RELAXIN DEFICIENCY IN THE PLACENTA AS POSSIBLE CAUSE OF CERVICAL DYSTOCIA

A case report

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Summary: In a case of cervical dystocia which had to be delivered by caesarean section, the placenta was examined for relaxin content. The placenta was processed by acidic acetone extraction and separation of the raw extract on a carboxymethylcellulose column. The activity of relaxin was estimated by means of heterologous porcine radioimmunoassay. The elution profile of the placenta extract showed significant differences to that of the placenta in normal deliveries i.e. there was no evidence of typical protein peaks with relaxin activity. It is suggested that there is possibly a causal relationship between the lack of placental relaxin and the pathological behaviour of the cervix during delivery.

INTRODUCTION

Relaxin, a peptide hormone with a molecular weight of approximately 6000, was originally known only as a pregnancy hormone in mammals.

After the development of sensitive radioimmunological detection methods, as compared with biological methods, it was also found present in non-pregnant women, in males and in non-mammals ([1]).

During pregnancy in humans, the corpus luteum graviditatis is the main source of relaxin, as reflected by serum values ([2]). As early at 14 - 21 days after conception, relaxin can be detected in serum ([3]). During the first trimester, the relaxin values are highest and then fall slightly during the second and third trimesters ([4, 5]). Post partum, the relaxin values sink within 72 hours parallel to those of progesterone. Generally, the serum relaxin values are relatively constant in human pregnancy without the rapid rise shortly before birth and the rapid fall post partum which are characteristic in pigs and rodents ([6]).

In addition to the corpus luteum graviditatis, relaxin has also been found in human placenta ([7, 8, 9, 10]). Placental relaxin seems to have mainly local functions ([11, 12]), especially during birth. It has been shown that a more rapid, easier birth takes place connective tissue metabolism high ([13]). Due to molecular changes in when the relaxin content of the placenta is high relaxin induces ripening and dilatation of the cervix ([14, 15, 16]), important conditions for a spontaneous birth without complications.

In case of cervical dystocia, however, deficiency of placental relaxin might be involved in the dysfunction of the cervix as the following case indicates.

CASE DESCRIPTION

A 27 year old 1st para was admitted to hospital in labor in the 42nd week of her 2nd pregnancy. The obstetrical history reported that the first child, one year earlier, was delivered by cesarean section because birth had not progressed in spite of labor. The present pregnancy had been uneventful. An obstetrical examination on admission indicated no pathological condition. The child was in the first vertex presentation, the true pelvis did not appear to be narrow so that a vaginal delivery seemed possible. In spite of strong spontaneous labor little progress in cervical dilatation occurred over the following 9 hours and finally a cesarean section had to be carried out. A healthy male child was born with a weight of 3440 g and a

length of 49 cm. The Apgar score was 8, 9, 10 after 1, 5 and 10 min. respectively. The placenta weighed 680 g and macroscopically showed no abnormal features. It was at once collected for extraction of relaxin.

MATERIAL AND METHODS

Immediately after delivery the placenta was added to ice-cold distilled water and homogenised in the presence of sodium azide and protease inhibitors. By acidic acetone extraction, modified according to Doczi ([17]), a relaxin raw extract was obtained in which all non aqueous soluble proteins, as well as lipids and low molecular weight substances were separated. The raw extract was run on a carboxymethylcellulose column (40 cm × 2.6 cm) in 50 mM ammonium acetate buffer, 0.02% NaN_3, pH 5.3 and eluted with a linear sodium chloride gradient (0.2 M NaCl to 0.5 M NaCl in the same buffer). The protein was detected in the fractions at 280 nm. From the elution profiles, the relaxin content of the placenta was estimated.

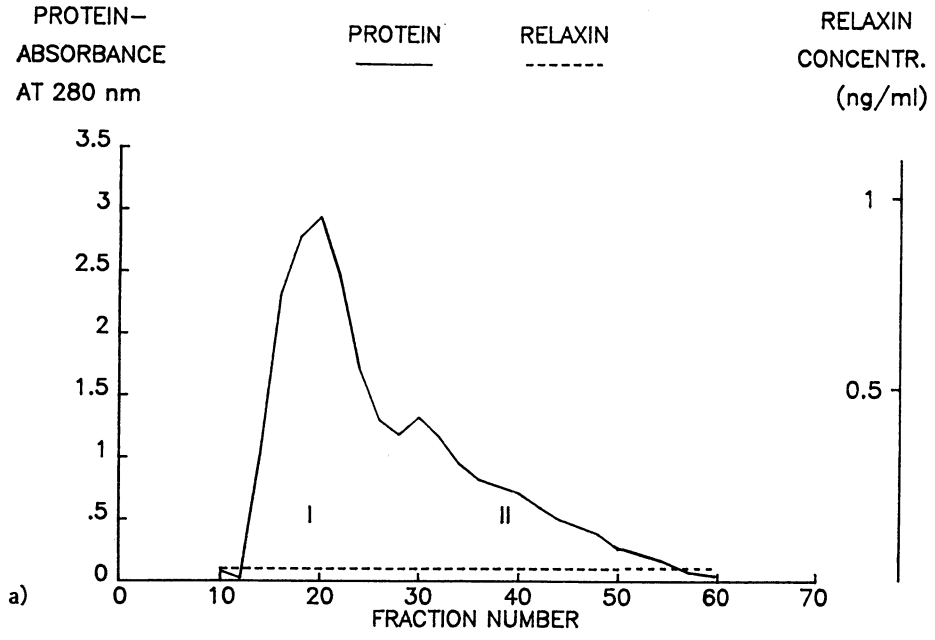
The immunological relaxin activity of the raw extracts and each eluted fraction was estimated using a heterologous radioimmunoassay (RIA) for porcine relaxin, modified according to Sherwood *et al.* ([18]). Due to cross reaction this test is suitable for estimation of human relaxin also. After preincubation of the samples with the antibody (antiporcine relaxin from sheep in a dilution 1:500,000) ^{125}I-tyrosylporcine relaxin with a specific activity of 150 mCi/mg was added and incubated for a further 24 hours at room temperature. The antigen/antibody complex was precipitated by 17.5% polyethyleneglycol. For the standard curve, 6 different concentrations were prepared with standard porcine relaxin in the range 15.6 -500 pg and evaluated with the computer model logitlog. The non-specific binding of this assay was 12-14%, the zero binding which was corrected by the non-specific binding was 50-60%. The interassay variation was 10-15%, the intra-assay variation was 7-12%. The sensitivity of the RIA i.e. the lowest limit of detection, was 30 pg/ml. The protein content was estimated with bovine albumin according to Lowry ([19]).

RESULTS

Neither in the relaxin raw extract, nor after purification of the raw extract by cation exchange chromatography on carboxymethylcellulose, could immunologically active relaxin be found in this placenta. This was particularly surprising because in an earlier study using the same method in 100 placentas after spontaneous delivery and cesarean sections, immunologically active relaxin was always present both in the raw extract and in the elution profile ([21]). There were certainly large individual differences in relaxin content but complete absence of relaxin was never observed. The mean relaxin content of the previously examined placentas was 20.1 ng with a standard error of the mean of 7.6 ng. The physiological variation was very large, from 2 ng to 196 ng. The relationship of relaxin content with clinical parameters has been discussed elsewhere ([13]). The elution profiles in these 100 placentas were relatively similar and only the absorbance values, corresponding to the protein content, and the immunological relaxin activity of the individual peaks differed from each other. However, the elution profile of the patient with cervical dystocia was dissimilar.

Fig. 1 shows a comparison of the elution profile of the placenta from the patient with cervical dystocia with a characteristic elution profile of a placenta after spontaneous delivery from the previous study. The second diagram presents a high symmetrical protein peak I, which is followed by two smaller sharp protein peaks containing the immunological relaxin activity. The highest immunological relaxin activity is as usual in protein peak III. The relaxin containing fractions were eluted only after rise of the NaCl gradient, i.e. relaxin was bound on the carboxymethylcellulose columns. The protein peak I on the other hand, is not bound and can be eluted with pure buffer solution.

When the elution diagram of the placenta in the case of cervical dystocia is compared with the second elution diagram it can be seen that it consists almost exclusively of the non-cation exchange bound protein peak I i.e. of non-charged protein. In the usual relaxin region there



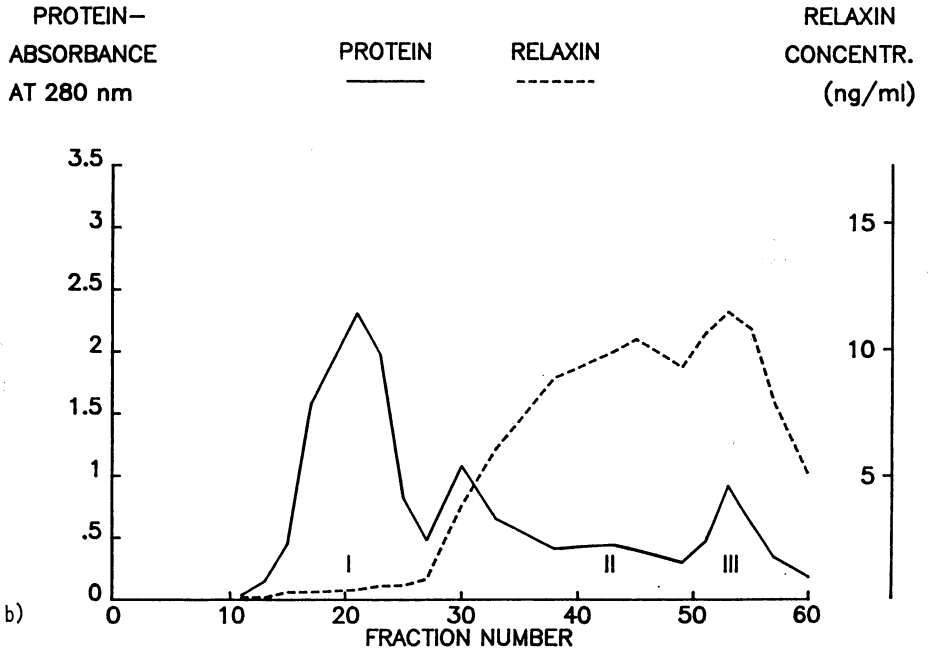


Fig. 1. — Comparison of the elution profile of the placenta extract from the patient with cervical dystocia (*a*) with a characteristc elution profile after uncomplicated spontaneous delivery (*b*).

were neither protein peaks nor immunological relaxin activity. Both the shape of the elution diagram and the lack of relaxin activity in the fractions, as well as in the raw extract, indicate the complete absence of relaxin in this placenta.

DISCUSSION

It may be suspected that the total lack of relaxin in the placenta is associated with the deficient dilation of the cervix in the described case.

In rodents, the action of relaxin during delivery is to induce stretching of the cartilaginous, rigid symphysis to a broadened symphysis ligament.

Combined with the increased ability of the cervix to stretch, the birth of the fetus is facilitated. The serum relaxin values in rodents run parallel to widening of the symphysis ligament and stretching of the cervix. The changes in the serum relaxin correspond with biological activity in pigs also. In humans, however, the serum relaxin values show little variation ([4, 5, 6]) therefore softening of the cervix seems to be influenced by relaxin produced or stored in the placenta ([11, 12]), and released during labor. The significance of placental relaxin for spontaneous delivery is supported by the description of another case, by Yki-Jaervinen *et al.* ([22]).

In a pregnant patient in the 2nd trimester, the ovary which contained the corpus luteum graviditatis had to be removed because of an ovarian tumor. Nevertheless spontaneous delivery followed at term without complications. The placenta was examined immunohistologically for its relaxin content and showed no deviation from the normal. Thus, the relaxin present in the placenta must have been sufficient to support spontaneous delivery without complications.

Further indication that placental relaxin may have a local action on the cervix is provided by the work of Maillot *et al.* ([14]) who found the highest relaxin values in cervical tissue at the time of delivery although the serum values remained unchanged.

In rats it has been shown that locally administered relaxin led to increased cervical stretching ([13]). In humans in a study by McLennan *et al.* ([23]) the local use of purified porcine relaxin led to softening of the cervix. Evans *et al.* ([24]) also administered purified porcine relaxin locally and found that both cervical score and time for delivery were significantly shortened compared with controls.

Experiments with local administration of relaxin also indicate that cervical dilation depends on supply of relaxin. In severe cervical dystocia as described in the present case the cause of the pathological condition could be the lack of relaxin in the placenta. However, further study is required to clarify this concept.

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