Relaxin-induced changes in adenosine 3',5'-monophosphate levels in the human cervix

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Abstract. The effects of porcine relaxin on the levels of cAMP in human cervical tissue were studied in vitro. The specimens were obtained by needle biopsy from women undergoing hysterectomy, legal abortion in the first trimester or elective Caesarean section at term, and were incubated in Krebs-Ringer buffer for 15 min in the presence of porcine relaxin (5 μg/ml, 3000 GPU/mg). cAMP was determined using a modified protein binding assay. The concentration of cAMP was higher in pregnant than in non-pregnant women. Relaxin stimulated the production of cAMP in the 7th–8th week of gestation and at term but did not significantly alter the cervical cAMP levels in neither non-pregnant women nor in women in the 10th–12th week of pregnancy. Previous studies have shown that porcine relaxin reduces collagen synthesis in tissue from the human cervix and lower uterine segment. The present observations indicate that these effects can be mediated by cAMP.

Several hormones have been shown to promote the ripening process of the uterine cervix (Uldbjerg et al. 1983). One of them is relaxin. Recent clinical studies, using purified relaxin, indicate that this polypeptide hormone may induce softening of the cervical tissue, thereby shortening the induction-delivery period (Mac Lennan et al. 1980; Evans et al. 1983). Experimental work in this laboratory on human cervical tissue has demonstrated that relaxin influences collagen metabolism (Wiqvist et al. 1984) and inhibits cervical smooth muscle activity during pregnancy (Norström et al. 1984). Studies on rat uterine tissue suggest that the biological effects of relaxin are exerted through cyclic adenosine 3',5'-monophosphate (cAMP) (Cheah & Sherwood 1980; Judson & Bhoola 1980; Sanborn et al. 1980). The present investigation on human cervical tissue gives further support for that concept.

Material and Methods

Hormones and chemicals
Porcine relaxin (3000 GPU/mg) was kindly supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK), University of Maryland, School of Medicine, Baltimore, Maryland, USA, and by Dr. B. Steinetz, Research Department Pharmaceutical Division, Ciba-Geigy Co., Ardsley, N.Y. 10502, USA. Cyclic [3H]adenosine 3',5'-monophosphate [2,8-H]cAMP, Na salt, 31.5 Ci/mmol was purchased from New England Nuclear Co., Boston, Mass., USA. Adenosine 3',5'-cyclic monophosphate acid (cAMP) and 3-isobutyl-1-methyl-xanthine (IBMX) were obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Tissue material
Cervical tissue was obtained by needle biopsy (Tru Cut, Travenol, Deersfield, Illinois, USA) from women undergoing hysterectomy (non-malignant disorders not involving the cervix) (n = 6), legal abortion in the 1st trimester (n = 12) or elective Caesarean section before start of labour (n = 6). The specimens (1.5 × 15–20 mm), excised from the middle portion of the anterior cervical lip, were immediately transferred to ice-chilled, oxygenated (5% CO2 in O2) Krebs-Ringer bicarbonate (KRB) buffer and transported to the laboratory.

Each patient had given her informed consent and the tissue sampling technique was approved by the Ethical Committee, The Medical Faculty, University of Göteborg.
Incubation procedure
Each tissue strip was divided into 6–7 smaller pieces, which were preincubated for 15 min at 37°C in freshly oxygenated KRb buffer, containing 10 mM glucose in the presence of IBMX (100 μM). Incubation proceeded for another 5 min and relaxin (5 μg/ml) was then added to the vials of the experimental group. Incubation was finished 15 min later.

Analytical procedure
The tissue pieces were homogenized in ice-chilled 80% (v/v) ethanol containing 5 mM HCl (Rani et al. 1983). The supernatant, obtained at 4000 × g for 20 min, was evaporated to dryness under an air-stream. The dried material was dissolved in 0.05 M sodium acetate buffer at pH 4.0 and was analyzed for cAMP content using the protein binding assay of Gilman (1980) as modified by Khan (1979). The pellet was dissolved in 1 M NaOH for determination of protein according to Lowry et al. (1951).

Statistics
The mean values were calculated for each experiment and then pooled to form control and relaxin groups. Student’s t-test was used for comparison between two groups.

Results
Cervical tissue was homogenized in 80% ethanol-5 mM HCl containing [3H]cAMP. The radioactive cAMP, recovered in the supernatant was measured. The mean recovery was 83.0 ± 3.9% (9 experiments on tissue specimens from 2 women).

The basal level of cAMP was higher in the 7th–8th week of pregnancy than that in non-pregnant subjects (P < 0.01). A further increase (P < 0.001) occurred in the 10th–12th week. The cAMP levels at term were in the same range as those found at the end of the 1st trimester (Fig. 1).

Different concentrations (0.05–10 μg/ml) of relaxin were tested. Reproducible effects of the compound were obtained at a concentration of 5 μg/ml which was chosen in the main experimental series (Table 1).

The effect of relaxin on cervical levels of cAMP was investigated at 5 and 15 min of incubation. In both control and experimental groups the levels increased by approximately 50% during this time interval. On the basis of these experiments and earlier experiences with the same system (Norström et al. 1983) an incubation time of 15 min was chosen for the main experimental series.

![Graph](image-url)

Fig. 1.

Levels of cAMP in human cervical tissue from non-pregnant (N.P.) and pregnant women (gestational week (w) indicated). Relaxin (R, hatched bar) significantly increases cAMP levels over control values (C, open bar) in the 7th–8th week (P < 0.05) and at term (P < 0.001). Each bar is the pooled means ± SEM.
Table 1.

Concentrations of cAMP (pmol/mg protein) in cervical tissue from 3 women at term pregnancy after incubation for 15 min in the presence of relaxin. Number of tissue pieces in each group within parenthesis. Means ± SEM in each group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Relaxin</th>
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<tbody>
<tr>
<td></td>
<td>0.5 µg/ml</td>
<td>5 µg/ml</td>
</tr>
<tr>
<td>17.9 ± 5.6 (6)</td>
<td>15.0 ± 3.9 (6)</td>
<td>28.4 ± 6.4 (7)</td>
</tr>
<tr>
<td>17.4 ± 2.9 (6)</td>
<td>17.5 ± 1.7 (6)</td>
<td>20.0 ± 2.1 (6)</td>
</tr>
<tr>
<td>10.8 ± 1.9 (7)</td>
<td>17.4 ± 2.0 (5)</td>
<td>18.6 ± 2.2 (6)</td>
</tr>
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</table>

When cervical tissue was incubated in the presence of the phosphodiesterase inhibitor IBMX, the levels of cAMP increased in control and relaxin-incubated tissue by 95 and 75%, respectively. Since the cAMP levels in cervical tissue were low, IBMX was added to the control as well as experimental groups to improve the differentiation of the results.

Under the experimental conditions mentioned above, relaxin did not influence the tissue content of cAMP in cervixes of non-pregnant patients. In the 7th–8th week of gestation relaxin slightly increased cervical cAMP (P < 0.05) over control levels but there was no such response in the 10th–12th week. However, at term, cAMP levels significantly increased in response to relaxin (P < 0.001, Fig. 1).

Discussion

Due to limitation with respect to the cervical tissue available, time-course and concentration-effect studies could not be performed in each patient. The dose of relaxin used (5 µg/ml) is, however, effective in reducing collagen synthesis (Wiqvist et al. 1984) and inhibiting spontaneous smooth muscle activity (Norström et al. 1984) in vitro in the strips from the human cervix. This concentration is by far higher than that reported for human serum (Quagliarelli et al. 1979) but it appears possible that the contribution of decidual relaxin in the in vivo situation could yield a considerably higher local concentration (Bigazzi et al. 1980). Although relaxin can be demonstrated in ovaries of non-pregnant women (Thomas et al. 1982), this polypeptide hormone is mainly produced in the corpus luteum of pregnant women (O'Byrne et al. 1978). Relaxin does not affect collagen synthesis in non-pregnant subjects as evaluated by incorporation of [3H]proline (Wiqvist et al. 1984). The demonstrated unchanged levels of cAMP after incubation of cervical tissue of non-pregnant women in the presence of relaxin is in line with those results and point to a less important role of this hormone in non-pregnant women.

We recently observed that the tissue response to relaxin changed between the early and the late stages of the 1st trimester (Wiqvist et al. 1984). Thus the incorporation of [3H]proline was decreased by relaxin in the 7th–9th week whereas relaxin was without effect in the 10th–15th week. The difference in cAMP response to relaxin in these stages of the 1st trimester is thus in complete agreement with previous findings. This shift in response could possibly be explained by down-regulation of relaxin receptors as induced by endogenous relaxin (Bryant-Greenwood et al. 1982) since the serum levels of relaxin peak at the end of the 1st trimester (Quagliarelli et al. 1979).

Regarding cervical cAMP-levels as indicators of metabolic activity the increased levels of cAMP during pregnancy delineate the changes in shape and consistency of the cervix during pregnancy as an expression of increased cellular activity. Not only prostaglandins (PGs) (Norström 1982) but also relaxin (Wiqvist et al. 1984) seem to contribute to this process. The action of PGE2 on cervical tissue may be mediated by cAMP (Norström et al. 1983). The present data suggest that the effect of relaxin could also be mediated by cAMP. In this context it should be emphasized that the action of relaxin on the mouse pubic symphysis is associated with increased levels of cAMP (Braddon 1982).

In addition to connective tissue the human cervix contains 10–15% smooth muscle. It is a general finding that relaxation of smooth muscle is associated with increased levels of cAMP. In rat uterine tissue the muscle relaxing effect of relaxin is accompanied by increased cAMP values (Cheah & Sherwood 1980; Judson et al. 1980). Relaxin has a contractility inhibiting effect on human cervical smooth muscle (Norström et al. 1984). The question as to whether the relaxin-induced increase in cervical cAMP refers to alterations within the predominant connective tissue or within the smooth muscle elements presently remains unknown.
Acknowledgments

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