Polymorphism of human pituitary lutropin (LH)

Isolation and partial characterization of seven isohormones

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The complete microheterogeneous system of human pituitary lutropin was demonstrated by gel isoelectric focusing. In addition 7 LH isohormones could be isolated by preparative column focusing and characterized with respect to physicochemical, biological and immunological properties. The in vivo biopotencies ranged from 4.50–11.50 IU/mg, the in vitro bioactivity was from 1.20–10.10 IU/mg, and the immunological activity was from 3.10–7.55 IU/mg. The sialic acid content was found to be 1.8–3.2%. Treatment with neuraminidase resulted in a shift of all bands to the alkaline region, however the 7 LH forms were still present.

Human lutropin (LH) *Pituitary* *Isohormone* *Isoelectric focusing* *Microheterogeneity*

1. INTRODUCTION

Human pituitary luteinizing hormone was recognized to be heterogeneous from the very first attempts of purification. Subsequent reports confirmed the microheterogeneity of pituitary LH by demonstration of 3 or 4 forms of LH in highly purified preparations of the hormone [1–3]. More recently, analysis of radioimmunological and in vitro biological activities of crude pituitary extracts following column isoelectric focusing has revealed a polymorphism of far greater extent. For example, authors in [4] described at least 20 forms of human LH in crude pituitary extracts which could be attributed to 7 main regions of LH activity. However, the characterization of LH heterogeneity is still incomplete and discrepancies exist with respect to the number of LH species detected, the biological and immunological activities of these species and their physico-chemical characteristics.

Here, we demonstrate and characterize the entire polymorphism of human pituitary LH by gel isoelectric focusing and analysis of the separated forms by radioimmunoassay and in vitro bioassay. In addition, the 7 hormone forms detected by gel electrofocusing were isolated in mg amounts by column isoelectric focusing and then partially characterized.

Abbreviations: LH, luteinizing hormone; FSH, follicle-stimulating hormone; IEF, isoelectric focusing; NANA, *N*-acetyl neuraminic acid

2. MATERIALS AND METHODS

2.1. *Isolation procedures*

Crude LH was prepared from frozen human pituitaries by the extraction procedure in [5]. Gel filtration was performed on Sephadex G-100 (column size: 2 × 140 cm) using 0.2 M ammonium hydrogen carbonate as eluent.

Purification of LH by immunoaffinity chromatography was carried out using anti-hCG-IgG from rabbits coupled to CNBr–Sepharose (Pharmacia). The pituitary extract was passed through the immunoadsorbent column using 0.1 M Tris–HCl (pH 8.0) containing 0.5 M NaCl as eluent. The retained antibody-bound LH was then eluted using 0.1 M glycine–HCl (pH 2.8) containing 0.5 M NaCl.

Preparative isoelectrofocusing in a sucrose density gradient was carried out in a 440 ml column

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(LKB Produkter, Sweden). The pH gradient was formed using the following mixture of Ampholines: 3.5–10, 8–9 and 5–8; in a ratio of 3:6:8 (by vol.). The carrier ampholyte concentration was 12 g/l and the focusing run was performed for 60 h at 1200 V.

2.2. *Analytical characterization*

Polyacrylamide gel electrophoresis was carried out at pH 8.9 with a polymer concentration of 7.5%. Analytical polyacrylamide gel isoelectric focusing was performed in Ampholine PAG plates using the Multiphor electrofocusing equipment (LKB Produkter). The pH gradient was measured directly using a special surface electrode (403-8298 K7, Dr Ingold, Frankfurt/Main). Protein bands were visualized either by staining with 0.1% Coomassie brilliant blue R-250 in water: ethnol:acetic acid (11:8:1, by vol.) or by immersion of the slabs in 15% trichloroacetic acid for 20 min.

Two-dimensional immunofocusing was carried out by analytical gel electrofocusing in the first dimension and subsequent electrophoresis at right angles into a 1.25% agarose gel containing 5% rabbit anti-hCG serum. Sialic acid was determined as in [6].

2.3. *Assays*

Radioimmunological LH activity was determined using the NIAMDD-kit and 68/40 as a standard. FSH activity was determined using the Serono FHS RIA kit and 69/104 as a standard. In vitro bioactivity of LH was measured as in [7]. LH-bioactivity was determined as in [8] using Wistar rats and 68/40 as a standard.

3. RESULTS AND DISCUSSION

The extraction procedure described in [5] yielded 1600 mg crude gonadotrophin from 2000 human pituitaries. The specific activities of LH and FSH determined by RIA were 1750 IU/mg and 750 IU/mg, respectively.

Polymorphism of LH in this crude preparation was investigated by immunofocusing using anti-hCG. Six precipitation arcs over pH 6–9 were obtained. LH in the crude preparation reacting with the anti-hCG was then isolated by immunoaffinity chromatography as in section 2. Crude LH (80 mg) yielded 4.5 mg of highly purified lutropin in this one-step procedure. This preparation had spec. act. of 6050 and 6300 IU/mg determined by RIA and in vitro bioassay, respectively. The specific activity of the protein fraction which did not bind to the column was 3 IU/mg. The FSH content of the purified LH was 55 IU/mg.

Gel isoelectric focusing of the immunoaffinity-purified LH revealed 7 or 8 bands as shown in fig.1 (the band with the most alkaline pI being very faint). However, polyacrylamide gel electrophoresis of this highly potent LH preparation showed only one slow migrating zone. As sufficient material for the attempted isolation of the individual LH forms could not be obtained by the immunochromatography technique, purified LH containing a mixture of the isohormones was prepared in larger amounts by another approach. Starting with 1200 mg of crude LH, gel filtration on Sephadex G 100 yielded 145 mg of purified hormone. Analysis of this material by poly-

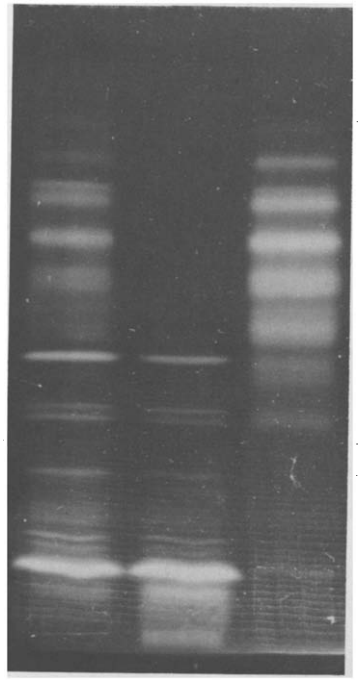

Fig.1. Analytical gel isoelectric focusing pattern of crude human pituitary gonadotrophin (left position), of unabsorbed protein fraction (middle), and of highly purified LH complex after isolation by immunochromatography (right position); pH 3.5–9.5; cathode at the top; visualization by trichloroacetic acid precipitation.

acrylamide gel electrophoresis showed, in addition to the LH, only one fast-migrating zone of contaminants. Gel electrofocusing followed by trichloroacetic acid precipitation revealed 7 bands, corresponding to the 7 bands seen with the immunoaffinity purified LH preparation. Since the contaminating proteins were found to focus outside the pI range of LH activity, this material was considered suitable for the purification of the isohormones by column isoelectric focusing without the use of additional purification procedure. When 110 mg of the purified LH complex were focused in a pH 4–9.5 gradient 7 peaks of LH were detected by RIA in the pI range 5.9–8.8 (fig.2). The profile for immunoreactive LH correlated with the absorbance profile (280 nm). Following dialysis and lyophilisation, between 2–9 mg of each LH form was obtained. Homogeneity of the isolated hormones was demonstrated by analytical focusing in thin-layer gels (fig.3). The biological, immunological and physicochemical properties of the isohormones are listed in table 1. The highest in vitro biological as well as immunological activities were found in the more alkaline forms with a stepwise decrease of both activities concomitant with falling pI. The radioimmunological FSH

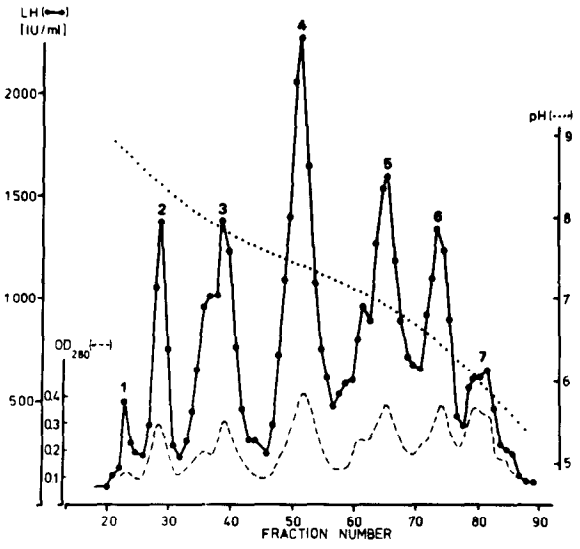

Fig.2. Preparative isoelectric focusing in sucrose density gradient (440 ml column; pH 4–9.5) of 110 mg purified human pituitary LH complex. Profiles of RIA (——) and UV absorption (– – –) of the different LH isohormones are shown.

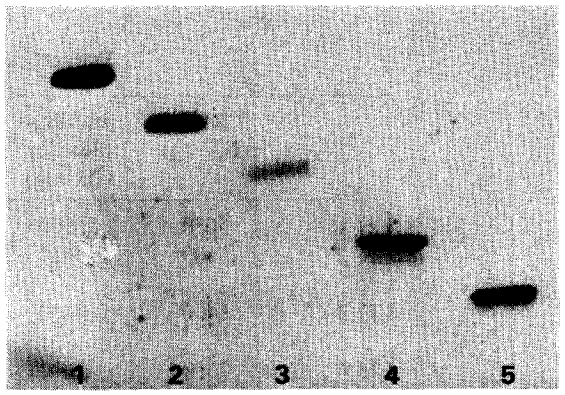

Fig.3. Analytical gel refocusing of aliquots (50 μg) of the different LH isohormones isolated by column isoelectric focusing. Positions 1–5 correspond to LH 2–LH 6. Visualization by trichloroacetic acid precipitation; cathode at the top.

potency for all forms was <20 IU/mg. The sialic acid content was found to vary between 0.9 and 2.9%.

Although human pituitary LH was first purified more than 20 years ago, the heterogeneity of this hormone has not been fully characterized until now. The reason for this may be that most of the purification procedures may have favoured the selection of certain forms by 'chromatographical cuts'. Thus, in this study, ion-exchange chromatography was omitted to preserve the microheterogeneity of the LH. Another major problem was the visualization of LH bands after gel isoelectric focusing. Unlike hCG which is chemically closely related to LH and shows a similar molecular polymorphism [9], LH bands do not stain well with Coomassie brilliant blue, the commonly used dye for gel isoelectric focusing procedures. This problem has been circumvented by trichloroacetic acid precipitation. Since the polymorphism of hCG has been shown to be due only to differences in the NANA content [9] the sialic acid content of the LH complex and the different isohormones were investigated. After enzymatic degradation of the LH complex with neuraminidase 7 forms were still present, however, there was a clear shift of all bands to the alkaline region, of pI 8.5–9.5. Thus, charge heterogeneity of the several LH isohormones in contrast to that of hCG, is not only due to differences in the sialic acid content.

Table 1

Properties of LH isohormones

	RIA (IU/mg)	In vitro bioassay (IU/mg)	OAAD assay (IU/mg)	NANA (%)	pI
LH 1	5050	5900	n.d.	n.d.	8.8
LH 2	5100	6450	10400 (8400–13200)	1.8	8.4
LH 3	6400	10100	10600 (7800–12800)	2.0	7.8
LH 4	7550	7600	11500 (7300–13700)	2.5	7.4
LH 5	3740	3650	10100 (7400–16500)	3.1	6.9
LH 6	3520	2500	7300 (4300–10200)	3.2	6.5
LH 7	3100	1200	4500 (3600–6800)	3.2	5.9

n.d., not determined

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