Multiple molecular forms of prolactin during pregnancy in women

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ABSTRACT

The distribution of isomorphic forms of prolactin in the serum of pregnant women was studied by gel filtration chromatography. Using this technique we were able to resolve three peaks, detected by radioimmunoassay: they were termed 'big-big', 'big' and 'little' prolactin in order of decreasing size, with approximate molecular weights > 100 000, 50 000 and 21 000 respectively. They displayed a comparable immunoreactivity to the antiserum employed in the radioimmunoassay, as determined in competition experiments. The relative amount of each hormone form in serum changed during the third trimester of pregnancy. At week 33 of pregnancy, 'little' prolactin accounted for 63.2 ± 7.7% of the total circulating hormone present in the serum of five normal pregnant women. During the progression of pregnancy, there was a gradual increase in the low molecular weight prolactin, so that, at the time of delivery, the larger forms of the hormone were present only in small amounts.


INTRODUCTION

As measured in gel filtration experiments, prolactin appears to be present in multiple forms of different size in maternal and fetal serum, as well as in cerebrospinal and amniotic fluid (Fang & Kim, 1975; Keiffer & Malarkey, 1978). Two peaks of radioimmunoassayable activity have consistently been found: the elution volume of the prevalent forms coincides with the chromatographic position of an iodine-labelled monomeric prolactin tracer ('little' prolactin, mol. wt = 21 000), while the residual activity emerges in a smaller peak of apparent molecular weight of 50 000 ('big' prolactin). Only in some patients is a third form present with an estimated molecular weight greater than 100 000 ('big-big' prolactin).

Very little is known about these isomorphic forms of prolactin and it has been suggested that the larger forms may be less active biologically, since they display lower affinity for the prolactin receptor (Farkouh, Packer & Frantz, 1979). In any case little information is available on the relative distribution of the larger prolactin forms in physiological conditions. The aim of the present investigation was to verify the composition of serum prolactin immunoreactive material during normal pregnancy. Our results demonstrate that large prolactin forms, 'big' and 'big-big' prolactin, are detectable in serum as early as week 33 of pregnancy and that 'big' and 'big-big' prolactin are present in significantly smaller amounts at the time of parturition.

MATERIALS AND METHODS

Subjects and samples

Blood samples were taken from five pregnant women (between 25 and 35 years of age) every alternate week starting from week 33, after obtaining their consent. During the time of the experiment, no pathological alterations took place and the outcome of the pregnancies was satisfactory. In each case parturition was normal. A further sample of blood was withdrawn between days 4 and 7 post partum, in the morning and exactly 10 min after breast feeding started.

The serum, obtained by centrifugation, was either analysed immediately or stored frozen at −30°C. No alteration was detected either in the total concentra-
tion of immunoreactive prolactin or in the elution profile from the gel filtration column.

Chromatographic separation

Serum (0.5 ml) was fractionated at room temperature on a column of Ultragel AcA 54 (gel dimension 60 × 0.9 cm; Réactifs IBF, Villeneuve-la-Garenne, France) equilibrated with 0.04 M-K-phosphate buffer, pH 7.5, containing 5 mg bovine serum albumin (BSA)/ml. Elution was performed with 0.1 M-K-phosphate buffer, pH 7.5, containing 1 mg BSA/ml, exactly as described by Soong, Ferguson, McGarrick & Jeffcoate (1982). Fractions of 0.7 ml were collected at a rate of about 30 ml/h using an LKB 2112 fraction collector assisted by an LKB 2132 computerized microperspex peristaltic pump. The molecular weight of the multiple prolactin forms was calculated by reference to the elution volumes of human serum albumin, ovalbumin and cytochrome c, used as marker proteins in separate chromatographic runs.

To calculate the proportion of prolactin present in each form, identified by radioimmunoassay, all the fractions of each peak (2.8–4.2 ml) were pooled and lyophilized. The powder was then redissolved in 0.5 ml water and used for radioimmunoassay. By this means we determined a total recovery of radioimmunoassay-detectable hormone (defined as the ratio of the hormone present in the sum of the pooled fractions to the hormone originally loaded) of 91 ± 8% (s.e.m.). Only samples with prolactin concentrations in excess of 100 μg/l were subjected to chromatographic separations. This was because of the dilution factor of the column, and because it is impossible to detect prolactin reliably in the eluent at lower concentrations of prolactin in the starting samples.

Prolactin radioimmunoassay

This was performed on 0.1 ml aliquots of whole serum, column eluate or lyophilized pooled fractions, using the radioimmunoassay kit provided by Diagnostic Product Corporation, Los Angeles, CA, U.S.A. This procedure employs 125I-labelled prolactin tracer, purified by affinity chromatography on immobilized monoclonal antibodies (specific activity, 40 μCi/μg) and a double-antibody precipitating procedure, assisted by the addition of a diluted PEG solution. The first antibody was a rabbit-specific antibody employed at a 1:6–500 dilution in the assay tube. The concentration of 125I-labelled prolactin in the assay tube was 0.08 μg/l. The detection limit of the assay was 5 μg/l and the logit–log plot for calculation of the amount of prolactin in the sample was proportional to the prolactin added up to 200 μg/l. The intra- and interassay variations were less than 7 and 12%, respectively. The antisera used showed 1% cross-reaction to growth hormone and comparable reactivity towards each of the three prolactin fractions. This was checked in competition experiments with fixed concentrations of antibody, labelled tracer and increasing quantities of each of the purified prolactin forms.

RESULTS

It is well established that during normal pregnancy prolactin values increase enormously. This effect was also present in the five patients in the present study. At week 20 of pregnancy we measured serum prolactin values of 45 ± 12 (s.d.) μg/l which were clearly higher in comparison with normal values of age-matched control women of 22-7 ± 8.1 μg/l as reported by our group previously (Pansini, Bianchi, Zito et al. 1983).

A thorough investigation of the molecular variants of prolactin was, nevertheless, started only at week 33 since at this time all the patients had prolactin values greater than 100 μg/l, a value regarded as convenient for a successful estimation of prolactin in gel chromatography eluents. The results of these assays are summarized in Table 1 from which it is apparent that prolactin values are maintained at raised levels during the last 2 months of pregnancy. Nevertheless, we observed a progressive decrease in the large molecular size prolactins and a corresponding increase in the percentage of ‘little’ over total prolactin in all the reported cases. The proportion of ‘little’ prolactin was not modified during the first week of lactation, although a large scatter was observed in the total concentration of radioimmunoassayable prolactin in these patients’ serum.

The dynamics of the shift of prolactin isoforms is clearly shown in Fig. 1, with the progressive decrease of the contribution of ‘big’ plus ‘big-big’ prolactin and the corresponding increase of ‘little’ prolactin with respect to the total amount of circulating prolactin. The analysis of the variance of per cent increase of the little form of prolactin showed that the change was significant at the 0.05 value with an F coefficient of 4-16.

A preliminary molecular characterization of the prolactin forms was obtained by calibrating the separation column with standard proteins; in this way prolactin was eluted in three forms: the ‘little’ and the ‘big’ with molecular weights of 21 000 and 50 000 respectively, and ‘big-big’ which was present only in the serum of patient B.L., in the void volume of the column, corresponding to a molecular weight greater than 100 000. The position of the peaks was not altered when samples, lyophilized after separation, were rechromatographed even after dialysis against elution buffer containing 20 mM-2-mercaptoethanol. The results obtained with two runs of serum from patient
TABLE 1. Relative distribution of multiple forms of blood prolactin during normal human pregnancy

<table>
<thead>
<tr>
<th>Patient</th>
<th>33–34 weeks</th>
<th></th>
<th></th>
<th></th>
<th>35–36 weeks</th>
<th></th>
<th></th>
<th></th>
<th>37–38 weeks</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B.L.</td>
<td>207</td>
<td>52</td>
<td>13</td>
<td>35</td>
<td>255</td>
<td>43</td>
<td>20</td>
<td>37</td>
<td>256</td>
<td>39</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>B.S.</td>
<td>250</td>
<td>23</td>
<td>77</td>
<td></td>
<td>269</td>
<td>19</td>
<td>81</td>
<td></td>
<td>135</td>
<td>15</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>G.F.</td>
<td>135</td>
<td>26</td>
<td>74</td>
<td></td>
<td>142</td>
<td>24</td>
<td>76</td>
<td></td>
<td>110</td>
<td>12</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>C.K.</td>
<td>269</td>
<td>30</td>
<td>70</td>
<td></td>
<td>182</td>
<td>24</td>
<td>76</td>
<td></td>
<td>162</td>
<td>19</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>P.F.</td>
<td>200</td>
<td>40</td>
<td>60</td>
<td></td>
<td>170</td>
<td>36</td>
<td>64</td>
<td></td>
<td>182</td>
<td>16</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39–40 weeks</td>
<td></td>
<td></td>
<td></td>
<td>Post partum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.L.</td>
<td>187</td>
<td>36</td>
<td>21</td>
<td>43</td>
<td>120</td>
<td>33</td>
<td>11</td>
<td>56</td>
<td>166</td>
<td>14</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>B.S.</td>
<td>140</td>
<td>15</td>
<td>85</td>
<td></td>
<td>154</td>
<td>10</td>
<td>90</td>
<td></td>
<td>166</td>
<td>14</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>G.F.</td>
<td>153</td>
<td></td>
<td>100</td>
<td></td>
<td>95</td>
<td>7</td>
<td>93</td>
<td></td>
<td>214</td>
<td>15</td>
<td>85</td>
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<tr>
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<td></td>
<td></td>
<td>214</td>
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<tr>
<td>P.F.</td>
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W indicates prolactin concentration in whole serum, while BB, B and L indicate percentages of big-big, big and little forms respectively.

IR, immunoreactivity.

FIGURE 1. Per cent cumulative values (±s.e.m.) of (a) ‘big’ and ‘big-big’ and (b) low molecular weight forms of prolactin in serum throughout the last 8 weeks of human pregnancy and post partum (PP).

FIGURE 2. Gel filtration pattern of immunoreactive prolactin in the serum of patient B.L. at week 33 of pregnancy and in patient B.S. at week 36 of pregnancy.
B.L. (week 33) and from patient B.S. (week 37) are presented in Fig. 2.

The immunological cross-reactivity of these fractions was analysed both to validate our assay method on a quantitative basis and to obtain information about the interrelationship of these molecules, as shown by the antibody employed. As illustrated in Fig. 3, a close immunological similarity was found when all three isolated fractions of the hormone were assayed over a wide range of concentrations. In fact, the curves for 'big-big', 'big' and 'little' prolactin were all superimposable.

![Figure 3](Image)

**FIGURE 3.** Radioimmunoassay of 'big-big' (●), 'big' (○) and 'little' (△) prolactins, isolated by column chromatography. The lyophilized pooled fractions were analysed for their prolactin content by conventional radioimmunoassay and expressed as equivalent amounts of standard prolactin. Dilutions of these solutions were used for the competition experiments to test their ability to displace a labelled prolactin tracer from the specific antibody. Results are presented in the logit-log form.

**DISCUSSION**

Our data confirm the existence of multiple forms of prolactin of different molecular weights in the serum of pregnant women and further document significant changes in the distribution of these forms during pregnancy. In particular, a decrease in the large forms of the hormone and a corresponding increase in the concentration of 'little' prolactin towards term was evident (Fig. 1). The molecular basis for this phenomenon is unclear at present but it is evident that (i) these molecular forms have some antigenic determinant (recognized by the antiserum employed) in common, as demonstrated by immunological cross-reactivity and that (ii) all these forms bind to receptors present at the target tissue level, even though with different affinity (Kelly, Ferland, Labrie & De Lean, 1976; Farkouh et al. 1979; Dubé, Kelly & Pelletier, 1980; Shiu & Friesen, 1980). This is consistent with the hypothesis that the multiple circulating forms of prolactin are either secretion products (e.g. larger polypeptides not cleaved by proteases) or are generated in serum by dimerization or tetramerization of 'little' prolactin or by interaction with serum proteins to form tight binding complexes which survive during chromatography. In this context, it has been suggested that 'big' prolactin may be the result of an artifact due to oxidation of thiol groups to disulphide; indeed Benveniste, Helman, Orth et al. (1979) have shown that reduction of disulphide bonds converts 'big' to 'little' prolactin. However, our conclusions do not agree with this hypothesis because, as we have reported, when rechromatographed, the peaks were not altered after extensive reduction.

The physiological significance of the multiple prolactin forms, as well as their changes during pregnancy, is still unclear. However, the consistent appearance of a shift in their relative concentrations during normal pregnancy strongly suggests that this modification is a physiologically relevant event and may possibly be correlated with different actions of these prolactins. At the present time nothing is known about this, apart from the fact that large prolactin is apparently less biologically active, since it has a reduced binding activity for the prolactin receptor (Farkouh et al. 1979). In contrast with this observation of a different ability to bind to prolactin receptors, we must emphasize that a recent report has suggested a similar ability of each isomorphic form of prolactin to stimulate the growth of Nb2 rat lymphoma in vitro (Whitaker, Klee, Kao et al. 1984). Further experiments are needed to resolve this.

It is important to remember, in this perspective, that receptors for prolactin are present in tissues (uterus, breast, liver, fetal lung, chorion-leaf and amnion) (Leontic, Schruer, Andressen et al. 1979; Herington, Graham & Healy, 1980; Bigazzi & Nardi, 1981; Mendelson, Johnston, MacDonald et al. 1981) which are clearly involved in the profound changes which take place during pregnancy. According to this view it is conceivable that the presence of different forms of prolactin at different stages of pregnancy, and their different interactions with the cellular receptors, might be a mechanism to elicit selectively the different functions attributed to prolactin (control of uterine motility, carbohydrate and lipid metabolism, hydroelectrolytic balance in the amnion and the development of fetal functions).

On the clinical side, an intriguing feature of the hyperprolactinaemia in pregnancy is the absence of clinical signs of hyperprolactinaemia itself, and in particular the absence of galactorrhea. This effect is
usually ascribed to the action of high levels of circulating progesterone (Shiu & Friesen, 1980), but an alternative possible explanation for this finding would be the prevalence of high molecular weight forms of prolactin, with their low binding affinity to the prolactin receptor, but clearly this must be proven experimentally.

In conclusion, our data demonstrate a modification of levels of the isomorphic forms of prolactin during normal pregnancy, but the significance of this observation cannot be appreciated until the exact physiological role of the multiple prolactin forms is clearly understood. One possibility to be explored would be to correlate known physiological or pathological changes in prolactin function with the serum patterns of isoprolactins.

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REFERENCES


