RADIOIMMUNOASSAY OF LUTEINIZING HORMONE RELEASING FACTOR

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The decapptide luteinizing hormone releasing factor (LH-RF) has recently been isolated, sequenced and synthesized (Schally, Arimura, Kastin, Matsuo, Baba, Redding, Nair, Debeljuk & White, 1971). This has made possible the development of radioimmunoassays for this factor enabling it to be measured in biological fluids in vivo and in vitro.

Two milligrammes of the synthetic decapptide (Hoechst) were conjugated to 2 mg bovine serum albumin (BSA) using 75 mg 1-ethyl-3-(3-dimethylaminopropyl)-carbo diimide in 0·25 ml water (Goodfriend, Levine & Fasman, 1964). After dialysis overnight, 1 mg of the conjugate in 2 ml water, emulsified in Freund’s complete adjuvant, was injected into 20 intradermal sites in a white New Zealand rabbit. A blood sample was obtained 8 weeks after this primary immunization and the assay developed using this antiserum.

LH-RF (0·1–1 µg) was iodinated with 125I (0·5–1 mCi) by the chloramine-T technique (5 µg of chloramine-T), specific activities between 100 and 500 µCi/µg being obtained. 125I-Labelled LH-RF was separated from unreacted 125I by gel filtration on a 10 × 1 cm column of Sephadex G-10 or G-15. There was a single peptide peak on G-10 but two peptide peaks on G-15. The latter were eluted before the iodide peak and they were immunoreactive. The C-terminal octapeptide (Hoechst) was also iodinated, with similar results, and was found to be more immunoreactive than the decapptide. For both the decapptide and octapeptide the most immunologically active fractions from the Sephadex columns were frequently eluted after the peak of unreacted iodide.

The iodination mixtures could also be separated by silica gel thin-layer chromatography (Merck pre-coated plates; solvent system, chloroform:methanol:acetic acid:water, 60:45:5:10, by vol.). The $R_f$ values of the two gel filtration peaks from the G 15 column were 1·0 and 0·43 respectively and the $R_f$ of iodide 0·51.

The radioimmunoassay employed a coated-tube technique. Polystyrene precipitin tubes, 1·5 × 0·25 in. (M and H Plastics Ltd), were coated with 0·2 ml of the antiserum, diluted 1:50000 in carbonate buffer (pH 9·6), at 4 °C overnight. After aspiration of the antiserum and washing with 0·2 ml of buffer (0·04 M-phosphate, pH 7·4, containing 0·6% NaCl and 0·1% BSA), 0·1 ml of standards in buffer and 0·1 ml of diluted tracer (100 pg approximately) were added to the tubes. After mixing, the tubes were incubated at 4 °C overnight and then aspirated, washed once with water and counted in an automatic gamma counter.
At a dilution of 1:50000, the binding of tracer in the zero (no added antigen) tube was 11% for $^{125}$I-labelled octapeptide and 7% for $^{125}$I-labelled LH-RF. The binding of these tracers to the tubes in the absence of antibody (antibody control tubes) was 0-9% and 0-4% respectively.

A standard curve of LH-RF using the $^{125}$I-labelled octapeptide as tracer is shown in Fig. 1. This is the mean of three standard curves each with triplicate tubes. Standard deviations at each point are shown. The detection limit of the assay was approximately 20 pg LH-RF per assay tube and the standard curve was linear up to 1000 pg. Cross-reaction from the C-terminal octapeptide was 100% using either the decapptide or octapeptide as tracer. This raises the possibility of immuno-logically active but biologically inactive fragments being detected in body fluids.

![Graph](image)

Fig. 1. Radioimmunoassay of luteinizing hormone releasing factor (LH-RF). Mean ± s.d. of three standard curves each with triplicate tubes at each point. The tracer used was $^{125}$I-labelled octapeptide and the radioactivity bound is expressed as a percentage of that bound in the zero (no added LH-RF) tube, $C_0$ being the counts in the zero tube and $C$ being the counts at each point.

Cross-reaction from thyrotrophin releasing factor was undetectable (less than 0-00001%). Cross-reaction from luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was also undetectable; 100 µu. of LH and 500 µu. of FSH per assay tube showed no reduction of binding of tracer.

Experiments are in progress to investigate the validation of the assay of LH-RF in human serum and urine samples using both unextracted samples and simple methanol extracts of serum.

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REFERENCES
