Plasma LRH levels in chronic renal failure before and during haemodialysis

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Abstract. Endogenous immunoreactive luteinizing hormone – releasing hormone (LRH) in plasma was determined in 6 male and 7 female patients with chronic renal failure before and during haemodialysis. Basal plasma LRH levels ranged from 5.8 to 23.0 pg/ml and, in 11 out of 13 patients, were above the levels seen in healthy subjects (less than 7 pg/ml for men and less than 8 pg/ml for women). This immunoreactivity was eluted in identical fractions with synthetic LRH and plasma extracts from climaacteric women on Sephadex G-25 chromatography, and the dilution gave a displacement curve parallel to the standard. Within 5 h of haemodialysis, these high LRH levels declined into the normal range. The concentrations of LH in plasma in these patients were also elevated, but those of testosterone in male patients were decreased. These results suggest 1) that elevated plasma LRH reflects decreased feedback inhibition by primary gonadal failure and might in turn be responsible at least in part for high concentrations of plasma LH in chronic renal failure, and 2) that plasma LRH is mainly not bound to plasma proteins.

In patients with chronic renal failure in whom various symptoms of gonadal dysfunction are observed (Schmitt et al. 1968; Swamy et al. 1979), elevated LH with normal or slightly elevated FSH levels and low testosterone (T) concentrations in plasma have been reported (Sawin et al. 1973; Bentley et al. 1974; Distiller et al. 1975; Lim & Fang 1975). With regard to LRH, Pimstone et al. (1977) observed delayed catabolism of exogenous LRH, but there has been no report of endogenous LRH levels in this condition. We report here on plasma levels of endogenous LRH before and during haemodialysis in patients with chronic renal failure.

Materials and Methods

Thirteen uraemic patients (6 males, 7 females) between 27–48 years of age were studied. They had undergone chronic intermittent haemodialysis for 5 h three times a week for 1 to 4 years with C-DAK 3500 (Cordis Dow Corp., USA) dialyzers, and their serum creatinine levels before dialysis were 10–17 mg/100 ml. All male patients complained of decreased libido and female patients had amenorrhea. Blood samples for LRH, LH, FSH and T determinations were obtained immediately before and 30, 60, 120, 180, 240 and 300 min after the beginning of haemodialysis and the plasma was stored at −20°C until assay.

To examine the dialysability of LRH, a blood sample from a healthy male 10 min after iv injection of 100 µg LHR (Tanabe Pharmaceutical Company, Osaka, Japan) was introduced into the dialyzer used for patients in the present study.

For RIA of plasma LRH, 1 ml of plasma was extracted with an ice-cold mixture of 3 ml methanol and 0.1 ml 1/10 N acetic acid. The supernatant after centrifugation at 4°C, 12,000 × g for 20 min, was evaporated at 45°C under nitrogen and the residue was stored at −20°C until assayed. Anti-LRH serum was raised in rabbits by immunization with bis-diazotized benzidine-bovine serum albumin-LRH conjugate which was prepared according to the method of Bassiri & Utiger (1972) for thyrotrophin releasing hormone. Synthetic LRH was radioliodinated by the lactoperoxidase method (Arimura et al. 1973).
reaction mixture was transferred to a carboxymethyl cellulose column (0.5 x 30 cm) which had previously been equilibrated with 0.002 M ammonium acetate buffer, pH 4.6. The column was eluted with 0.1 M ammonium acetate buffer, pH 4.6, and fractions of 1 ml were collected. Labelled LRH which corresponded to 49th to 75th fractions was repurified prior to each assay by a Sephadex G-25 column (1 x 20 cm) prepared and eluted with 0.01 M phosphate buffered saline (PBS), pH 7.6, with 0.1% gelatin and 0.1% sodium azide. Each tube of the assay contained 0.4 ml of the buffer, 0.1 ml each of standard LRH or plasma extract, anti-LRH (1:6000) and 125I-labelled LRH, and was incubated at 4°C for 18 h. After the addition of 0.1 ml each of normal rabbit serum (1:100) and anti-rabbit γ-globulin goat serum, the mixtures were further incubated at 4°C for 24 h. The precipitate after centrifugation was counted with a gamma spectrometer. In the absence of unlabelled LRH, the antiserum bound 45–55% of the labelled LRH. Fig. 1 shows a displacement curve with unlabelled LRH. As little as 1.25 pg of unlabelled hormone produced a significant decline in binding of labelled LRH and 640 pg resulted in nearly complete displacement. The immunocross-reactivity of several kinds of LRH derivatives (Des pGlu1 LRH, [Tyr3, Trp5] LRH(2-10), LRH H-(3-8)-NH2, [Ile7] LRH and LRH-OH, generously supplied by Dr. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka, Japan) with the antiserum is shown in Fig. 2. Other hormones (TRH, hACTH, hGH, hPrl, HCG, thyroxine, insulin, oestrogen etc.) and various materials, such as dexamethazone, dopamine, clomiphene, chlorpromazine, sulpiride, nomifensine, furosemide, glucose and arginine had no cross-reactivity in this assay system. The recovery of 20 to 80 pg LRH added to plasma from healthy subjects was 99.4 ± 4.1% (mean ± st). The intra- and inter-assay coefficients of variation were 10.2% (n = 10) and 18.7% (n = 7), respectively. Plasma levels of immunoreactive LRH in healthy adults were less than 7 pg/ml for men (n = 17) and less than 8 pg/ml for women (n = 27). The levels in women increased in menstrual midcycle (9–18 pg/ml) and in the post-menopausal period (1.5–22, 10.2 ± 1.7 pg/ml). The concentrations of LRH were 12–18 pg/ml in hypergonadotrophic hypogonadism and less than 1.25 pg/ml in pregnancy.

**Fig. 1.** Displacement curves with unlabelled LRH (○—○) and plasma extracts from uraemic patients (○—○, ×—×). Each point represents the mean of triplicate determinations.
Immunocross-reactivity of LRH analogues and fragments.

To examine the identity of LRH immunoreactivity in plasma of patients with chronic renal failure with that of climacteric subjects, the extracts from 30 ml of plasma were applied to a 1 x 60 cm Sephadex G-25 column prepared and eluted with incubation buffer, and the LRH levels of each 1 ml fraction were measured by our RIA described above.

Plasma LH, FSH and T were measured by double-antibody RIA methods using kits purchased from Daiichi Radioisotope Laboratories, Tokyo, Japan for LH and FSH and those supplied from Eiken Immunochemical Laboratories, Tokyo, Japan for T. The normal ranges with these kits were 8.6 ± 0.6 mIU/ml for LH, 6.2 ± 0.5 mIU/ml for FSH and 8.6 ± 0.6 ng/ml for T (male).

All samples for a given subject were measured in an assay. Statistical analyses were performed using Student's t-test for paired and unpaired values.

Results

Plasma immunoreactive LRH levels before and during haemodialysis in 13 uraemic patients are presented in Fig. 3. The mean concentration before dialysis was 11.1 pg/ml with a SE of 1.4 pg/ml. The values in 11 out of 13 patients were over the upper limit for healthy subjects. There was no statistically significant difference between the male (10.0 ± 2.6 pg/ml) and female (12.0 ± 1.5) patients. The dilution of plasma extracts of 2 patients before
haemodialysis gave displacement curves parallel to the standard (Fig. 1), and this immunoreactivity was eluted almost in the same fractions as synthetic LRH and that from plasma of climacteric subjects on Sephadex G-25 gel chromatography (Fig. 4). Fractions before synthetic LRH fractions seen in patients with chronic renal failure represented 21.7% of total immunoreactivity. These might be fragments or precursor, but the same fractions were also found in climacteric subjects as 10.1%.

After the beginning of haemodialysis, plasma LRH levels declined even at 30 min and between 60 and 300 min reached a nadir of 4.8 ± 0.5 pg/ml (4.1 ± 0.9 pg/ml for males and 5.3 ± 0.7 pg/ml for females), which was significantly ($P < 0.005$) lower than the initial level. The mean concentrations at 120, 180, 240 and 300 min were also significantly ($P < 0.005$) lower than the predialysis value (Table 1).

When blood from a healthy man 10 min after iv injection of 100 µg of LRH was dialysed, plasma LRH levels declined very rapidly as shown in Fig. 5.

Plasma LH levels in these patients before dialysis ranged from 16.4 to 476 (112 ± 62) mIU/ml, and FSH from 5.9 to 17.8 (8.9 ± 1.7) mIU/ml. They remained in the range from 93 to 109% of the initial levels during the 5-h haemodialysis.

The predialysis plasma levels of T in the male patients were 2.7 ± 1.3 (1.0–5.3) ng/ml, which were significantly ($P < 0.0001$) lower than those in healthy men. These were not affected by 5 h of haemodialysis.

Fig. 4.
Gel filtration chromatography on Sephadex G-25 (1 x 60 cm) of synthetic LRH (600 pg) (a) and acid-methanol extract of 30 ml of plasma from patients with chronic renal failure (b) and climacteric women (c).

Fig. 5.
Effect of haemodialysis on LRH levels when a blood sample obtained from a healthy man 10 min after the iv injection of 100 µg of LRH was introduced into the dialyzer.
Table 1.
Mean plasma LRH levels in 13 patients with chronic renal failure before and during haemodialysis.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Before</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>Nadir</th>
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<tr>
<td>Plasma LRH (pg/ml)</td>
<td>11.1 ± 1.4</td>
<td>7.9 ± 0.7</td>
<td>7.6 ± 0.9</td>
<td>5.9 ± 0.6*</td>
<td>5.8 ± 0.6*</td>
<td>5.7 ± 0.6*</td>
<td>4.8 ± 0.5*</td>
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Mean ± se. * P < 0.005.

Discussion

In patients with chronic renal failure, elevated plasma LH with normal or elevated FSH levels has been reported (Swamy et al. 1979; Sawin et al. 1973; Bentley et al. 1974; Distiller et al. 1975; Lim & Fang 1975). However, there has been no report on endogenous LRH levels in this condition. In the present study a sensitive RIA was used to demonstrate elevated plasma LRH concentrations in 11 out of 13 patients with chronic renal failure.

Elevated immunoreactive LRH in plasma might represent biologically inactive metabolite(s) of LRH. The dilution of plasma gave displacement curves parallel to that with standard LRH. However, some analogues and fragments of LRH, especially those with intact COOH terminal amino acids, showed essentially identical displacement curves with LRH as presented in Fig. 2. Therefore we examined the elution pattern of the plasma extract from patients on Sephadex G-25 column chromatography. The immunoreactivity was eluted at the same fraction as synthetic LRH and that from climacteric women. This strongly suggests that the elevated immunoreactivity in the plasma of patients with chronic renal failure is genuine LRH with full biological activity. However, complete proof awaits the bioassay of the material.

The elevation of plasma LRH may be the reflection of decreased MCR and the prolongation of t½, as shown with exogenously administered LRH by Pinstone et al. (1977). In this case, the elevated endogenous LRH should cause an elevation of LH and then of T in males. Plasma LH was certainly increased in our patients and those of others (Sawin et al. 1973; Bentley et al. 1974; Distiller et al. 1975; Lim & Fang 1975). However, plasma T levels have been reported to be decreased (Sawin et al. 1973; Bentley et al. 1974; Distiller et al. 1975) and this was confirmed in our present series. Therefore the primary site of disturbance should be in the gonads, a possibility suggested by Sawin et al. (1973), and the increased LRH levels would be the reflection of decreased inhibitory feedback. The present study thus completes the total picture of the hypothalomo-pituitary-gonadal axis in patients with chronic renal failure.

The high levels of plasma LRH in patients with chronic renal failure decreased to normal during haemodialysis. We also showed that exogenously administered LRH was easily dialysed from the blood of a healthy man with the dialyzer used for the patients. As the dialysis membrane of this apparatus is said not to be permeable to substances larger than about 25 000 dalton, the above result would indicate that LRH is mainly not bound to molecules larger than this size. On the other hand, plasma LH concentrations did not change significantly with haemodialysis, confirming other reports (Swamy et al. 1979; Sawin et al. 1973; Bentley et al. 1974; Distiller et al. 1975).

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References


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