Regulatory influence of relaxin on human cervical and uterine connective tissue

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Abstract. Cervical tissue was obtained from women undergoing legal abortion in the 7th–15th week of gestation and tissue from the lower uterine segment was excised at elective Caesarean section in the 38th–40th week. The specimens were incubated with [3H]proline in the presence of relaxin or prostaglandin E2 (PGE2). Relaxin had a concentration related inhibitory effect on the radiolabelling in the 7th–9th week but failed to influence the amino acid uptake in the 10th–15th week of pregnancy. PGE2 had the inverse effect, i.e. no influence in the former group but reduced incorporation of proline in the latter group of patients. Incubation of tissue from the lower uterine segment showed a similar response as that of the early pregnant cervix. It is concluded that relaxin has a significant influence on [3H]proline incorporation by cervical and uterine tissue under in vitro experimental conditions.

Softening and lengthening of the ligament of the pubic symphysis was the first identified effect of the ovarian polypeptide hormone relaxin (Hisaw 1926). It was not until considerably later it was realized that relaxin also had a softening and dilating action on another collagenous structure of the reproductive tract, the cervix. This action has been demonstrated in a variety of species, such as the rat, mouse, sow and monkey (Graham & Dracy 1952; Zarrow et al. 1956; Steinetz et al. 1957; Kroc et al. 1959; Zarrow & Yochim 1961; Hisaw & Hisaw 1964). There also appeared some clinical reports claiming that administration of relaxin induced cervical softening in the human female (Stone et al. 1959). These studies were, however, hampered by the fact that the relaxin preparations available were of low potency, generally not exceeding 150 GPU/mg. Considerable progress within the biochemical field, including identification of the molecular structure of the substance has recently provided samples of highly purified porcine relaxin with a potency of 3000 GPU/mg (Sherwood & O'Byrne 1974).

The possibility of using purified relaxin for induction of cervical softening in the human has so far been tested in two controlled clinical studies. The results indicated that the drug induced an increase in the cervical score and shortening of the induction-delivery interval (MacLennan et al. 1980; Evans et al. 1983).

Relaxin induced alterations in human cervical and uterine connective tissue may also be studied by biochemical methods using in vitro techniques. The incorporation of [3H]proline, a precursor of collagen, in the presence or absence of relaxin may provide evidence of the regulatory influence of this hormone on connective tissue metabolism.

Material and Methods

Specimens of cervical tissue were obtained by needle biopsy (TruCut. Travenol, Deersfield, Ill., USA) from non-pregnant patients and from patients undergoing legal abortion in the 7th–15th week of gestation. Tissue from the lower uterine segment was excised from the lower flap at the site of transverse incision at elective Caesarean section in the 38th–40th week. Each patient...
had given her informed consent and the sampling procedures had been accepted by the Ethical Committee of the Medical Faculty, University of Göteborg.

The tissue was immediately placed in chilled, oxygenated Krebs Ringer bicarbonate (KRB) buffer, divided into smaller pieces (5–10 mg wet weight) and pre-incubated for 60 min in an atmosphere of 5% CO₂ in oxygen in KRB buffer fortified with glucose (10 mM) and different concentrations of relaxin (1 ng–5 µg) or PGE₂ (300 ng/ml). The specimens were incubated for 2 h in gassed KRB buffer together with [³H]proline and the corresponding concentration of relaxin or PGE₂. The incubation was followed by washing the tissue in chilled KRB buffer. Precipitation of proteins was performed in 12% trichloroacetic acid and lipids were extracted by chloroform-methanol (2:1, v/v) before drying the samples in an oven at 50°C for a period of 24 h. Dry weight was determined using a Cahn microbalance (model 4700). Radioactivity was extracted by 0.25 ml Soluene (Packard Co., USA) at 50°C overnight and counted (cpm/mg dry weight) in a Packard liquid scintillation counter (model 2400 Tri Carb) using Permablend III (Packard Co., USA) in toluene as a scintillator. Incorporated radioactivity was expressed as per cent of control levels in each experiment.

A separate batch of relaxin (3000 GPU/mg) and an inactivated sample was tested on tissue from the lower uterine segment of term-pregnant women. Inactivation of relaxin was accomplished by reduction and alkylation according to the procedure of Crestfield et al. (1963).

The reduced alkylated porcine relaxin was hydrolyzed with 6 M HCl; amino acid analysis indicated that cysteine residues had been converted to carboxymethyl cysteine. The reduced and alkylated relaxin was inactive in guinea pig palpation and mouse pubic ligament bioassays and in the R₆ antibody radioimmunoassay (Sherwood & O’Byrne 1974).

**Chemicals**

Relaxin 3000 GPU/mg¹ was dissolved in KRB buffer (500 µg/ml) and stored at −20°C; PGE₂ (Upjohn Company, Kalamazoo, Mich, USA) was dissolved in 99.5% ethanol and stored as stock solution (1 mg/ml) at −20°C; [³H]proline (139 Ci/mмole, 0.01 µm) was purchased from New England Nuclear Company, Boston, Mass, USA.

**Statistical procedure**

Mean values ± SEM were calculated and the significance of variations was determined using analysis of variance according to Student-Neuman-Keul’s multiple range test (Wolf 1968).

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**Results**

The study was initiated by a series of control experiments. Specimens from the lower uterine segment of women (n = 4) undergoing elective Caesarean section near term were incubated with relaxin to investigate dose-response relationships. There was a concentration dependent decrease in the incorporation of [³H]proline in all four cases at concentrations between 0.5–5 µg/ml (Fig. 1).

In another set of control experiments biopsies from the cervix as well as from the lower uterine segment of 2 women undergoing elective Caesarean section, were incubated with relaxin (5 µg/ml). The incorporation of [³H]proline into the control specimens was lower in tissue from the lower uterine segment that in biopsies from the cervix (64 and 84%, respectively). However, relaxin decreased the incorporation of [³H]proline by approximately 40% in both types of tissue. As a comparison cervical specimens from 4 non-pregnant women were incubated with relaxin (0.5 and 5 µg/ml) and [³H]proline. Relaxin did not influence the radiolabelling.

A separate series of experiments was carried out to test the effect of inactivated relaxin as compared to that of the active hormone. In specimens from the lower uterine segment of 6 term-pregnant women intact relaxin caused 34% decrease in proline uptake whereas the inactivated sample had no effect (Table 1).

In an extended series of experiments cervical tissue from early pregnant women was incubated with relaxin at a concentration of 5 µg/ml. With biopsies from 20 women in the 7th–9th week of pregnancy there was a significant decrease in the incorporation of [³H]proline but with biopsies

**Table 1.**

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<thead>
<tr>
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<th>Value (µg/ml)</th>
<th>SEM</th>
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<tbody>
<tr>
<td>Control</td>
<td>100 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Relaxin</td>
<td>66.2 ± 3.0*</td>
<td></td>
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<tr>
<td>Inactivated relaxin</td>
<td>102.1 ± 3.1</td>
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*P < 0.001.
Influence of relaxin on the incorporation of [3H]proline into tissue from the lower uterine segment of a term-pregnant patient. Each bar represents the mean ± SEM of 7 individually tested tissue pieces from the same subject. C = control. Significance of difference from control: *** P < 0.001.

from 15 cases in the 10th–15th week of gestation there was no effect (Fig. 2). Incubation of tissue from the lower uterine segment of term-pregnant women using a concentration of 0.5 and 5 µg relaxin per ml, resulted in reduction of radiolabelling with [3H]proline by 25 and 35% of the control level (Fig. 3).

Earlier studies from this laboratory showed that PGE2 increases [3H]proline incorporation into cervical tissue in the 7th–8th week but decreases the radiolabelling in the 12th–13th week of gestation (Norström 1982). The difference in response to relaxin and PGE2 at different weeks in the first trimester of pregnancy was re-investigated in the present study. Cervical specimens were incubated with relaxin or PGE2 in the way that each compound was tested on tissue from the same patient. The data on proline incorporation again showed that relaxin reduced the uptake in the beginning of the first trimester (P < 0.01) but had no effect in the 10th–13th week of gestation. The corresponding values with PGE2 were opposite to those of relaxin, i.e. no effect in the former and decreased uptake of [3H]proline (P < 0.05) in the latter period of gestation (Fig. 4).
Fig. 3.
Effect of relaxin on the incorporation of [3H]proline into specimens from the lower uterine segment as obtained near term. C = control, n = number of patients. *** P < 0.001.

Fig. 4.
Effects of relaxin and PGE₂ on the incorporation of [3H]proline into cervical tissue of women in early pregnancy. R = relaxin (5 µg/ml) and PGE₂ = prostaglandin E₂ (300 ng/ml). Each compound was tested on tissue from the same subject. Note the inverse effects of relaxin and PGE₂ at different stages of pregnancy. * P < 0.05; ** P < 0.01.

Discussion
Although the dilatability of the cervix in a variety of laboratory animals is enhanced by the addition of relaxin to regimens of steroid treatment the histological and ultrastructural features observed in these cervices are indistinguishable from those elicited by steroids alone. Hypertrophy of fibroblasts and separation and spacing of the collagen bundles seem to be common alterations (Leppi 1964; Leppi & Kinnison 1971). This remodelling of the collagenous framework is likely to involve both destruction and new synthesis of collagen. Relaxin appears to stimulate the production or release of collagenolytic enzymes and also to cause an increase in the non-collagenous matrix of cervical connective tissue (Weiss et al. 1979; Steinetz et al. 1980). These alterations are obviously of multiple nature and do not disclose the exact mechanism of action of relaxin. However, in view of the finding that human fibroblasts possess receptors for relaxin (McMurtry et al. 1980) and that the hormone causes hypertrophy of these cells it seems possible that relaxin exerts its effects on collagen through modulation of the activity of fibroblasts.

Most proteins contain proline but this amino acid is one of the dominating constituents of collagen. Moreover, proline is an essential precursor of collagen specific hydroxyproline. The radiolabelling with [3H]proline of specimens from the cervix and the lower uterine segment may therefore reflect metabolic events particularly within this tissue component. As demonstrated in 2 patients at term-pregnancy, tissue from the lower uterine segment did respond to relaxin in a similar way as cervical tissue. Therefore, for reasons of accessibility and comparability, tissue from the lower uterine segment was used in one of the main series of experiments. The fact that recovered net radioactivity was lower in the isthmus than in the cervix may reflect a relative predominance of collagen in the cervix.

The present results demonstrate that relaxin decreases the incorporation of [3H]proline into specimens from the cervix of women in the mid first trimester and into the lower uterine segment of women at term. This effect is reproducible and occurs in a concentration dependent manner.

Inactivation of the relaxin preparation abolished the response which demonstrates that the decrease in [3H]proline incorporation depends upon an action of the intact hormone.
Relaxin decreased proline uptake in the mid first trimester but failed to do so in the 10th–15th week of gestation. The difference in response, dependent upon gestational week, was highly significant. Evidence of a change in the biochemical properties of the early pregnant cervix as compared to that in the beginning of the second trimester has been demonstrated in a previous study from this laboratory. PGE₂ increased the incorporation of [³H]proline in early pregnancy but decreased uptake of this amino acid in the second trimester (Norström 1982). This observation was confirmed in the present study to the extent that PGE₂ had no effect in the 7th–9th week but decreased uptake in the 10th–13th week of gestation. Thus it is evident that relaxin and PGE₂ have opposite effects when tested on tissue from the same patients and at different stages of gestation.

Pre-treatment of mice with indomethacin prevented the increase in cervical dilatability in connection with relaxin administration (Kennedy 1976). These results indicate that the action of relaxin could be mediated by prostaglandins. The present results showing opposite effects of PGE₂ and relaxin in different stages of pregnancy support the view that the interaction between PGE₂ and relaxin is more complex. It may be possible that prostaglandin mediated effects depend on the release of prostaglandin compounds other than PGE₂.

The relaxin concentrations used in the present study are by far higher than those in the human circulation (Quagliarello et al. 1979). However, it should be remembered that relaxin is secreted from the decidua resulting in a high local concentration of relaxin (Bigazzi et al. 1980).

It is a long way between the biochemical observations presented in this study and effects of relaxin in the clinical situation. However, in view of the difficulties involved in the evaluation of clinical trials aiming at softening the human cervix by relaxin it might be of interest that human cervical and uterine connective tissue is influenced by relaxin under in vitro experimental conditions.

Acknowledgments

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References


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