Vol. 101, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1981 Pages 486-494

RAT TESTIS IMMUNOREACTIVE LH-RH DIFFERS STRUCTURALLY FROM HYPOTHALAMIC LH-RH.

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Received June 8, 1981

SUMMARY

Luteinizing hormone releasing hormone immunoreactivity (LH-RH-IR) has been identified in acetic acid extracts of adult rat testes and partially purified by immunoaffinity chromatography. On Sephadex G-100 this material separated into four major peaks of >100K, ~32K, ~5K and ≤4K daltons. The ≤4K peak of LH-RH-IR eluted later than synthetic hypothalamic LH-RH decapeptide on Sephadex G-25. Antibody binding studies on the various LH-RH-IR species with antisera specific for different regions of synthetic LH-RH decapeptide indicate that all the testicular LH-RH-IR molecules have C-terminal immunological homology with the hypothalmic decapeptide but differ towards the N-terminus of the decapeptide sequence.

LH-RH was originally isolated from porcine hypothalami owing to its ability to stimulate luteinizing hormone (LH) release from the anterior pituitary (1). Subsequently numerous studies have supported the concept that LH-RH is the major hypothalamic peptide responsible for stimulating the release of both folicle stimulating hormone (FSH) and LH from the anterior pituitary of a variety of mammalian species (2).

Recently, however, a number of extrapituitary effects of LH-RH have been reported, and in particular an inhibition of gonadal function of various species has been shown after repeated or prolonged administration of the peptide or its analogues (3, 4). These effects are partly due to "down regulation" of gonadal gonadotrophic hormone receptors resulting from LH-RH induced elevation of pituitary FSH and LH levels (5), but direct effects of LH-RH on the gonads have also been described (3). Thus, in hypophysec-

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tomized female rats LH-RH agonists are known to inhibit hormone stimulated steroidgenesis by acting through the receptor on the granulosa (6) and luteal cells (7, 8). Similar effects are obtained in hypophysectomized male rats where LH-RH agonist administration impairs testicular endocrine function and spermatogenesis (9-12). Recently, ^{125}I labelled LH-RH and its analogues have been shown to be taken up by the rat testis *in vivo* (13-15). Furthermore, specific, high affinity receptors for LH-RH have been demonstrated on the cell membranes of ovarian granulosa (7) and luteal cells (8) and on testicular interstitial cells (6, 14, 16). The occurrence of these LH-RH receptors in the gonads and the demonstration that gonadal function is altered by LH-RH administration argues for a direct involvement of LH-RH-like peptides in the control of ovarian and testicular function *in vivo*. Since hypothalamic LH-RH is released into the portal veins at low concentrations (17) and is rapidly degraded by the pituitary (18), these findings suggest that there may be a local source of LH-RH within the testis. Recently, testicular interstitial fluid has been demonstrated to contain a LH-RH-like species which has both Leydig cell receptor binding activity and pituitary LH releasing activity (19). Acetic acid extracts of rat testis have been shown to contain bio-active (20) and immunoreactive LH-RH (20, 21). We report here on the partial purification and characterisation of this testicular LH-RH-IR.

MATERIALS AND METHODS

390 rat testes were collected on solid CO_2 and stored at -20°C. The testes were homogenized in two volumes of 2N acetic acid at 4°C by three 5-10 sec. bursts with an Ultra-Turrax homogeniser. Homogenates were centrifuged at 18 000 g for 30 min at 4°C and

the clear supernatants were lyophilized. The lyophilized extract was reconstituted in 0.5 M ammonium acetate pH 7.0 and centrifuged at 18 000 g for 30 min at 4°C. The clear supernatant was subjected to batch-wise affinity chromatography using anti-LH-RH antiserum 1076 conjugated to Sepharose-4B (22). The testicular extract was shaken with the resin for 3 h at room temperature and non-immunoreactive material removed by washing with 0.5 M ammonium acetate pH 7.0. LH-RH-IR bound to the affinity gel was eluted with five washes of 80 ml of 1.5 M acetic acid (until pH 2.8 was reached) and lyophilized. The acid was then replaced by 0.5 M ammonium acetate and washing continued until the pH of the eluant reached pH 7.0.

The material partially purified by immunoaffinity was taken up in 2N acetic acid containing 8 M guanidinium chloride, incubated at 50°C for 3 h and subjected to Sephadex G-100 chromatography. Immunoreactive peaks were detected by radioimmunoassay using antiserum 1076 (24) and the relative interaction of the pooled immunoreactive peaks with antisera R-42 (25) (a kind gift from T.M. Nett and G.D. Niswender) and 744 (24) (a kind gift from A. Arimura) was tested to yield information on the molecular structure of the material.

The lower molecular weight immunoreactive species off the G-100 column were chromatographed on Sephadex G-25. Peptide concentration in the extracts and column fractions was determined according to the method of Lowry (23).

RESULTS AND DISCUSSION

The acid extract of 390 rat testes contained 54.5 ng of LH-RH-IR with a specific activity of 2.93 ng/g peptide. After affinity chromatography of 17.4 ng of the LH-R-IR, the specific activity increased 56-fold to 165 ng/g peptide. Sephadex G-100 chromato-

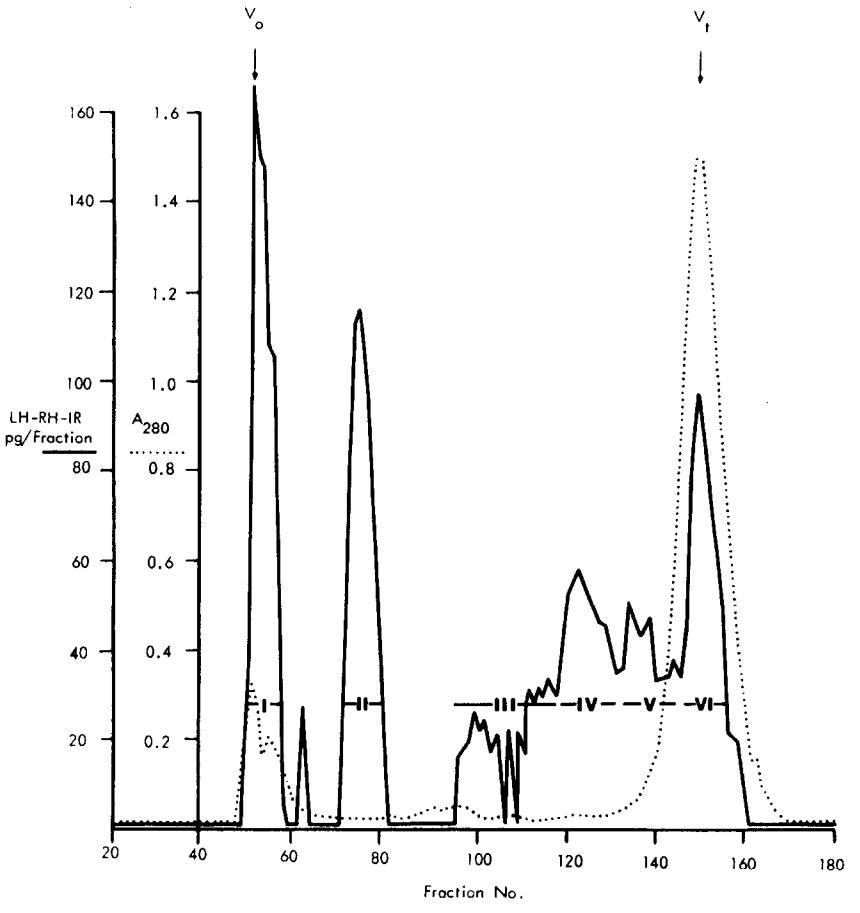


Fig. 1 Sephadex G-100 Chromatography of affinity purified testicular LH-RH-IR. 3.5 ng of LH-RH-IR partially purified by affinity chromatography was reconstituted in 2 N acetic acid, 8M guanidinium chloride, incubated at 50°C for 3 hrs and placed on a Sephadex G-100 column (86.5 cm x 5 cm) and eluted with 2 N acetic acid at a flow rate of 30 ml/hr at 4°C. 30 min fractions were collected, freeze dried and assayed with antiserum 1076.

graphy of a portion of the affinity purified material revealed four major LH-RH-like peptides (I, II, IV, VI) with apparent molecular weights of $>100\ 000$, $\sim 32\ 000$, $\sim 5\ 000$ and $\leq 4\ 000$ (Fig. 1). On Sephadex G-25 the major immunoreactivity of peak V emerged later than decapeptide LH-RH, as did peak VI (Fig. 2). These studies demonstrate therefore, that all of the major LH-RH-IR peaks

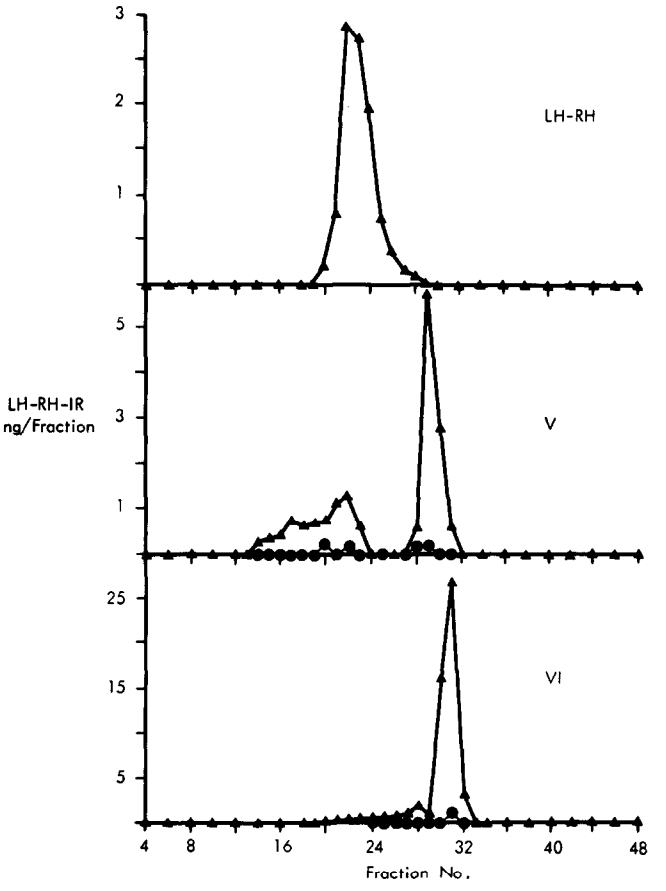
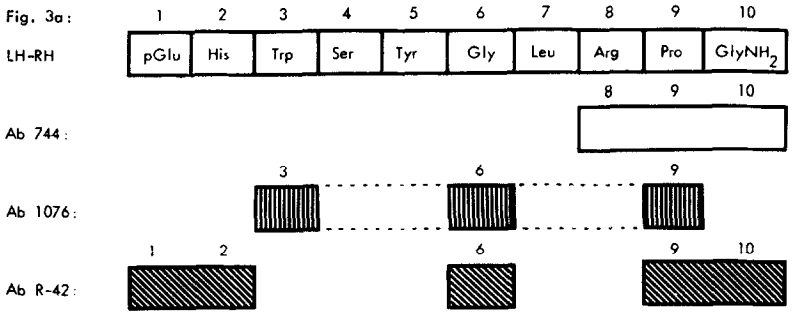


Fig. 2 Sephadex G-25 chromatography of LH-RH decapeptide and of pooled testicular LH-RH-IR peaks V and VI off Sephadex G-100 (Fig. 1).
The G-25 column (87.5 cm x 1.7 cm) was run at 4°C with a flow rate of 12 ml/hr and 30 min fractions were collected. Material placed on the column was in 2 N acetic acid, 8 M guanidinium chloride and eluted with 2 N acetic acid. The column fractions were freeze dried, reconstituted in 0.01 N acetic acid and assayed with antiserum 1076 (●—●—●) and antiserum 744 (▲—▲—▲). Estimations by both antisera of all LH-RH chromatography fractions were identical. For clarity the LH-RH-IR measurements by antiserum 1076 are therefore not shown for the LH-RH chromatography and beyond the peak fractions in the other two cases.

are chromatographically distinguishable from the hypothalamic decapeptide.

Comparison of the relative quantitation of the LH-RH-IR peaks with antisera specific for different regions of LH-RH decapeptide (Fig.



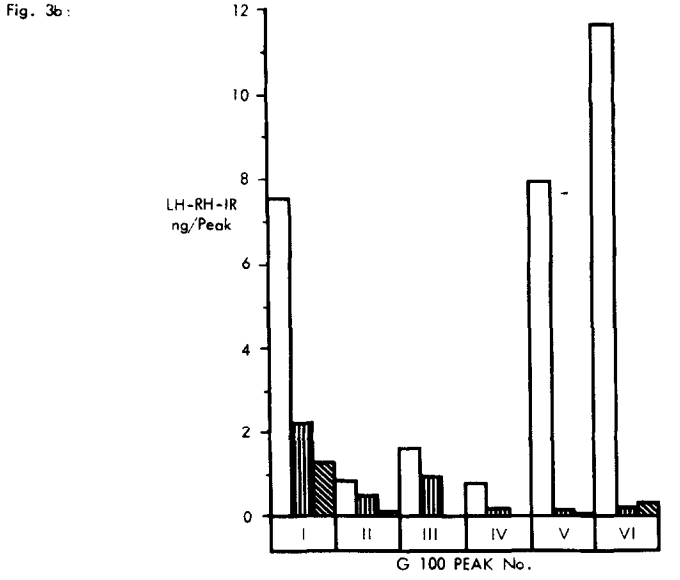


Fig. 3a Hypothalamic LH-RH decapeptide and the sections of the molecule to which the three antisera are directed. Specificity of the antisera was tested using analogues and fragments of the decapeptide sequence (24, 25).

Fig. 3b Estimation by three antisera of LH-RH-IR in pooled peak fractions of Sephadex G-100 chromatographed LH-RH-IR. The LH-RH-IR's were pooled as indicated in Fig. 1, reconstituted in 0.01 N acetic acid and assayed in serial dilution with each antiserum.

Identification of antisera in Fig. 3b as indicated in Fig. 3a.

3) demonstrated that highest interaction occurs consistently with antiserum 744 which is directed against the -COOH terminus of the hypothalamic LH-RH decapeptide (24). Antiserum 1076 which is directed towards the middle region of LH-RH (24) and antiserum R-42 which requires both the $-NH_2$ and -COOH termini of LH-RH (25)

for effective binding, yielded much lower results. These data suggest that the high molecular weight species are probably $-NH_2$ terminally extended forms but may also differ from hypothalamic LH-RH within the amino acid sequence 1-7 of the decapeptide. Peaks V and VI eluted later than decapeptide LH-RH on Sephadex G-25 suggesting that they are unlikely to be $-NH_2$ terminally extended. However, they clearly lack the middle and $-NH_2$ terminal features of LH-RH required by antisera 1076 and R42. In view of their high interaction with the -COOH terminally directed antiserum 744 these species are either smaller -COOH terminal fragments or molecules of similar size to LH-RH, but with considerable differences in the middle region and/or $-NH_2$ terminus.

These findings have shown the presence of a number of molecular forms of LH-RH-IR in the rat testis suggesting that the direct effects of exogenously administered synthetic LH-RH on gonadal function reflect an in vivo physiological mechanism, in which a locally produced source of LH-RH-like material regulates gonadal function through a receptor mediated mechanism. We are presently investigating the binding of the different forms of testicular LH-RH-IR to testicular Leydig cell receptors.

ACKNOWLEDGEMENTS

We are grateful to Prof. M.C. Berman for his support, to Dr. E. Polakow (Ayerst Laboratories) for synthetic LH-RH, to Dr. T. Nett and Dr. A. Arimura for antisera and C. Tobler and J. Morta for technical assistance. The study was supported by grants from the Medical Research Council, Atomic Energy Board, J.W. Duncan Baxter Trust, Harry Crossley Foundation, Nellie Atkinson Bequest and the University of Cape Town.

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