Relaxin Activity in the Pregnant Cat

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ABSTRACT

Plasma relaxin activity was measured by radioimmunoassay (RIA) in the domestic cat utilizing two different antisera developed against highly purified porcine relaxin. One was the 5858 antiserum from our laboratory and the other was the R6 antiserum of Dr. Bernard Steinetz. Relaxin activity could not be detected during the estrous cycle or during pseudopregnancy. Relaxin immunoactivity during early gestation was not detected by either antiserum. Plasma relaxin immunoactivity was first detected by both antisera on about Day 25 of gestation. Relaxin concentrations then increased rapidly, with a plateau reached between Days 30 and 35 that was maintained until 10–15 days prepartum. Relaxin concentrations then declined gradually until parturition. No prepurin increase was observed. Relaxin concentrations were undetectable within 24 h of delivery. Although amounts of immunoactivity measured with the R6 antiserum were consistently higher than measurements with the 5858 antiserum, the patterns of secretions observed were similar for both antisera.

INTRODUCTION

Relaxin is a polypeptide hormone found during pregnancy in many species. It has several actions related to the maintenance of pregnancy and is involved in the prepartum softening of the tissues that surround the pelvic canal (see Porter, 1979; Bryant-Greenwood, 1982).

Plasma relaxin concentrations have now been determined in a number of species, including the human. From these studies, several patterns of secretion have been identified. The sow represents a class where plasma relaxin is only found in high levels during the last few days of pregnancy (Sherwood et al., 1975). The rat and mare represent a group of animals in which relaxin is undetectable in early pregnancy; concentrations rise before midgestation and remain elevated for the duration of pregnancy (Sherwood et al., 1980; Stewart and Stabenfeldt, 1981). In contrast to the above, relaxin is present throughout gestation in the human (O’Byrne et al., 1978).

Hisaw (1929) reported relaxin biologic activity in pregnant cat blood, but to our knowledge no further reports on the secretion of relaxin in the cat have been made. We have found that plasma obtained from cats during pregnancy contains material that cross-reacts in the porcine relaxin radioimmunoassay. Utilizing this system, we have determined the profile of plasma relaxin in the domestic cat throughout gestation.

MATERIALS AND METHODS

Animals

Domestic cats (3.2—4.5 kg) of mixed breeds were used in this study. Groups of females were maintained in rooms (8' x 9', 6 per room) with controlled temperature (22°C) and lighting (14L:10D). Cats were fed Purina Cat Chow and water ad libitum. Eight females were bred an average of 4 times on either the third or fourth day of estrus and all became pregnant. Blood samples were taken daily until Day 10 of gestation, then 3 or 4 times weekly for the first 7 wk. Beginning about Day 50 of gestation samples were collected daily. When the first appearance of milk was observed, samples were collected twice daily through parturition. Two additional cats with unknown breeding dates were included in the study. Two cats were bred with a vasectomized male cat to induce pseudopregnancy. One cat was sampled for three pseudopregnancies and a second cat was sampled for one pseudopregnancy. Blood was collected in heparinized syringes by jugular venipuncture and centrifuged, and the plasma was stored at −20°C.

Radioimmunoassays

Plasma relaxin immunoactivity was measured utilizing a porcine relaxin radioimmunoassay (RIA). Porcine relaxin was purified from ovaries of pregnant cat.
sows by the method of Sherwood and O'Byrne (1974). The three forms of relaxin obtained by this purification were combined for further use and had a potency of 2660 U/mg (Stewart and Stabenfeldt, 1981). Antibodies to porcine relaxin were raised in two rabbits and both animals developed antisera that detected feline relaxin. Antiserum 5858 was used at a final dilution of 1:45,000 (24.3% binding). Antiserum R6, donated by Dr. Bernard Steinetz, was also generated in rabbits against porcine relaxin (O'Byrne and Steinetz, 1976) and was used at a final dilution of 1:90,000 (32.5% binding). The assays were then conducted as previously described (Stewart and Stabenfeldt, 1981), with both assays using the 2660 U/mg porcine relaxin for tracer.

Since plasma relaxin activity could not be detected in male cat plasma, porcine relaxin standards were diluted in male cat plasma. One hundred μl of cat plasma were used for determination of unknowns with the 5858 antiserum and 50 μl were utilized for the R6 antiserum. Unknowns were assayed in triplicate and reported in ng equivalents of porcine relaxin/ml. All pregnancy samples were determined in one assay for each antiserum. All estrous cycle and pseudopregnancy samples were run in a third assay using the R6 antiserum. Dose-response curves were transformed to logit log coordinates. The best-fit straight line was calculated by weighted least-squares linear regression with weights calculated by the method of Rodbard and Lewald (1970) using an IBM PC with a computer program written by one of the authors (D.R.S.). The least detectable dose was the amount resulting in a response 2 standard deviations from the 0-dose response. Lines were checked for parallelism by the method of Snedecor and Cochran (1967).

Plasma progesterone was measured by RIA methods previously described (Shille and Stabenfeldt, 1979).

RESULTS

Assay Characterization

When using the 5858 antiserum, dose-response curves of dilutions of cat plasma containing relaxin activity were not significantly different from porcine standards \((P<0.05)\) from 10 through 160 μl. When using the R6 antiserum, dilutions of plasma containing relaxin activity were not parallel with standards \((P>0.05)\). The least detectable doses were 0.17 ng/ml for the 5858 antiserum and 0.43 ng/ml for the R6 antiserum. Fig. 1 presents a correlation of relaxin concentrations measured in the two assays and shows a general agreement as to relative amount of hormone.

**CORRELATION OF RIAS**

\[
Y = (1.67 X) + 0.008 \quad r = .90
\]

![Graph](image)

FIG. 1. Correlation of plasma relaxin immunoactivities measured with 5858 and R6 antisera.
Estrous Cycle and Pseudopregnancy

All estrous cycle and pseudopregnancy samples were measured by the R6 antiserum. Relaxin activity was not detectable in any samples taken from the estrous cycle or pseudopregnancy (data not shown).

Pregnancy

Relaxin activity measured during pregnancy with both assays in three individual cats is shown in Fig. 2. Relaxin activity remained below detection in both assays until between Days 20 and 25 of gestation. Relaxin activity then rose to plateau concentrations by Days 30 to 35 of gestation. Starting approximately 15 days prior to parturition a downward trend in relaxin activity was seen with the R6 antiserum. This was not noted with the 5858 antiserum, where concentrations did not decline until the last few days of pregnancy. No prepartum surge in relaxin was observed with either antiserum. Relaxin activity fell at parturition to undetectable concentrations within 24 h of delivery and remained below detection in the immediate postpartum period.

A composite of relaxin concentrations measured in all cats by both antisera (Fig. 3) reveals that the profiles are similar but the R6 antiserum consistently detected higher amounts of relaxin activity. The one time during pregnancy when the two antisera seemed to diverge was in the last 15 days, where the mean relaxin concentrations measured by the R6 antiserum dropped from 7 ng/ml to less than 4 ng/ml, while mean relaxin concentrations measured by the 5858 antiserum did not show such a decline.

Plasma progesterone increased early in pregnancy (Fig. 4) and peaked around Day 25 of gestation. Progesterone then slowly declined throughout the remainder of pregnancy. These progesterone data have been published previously (Banks et al., 1983).

DISCUSSION

Most assays for relaxin are based upon porcine relaxin because relaxin has been purified from only a small number of species. Relaxins isolated from different species have been found to have significant biochemical and immunologic differences. This suggests that the porcine assay may not be the optimal means for feline relaxin determinations. A homologous feline assay is not currently available and, although the porcine relaxin assay does not permit determination of absolute amounts of feline relaxin, it does provide the profile of secretion for comparisons between various stages of pregnancy. This situation may prove similar to that seen in the horse, where plasma relaxin activity was determined using a porcine relaxin RIA (Stewart and Stabenfeldt, 1981) in which plasma dilutions were not parallel with porcine standards. Relaxin concentrations in the same samples were also determined using a homologous equine relaxin RIA (Stewart, 1984). The two assays gave similar profiles of relaxin concentrations during gestation, but concentrations measured in the homologous assay were much higher than that measured in the porcine RIA.

Because of potential problems with heterologous relaxin assays, it was of interest to us to be able to measure relaxin in the cat with more than one antiserum. Both antisera were raised in rabbits against porcine relaxin prepared in a similar manner. Although there were large differences in concentrations measured by these two antisera, the profiles were similar. The one point of divergence was in late pregnancy, where the concentrations measured by the R6 antiserum declined in the last 15 days prior to parturition while the values determined by the 5858 antiserum were essentially unchanged. One explanation for this could be that the two antisera were detecting different forms of relaxin. Multiple forms of 6000-Dalton relaxin have been purified from tissue sources from the sow (Sherwood and O’Byrne, 1974) and rat (Sherwood, 1979). Oliver et al. (1978) reported that the relative proportions of relaxin fractions found in the ovary of the pig changed as pregnancy progressed. Whether or not multiple forms of 6000-Dalton relaxin are found in plasma is not known. Sherwood et al. (1984) reported dynamic changes in large molecular weight forms of relaxin in the plasma of rats during gestation. However, the physiologic significance of the larger molecular weight forms is not known.

Relaxin immunoactivity was not found during early pregnancy in the cat, a time when the corpora lutea are known to be active. This suggests that the corpora lutea are not secreting significant amounts of relaxin, at least in early pregnancy. The corpora lutea of pseudopregnancy also do not seem to secrete relaxin. The onset of relaxin secretion in the cat between Days 20 and 25 of gestation suggests that the placenta either is secreting relaxin or is stimu-
FIG. 2. Relaxin immunoactivity measured with two antisera in three individual cats during pregnancy. Parturition is indicated by the vertical line. Relaxin measured by the R6 antiserum is in the upper line and relaxin measured by the 5858 antisera is in the lower line in each panel.
FIG. 3. Composite of relaxin concentrations measured in all cats with both antisera. Parturition is indicated by the dashed vertical line. Concentrations are shown ± SEM.

Lating relaxin production by the ovary. The latter situation is believed to occur in the rat, where a placental substance is responsible for luteal production of relaxin (Goldsmith et al., 1981). In the rat, enhanced progesterone production occurs in the second half of pregnancy (Sanyal, 1978). However, there is no increase in progesterone concentrations as relaxin production begins in the cat (Fig. 4). Therefore, if the placenta is controlling ovarian relaxin production, it does not alter progesterone production. The relaxin pattern in the cat is similar to that in the mare, in which relaxin secretion begins relatively early in pregnancy (Stewart and Stab-
enfeldt, 1981). In the mare, the placenta has been shown to be the source of relaxin (Stewart et al., 1982b).

Prepartum surges in relaxin have been observed in animals such as the sow (Sherwood et al., 1975) and rat (Sherwood et al., 1980, 1983). However, the cat is like the mare (Stewart et al., 1982a) and human (Quagliarello et al., 1980) in that it does not seem to have such surges. In fact, there seemed to be a steady decline in mean concentrations of relaxin during the last 10 to 15 days of pregnancy. The reason for this decline is unknown. In all animals, a rapid decline in relaxin concentrations was observed postpartum. In most animals, relaxin was undetectable in the first postpartum sample.

The function of relaxin in the cat has not been investigated. Its presence during the latter two-thirds of pregnancy would suggest that it may be important during this time, perhaps for a uterine quieting effect. Because relaxin can inhibit spontaneous uterine contractions in laboratory animals, it may be synergistic with progesterone in the maintenance of pregnancy in the cat.

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