthalein, Sigma Chemical Co., St. Louis, MO; bacterial collagenase, Millex-GV sterilizing filter units, Millipore Corp., Bedford, MA; Dulbecco-Vogt’s modified Eagle’s medium (DMEM), penicillin-streptomycin (10 000 U-10 000 μg sulphate base/ml), Grand Island Biological Co., Santa Clara, CA; Na2 35SO4, Amersham-Searle Corp., Arlington Hts., IL; [1-14C]acetic anhydride, New England Nuclear, Boston, MA.

All other chemicals were of analytical grade.

Treatment of animals and tissues

Rat uterine and cervical tissues were obtained from mature Wistar rats (180–220 g) at different stages of pregnancy and puerperium and after treatment of cyclic animals with E2-17β in oil (200 μg/rat sc) alone or followed by Rx in saline (20 μg/rat ip) 24 h later.

Tissue enzyme levels during pregnancy and puerperium

Rats were mated and the morning when sperm plugs were observed was designated as day 1 of pregnancy. Birth occurred on day 22 and the following day was designated day 1 post-partum. Animals were killed with ether on days 5, 10, 14, 17, 19 and 21 of pregnancy and on days 1, 3 and 5 post-partum. A baseline for enzyme level was obtained from cyclic rats whose stage of oestrous cycle was not determined. A minimum of 3 rats were used for each day of study.

The uteri and cervices were dissected free of mesenteries and exogenous fat. The former were slit open lengthwise to remove the foetuses and placenta. Both tissues were washed in ice-cold PBS (Dulbecco-Vogt’s), blotted dry, weighed, wrapped in Parafilm and placed in a plastic bag to reduce dessication and stored at -20°C until further processing. The method of Weeks et al. (1976) was used for the preparation of tissues for enzyme assays without enzyme extraction at 60°C. Briefly, uterine and cervical tissues were thawed, finely minced with a pair of scissors and homogenized in 5 ml (cervix) or in 10 vol (uterus) of ice-cold 0.01 M CaCl2 containing 0.25% Triton X-100. The homogenates were centrifuged at 6000 x g for 20 min at 2°C. In a preliminary experiment, using the uterus from a day 20 pregnant rat, approximately 97% of the total collagenase activity, measured as hydroxyproline released after trypsin activation (Weeks et al. 1976), detectable in the whole homogenate was recovered in the 6000 x g pellet (data not shown). This is in agreement with the average recovery of 95% to the 6000 x g pellet of a day 1-2 post-partum uterus as reported by Weeks et al. (1976). Consequently, the 6000 x g supernatants were discarded. The 6000 x g precipitates were resuspended by a brief homogenization to the original volume in ice-cold 0.05 M Tri-HCl, pH 7.4 containing 0.1 M CaCl2. This tissue suspension was used in the enzyme assays and protein determination (Lowry et al. 1951). Earlier experiments showed that with or without a 4 min extraction at 60°C, collagenase activities, measured either with DNP-peptides (Masui et al. 1977) or with [1-14C]collagen (Cawston & Barrett 1979) as substrates in the presence of AMP for enzyme activation, in the 6000 x g suspension of a day 20 pregnant uterus were comparable (data not shown).

Tissue enzyme levels after hormone treatment in vivo

Adult cyclic rats were primed with E2-17β (200 μg in 0.2 ml peanut oil, sc) for 24 h and then given either Rx in 20 μg in 0.1 ml saline/rat, ip) or saline. Rats were killed by cervical dislocation 0, 6, 12, 24, 48, 72 and 96 h after Rx treatment. Uteri and cervices were removed into ice-cold PBS, dissected free of mesenteries and fat, blotted dry and weighed. The tissues were extracted by the method described above and the suspensions were used for the enzyme assays and protein determination.

Enzyme production in vitro

Adult cyclic rats were primed with E2-17β (200 μg in 0.2 ml peanut oil/rat, sc) for 24 h and killed by cervical
dislocation. The uterine horns and cervixes were removed under sterile conditions and placed in DMEM buffered with 15 mM HEPES, pH 7.4, containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Tissues were cleaned of mesenteries and fat and were thoroughly washed in the above medium. Uniform pieces of explants, approximately 2 mm² were mixed and put into wells (16 mm × 10 mm, diameter × depth) of a tissue-culture plate, under sterile conditions. The tissues were cultured in the above medium which was further supplemented with ascorbic acid, t-alanine, t-aspartate, t-glutamate, t-proline, t-serine, glycine, all at 0.1 mM, t-asparagin at 0.01 mM, cysteine-HCl 90 mg/ml, calcium acetate 0.55 g/l, histidine-HCl 110 mg/l, and sodium pyruvate 50 mg/l. Tissues were cultured with or without Rlx (5 µg/ml, pre-filtered through a Millex-GV sterilizing filter unit) for 7 days in a humidified atmosphere of 95% air: 5% CO₂ at 37°C with daily changes of media. At the end of the 7 days, the pieces of tissue in each well were collected, washed thoroughly in distilled water to remove the DMEM phenol red coloration, and hydrolyzed in 1 M NaOH for protein estimation.

Collagenase, proteoglycanase and β-glucuronidase activities were assayed in the harvested media and expressed as enzyme activity per mg tissue protein.

**Enzyme assays**

**Collagenase.** Total (active and latent) collagenase activity was assayed using the micro-tube method of Harris et al. (1967), by measuring the release of radioactivity from thermally reconstituted fibrils (Webb & Burleigh 1974) of Type I collagen prepared from foetal bovine skin and labelled with [1-14C]acetic anhydride (Cawston & Barrett 1979). The presence of APMA in the assay activated the latent form of the enzyme. Collagenase-dependent proteolytic activity was expressed as a percentage of collagenolysis by bacterial collagenase/mg tissue protein/20 h at 37°C. Hydrolysis by trypsin gave a collagenase-independent release of radioactivity of approximately 15%. Corrections were made for buffer alone and for unincubated culture media, with or without the addition of hormones.

**Proteoglycanase.** Total proteoglycanase activity was assayed by the 35S-proteoglycan disc method of Sandy et al. (1981) with a minor modification. The assays were carried out in screw-top plastic vials (1.2 × 0.5 cm) containing 0.05 mmol Tris-HCl buffer, pH 7.4, 5 µmol CaCl₂, 5 × 10⁻⁴ mmol APMA as enzyme activator, enzyme extract and one 35S-proteoglycan polycrylamide disc, in a total volume of 0.5 ml. Total proteoglycanase activity was expressed as percentage hydrolysis/mg tissue protein/20 h at 37°C. Correction was made for non-enzymic release of 35S-proteoglycan (approximately 10%).

β-glucuronidase. β-glucuronidase activity was measured by the method of Jacox & Feldman (1955). Briefly, enzyme extract was added to 0.1 M acetate buffer, pH 4.5 to give a total volume of 0.9 ml. Blanks consisted of the same volume of enzyme extract but in a total volume of 1.0 ml with 0.1 M acetate buffer, pH 4.5. After temperature equilibration at 37°C for 10 min, 100 µl of 0.005 M phenolphthalein-mono-β-glucosiduronic acid was added to the samples only. Samples and blanks were incubated at 37°C for 16 to 20 h. One ml of 0.4 M glycine – NaOH buffer, pH 10.5 was added to both the samples and the blanks. Phenolphthalein released by β-glucuronidase was determined from a standard graph with a phenolphthalein concentration ranging from 0–20 µg/ml in 80% ethanol. One ml of 0.4 M glycine-NaOH buffer, pH 10.5 was added to the standards. Absorbance at 540 nm was measured. β-glucuronidase activity was expressed as µg phenolphthalein released/mg tissue protein/20 h at 37°C, after subtraction of the respective blanks.

**Results**

The wet weight of rat uteri and cervixes was measured during pregnancy and the puerperium. The uterine wet weight increased from day 10 of pregnancy (0.46 ± 0.04 g) and maximum weight was reached by day 19 (3.45 ± 0.75 g). The increase in cervical wet weight was more gradual and occurred from day 14 onwards (0.09 ± 0.04 g on day 10 and 0.18 ± 0.01 g on day 19). The maximum weight recorded was on day 1 post-partum (0.29 ± 0.08 g). Thereafter the wet weight of both tissues decreased over a 4-day period and returned to those of the cyclic animal.

1. **Endogenous tissue enzyme levels in the uterus and cervix in different reproductive states**

There were marked differences in the levels of collagenase in the uterus and cervix during the course of pregnancy and in the first few days post-partum when compared to levels in cyclic animals (Fig. 1). Pregnancy significantly increased the levels of uterine collagenase above cyclic levels (P < 0.05). The increased activity was maintained through mid-pregnancy with a further, but not significant, increase during late pregnancy. The enzyme activity was again elevated on day 1 post-partum and this was significantly higher than the levels of mid-pregnancy (P < 0.05). The activity of uterine proteoglycanase increased much more gradually, with the activity in mid-pregnancy being significantly higher than that of the cyclic
Uteri and cervices were removed from cyclic rats (n = 3), during pregnancy and post-partum (n = 3). Collagenase (O—O), proteoglycanase (●—●) and β-glucuronidase (Δ—Δ) activities were measured in the 6000 × g suspension from each uterus (A) and cervix (B). The mean and sd at each point was used to determine levels of significance. The mean values during pregnancy and the puerperium were expressed as a percentage of the mean cyclic enzyme level in the uterus or cervix respectively. The levels of significance are given in the text.

animals (P < 0.05). Thereafter, the enzyme activity decreased to a level, on day 1 post-partum, which was significantly lower (P < 0.05) than that of late pregnancy. However, from day 1 post-partum the enzyme activity again increased significantly (P < 0.05) over the next 2 days.

In the cyclic animals, cervical concentrations of collagenase (145.2 ± 20.4 U) and proteoglycanase (90.4 ± 37.1 U) markedly exceeded the uterine levels, (4.4 ± 1.8 and 24.8 ± 2.2 U, respectively) (P < 0.01). There was a converse effect of pregnancy on cervical collagenase and proteoglycanase but only collagenase was significantly lowered (P < 0.05) by day 10 of pregnancy. The decrease in cervical collagenase and proteoglycanase during early pregnancy was followed by a brief increase during mid-pregnancy whereupon it fell again, significantly (P < 0.01), until day 1 post-partum. From day 1 to day 3 post-partum both cervical collagenase and proteoglycanase levels were significantly elevated (P < 0.01 and 0.05, respectively).

β-glucuronidase activities in the uterus (48.3 ± 7.5 U) and cervix (52.9 ± 16.4 U) of cyclic animals were similar. Pregnancy significantly lowered the level in both the uterus (P < 0.01) and cervix (P < 0.05). However, during the course of pregnancy uterine β-glucuronidase increased to again reach cyclic levels by late pregnancy. The cervical enzyme increased significantly from early to mid-pregnancy (P < 0.05). However, this cervical increase then returned to early pregnancy levels. After parturition, there was a significant rise in β-glucuronidase activities in both the uterus
Data based on the observation of changes in tissue enzyme levels during the course of pregnancy cannot be readily related to any one particular hormone because of the multiple hormone changes occurring. Accordingly, both in vivo and in vitro models were used in an attempt to detect the changes observed in the tissue enzymes in the reproductive cycle and to relate these with more precision to the effect caused by Rlx.

II. Endogenous tissue enzyme levels after treatment with oestrogen and Rlx in vivo

The cyclic animal primed with oestrogen or oestrogen and Rlx was used in an attempt to isolate the contributions of these hormones to the changes in tissue enzyme concentrations noted above in the oestrus cycle, pregnancy and the puerperium.

Figs. 2 and 3 show the lability of the enzyme concentrations over a 4-day period in response to a single sequential bolus of oestrogen followed by Rlx or by saline (control). The changes induced are in the same order of magnitude as the range of concentrations observed in pregnancy, but in general higher concentrations could be induced by the hormone injections.

The direction of the changes in the uterus for collagenase and proteoglycanase were similar; oestrogen lowered the concentration and oestrogen plus Rlx increased them again. In the oestrogen treated animals there were few changes in either uterine or cervical collagenase or proteoglycanase but with oestrogen and Rlx these enzymes increased significantly in both tissues above the oestrogen treated controls. In some instances the response (Fig. 2A and B; Fig. 3A and B) was biphasic with rapid changes in the first 24 h and a further period of change in the following 48 h. Contrary to the effects observed for uterine and cervical collagenase and proteoglycanase, oestrogen alone caused significant increases in uterine and cervical β-glucuronidase activity. However, this increase was abolished when oestrogen and Rlx were used in combination. A biphasic response to oestrogen was observed in the cervix but not the uterus.

III. Enzyme release from uterine and cervical explants in vitro: effect of added hormones

An attempt was made to further refine a model for isolating the role of Rlx in controlling the enzyme levels. The effect of Rlx in vivo on the
release of these enzymes from the uterus and cervix was therefore investigated.

It has been shown that uterine explants remain viable for at least 10 days when cultured in vitro and are able to secrete enzymes in tissue culture (Jeffrey et al. 1971). This affords a model system to isolate the effects of different hormones including RLx. In the following experiments uterine and cervical tissues used for culture were obtained from animals given oestrogen in vivo since oestrogen has been shown to maximize the number of RLx receptors in the uterus (Mercado-Simmen et al. 1982).

Collagenase activity was progressively released from both uterine and cervical cultures for up to 7 days. This release was partially inhibited in the uterus when the animal was pretreated with oestrogen, the cervical response being unaffected. The addition of RLx in vitro partially overcame the inhibitory effect of oestrogen on the uterine release of collagenase. In the cervix, however, RLx further inhibited the release of collagenase.

Proteoglycanase activity was progressively released from both uterine and cervical cultures for up to 7 days. This release was partially inhibited in the uterus when the animal was pretreated with oestrogen and further inhibited in the uterus when the animal was pretreated with oestrogen and further inhibited when RLx was added in vitro. A converse response of the cervix to oestrogen priming was seen; there was an increased release of proteoglycanase in culture, but this was inhibited by the addition of RLx in vitro.

β-glucuronidase activity progressively and rapidly decreased in media from cultures of both uterus and cervix and was not affected by either oestrogen-priming in vivo or by the subsequent addition of RLx in vitro.

**Fig. 3.**

Cervical enzyme activities after hormone treatment in vivo. E2-17β was injected into cyclic rats followed 24 h later by either RLx (---) or saline (0-0). Animals were killed and cervices removed at the specified times. A. Collagenase. B. Proteoglycanase. C. β-glucuronidase activities were measured in the tissues. Mean ± SD (n = 4). Levels significantly higher or lower (P < 0.01) than the respective control (+). The range of enzyme activity in the cervix during pregnancy is represented as a bar.
Uterine enzymes released into medium in vitro: effect of oestrogen priming in vivo and the addition of Rlx in vitro. Uteri from cyclic rats (○), cyclic rats treated with E2-17β in vivo (▲), and with Rlx added in vitro (■). Mean enzyme activities ± SD (n = 3).

A. Uterine collagenase: levels significantly lower (P < 0.05) in media from Rlx-treated uteri (●) than in media from non-treated cyclic uteri (X). Levels significantly higher (P < 0.05) than in media from uteri of rats treated with E2-17β in vivo only (*). B. Uterine proteoglycanase: levels significantly lower (P < 0.01) than in media from non-treated cyclic uteri (○). Levels significantly lower (P < 0.05) than in media from uteri of rats treated with E2-17β in vivo only (#). C. Uterine β-glucuronidase: levels significantly lower (P < 0.01) than in media from non-treated cyclic rat uteri (**).

Cervical enzymes released into medium in vitro: effect of oestrogen priming in vivo and the addition of Rlx in vitro. Cervices from cyclic rats (○), cyclic rats treated with E2-17β in vivo (▲) and with Rlx in vitro (■). Mean enzyme activities ± SD (n = 3).

A. Cervical collagenase: levels significantly lower (P < 0.05) in media from oestrogen and Rlx-treated cervixes than in media from cervixes of oestrogen-treated animals (*). B. Cervical proteoglycanase: levels significantly lower (P < 0.05) than in media from cervixes which were oestrogen-treated only (#). C. Cervical β-glucuronidase: no significant differences between the three groups.
Discussion

It was the purpose of this study to attempt to delineate the actions of oestrogen and Rlx upon the collagen in the uterus and cervix during pregnancy and the puerperium in the rat. Although synthesis and degradation of collagen and proteoglycan may occur simultaneously, their relative rates are direct determinants of the structure of the tissues and therefore their function. The measurement of tissue levels of collagenase, proteoglycanase and β-glucuronidase might be expected therefore to provide an index of collagen, proteoglycan and hyaluronate breakdown during pregnancy and the puerperium. This does not preclude, however, simultaneous changes taking place in the synthesis of these two components.

The major structural components of the myometrium and cervix are muscle cells embedded in connective tissue, however they differ in their relative proportions both between the tissues and in different regions of the same tissue (Huszar & Naftolin 1984). Our observations on simple wet weight changes of these tissues during pregnancy are in agreement with those reported by Montfort & Perez-Tamayo (1961). We verify that each tissue reaches its maximum wet weight at a different stage of pregnancy. Since both the uterus and cervix are exposed to the same endogenous hormonal milieu, it appears that they respond differently to the circulating hormones reflecting their different hormone receptor number and population. It is generally accepted that oestrogen and progesterone together with systemic Rlx from the corpus luteum and local prostaglandins are largely responsible for the orchestration of uterine accommodation and myometrial contractility, as well as cervical dilatation during pregnancy (Huszar & Naftolin 1984). Rlx levels in the rat begin to rise on day 10 of pregnancy and reach high levels by day 14 (Sherwood et al. 1980), at which time tissue oestrogen levels begin to rise (Yoshinaga et al. 1969). This is in accord with the observation that the extensibility of the rat cervix does not change until day 16 of pregnancy (Huszar & Naftolin 1984). We have demonstrated the requirement of oestrogen for the expression of the relaxin myometrial receptor in the rat and it would seem likely that a similar relationship exists in the cervix (Mercado-Simmen et al. 1982).

From our data on the endogenous tissue enzyme levels during pregnancy and the puerperium, it is difficult to relate these to any one hormone of pregnancy. It also appears likely that hormones other than oestrogen and Rlx are involved in the changes recorded. There are, however, marked differences in the tissue enzyme levels in the uterus and cervix during pregnancy which then undergo similar changes immediately post-partum associated with involution. Tissue enzyme levels do appear to reflect changes in uterine and cervical collagen and proteoglycan content, but are not simple indices. For these reasons two models were used to test the effect of Rlx on the production of these enzymes in vivo and in vitro.

In the first model, cyclic animals were treated with oestrogen or oestrogen and Rlx in vivo and tissue enzyme levels measured. The magnitude of the changes induced after a single bolus of the hormones suggest that they are both potent controlling factors for uterine and cervical collagenase and proteoglycanase activities. Indeed the addition of Rlx to the oestrogen treatment reversed the inhibitory effect of oestrogen on uterine and cervical collagenase and proteoglycanase activities and completely obliterated the stimulating effect of oestrogen on uterine and cervical β-glucuronidase activity.

In comparison with these results, the endogenous tissue enzyme changes during pregnancy, with the increase in collagenase and proteoglycanase activities in the uterus and a decrease in the cervix suggest the influence of both oestrogen and Rlx on the uterus and by oestrogen alone on the cervix. In addition, the effect of Rlx to completely inhibit the stimulatory effect of oestrogen on β-glucuronidase activity in both the uterus and cervix suggests that the decrease in this enzyme in the uterus from early to mid-pregnancy and in the cervix from early and late pregnancy could be due to Rlx. It has recently been reported that Rlx exerts a dose-dependent inhibiting effect on collagen synthesis measured by [3H]proline incorporation by human uterus and cervix in the 7th-9th week of pregnancy (Wiquist et al. 1984). This, together with our demonstration that a combination of oestrogen and Rlx could increase uterine collagenase activity, suggests that Rlx is involved in the turnover of collagen in these tissues.

The in vivo model was further refined by oestrogen treating in vivo but studying the effect of Rlx in vitro upon the enzymes secreted from...
the tissues into the medium. The results clearly show that oestrogen and Rlx are important hormones determining the release of collagenase and proteoglycanase; however the effect of the hormones on the two tissues differed.

The level of collagenase secreted into the media by uterine cultures was approximately twice as high as the tissue enzyme activity measured in the pregnant animal. This agrees with the data of Jeffrey & Koob (1973) who reported that the amount of collagenase found in the medium between days 4–7 from rat uterine tissue exceeded the amount of enzyme bound to endogenous collagen at any time. This, together with the inhibition of collagenase production in vitro by inhibitors of protein synthesis or by the freeze-thawing of uterine tissues (Eisen et al. 1970; Jeffrey et al. 1971), was taken as additional evidence that active collagenase was not stored in the tissue and that the appearance of collagenase activity in the culture system was a reflection of continuing protein synthetic processes in cultured tissues (Jeffrey & Koob 1973). It is also likely that lysosomal enzyme activities would increase during tissue death, thus β-glucuronidase, a lysosomal enzyme (Barrett 1977), would be a marker for such an occurrence. In our study β-glucuronidase activity was high on the first day of uterine culture but declined thereafter, suggesting that after stabilization there was no further cell death. Collagenase activity released by the cervical tissues in culture was lower than that measured in the same tissue from pregnant animals.

In any event, the results show that using tissue from an oestrogenized animal Rlx could partially overcome the inhibitory effect of oestrogen on uterine collagenase but reinforced the oestrogen inhibition of uterine proteoglycanase without affecting the levels of β-glucuronidase. In contrast, in the cervix, Rlx decreased cervical collagenase and proteoglycanase activities whilst having no effect on β-glucuronidase.

We have demonstrated that by using three different systems on two tissues that it is possible to define the limits of each system in an attempt at dissecting complex hormonal interactions resulting in complex structural changes. We have been able to demonstrate that Rlx clearly has an effect on the enzymes involved in both collagen and proteoglycan degradation, thus adding to the known changes caused by oestrogen and progesterone. However, there is a considerable need for more precise methods for the measurement of turnover of the individual constituents of connective tissues in order to be able to fully understand the role of the pregnancy hormones on the remodelling of the uterus and cervix which occurs during pregnancy and immediately post-partum.

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