Polymorphism of human pituitary lutropin (LH)

Effect of the seven isohormones on mouse Leydig cell functions

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The effect of the 7 different molecular forms of human pituitary lutropin (LH) on mouse Leydig cells as a model of a target organ were studied. The hormone functions have been characterized by receptor binding, cAMP accumulation and testosterone production. One important finding was the similar intrinsic in vitro biological activity for all isohormones. Quantitatively, however, the potencies of the 7 hormone forms did not correlate with the activities obtained by radioimmunoassay: there was a dramatic decrease of receptor binding activity and biological activity compared to immunoactivity from the more alkaline to the more acidic LH isohormones.

Human lutropin *Isohormone* *Leydig cell* *Receptor binding* *cAMP production* *Testosterone production*

1. INTRODUCTION

Several studies have indicated that pituitary lutropin (LH) from human and other species exists as a number of differently charged molecular forms in unprocessed aqueous pituitary extracts [1,2]. Recently, we were able to demonstrate the complete microheterogeneous system of human pituitary LH by gel isoelectric focusing. Furthermore, the 7 LH forms could be isolated by preparative column focusing and partially characterized [3]. However, it is not known whether the various isohormones play different roles in the regulation of target organ function. To answer this question we have studied the effect of the 7 molecular forms of human LH on receptor binding, cAMP accumulation and testosterone production in mouse Leydig cells. Mouse Leydig cells were chosen as a target organ model for the different LH forms because this in vitro system is well defined with respect to responsiveness to the multi-component system of human chorionic gonadotropin and lutropin [4].

Abbreviations: cAMP, adenosine 3′,5′-cyclic phosphoric acid; hCG, human chorionic gonadotropin; hLH, human luteinizing hormone; MIX, 1-methyl-3-isobutylxanthine; MEM, Eagle's minimum essential medium; RRA, radioreceptor assay

2. MATERIALS AND METHODS

2.1. *Preparation of hLH isohormones*

LH isohormones were prepared from frozen human pituitaries by extraction, gel filtration and preparative column isoelectric focusing as in [3].

2.2. *Immunological characterization of the hLH forms*

Radioimmunological hLH concentration was measured using the NIAMDD-hLH kit. The LH preparations I-1 and MRC 68/40 were used for iodination and as a standard, respectively. All assays were performed with antiserum to hLH, batch no.2. Complete dose-response curves for all 7 isohormones were constructed by serial dilutions.

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Parallelism between the different LH isohormones was tested by analysis of variance comparing the slopes of the logit-log regression lines.

2.3. *Preparation of Leydig cells*

A crude mouse Leydig cell suspension was prepared by a non-enzymatic procedure as in [4] and purified (90–95% Leydig cells) by Percoll® density gradient centrifugation according to [5].

2.4. *Receptor-binding experiments*

Highly purified hLH (I-1, NIAMDD) was radioiodinated with ^{125}I by the lactoperoxidase method. The specific activity was 40–60 mCi/mg and the maximal binding activity of the labeled hormone with an excess of receptor sites was about 40%. The initial ratio of bound to total counts was 15–20% and the nonspecific binding 1–2%. Approx. 10^5 Leydig cells in 500 μl MEM were incubated with serial dilutions of identical radioimmunological concentrations of the 7 LH isohormones in the presence of 20000 cpm ^{125}I-hLH for 2 h at 37°C. The incubation was terminated by dilution and centrifugation, and the radioactivity bound to the cell pellet was measured in a gamma-counter. Parallelism of the dose-response curves was tested as described in section 2.2.

2.5. *cAMP accumulation*

Approx. 10^4 Leydig cells in 300 μl MEM were incubated for 3 h at 37°C with the 7 LH isohormones in the presence of 0.5 mM MIX. Incubations were stopped by addition of ethanol (80% final concentration). After centrifugation the supernatant was evaporated and the cAMP redissolved in water. Total cAMP (intra- and extracellular) was measured by specific RIA. Complete dose-response curves were constructed using multiple doses of the LH isohormones.

Table 1

Isoelectric points (p*I*) and slopes of radioimmunoassay (RIA) and radioreceptor assay (RRA) dose-response curves for the 7 LH isohormones

LH component no.	p*I*	RIA slope	RRA slope
1	8.8	0.91 ± 0.05	1.36 ± 0.12
2	8.4	0.87 ± 0.06	1.35 ± 0.09
3	7.8	0.91 ± 0.06	1.26 ± 0.05
4	7.4	0.90 ± 0.03	1.16 ± 0.02
5	6.9	0.92 ± 0.05	1.13 ± 0.06
6	6.5	0.92 ± 0.03	1.07 ± 0.07
7	5.9	0.90 ± 0.07	1.04 ± 0.03

Values are the means ± SD of 4 assays

2.6. *Testosterone production*

The incubation conditions were the same as described in section 2.5 but without addition of MIX. Incubations were stopped by dilution with 10 vols cold MEM. Testosterone production was measured by RIA of unextracted cell medium as in [6]. A parallel line bioassay design was employed to test parallelism and potencies for the cAMP and testosterone production assay.

3. RESULTS

When serial dilutions of the 7 isohormones separated by column isoelectric focusing of a purified LH preparation were tested in the hLH-RIA, all dose-response curves were parallel to each other and to the MRC 68/40 LH standard as shown in table 1. These results indicate that the immunochemical nature of the different hLH components is identical at least for the anti-hLH serum used here.

On the basis of identical immunological hormone concentrations, inhibition curves of the 7 isohormones were constructed in a radioreceptor assay system using isolated intact Leydig cells as the receptor source. As shown in fig.1A all LH species were able to compete with the radiolabeled LH on the receptor sites. However, there was a stepwise decrease of the slopes of the dose-response curves from 1.36 to 1.04 with decreasing p*I* values of the isohormones (table 1). Thus, a higher relative affinity to the receptor of the more alkaline hormone forms is indicated. This correlation was also revealed by a stronger receptor-binding activity for the more alkaline LH forms as calculated from the 50% intercept (ED_{50} = 100 mIU/ml for the 68/40 standard) of the various dose-response curves. The ratios of receptor-binding activity to radioimmunoactivity ranged from 2.34 to 0.23 (fig.2).

Stimulation of purified Leydig cells with the different isohormones in the presence of the phosphodiesterase inhibitor MIX resulted in iden-

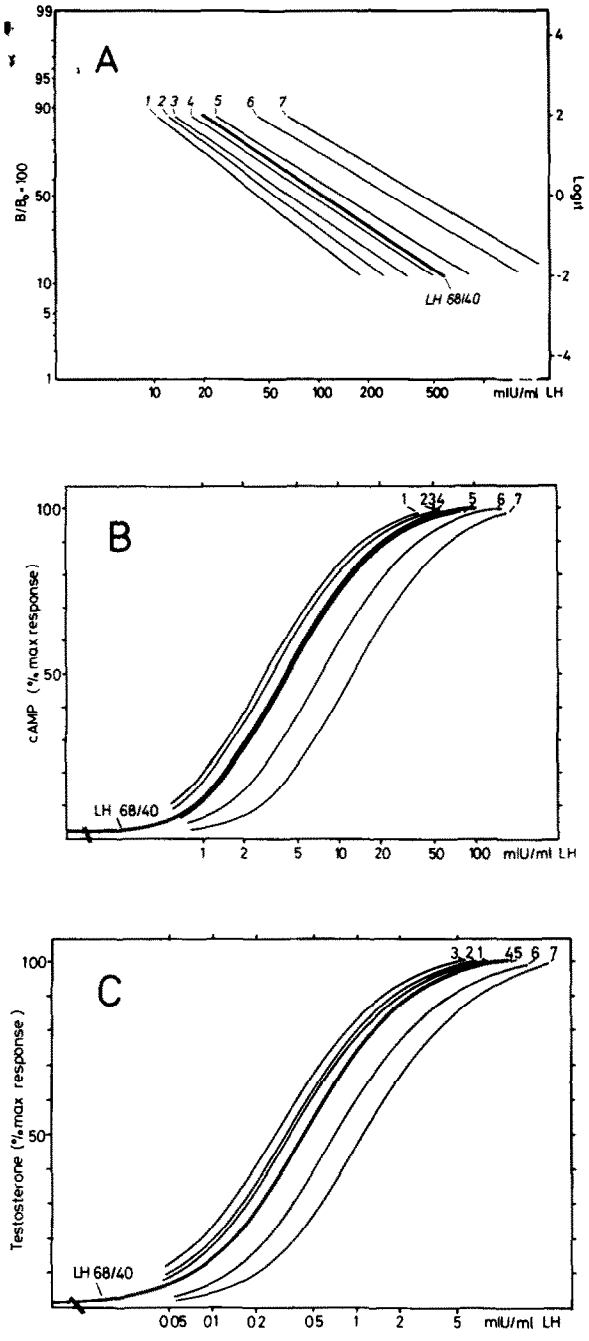


Fig.1. Effect of increasing immunoreactive concentrations of the 7 LH forms (1–7) on ^{125}I-hLH binding to mouse Leydig cells (A), cAMP accumulation (B) and testosterone production (C) by mouse Leydig cells. Dose-response curves were constructed from the mean values of 4 doses for each LH form in 4 different assays.

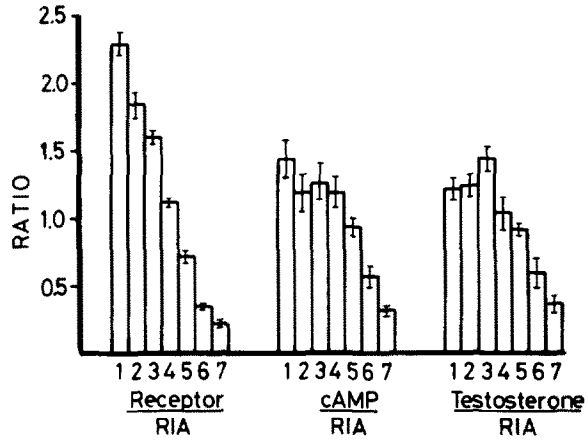


Fig.2. Relationship between biological activities (receptor-binding activity, cAMP and testosterone production activity) and radioimmunoactivity (RIA) of the 7 LH isohormones (1–7). Results are means ± SD of 4 assays.

tical maximum cAMP accumulation (20 pmol/tube) after 3 h incubation. Furthermore, all dose-response curves were parallel to each other and to the 68/40 standard preparation (ED_{50} = 4 mIU/ml). However, a discrepancy between bioactivity (B) with respect to cAMP accumulation and immunoreactivity (I) could be observed. The $B:I$ ratios for hLH components 1–7 ranged between 1.44 and 0.33.

Finally, determination of the androgen-producing activity of the LH isohormones showed that Leydig cells were stimulated maximally by the different LH forms. This was expressed in identical maximal testosterone production rates, as shown in fig.1C. All dose-response curves were parallel, the ED_{50} being 0.5 mIU/ml (68/40) and maximal testosterone production 20 ng/tube. The $B:I$ ratios for the isohormones were in the same range (1.47–0.38) as for cAMP accumulation (fig.2).

4. DISCUSSION

The responses of rat and mouse Leydig cells to gonadotropic hormones in vitro have been extensively evaluated (review [7]). The stimulating hormones used in these studies were hCG or LH. However, it must be borne in mind that these hormones, even in their highly purified form, consist of 6 (hCG) and 7 (LH) differently charged forms.

We have here demonstrated that all 7 immunoreactive human LH isohormones appear to have similar intrinsic biological potencies at least with respect to maximal cAMP accumulation and testosterone production of isolated mouse Leydig cells. However, all LH components showed small but significantly different relative affinities to the receptors of intact Leydig cells. These minor differences have no influence on the maximum amount of testosterone secreted by the Leydig cells, as it is well known that only 1% of all receptor sites need to be occupied to elicit the steroidogenic maximum [7].

Besides maximal response a further component of responsiveness is the sensitivity of the response (ED_{50}) [8]. The absolute ED_{50} values for hormone competition, cAMP accumulation, as well as testosterone production are not deducible from the present data because potency comparison between the isohormones was made on the basis of identical immunoreactive concentrations rather than on a mass basis. Thus, the relative biological potencies were expressed as $B:I$ ratios. As shown in fig.2 there was a dramatic decrease in the ratio between receptor-binding activity and immunoreactivity from the most alkaline (component 1) to the more acidic (component 7) hLH isohormone. These results largely agree with those reported for rat LH isohormones [2]. The high receptor-binding activity of hLH 1 and hLH 2 did not correlate with the cAMP accumulation and testosterone production assay. Nevertheless, a significant decrease in the $B:I$ ratios was found for LH components 5–7. This is in accordance with [1] in which lower $B:I$ ratios were also reported for the more acidic LH forms of crude pituitary extracts. The low $B:I$ ratios of the more acidic LH forms indicate that the physico-chemical properties of these forms may differ in such a way that they may have an effect on the stimulation of the cell rather than on the affinity to the antibody. From our data we conclude that the immunological nature of the isohormones is identical. In this context it would be of interest to determine whether monoclonal antibodies are able to differentiate between isohormones.

The results discussed here deal only with in vitro effects of the different hLH forms on the target cells and not with the survival of the components in the circulation. Such studies in progress.

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