Serum prolactin in uraemia: correlations between bioactivity and activity in two immunoassays

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Abstract. The clinical significance of hyperprolactinaemia in uraemic patients is uncertain and discrepancies between immunoreactivity and biological activity of serum hPRL have been reported. We have modified the Nb2 cell bioassay to improve specificity for hPRL and used this assay to measure hPRL bioactivity in sera from 26 uraemic patients and 40 control subjects. Seventeen patients were receiving regular haemodialysis and 9 continuous ambulatory peritoneal dialysis. Levels of hPRL bioactivity were compared with hPRL immunoreactivity measured by RIA (PRL-RIA) and by immunoradiometric assay (PRL-IRMA). Serum hPRL levels measured by all three assays were significantly elevated in uraemic patients compared with control subjects (P<0.001). The immunoradiometric method gave significantly lower results than RIA in control subjects but not in uraemic patients (P<0.05). There was no significant difference in mean ratio of hPRL bioactivity to PRL-RIA between patients and control subjects (1.18 ± 0.05 vs 1.11 ± 0.03, mean ± SEM). The ratio of hPRL bioactivity to PRL-IRMA was slightly decreased in uraemic patients compared with controls (P = 0.05). Serum hPRL bioactivity was closely correlated with immunoreactivity in both immunoassays (r = 0.96) in patients and controls. These results confirm that elevated serum hPRL levels in uraemic patients represent biologically active hormone which may contribute to hypogonadism.

Hyperprolactinaemia occurs in 50 to 80% of patients with end-stage renal failure (ESRF) treated with either haemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD) (Cowden et al. 1978; Hou et al. 1985). It persists despite adequate dialysis and is usually improved only by renal transplantation (Lim et al. 1979; Holdsworth et al. 1978). Uraemic hypogonadism, which is almost universal in dialysed patients, is multifactorial, with evidence of defects at hypothalamic, pituitary and gonadal levels (Handelsman 1985; Gomez et al. 1980; Holdsworth et al. 1977; Lim et al. 1980). The role of hyper-prolactinaemia is uncertain, and it has been suggested that the high levels of hPRL detected by RIA may not represent intact, fully biologically active hormone (Mooradian et al. 1985).

We have modified the Nb2 rat lymphoma cell bioassay for serum lactogens (Tanaka et al. 1980) by the use of monoclonal antibodies to make the assay specific for hPRL bioactivity, and used it to study sera from uraemic patients receiving treatment with HD or CAPD. The precision and reproducibility of the Nb2 bioassay, modified in this way, are comparable with those of immunoassay (Smith et al. 1988).

Patients and Methods

Patients
We studied 26 patients with ESRF who had been receiving regular dialysis for at least 3 months at the time of the study and were in good general health. Seventeen patients (7 men, 10 women) were treated with HD three times weekly in the Renal Unit and 9 patients (7 men, 2 women) were treated with CAPD. No patient was taking drugs known to affect PRL secretion, but 5 patients were...
taking low-dose prednisolone (<10 mg/day) and 12 were taking ranitidine. Six were diabetic. The mean age of the whole patient group was 52 ± 15.7 (SD) years (range 23–77). There was no significant difference in age between patients treated with HD and CAPD. Blood was taken for this study immediately before HD (at least 3 h after patients had woken) or at an afternoon clinic.

Control subjects were healthy and not taking any medications. There were 22 men and 18 women. The mean age of control subjects was 42 ± 13.8 years (range 23–68).

**Nb2 cell bioassy**

The Nb2 cell line was established from a malignant lymphoma which arose in an oestrogen-treated male rat (Gout et al. 1980). Cellular replication is stimulated by lactogens (hPRL, hGH and human placental lactogen) and this forms the basis of a highly sensitive bioassay for lactogens in serum. The addition of antiserum or monoclonal antibodies (Mab) to other lactogens can make the assay specific for hPRL. Polyclonal antisera used in this assay may distort results due to cross-reactivity between anti-hGH antiserum and hPRL or between anti-hPRL antiserum and hGH; the use of Mab avoids these problems (Baldwin et al. 1988). It has recently been shown (Rayhel et al. 1988) that the lymphokine interleukin-2 may also stimulate Nb2 cell mitogenesis; the use of Mab to both hPRL and hGH in control wells allows any such activity to be detected.

**Nb2 cell culture**

Nb2 cells were maintained in suspension culture in RPMI-1640 medium supplemented with 10⁻⁴ mol/l 2-mercaptoethanol, 10% fetal calf serum (FCS) and 10% horse serum. Media and sera were purchased from Gibco Ltd, Paisley, Scotland. Twenty-four hours before use in an assay, cells were transferred to medium without FCS to arrest population growth.

**Bioassay protocol**

The bioassay was carried out as described previously (Smith et al. 1988). Samples, standards and Mab were added to 24-well tissue-culture plates (Nunc, Gibco, Paisley, UK). Serum samples were diluted with RPMI-1640 (without FCS) as necessary to bring hPRL concentrations within the working range of the assay (25–800 mU/l). All samples were assayed in duplicate at two or more dilutions. hPRL standard 85/562 (NIBSC, Potters Bar, Hertfordshire) was diluted in RPMI-1640 for use in the bioaassay. Mitogenic activity due to hGH was blocked by using a Mab to hGH (Mab NA71). Control wells were set up for each sample with appropriate dilutions of Mab to both hGH and hPRL (Mab NA71 and NEO2, the kind gift of Dr F. Shand, Wellcome Research Laboratories, Beckenham, Kent). Nb2 cells were pelleted by centrifugation, resuspended in RPMI-1640 without FCS, and added at a concentration of 2 × 10⁵ cells/well. The final volume was adjusted to 1.65 ml/well and plates incubated for 72 h in an atmosphere of humidified air/5% CO₂. Cell number was determined using a Coulter counter (Coulter Electronics, Luton, Beds., UK). The mean of three counts was taken for each well and hPRL concentration read from the standard curve.

We noted that some sera, from both uraemic and control subjects, gave higher results of hPRL bioactivity when assayed at a final dilution of less than 1:33 than they did when assayed at a greater dilution, even when there was no growth in control wells containing Mab to hGH and hPRL. This effect was first noted by McNeilly & Friesen (1985) and appears to be due to a non-lactogenic factor which acts synergistically with lactogens in the assay. All sera were thus assayed at a final dilution of at least 1:40 and at one or more further dilutions so that any deviation from parallelism between sera and standard could be assessed. No sera were found to give non-parallel results except as noted above, so the hPRL bioactivity of a sample was taken to be the mean of results read from

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**Table 1.**

Serum PRL concentration (mU/l, median and observed range) measured in the Nb2 assay (PRL-BA), RIA (PRL-RIA) and IRMA (PRL-IRMA) in uraemic patients and control subjects. Mean ratios of bioactivity (BA) to immunoactivity in RIA and IRMA are also shown.

<table>
<thead>
<tr>
<th></th>
<th>BA</th>
<th>RIA</th>
<th>IRMA</th>
<th>BA/RIA</th>
<th>BA/IRMA</th>
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</thead>
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<td></td>
<td></td>
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<td>N = 26</td>
<td>627</td>
<td>595</td>
<td>504</td>
<td>1.18</td>
<td>1.25</td>
</tr>
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<td></td>
<td>(267–1563)</td>
<td>(150–1420)</td>
<td>(137–1124)</td>
<td>(0.76–1.6)</td>
<td>(0.8–1.6)</td>
</tr>
<tr>
<td><strong>Control subjects</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N = 40</td>
<td>193</td>
<td>185</td>
<td>130</td>
<td>1.11</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
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<td>(50–380)</td>
<td>(0.83–1.6)</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>0.05</td>
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</tbody>
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the standard curve for final dilutions of serum of more than 1:33.

The detection limit for hPRL was 25 mU/L. Within-batch cv was <5% over the range 25–1100 mU/L and between-batch cv 10% at 100 mU/L, 6% at 310 mU/L and 8.6% at 2000 mU/L (measured at appropriate dilutions).

Radioimmunoassays
Reagents were obtained from NETRIA (St. Bartholomew's Hospital, London). The standards were calibrated against IRP 83/562. Between batch cv were 10% at 130 mU/L, 6.7% at 540 mU/L and 5.8% at 1150 mU/L.

Immunoradiometric assay (IRMA)
Samples were assayed using the 'Sucrosep' system from Boots-Celltech Diagnostics Ltd (Slough, UK). Standards were calibrated against IRP 83/562. Between batch cv were 4.5% at 94 mU/L, 4.5% at 460 mU/L and 5.1% at 1360 mU/L.

Statistics
Data were analysed using OXSTAT II (Microsoft, UK). Since the distribution of serum hPRL values was skewed, results are presented as median and range. Reference ranges were constructed for the three assays by calculating the mean ± 2 SD of logarithmically-transformed data from the control subjects (male and female combined). Log-transformation of the data produced an approximately normal distribution. Correlations between results given by different assays were calculated using linear regression analysis of logarithmically-transformed data. Comparisons between hPRL values in different groups were made using the Mann-Whitney U-test, and proportions of patients with hyperprolactinaemia were compared using Fisher's exact test.

Results
Serum PRL immunoactivity and bioactivity were significantly raised in uraemic patients compared with control subjects ($P < 0.001$). There was no significant difference in serum hPRL levels as measured by all three assays between patients treated with ranitidine or prednisolone and those not receiving these drugs. Mean serum hPRL bioactivity and immunoactivity did not differ significantly between diabetic and non-diabetic subjects. Similarly, although patients treated with CAPD had higher hPRL levels than did HD-treated patients ($P < 0.02$), there was no significant difference between the proportion of hyperprolactinaemic patients in the two groups (67 and 70%, respectively). Since the number of CAPD-treated patients was so small ($N = 9$), results for all ESRF patients were combined for comparisons with the control group.

Correlations between a) hPRL concentrations measured by bioassay and RIA; b) hPRL concentrations measured by bioassay and IRMA; c) hPRL concentrations measured by RIA and IRMA. Closed triangles represent control subjects and open triangles uraemic subjects. Note logarithmic scales.
**PRL bioactivity**

The calculated reference range for serum hPRL bioactivity (hPRL-BA) in the control population was 110–470 mU/l. Concentrations of serum hPRL-BA in uraemic patients ranged from 267–1563 mU/l. Serum hPRL bioactivity was significantly higher in uraemic patients than in control subjects (median 627 mU/l vs 193 mU/l, P < 0.001, Table 1).

**PRL immunoactivity**

The calculated reference range for PRL-RIA was 70–425 mU/l and that for PRL-IRMA 50–330 mU/l. The median serum concentration of immunoactive PRL measured by both immunoassays was higher in uraemic patients than in control subjects (P < 0.001, Table 1). The ranges of hPRL concentrations measured by immunoassay in uraemic subjects are also shown in Table 1. IRMA results were significantly lower than RIA results in control subjects (P < 0.05), but not in patients.

**Bioactivity:immunoactivity correlations**

Table 1 shows the ratios of bioactivity to immunoactivity measured by RIA (hPRL-RIA) and IRMA (hPRL-IRMA). The mean ratio of hPRL-BA to hPRL-RIA in uraemic patients was 1.18; the ratio in normal subjects was 1.11 (not significantly different). The mean ratio of hPRL-BA to hPRL-IRMA was 1.25 in uraemic patients and 1.42 in control subjects. This difference just reached statistical significance (P = 0.05). Fig. 1 shows the highly significant positive correlations between PRL bioactivity and PRL-RIA (r = 0.97, P < 0.001, Fig. 1a) and between PRL bioactivity and PRL-IRMA (r = 0.97, P < 0.001, Fig. 1b). The correlation between RIA and IRMA estimates of serum PRL concentration is shown in Fig. 1c (r = 0.96, P < 0.001).

**Discussion**

In this study we have confirmed that a high proportion of patients treated with dialysis for ESRF have elevated serum levels of immunoactive hPRL. There was no significant difference in the incidence of hyperprolactinaemia between patients treated with HD and CAPD.

Patients treated with ranitidine had mean serum hPRL levels which were not significantly different from those in untreated patients. This is in accordance with previous studies which have shown that ranitidine given orally or intravenously does not influence basal or stimulated hPRL release (Bohnet & Riley 1981), whereas the H₂ antagonist cimetidine does cause a rise in serum hPRL levels (Carlson & Ippoliti 1977; Bohnet et al. 1978).

The major disadvantage of immunoassays is that they depend on the recognition of antigenic determinants which may not be important for the biological activity of the hormone. Immunoradiometric assays, which require binding by two Mab directed against different epitopes on the hormone molecule, should be more likely than RIAs to detect only intact biologically active hormone. While immunoassays are routinely used because of their sensitivity, specificity and ease and speed of use, biological assays retain an important place in investigating possible discrepancies between immunoactivity and biological activity of hormones.

We have addressed the question of a possible loss of biological activity of hPRL in ESRF, which might result from retention of molecular fragments normally cleared by the kidney, alterations in hPRL secretion from the pituitary, or peripheral blockade of hPRL action by uraemic toxins. Heterogeneity of hPRL in serum and in the pituitary is well established (Suh & Frantz 1974; Gudya 1975; Garnier et al. 1978) with two or three immunoreactive forms usually being detected. Monomeric (23K) hPRL is the predominant form in unstimulated normal subjects.

Evidence for reduced biological activity of hPRL in uraemia comes from a study by Mooradian et al. (1985). They found significantly lower levels of total serum lactogens (measured using the Nb2 assay) than of immunoactive hPRL (measured by RIA) in sera from men with ESRF. In sera from control subjects no such difference was found. They postulated that biological activity of hPRL was reduced in ESRF, possibly due to circulating fragments or precursors of PRL present in uraemic patients which remained immunoactive while losing bioactivity.

We however found the ratio of hPRL bioactivity to immunoassayable hPRL measured by RIA to be approximately 1:1 in both uraemic patients and control subjects. There are several possible reasons for the difference between Mooradian's results and our own. The bioassay protocol used in their study was similar to ours, but they measured total lactogenic potency of serum rather than specific hPRL bioactivity. Another possible reason for the discrepancy may be their sterilization of serum.
samples by filtration, which we have found may significantly reduce bioactivity in some sera (Smith, unpublished). It is also possible that the apparent difference between the two studies may relate to the immunoassay used.

Support for this last explanation is provided by our results. While there was no difference in the ratio of hPRL bioactivity to immunoactivity measured by RIA between patients and controls, the ratio of hPRL bioactivity to hPRL immunoactivity measured by IRMA was slightly lower in uraemic patients (1.25) than in control subjects (1.42); a difference significant at the level \( P = 0.05 \). The IRMA method gave consistently lower results for serum hPRL than did RIA. The difference was statistically significant \( (P < 0.05) \) for control subjects but not for uraemic patients. The IRMA was both more sensitive than RIA and more precise at low hPRL concentrations.

IRMA may not recognise biologically active high molecular weight forms of hPRL which are recognised by RIA. The difference between IRMA and IRA results may therefore be more marked in normal subjects than in uraemic patients because the proportion of monomeric hPRL is relatively increased in uraemia (Rodriguez-Puyol et al. 1986). The difference in ratio of bioactivity to immunoactivity measured by IRMA between patients and control subjects may be due solely to the greater specificity of the IRMA for monomeric hPRL. We cannot however exclude a true, although small, reduction in biological activity of hPRL in uraemic sera, which is demonstrated using IRMA but may be masked by the recognition by the RIA of biologically inactive fragments of hPRL. This uncertainty exemplifies the problems associated with the dependence of immunoassays on the characteristics of antibodies, especially when the hormone assayed is known to be heterogeneous. We have also not excluded the possibility that there may be substances in uraemic sera which inhibit the biological activity of hPRL in the Nb2 assay, but we found nothing to suggest a significant effect of this kind.

Studies of molecular heterogeneity of hPRL in ESRF (Rodriguez-Puyol et al. 1986; Sievertsen et al. 1980) have shown no evidence for retention of fragments or altered forms of the hormone in the circulation and hyperprolactinaemia is mainly due to an increase in circulating monomeric (23K) hPRL. Our results, demonstrating parallelism between dilutions of uraemic sera and standard, are in agreement with this.

There is strong clinical evidence, such as the improvement in symptomatic hypogonadism after reduction in serum hPRL with bromocriptine treatment in dialysed patients (Bommer et al. 1979), that hPRL is biologically active in uraemic patients. Our results show that the biological activity of serum hPRL in such patients is raised to a degree similar to that of immunoactivity, implying that hyperprolactinaemia is likely to be clinically significant.

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References


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