Distribution of relaxin between human maternal and fetal circulations and amniotic fluid

M. R. Johnson, A. Abbas*, K. H. Nicolaides* and S. L. Lightman

Neuroendocrinology Unit, Charing Cross and Westminster Medical School, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, U.K.
*Harris Birthright Research Unit in Fetal Medicine, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 8RX, U.K.

(Requests for offprints should be addressed to M. R. Johnson)

RECEIVED 10 December 1991

ABSTRACT
Relaxin was measured in maternal blood and amniotic fluid samples at 9–40 weeks and in fetal blood samples at 19–41 weeks of pregnancy. In amniotic fluid, concentrations of relaxin rose from 58 ng/l (geometric mean) at 10 weeks to 142 ng/l at 14 weeks and declined subsequently to 55 ng/l at 22 weeks. In maternal blood, mean relaxin concentrations were ten times greater than in amniotic fluid, and concentrations decreased with gestation. Since there was no significant association between the relaxin concentrations in the two compartments, relaxin in the amniotic fluid may be derived from the decidualized endometrium rather than the maternal circulation, alternatively its metabolism may be different in the two compartments. The absence of detectable concentrations of relaxin in any of the fetal blood samples demonstrates that there is no significant placental transfer or fetal synthesis of this peptide.


INTRODUCTION
Relaxin is detectable in the peripheral plasma between the late luteal and early follicular phases of the normal menstrual cycle (Stewart, Celnicker, Taylor _et al._ 1990). During pregnancy, plasma concentrations increase rapidly to a peak at 12 weeks of gestation, then fall to a level which is maintained for the remainder of pregnancy, with the exception of a transient increase at 32 weeks (Bell, Eddie, Lester _et al._ 1987). Circulating relaxin is derived exclusively from the corpus luteum (Johnson, Abdalla, Allman _et al._ 1991a) and concentrations increase in response to exogenous human chorionic gonadotrophin both in non-pregnant (Quagliarello, Goldsmith, Steinetz _et al._ 1980) and pregnant women (Johnson, Okokon, Collins _et al._ 1991b). While relaxin plays an essential role in some species (Downing & Sherwood, 1985), its role in human reproduction is unclear. Pregnancy can be established and maintained in its absence (Johnson _et al._ 1991a), but cervical compliance may be impaired (Eddie, Cameron, Leeton _et al._ 1990); thus relaxin may have a facilitatory role in the process of cervical dilatation, consistent with its ability to alter connective collagen content (Hwang & Sherwood, 1988).

The effect of relaxin on connective tissue and its inhibitory effects on myometrial contractility (Porter, Downing & Bradshaw, 1979) led to the hypotheses relating relaxin to the origins of diabetic embryopathy (Edwards & Newall, 1988), congenital dislocation of the hip and inguinal hernia (Uden & Lindhagen, 1988). However, such hypotheses rest on establishing whether or not relaxin is present in significant concentrations in the fetal circulation and/or in amniotic fluid. In one report, relaxin could not be detected in eight out of nine samples of amniotic fluid and in nine out of ten samples of cord blood at term (Weiss, O'Byrne, Hochman _et al._ 1985). We have investigated the distribution of relaxin between the maternal and fetal circulations and amniotic fluid at different stages of pregnancy in order to determine whether relaxin is present in the fetal circulation or amniotic fluid.
PATIENTS AND METHODS

Samples of umbilical cord blood were obtained by cordocentesis between 19 and 34 weeks of gestation (n = 24) and after delivery at term (n = 12). The indications for cordocentesis were: karyotyping of fetuses found to have a malformation on ultrasound (n = 11), prenatal diagnosis of hereditary blood disorders (n = 4) and assessment of fetal anaemia in rhesus isoimmunization (n = 9); the fetuses in the latter two groups were morphologically normal. Amniotic fluid was obtained at the time of cordocentesis (as above), elective Caesarean section and amniocentesis, the latter performed for fetal karyotyping because of advanced maternal age (n = 129, 102 of which were paired with maternal blood obtained from an antecubital vein immediately prior to the procedure). The amniotic fluid was frozen at −20 °C, and the plasma separated and stored at −20 °C within 60 min of being taken. Patients gave informed consent to taking part in the study, which had the approval of the King's College Hospital Ethics Committee.

The concentration of relaxin was measured in unextracted plasma by a non-competitive double-antibody enzyme-labelled immunosorbent assay (Ferraiolo, Winslow, Laramee et al. 1991). The polyclonal antibodies were raised in New Zealand White rabbits using synthetic relaxin (hRXN2) as the immunogen. The enzyme used in the assay is horse-radish peroxidase. Synthetic human relaxin was used to make up the standards in pooled normal male plasma at concentrations from 20 pg/ml to 1250 pg/ml. The working range of the assay (defined as the lowest and highest values with a coefficient of variation of <10%) was between 35 pg/ml and 1100 pg/ml. The interassay and intra-assay variation were 13-0% and 5-0% respectively. The cross-reactivity with insulin, nerve growth factor and porcine relaxin was <0-1%.

A standard curve made up in amniotic fluid diluted in parallel with that made up in plasma (Fig. 1). In samples of amniotic fluid left to stand at room temperature, mean relaxin concentrations declined in six samples as follows: baseline, 79-5 ng/l; 1 h, 78-6 ng/l; 2 h, 76-1 ng/l; 4 h, 73 ng/l; 8 h, 68 ng/l.

Statistics

Data are expressed as geometric means. Comparisons were made between amniotic fluid concentrations of relaxin at 10 weeks and subsequent weeks of gestation to 22 weeks using a Wilcoxon signed-rank test.

![Figure 1. Dilution curves of synthetic relaxin in plasma (○) and amniotic fluid (●) measured by an enzyme-linked immunoabsorbent assay, read at a wavelength of 492 nm.](image-url)
FIGURE 2. Concentrations of relaxin in (a) amniotic fluid during gestation in singleton pregnancies obtained at the time of amniocentesis, cordocentesis or Caesarian section and (b) in maternal plasma during gestation in samples obtained at the same time as the amniotic fluid samples. The solid lines represent the geometric mean.
RESULTS

Mean concentrations of relaxin in amniotic fluid rose significantly from 58 ng/l at 10 weeks to 142 ng/l at 14 weeks (P<0.01) and 127 ng/l at 16 weeks (P<0.01), and declined subsequently to 55 ng/l at 22 weeks (Fig. 2a). In maternal blood, mean relaxin concentrations were ten times greater than in amniotic fluid, and decreased with gestation (Fig. 2b). There was no correlation between relaxin concentrations in maternal plasma and amniotic fluid (r=0.02). In the amniotic fluid obtained at the time of Caesarean section from three patients who had become pregnant by ovum donation, relaxin was absent in one and present at 35 and 42 ng/l in the other two. In the paired maternal and fetal blood samples, relaxin was detected in all maternal samples (geometric mean 810 ng/l, range 159-3696) but in none of the fetal samples.

DISCUSSION

Relaxin is present in amniotic fluid from at least 9 weeks of gestation at a concentration ten times lower than in maternal blood. The difference in relaxin concentrations in the two compartments, and the similarity in the behaviour of the concentrations would suggest that amniotic fluid relaxin is a product of passive diffusion. However, the absence of a significant association between relaxin concentrations in amniotic fluid and maternal plasma suggests that this is not the case, and that the diffusion of plasma relaxin into the amniotic fluid may be by partial exclusion rather than simple passive diffusion. Alternative explanations are that the metabolism of relaxin in amniotic fluid may be more rapid or that it may be derived from another source, the most probable of which is the decidualized endometrium, in which immunoreactive relaxin has been identified (Koay, Bagnell, Bryant-Greenwood et al. 1985), even in women who had undergone oophorectomy (Sakbun, Ali, Greenwood & Bryant-Greenwood, 1990), and hence have no circulating concentrations of relaxin (Johnson et al. 1991a). Thus, like other amniotic fluid constituents such as prolactin, placental protein 12 and placental protein 14, relaxin may be derived from the endometrium. Indeed, that pregnancy can be achieved by ovum donation and embryo transfer in the absence of circulating relaxin, when relaxin is known to influence endometrial structure and function both in vivo (Hisaw & Hisaw, 1964) and in vitro (Bell, Jackson, Ashmore et al. 1991), would support this. The precise mechanisms that control amniotic fluid concentrations of relaxin may be determined only after the clarification of its source.

While the decline in material plasma relaxin confirms the findings of previous studies and is compatible with the declining function of the corpus luteum with gestation, the absence of detectable concentrations of relaxin in any of the fetal samples was unexpected. Relaxin concentrations in the fetal circulation of rhesus monkeys during maternal infusions of human relaxin was found to be 1% of the concentration in the maternal circulation (Cossum, Hill, Bailey et al. 1991). It was concluded that the placental transfer of relaxin is limited and that relaxin circulates in the fetus only at low concentrations. In the present study, the highest relaxin level measured in maternal plasma was 3696 ng/l, yet none was detected in the associated fetal plasma, suggesting that if placental transfer of relaxin does occur in women, then it results in fetal concentrations which are lower than the predicted 1% of circulating maternal concentrations. The absence of detectable concentrations of relaxin in the fetal circulation excludes the fetus as a source of relaxin and reduces the likelihood that endogenous relaxin is embryopathic. Nevertheless, the possibility that the relaxin present in amniotic fluid diffuses through fetal skin before it is keratinized (20-24 weeks) and influences fetal development, cannot be excluded.

ACKNOWLEDGMENTS

The authors are grateful for the gift of reagents for the measurement of relaxin from Genentech Inc., San Francisco, CA, U.S.A. M.R.J is supported by a Williams Fellowship from London University, and receives a Joint Research Committee grant from the Trustees of the Westminster Hospital Group.

REFERENCES