Distribution of immunoreactive relaxin in the genital tract and in the mammary gland of non-pregnant women

S. BONGERS-BINDER (*) - A. BURGARDT (**) - H. SEEGER (*)
W. VOELTER (**) - T. H. LIPPERT (*)

Summary: Relaxin was determined in healthy and pathological tissue samples from mammary gland, ovary, myometrium and cervix uteri of non-pregnant women. Relaxin could be detected in all groups of tissues examined. The highest values were found in healthy ovary, the lowest concentrations in the cervix, which is known as a target organ for relaxin activity during pregnancy. Mammary parenchyma showed the highest specific activity of all healthy tissues examined. The relaxin concentration in myoma tissue is significantly higher than in healthy myometrium.

Key words: Relaxin; Tissue content; Uterus; Ovary; Mammary gland.

INTRODUCTION

Relaxin is a protein hormone produced mainly in the corpus luteum graviditatis. Using a sensitive radioimmunological assay (RIA), it has been found present in very small amounts in non-pregnancy also. In a few studies, it could be detected in blood ([1, 2]), in the ovary ([3]) and in the endometrium ([4]) of non-pregnant women. The most important actions of relaxin are softening of the cervix and relaxation of the uterine muscle during pregnancy ([5]). Its functions in the nonpregnant organism are still unknown.

In the present study, tissue samples from the genital tract and mammary gland of non-pregnant women were examined radio-immunologically for the presence of relaxin. In addition, samples of healthy tissue were compared with those of pathological tissues, for relaxin content.

(*) Departments of Obstetrics and Gynecology
(**) and Biochemistry
University of Tübingen, (FRG)

MATERIALS AND METHODS

Tissue samples with a weight of at least 0.5 g were taken from the mammary gland, ovary, myometrium and cervix uteri.

All samples were obtained from the University Women's Hospital ,Tübingen, after clinically-indicated operations.

The tissue samples were frozen in liquid nitrogen shortly after excision and stored at −70° C until required for estimation. Parts of the samples were examined histologically and the results used to allocate the samples into healthy and pathological groups. The method of relaxin extraction has been described by Bigazzi *et al.* ([6]). In summary, the tissue, still half-frozen, was cut into small pieces with a scalpel and added to ice-cool 10 mM phosphate buffer 0.9% NaCl, 0.02% sodium acid (NaN3) pH 6.8, homogenised by means of an ultraturrax and brought to a buffer end volume of 1:10.

Table 1. – *Relaxin concentrations (X±SEM) in healthy tissue samples of the human genital tract and mammary gland.*

Tissue type	No. of samples	Age of patient (years) X±SEM	pg relaxin per g tissue X±SEM	pg relaxin per mg protein X±SEM
Myometrium	19	51±3	732±187*	52±30
Cervix	10	46±3	160±90	5±3
Ovary	10	51±3	1493±679*	45±23*
Mammary	12	53±4	1095±187**	190±114*

Statistical calculations compared the cervix with the other tissues.
*$p<0.05$ **$p<0.01$

Thereafter the extract was shaken and centrifuged at 35,000 rpm at 4° C. The lyophilisate filtrate was estimated for protein content and relaxin concentration by radioimmunoassay. Protein estimation was carried out according to the method of Lowry, modified by Bandadoun and Weinstein ([7]) and by Hess *et al.* ([8]). The protein was precipitated with 25% TCA, concentrated and separated from foreign particles in the test solution, so leading to an improved sensitivity over previous methods. After addition of 1% sodium desoxycholate, there followed precipitation with 24% trichloracetic acid.

Finally, the filtrate was removed, the protein coloured with Lowry reagents and, by means of a standard curve, measured spectrophotometrically.

The immunological relaxin activity was determined with a heterologous RIA for porcine relaxin according to Hunter and Greenwood ([9, 10]) which is valid for human relaxin by cross-reaction. As tracer, 125I-labelled tyrosilated porcine relaxin was used, iodated by the chloramine-T-method.

Antiporcine sheep antiserum was used as antibody. The sensitivity limit of the RIA is 10 pg/tube. Two measurements were made from each sample, and evaluated by computer using the logit-log-transformation of the standard curve. The interassay variation coefficient was 10-15%, the intra-assay variation coefficient was 7-12%. Significance calculations were made using the Student-T-test-for random samples.

RESULTS

Table 1 shows the relaxin concentrations in healthy tissue samples from myometrium, cervix uteri, ovary and mammary gland. Relaxin was found in all tissues examined. The highest values were found in the ovary (1493 pg/g tissue), although the women were no longer in the child-bearing age group (x = 51 ± 3 years).

The lowest concentrations were found in the cervix, a target organ for relaxin activity (160 pg/g tissue). In a further target organ, the myometrium, values of 732 pg/g tissue were obtained.

Mammary parenchyma with 190±114 pg/mg protein, showed the highest specific activity of the healthy tissues examined while the lowest specific activity (5±3 pg/ml protein) was measured in the cervix uteri.

Significant differences in relaxin activity from the cervix were found in myometrial, ovary and mammary tissue. Significant differences from the cervix were also found in specific activity in ovary and mammary tissues.

Table 2 shows the mean relaxin concentrations in the pathological tissues. The largest group (n=11) was that of the myoma (fibroid). In addition, single samples of neoplasms from ovarian, myometrial and mammary tissue were examined. The highest mean relaxin activity (2779 pg/g tissue) from all types of tissues examined in the study was found in the fibroid group.

Table 2. – *Relaxin concentration (X±SEM) in pathological tissue samples.*

Tissue type	N. of samples	(years) patient Age of X±SEM	per g tissue pg relaxin X±SEM	pg relaxin per mg protein X±SEM
Myoma tissue	11	45±2	2779±856	203±98
Myosarcoma	2	48	1953	7.9
		48	2354	11.4
Mammary carcinoma	2	56	0	0
		44	902	54
Ovarian carcinoma	2	44	1201	6.6
		44	2465	6.2

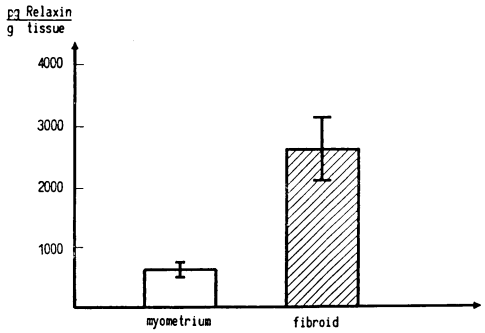

Fig. 1. — Comparison of relaxin content in myometrium and fibroid (myoma tissue).

In one mammary carcinoma tissue relaxin was present (902 pg/g tissue), in the second mammary carcinoma sample examined, no immunological activity could be found.

The relaxin values of the myosarcoma and ovarian carcinomas were only slightly lower than the values from the fibroid tissue.

The highest mean specific activity of all tissues examined was also the fibroid tissue (203 ± 98 pg/mg protein). The lowest specific activity (6.6 and 6.2 pg/mg protein) was found in the two ovarian carcinoma tissues.

Fibroid tissue had considerably more relaxin than normal healthy myometrium. Figure 1 illustrates the comparison of relaxin concentrations in healthy and in fibroid tissues of the myometrium. It shows that the relaxin content in fibroids is almost 4 times higher than that of healthy tissue. This difference is highly significant ($p < 0.01$). The relaxin content of the mammary carcinomas, was below the values measured in healthy mammary tissue. In ovarian carcinoma, a slightly higher relaxin content was found in comparison with the non-pathological ovary. However, the specific activity in carcinoma (6.4 pg/mg protein) was approximately 7 times lower than that of the healthy ovary (45 pg/mg protein).

DISCUSSION

In the present study, relaxin estimations were carried out with a heterologous radioimmunoassay for porcine relaxin suitable for human relaxin also, due to cross-reaction.

Relaxin was found in all groups of tissues examined; the highest values were measured in fibroid tissue. It is thought that relaxin may be involved in the regulation of growth in the myometrial tissue but comparative investigations have not been found in the literature.

The lowest relaxin concentrations were found in cervical tissue which is known to be an important target organ for relaxin action during pregnancy. It seems likely that relaxin is not produced in the cervix itself but is brought there by the blood when the need arises, e.g. during pregnancy and prepartal. This is indicated by investigations using biological methods by Maillot *et al.* ([11]). They found that the relaxin content of cervical tissues from pregnant women rose during gravidity from 0.40 GPU to 0.94 GPU/mg tissue protein (GPU = guinea pig unit). However, during delivery and immediately post partum, the tissue values returned to about 0.39 GPU.

Relaxin has been shown present in the mammary parenchyma for the first time. Compared to the other tissues examined, it showed a relatively high specific activity. Thus, it can be assumed that the breast gland tissue is an unrecognised site of relaxin production and this could explain the decrease in serum relaxin values below the detection limit of the radioimmunoassay from the 2nd week post partum and simultaneous increase of relaxin concentration in maternal milk, shown by Lippert *et al.* ([12]).

The mean relaxin concentration of 1.5 ng/g tissue and 0.045 ng/mg protein in ovarian tissue found in this study is markedly lower than the concentration measured by O'Byrne *et al.* ([39]) in the corpus

luteum of non-gravid young women. Their measurements gave values of about 4 ng/mg protein. However, the tissues were obtained from women in the reproductive age group whereas the average age of the patients in the present study was 51 years.

The presence of relaxin in the genital tract and in the mammary parenchyma presents a series of questions. The function of relaxin in tissues and its site of production, especially during the climacterium and in the post-climacteric phase, is still unknown.

REFERENCES

1) Thomas K., Loumaye E., Ferin J.: "Relaxin in nonpregnant women during ovarian stimulation". *Gyn. Obst. Invest., 11,* 75, 1980.
2) Quagliarsello J., Goldsmith L., Steinetz B., Lustig D. S., Weiss G.: "Induction of relaxin secretion in nonpregnant women by chorionic gonadotropin". *J. Clin. Endocr. Metab., 51,* 74, 1980.
3) O'Byrne D. M., Flitcraft J. F., Sawyer W. K., Hochmann J., Weiss G., Steinetz B. G.: "Relaxin bioactivity and immunoactivity in human corpora lutea". *Endoc., 102,* 1641, 1978.
4) Yki-Jaervinen H., Wahlstroem T., Sappaelae M.: "Immunhistochemical demonstration of relaxin in the genital tract of pregnant and non-pregnant women". *J. Endoc. Metab., 57,* 451, 1983.
5) Porter D. G.: "Myometrium of the pregnant Guinea Pig: The probable importance of relaxin". *Biol. Reprod., 7,* 458, 1972.
6) Bigazzi M., Nardi E., Bruni P., Petrucci F.: "Relaxin in human decidua". *J. Clin. Endocr. Metab., 51,* 939, 1980.
7) Bansadoun A., Weinstein D.: "Assay of proteins in the presence of interfering materials". *Anal. Bioch., 70,* 241, 1976.
8) Hess H. H., Lees M. B., Derr J. E.: "A linear Lowry-folline assay for both water-soluble and sodium dodecyl sulfate-solubilized proteins". *Anal. Bioch., 85,* 295, 1978.
9) Sherwood O. D., Dosentreter K. R., Birkhimer M. L.: "Development of radioimmunoassay for porcine relaxin using 125-J-labelled polytyrosyl-relaxin". *Endocr., 96,* 1006, 1975.
10) Hunter W. M., Greenwood F. C.: "Preparation of Jodine-131-labelled human growth hormone of high specific activity". *Nature, 194,* 495, 1962.
11) Maillot von K., Weiss M., Nagelschmidt M., Struck H.: "Muttermundseröffnung und Relaxin". *Arch. Gynäk., 233,* 323, 1977.
12) Lippert T. H., Göd B., Voelter W.: "Immunoreactive relaxin-like substance in milk". *IRCS Medical Science, 9,* 295, 1981.

Addres reprint request to:
Prof. T. H. LIPPERT
Universitäts-Frauenklinik
D-7400 Tübingen