Immunohistochemical demonstration of relaxin in the placenta after removal of the corpus luteum

H. Yki-Järvinen¹ and T. Wahlström²

¹Third Department of Medicine and ²Department I of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki

Abstract. We studied the occurrence of relaxin in the term placenta from a woman who had normal pregnancy after her corpus luteum had been removed at the beginning of the second trimester. Two antisera (antiserum R6 and anti-NIH-RXN-P1, 3000 U/mg) against highly purified porcine relaxin and the avidin-biotinylated anti-rabbit IgG method were used to detect relaxin-like immunoactivity. Specific staining for relaxin was seen in the placental syncytiotrophoblasts. Comparison to a placenta from a normal term delivery revealed no differences in the intensity or distribution of staining. Our case substantiates that the corpus luteum is not the only tissue producing relaxin during pregnancy.

The corpus luteum of pregnancy is not the only source of relaxin in serum in women (Weiss et al. 1976). If the corpus luteum was the sole source of relaxin during pregnancy, it would mean that relaxin is not necessary for a successful delivery in women since the corpus luteum normally regresses after the first trimester, and it can be removed without interrupting pregnancy. Recently, two groups have independently found relaxin-like immuno- and bioactivity in the placenta (Fields & Larkin 1981; Yamamoto et al. 1981). Subsequent demonstration of relaxin receptors in foetal membranes raised the possibility that placental relaxin acts as a local hormone without reaching the systemic circulation (Koay et al. 1983). It is not clear, however, whether relaxin is synthesized or bound by receptors in the placenta, and whether or not the placenta contains relaxin in the absence of the corpus luteum. We had a unique opportunity to study the occurrence of relaxin in the placenta from a woman who had a normal pregnancy after the corpus luteum had been removed at the beginning of the second trimester.

Materials and Methods

Antiserum

Immunohistochemical staining was carried out using two anti-porcine relaxin antisera. One was raised in a rabbit against highly purified porcine relaxin (NIH-RXN-P1, 3000 U/mg) (Yki-Järvinen et al. 1983a). The other was rabbit anti-porcine relaxin antiserum R6 kindly provided by Dr. E. M. O’Byrne (Ciba-Geigy Corporation, Ardsley, NY). This antiserum has been prepared against highly purified relaxin isolated from pregnant sow ovaries (Sherwood & O’Byrne 1974). Antiserum R6 inhibits the pubic symphysis-relaxing activity of extracts of human corpora lutea of pregnancy (O’Byrne et al. 1978) and human corpora lutea extracts and extracts of human pregnancy plasma yield concentration-dependent curves parallel to those obtained with porcine relaxin standards in two different RIA-systems employing this antiserum (O’Byrne et al. 1978; Loumaye et al. 1978).

Patient characteristics

The patient was an apparently healthy primigravid aged 27 years. At 15 weeks a routine examination disclosed a tumour with a diameter of 10 cm in the right ovary. At 16 weeks, the right ovary containing a large tumour (950 g) and the corpus luteum were removed. The left ovary was normal. Histologically the tumour was a semimalignant serous papillary cystadenoma. The corpus luteum had a normal structure. At 40 weeks she delivered a healthy girl weighing 3860 g.
Tissue specimens
Immediately after delivery, tissue specimens from the placenta were fixed in buffered (0.1 M sodium phosphate, pH 7.4) 10% formalin and embedded in paraffin according to routine histological procedures.

Immunoperoxidase staining
The biotin-avidin immunoperoxidase method was used as previously described (Hsu et al. 1981; Yki-Järvinen et al. 1983a, b). The antiserum against NIH-RXN-P1, 300 U/mg and the R6 antiserum were used at dilutions of

Fig. 1.
Immunohistochemical demonstration of relaxin in placenta at term after removal of corpus luteum in the beginning of the second trimester.
A) Haematoxylin-eosin.
B) Anti-relaxin antiserum
(antiserum against NIH-RXN-P1, 3000 U/mg).
C) Control immunoperoxidase staining
with antirelaxin serum adsorbed with relaxin
(original magnification × 375).
1:50 and 1:200, respectively. The following controls were included: the anti-relaxin antiserum was replaced by the same antiserum after absorption with purified relaxin (NIH-RXN-P1, 300 U mg, 167 μg ml) or by normal rabbit serum, or by omitting the first or the second antiserum.

Results

**Immunoperoxidase staining**

In the placenta, specific staining for relaxin was seen in the syncytiotrophoblast (Fig. 1). Comparison with normal placentae (n = 10) from 30–40 weeks revealed no difference in the distribution or intensity of staining. Control tissue specimens, stained after the anti-relaxin antiserum had been absorbed with purified relaxin, were negative.

Discussion

Our case shows that relaxin-like immunoactivity is present in the term placenta even after removal of the corpus luteum. This substantiates that the corpus luteum is not the only source of relaxin during pregnancy in the human.

Antiserum R6 used in the present study is the best characterized anti-porcine relaxin antiserum with respect to its ability to cross-react with relaxin-like immunoactivity in the human plasma and tissues. This antiserum also inhibits bioactivity of human relaxin (O'Byrne et al. 1978; Loumaye et al. 1978; Steinetz et al. 1981). The other antiserum was raised in our laboratory against NIH-RXN-P1, 3000 U/mg, the standard preparation now recommended for relaxin bioassays (Bryant-Greenwood 1982). We found similar staining in the placental syncytiotrophoblast with both antisera.

The origin of relaxin shows considerable interspecies variation. In the pregnant pig and rat, the only known source of relaxin is the corpus luteum (Schwabe et al. 1978; Steinetz et al. 1981b; Goldsmith et al. 1981), whereas in the pregnant mare, the major source is the placenta (Stewart et al. 1982). In the guinea pig, the major source of relaxin is the endometrium (Pardo & Larkin 1982). In pregnant women, relaxin has been demonstrated in the placenta, and isolated human placental relaxin is biologically active (Fields & Larkin 1981; Yamamoto et al. 1981). These differences in the tissues producing relaxin may reflect interspecies variation in the importance of these tissues for maintenance of pregnancy (Bryant-Greenwood 1982). Thus, in the rat, the corpus luteum is necessary for successful delivery whereas in women, the corpus luteum can be removed after the first trimester without interfering the pregnancy, indicating that relaxin produced by the pregnant corpus luteum is not necessary for maintenance of late pregnancy in women. Since serum relaxin is mainly of luteal origin, measurement of relaxin from serum samples cannot be used to monitor relaxin production from placental tissue during pregnancy (Weiss et al. 1976). It has recently been shown that the foetal membranes may be target tissues for relaxin in women (Ueno & Bryant-Greenwood 1981), and that this relaxin may be locally produced in the placenta (Koay et al. 1983). Our finding of relaxin in the placenta in the absence of the corpus luteum is consistent with this theory.

Acknowledgments

This study was supported by the Cancer Society of Finland and the Sigrid Juselius Foundation, the Research Council for Medical Sciences, Academy of Finland. We wish to thank Ms. Tuula Halmesvaara for technical assistance.

References


