Plasma relaxin immunoactivity in the pig at parturition and during nuzzling and suckling

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Summary. One sow bled at 30–60-min intervals for 48 h at 5 and 4 days before parturition had mean ± s.e.m. relaxin levels of 5.0 ± 0.48 ng/ml and 5.5 ± 0.44 ng/ml for each 24-h period respectively. This sow and another were bled at frequent intervals during parturition; both showed considerable fluctuations in their relaxin levels but no consistent peaks in relation to each birth. Mean levels during parturition were 10.7 ± 0.46 ng/ml and 13.4 ± 0.81 ng/ml respectively, both significantly higher than the levels at 4 and 5 days before birth.

Relaxin levels in two lactating sows rose acutely during nursing, showing a 3-fold rise in one animal and an 8-fold rise in the other. Results from a third sow during an extended period of nuzzling and suckling by the piglets showed multiple peaks of relaxin immunoactivity associated with each nuzzling/suckling stimulus.

Introduction

The application of radioimmunoassay methods to the measurement of relaxin has stimulated a resurgence of interest in this peptide hormone. In addition, full elucidation of its amino acid sequence and comparison with insulin and other growth factors have firmly established relaxin as a member of the insulin-like family of the peptide hormones (Schwabe & McDonald, 1976; James, Niall, Kwok & Bryant-Greenwood, 1977; Schwabe, McDonald & Steinetz, 1977). Relaxin has been found in several species, but is present in greatest quantity in the corpus luteum of the pregnant sow ovary (Hall, 1960), which has therefore been used as the primary source of the hormone.

The first radioimmunoassay reported used a label selected from the crude NIH-R-P1 preparation; after iodination selection was based upon known bioassay data, high levels in pregnancy and the absence of relaxin in ovariectomized and hysterectomized animals and the radioimmunoassay was then used to study its own specificity (Bryant, 1972; Bryant & Stelmasiak, 1974). This assay system was then applied to the measurement of relaxin in sheep plasma during the oestrous cycle (Chamley, Stelmasiak & Bryant, 1975), during pregnancy and parturition (Bryant & Chamley, 1976a) and in response to suckling (Bryant & Chamley, 1976b). These studies indicated that this hormone was secreted in a pulsatile manner, was cleared rapidly from the circulation, and highlighted the fact that frequent blood sampling must be carried out in order to obtain meaningful relaxin profiles.

In the pig, relaxin secretion has been studied throughout pregnancy by bioassay (Belt, Anderson, Cavazos & Melampy, 1971) and more recently by radioimmunoassay (Sherwood, Chang, Bevier & Dziuk, 1975) with sampling at 4-h intervals from 50 h until 2 h before parturition.
In this present study we report some detailed profiles of relaxin secretion in the sow at parturition and during nuzzling and sucking, using a radioimmunoassay for highly purified porcine relaxin.

Materials and Methods

Animals
Plasma samples were collected in a series of experiments to study the release of neurophysins at parturition and during suckling (Dax, Cumming, Lawson & Johnson, 1977). For all experiments, the three-quarter White and one-quarter Berkshire sows were anaesthetized about 1 week before experimentation and a catheter was inserted into a jugular vein. At the time of blood sampling the animals were in single pens in familiar surroundings to minimize stress. Blood samples (10 ml) were collected into heparinized syringes and the plasma was immediately separated and stored frozen until assay. The samples were thawed only once before being shipped on solid CO₂ for relaxin assay in Hawaii.

Parturition. Blood was collected from 1 sow at approximately 30–60-min intervals for 48 h at 5 and 4 days before parturition. Samples were then collected from the same animal at approximately 2–5-min intervals throughout the birth of 7 piglets. Samples were collected over this period as close as possible to the moment just before each birth and then again just after. A second sow had blood samples collected at 2-min intervals during the birth of her 1st, 3rd, 4th and 5th piglets.

Suckling. Blood was collected from 2 sows at 1-min intervals before and during the nursing period as indicated by milk let-down and characteristic sucking behaviour of the piglets; blood collection was begun at 10:00 h in each case. In a third sow blood samples were obtained throughout 5 suckling and/or nuzzling episodes which occurred during a morning between 09:27 and 12:38 h. All 3 sows were in well established lactation, the 2 former animals were at 10 and 14 days respectively, whilst the third sow studied was 4 weeks post partum.

Relaxin assay
Relaxin was purified from freshly frozen pregnant sow ovaries according to the method of Sherwood & O'Byrne (1974). Biological activity was assessed by the mouse interpubic ligament bioassay (Steinetz et al., 1960). The preparation used for this study (designated CM-a' by Sherwood & O'Byrne, 1974) had a potency equivalent to 2·87 × NIH-R-P1 (442 GPU/mg) and is subsequently referred to as relaxin.

Purified porcine insulin and proinsulin were generous gifts from Dr R. Chance, Lilly Pharmaceutical Company. Neurophysins I and II were purified by the method of Uttenthal & Hope (1970).

Antiserum. Antiserum to CM-a' relaxin was prepared in a New Zealand albino rabbit as described by Vaitukaitis, Robbins, Neischlag & Ross (1971) and was titrated with 125I-labelled relaxin (1 ng/ml) before use in the radioimmunoassay.

Radioiodination. Since highly purified porcine relaxin contains no tyrosine residue, the iodination method of Bolton & Hunter (1973) was employed, using the modification previously described (McMurtry, Kwok & Bryant-Greenwood, 1978). Separation of 125I-labelled succinylated relaxin from unreacted succinamide ester and from free 125I was accomplished by passage through a 2 g Sephadex G-25 column presaturated with 0·05% gelatin in 0·05 M-sodium phosphate buffer, pH 7·5. Specific activities between 13 and 60 μCi/μg were obtained during a 6-month period.

Method of assay. Standard solution of porcine relaxin (CM-a') were made up to contain 0, 26, 53, 106, 312, 625 and 1250 pg/ml in a 1:2 dilution of horse serum (Grand Island Biological Company) in 0·05 M-barbitone buffer, pH 8·6, and 0·2 ml was used for assay. Plasma samples
Relaxin levels in the pig

were made up as 8 dilutions from 1:4 to 1:512 in 1:2 horse serum in barbitone buffer. A 0.2 ml volume of relaxin or plasma was added to polystyrene incubation tubes (6 × 1 cm; Luckhams Ltd) and the antiserum to porcine relaxin (0.05 ml of a 1:3000 dilution) was added. The contents of the tubes were mixed gently and the tubes capped and incubated at 4°C. After a 48-h incubation, 125I-labelled porcine relaxin (50 pg in 0.05 ml diluent) was added, the contents mixed gently, the tubes again capped and the incubation continued for 48 h. The diluent was 0.5 mg bovine γ-globulin (Fraction II: Nutritional Biochemicals Corporation)/ml.

All variables influencing the assay were studied in detail before its application for the measurement of pig relaxin in plasma. Separation of bound and free hormone was carried out by a conventional double-antibody procedure. To each tube was added 0.05 ml normal rabbit serum (1:20 dilution in 0.05 M-barbitone buffer, pH 8.6, and 0.1 ml of 1:8 sheep anti-rabbit γ-globulin in barbitone buffer, pH 8-6, containing 370 mg EDTA/100 ml). The tubes were mixed gently, recapped and replaced at 4°C for a further 24 h. The tubes were then centrifuged in a refrigerated Sorvall RC-2 at 200 g and the supernatants carefully decanted. The radioactivity in the precipitates was counted in a Packard Auto Gamma Counter. The percentage bound was calculated (counts bound/cnts bound + free) and plotted against the amounts of standard or plasma added.

**Precision and sensitivity.** In routine use, by using a log/linear plot of amounts of relaxin against % labelled relaxin bound to antibody, the best straight line could be fitted over the range 1250–26 pg porcine relaxin/ml. The between-assay variability of the standard curve during a 6-month period is shown in Table 1. The within-assay coefficient of variation was 7.74% (n = 8). The quantitative sensitivity of the assay in routine use was 212 pg/ml.

<table>
<thead>
<tr>
<th>Amounts of relaxin (pg)</th>
<th>% bound (n = 6)</th>
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<tbody>
<tr>
<td>1250</td>
<td>15.1 ± 7.3</td>
</tr>
<tr>
<td>625</td>
<td>24.5 ± 7.7</td>
</tr>
<tr>
<td>312</td>
<td>29.8 ± 4.9</td>
</tr>
<tr>
<td>106</td>
<td>35.6 ± 5.3</td>
</tr>
<tr>
<td>53</td>
<td>34.6 ± 10.6</td>
</tr>
<tr>
<td>26</td>
<td>44.0 ± 8.9</td>
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**Specificity.** The specificity of the assay was such that none of the pituitary hormones tested showed any cross-reaction at concentrations of 1 µg/ml. Because of the similarities in structure between porcine relaxin and porcine insulin (James et al., 1977), porcine proinsulin and insulin were tested. Porcine proinsulin, but not insulin, inhibited the assay in the µg/ml range. This is not readily explicable in the absence of sequence data for prorelaxin; however, physiological concentrations of porcine proinsulin in plasma would not be inhibitory in the radioimmunoassay for relaxin. Neurophysins I and II showed no cross-reactivity in the relaxin radioimmunoassay at concentrations up to 5 µg/ml.

**Results**

**Parturition**

The sow bled throughout a 48-h period between 5 and 4 days before parturition had mean ± s.e.m. levels of 5.0 ± 0.48 ng/ml (n = 23) and 5.5 ± 0.44 ng/ml (n = 27) respectively over each 24-h period. There was no significant difference between these mean values (P > 0.05).

The same sow was bled at 2–5 min intervals during the birth of her 6th to 12th piglets (Text-fig. 1) and relaxin levels fluctuated during this period but showed no consistent peaks in relation to each birth. The mean level over the birth interval was 10.7 ± 0.46 ng/ml (n = 17). This was significantly higher than mean relaxin levels at either 5 or 4 days before birth (P < 0.001).
Text-fig. 1. Relaxin levels in a pig bled at 2–5 min intervals during the birth of her 6th to 12th piglets.

The second sow was bled during the birth of some of her piglets during which time strong uterine contractions were noted (Text-fig. 2). Throughout this period, there were several marked peaks of relaxin secretion that were not readily associated directly with each birth. The mean relaxin secretion during the period studied was $13.4 \pm 0.81$ ng/ml ($n = 28$), which was higher than the mean level at this time in the first pig ($P < 0.05$).

Text-fig. 2. Relaxin levels in a pig bled at 2 min intervals during the birth of her 1st, 3rd, 4th and 5th piglets. A period of strong uterine contractions occurred as shown.

Suckling

The relaxin levels in two lactating sows during periods of nursing and milk let-down are shown in Text-figs 3(a) and (b). The suckling stimulus caused relaxin levels to more than treble. within 0.5–1.0 min in one animal (Text-fig. 3a) and caused an 8-fold rise within 1 min in the other animal (Text-fig. 3b). Relaxin levels then dropped precipitously within 0.5 min, consistent with its short half-life in plasma (Bryant-Greenwood, 1977).

Results from the third sow during periods of sucking and nuzzling by her piglets are shown in relation to these events in Text-fig. 3(c).
Text-fig. 3. Relaxin levels in lactating sows during (a and b) suckling and milk let-down (S), samples collected at 0-5-1-0 min intervals, and (c) periods of sucking (S) and/or nuzzling (N) by piglets between 09:27 and 12:38 h one morning.

Discussion

A radioimmunoassay has been developed using highly purified porcine relaxin (CM-a’) both for labelling with iodine-125 and for antiserum production. This assay has then been applied to the measurement of relaxin in pig plasma during late pregnancy, during parturition and during established lactation.

Our results for relaxin concentration in sows bled on Days 5 and 4 before parturition are in good agreement with those of Sherwood et al. (1975) who assayed single plasma samples from 6 sows at daily intervals before parturition, with a similar radioimmunoassay. As stated by Dziuk (1977), “corticoids and relaxin follow the same pattern of secretion and at essentially the same time scale; cause and effect relationships between any of the hormonal changes prior to parturition have not as yet been clearly established”. Indeed the function of relaxin at this time is unknown; it may act on the pubic symphysis in preparation for birth or on the uterus, or both of these actions may be central to the birth process itself.

We have extended previous studies to ascertain whether, during parturition in the pig, acute elevations in plasma relaxin occur, as has been reported in the ewe (Bryant & Chamley, 1976a). Relaxin levels in sows were raised acutely during the actual birth process; however, due to the
very short half-life of the hormone in plasma (Bryant-Greenwood, 1977) and the less than continual blood sampling, it is impossible to say whether the actual births or uterine contractions per se were the cause of these relaxin elevations. Once again, the function of relaxin at this time is not clear. Most of the relaxin which has been stored in the corpus luteum during pregnancy is released before the piglets are expelled from the uterus (Sherwood et al., 1975). However, our results indicate that some secretion is still maintained during the birth process. The pig proved to be more difficult to study than the sheep because multiple births occur and the first born piglets suck before the last ones are born.

Sucking and nuzzling by the piglets caused an acute and consistent rise in peripheral plasma relaxin levels, as occurs in ewes (Bryant & Chamley, 1976b) and women (Bryant, 1973). Relaxin was secreted in response to these stimuli over a period of several hours, and the lack of depletion of relaxin indicates that synthesis was equivalent to release or, as in pregnancy, that the relaxin is present in storage granules. The tissue of origin of this relaxin is not known, since the principal source of relaxin in the pregnant pig is the corpora lutea (Sherwood, Martin, Chang & Dziuk, 1977) and these regress at the time of parturition (Dziuk, 1977). However, possible additional sources, whether ovarian or extra-ovarian, are suggested by our results but have not been studied further in the post-partum state. Clearly further study is required to try to answer this question and indeed to ascertain the function(s) of this hormone in late pregnancy, parturition and in lactation.

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References


levels through pregnancy and at parturition in the pig. *Endocrinology* 97, 834–837.


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