Action of some luteinizing hormone derivatives in ovaries from pseudopregnant rats: dissimilarities between their activities on this organ and on Leydig cells

M.P. de la Llosa-Hermier, C. Tertrin-Clary, M. Evrard-Hérouard, C. Hermier
and P. de la Llosa

Groupe de Recherches 34, Laboratoire des Hormones Polypeptidiques, CNRS,
91190 Gif-sur-Yvette, France

(Received 15 March 1982)

SUMMARY

Biological activities of several derivatives of ovine LH obtained by chemical modification of the amino groups were investigated using ovaries from pseudopregnant rats. Binding-inhibition activities and steroidogenic potencies of ethylated, isopropylated and guanidinated LH were in good agreement, whereas adenylate cyclase activities were relatively greater. When compared with previous results on binding-inhibition activities and steroidogenic potencies using isolated rat Leydig cells, the ovaries from pseudopregnant rats appeared to be more discriminating. Ethylated and isopropylated derivatives exhibited lower binding-inhibition activities and steroidogenic potencies in female gonads. This difference was particularly evident in the case of guanidinated LH which exhibited a very low binding-inhibition activity and consequently was unable to act as an inhibitor of the action of LH on the ovaries.

Guanidinated porcine LH (in which all the lysine residues of the α-subunit were transformed into homoarginine, without modification of the β-subunit which does not contain lysine) showed similar biological activities to guanidinated ovine LH in the isolated Leydig cells as well as in pseudopregnant ovaries. It can, consequently, act as an inhibitor of LH action on Leydig cells but not on the ovary of the pseudopregnant rat. Thus, the inhibitory properties of this derivative can be ascribed to the modification introduced in the α-subunit.

INTRODUCTION

We recently reported on the biological activities of several luteinizing hormone (LH, lutropin) derivatives when tested at different steps in the sequence of action of this hormone on rat Leydig cells, i.e. binding to the receptors, stimulation of adenylate cyclase and of steroidogenesis (de la Llosa-Hermier, Tertrin-Clary, Evrard-Hérouard, Colléaux, Hermier & de la Llosa, 1980). The relative activities found were not necessarily the same; some derivatives, for instance, are able to bind to receptors and to stimulate adenylate cyclase without a proportional stimulation of steroidogenesis, and can consequently act as antagonists of LH action (e.g. guanidyl LH). We also observed that the binding-inhibition activities of these derivatives to homogenates of corpora lutea and of testes may differ, clearly suggesting that these derivatives may help to reveal subtle differences in the behaviour of various gonadal tissues with regard to LH. We have now investigated the action of some of these derivatives in the ovaries of pseudopregnant rats in order to understand some of these differences more clearly.
MATERIALS AND METHODS

Highly purified ovine LH and porcine LH were prepared in our laboratory (Jutisz & Courte, 1968; Maghuin-Register, 1974). The preparation of LH derivatives has been described previously (de la Llosa, Durosay, Tertrin-Clary & Jutisz, 1974). Guanidination was performed for two different times to obtain two different degrees of guanidination (de la Llosa-Hermier et al. 1980). Tritiation of LH was achieved by reductive methylation (de la Llosa, Marche, Morgat & de la Llosa-Hermier, 1974).

Juvenile, 23-day-old, female Sprague-Dawley rats were primed using pregnant mare serum gonadotrophin (50 i.u. per rat) and human chorionic gonadotrophin (50 i.u.). For binding-inhibition experiments, the primed ovaries were homogenized in 0-1 M-Tris–HCl (pH 7-4) containing 5 mM-MgCl2, 0·1 M-sucrose and 0·1% (w/v) bovine serum albumin (BSA), and incubated with labelled LH and increasing amounts of LH or LH derivatives for 30 min at 37°C. In the experiments using Leydig cells, dispersed interstitial cells were incubated in a Dubnoff shaker for 90 min at 35°C with labelled LH and increasing amounts of LH or of LH derivatives. The preparation of cells and the determination of bound radioactivity were performed according to the methods described by de la Llosa-Hermier, de la Llosa & Hermier (1977) and de la Llosa-Hermier et al. (1980). Stimulation of adenylate cyclase was measured in homogenates consisting of 1 g wet tissue in 10 ml buffer containing 10 mM-Tris–HCl (pH 7·5), 1 mM-EDTA and 27% sucrose. Incubation (15 min at 37°C) was carried out in a medium containing 39 mM-Tris–HCl (pH 7·4), 5 mM-MgCl2, 0·15% BSA, 100 μM-GTP, 8 mM-phosphoenol pyruvate, 80 μg pyruvate kinase/ml, 2 mM-ATP and 8 mM-theophylline (Birnbaumer, Yang, Hunzicker-Dunn, Bokckaert & Duran, 1976). The amount of cyclic AMP was determined by the method of Gilman (1970) under conditions reported previously (Tertrin-Clary & de la Llosa, 1978). Stimulation of steroidogenesis was investigated in sliced ovaries or collagenase-dispersed Leydig cells of rats by measuring the biosynthesis of progesterone or testosterone (Hermier, Evrard & de la Llosa, 1977) using a radioimmunoassay as described by Evrard-Hérouard, de la Llosa-Hermier, Martinet, Mauléon, de la Llosa & Hermier (1981).

RESULTS

The values concerning the binding-inhibition activities, the relative potencies (in terms of native LH) calculated from the concentrations of hormone (or derivatives) required to

Table 1. Biological activities of various ovine LH derivatives in ovaries from pseudopregnant rats in vitro. Values are given relative to that of native LH ± S.E.M.

(Binding-inhibition activities were calculated by comparison of the concentrations of the hormone and derivative required for 50% inhibition of specific binding. Steroidogenic activity was calculated by comparison of the concentrations of the hormone and the derivative required to obtain a similar steroidogenic response.)

<table>
<thead>
<tr>
<th>LH derivatives</th>
<th>Binding inhibition</th>
<th>Cyclic AMP accumulation</th>
<th>Progesterone biosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylated LH</td>
<td>1·00±0·01</td>
<td>A50* 1·00±0·05</td>
<td>Vm† 1·00±0·08</td>
</tr>
<tr>
<td>Ethylated LH</td>
<td>0·26±0·03</td>
<td>0·95±0·19</td>
<td>0·92±0·07</td>
</tr>
<tr>
<td>Isopropylated LH</td>
<td>0·13±0·03</td>
<td>0·91±0·09</td>
<td>0·92±0·07</td>
</tr>
<tr>
<td>Guanidinated LH (G1)</td>
<td>0·34±0·09</td>
<td>0·39±0·11</td>
<td>0·75±0·08</td>
</tr>
<tr>
<td>Guanidinated LH (G2)</td>
<td>0·13±0·02</td>
<td>0·26±0·06</td>
<td>0·76±0·07</td>
</tr>
</tbody>
</table>

*A50*, values of the ratio between the concentration of the derivative and the concentration of the native LH needed to obtain half-maximal activation of the adenylate cyclase.

†Vm, ratio between the maximal activation obtained with the derivative and with the native hormone.

‡ Relative potencies with confidence limits.

§ G1, 9 lysine residues modified out of 12; G2, 11 lysine residues modified out of the 12.
induce half-maximal stimulation of the ovarian adenylate cyclase, and the potencies obtained from stimulation of progesterone biosynthesis, are summarized in Table 1. Figure 1 shows examples of typical dose–response curves for the three parameters measured. The basal production of cyclic AMP was 40–45 pmol/mg tissue protein and the maximal response was about three times this value for a hormone concentration of approximately $10^{-7}$mol native LH/l. The maximal response for progesterone formation (about 200–300 µg/g tissue) was obtained at a lower concentration of hormone. Generally, the maximal stimulation of steroidogenesis is observed when there is still a submaximal accumulation of cyclic AMP. It should be pointed out, however, that the conditions used for cyclic AMP and progesterone experiments were different. Homogenates were used for the study of the accumulation of cyclic AMP in order that the composition of the incubation medium could be strictly controlled (by the introduction of theophylline, EDTA etc.). In contrast, intact cells or tissue slices as well as longer incubation times (3 h steroidogenesis, 15 min adenylate cyclase) were needed for inducing steroidogenesis.

![Graph](image_url)

**Fig. 1.** Dose–response curves obtained with native LH and guanidinated LH (G2) for (a) binding inhibition, (b) accumulation of cyclic AMP and (c) production of progesterone in the pseudopregnant rat ovary.
Fig. 2. Production of progesterone induced in ovaries from pseudopregnant rats by ovine LH (oLH) and ovine LH plus guanidinated ovine LH (Gu oLH). No inhibition of the LH action by the derivative was observed. Vertical lines represent s.e.m.

Fig. 3. Production of testosterone induced in isolated Leydig cells from rat testes by the addition of porcine LH (pLH) or by the addition of porcine LH plus guanidinated porcine LH (Gu pLH) to the medium. The derivative inhibits the stimulating action of LH. Vertical lines represent s.e.m.
Table 2. Biological activities of guanidinated porcine LH when tested on pseudopregnant rat ovaries in vitro

(All ten of the lysine residues were guanidinated in this derivative. Results may be compared with guanidyl ovine LH G2 of Table 1. The activities were calculated as in Table 1. Values are in terms of native LH ± s.e.m.)

<table>
<thead>
<tr>
<th>Homogenized organ</th>
<th>Binding inhibition</th>
<th>(A_{50}^{*})</th>
<th>(V_m^{\dagger})</th>
<th>Steroidogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>0.24 ± 0.03</td>
<td>0.41 ± 0.15</td>
<td>0.72 ± 0.16</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td>Primed ovaries</td>
<td>0.09 ± 0.02</td>
<td>0.38 ± 0.04</td>
<td>0.65 ± 0.04</td>
<td>0.08 ± 0.01</td>
</tr>
</tbody>
</table>

*\(A_{50}\), values of the ratio between the concentration of the derivative and the concentration of the native LH needed to obtain half-maximal activation of the adenylate cyclase.
\(V_m\), ratio between the maximal activation obtained with the derivative and with the native hormone.

The activities of the derivatives (except methylated LH) were lower than those of native LH, but the decrease in potency obtained in some steps as a consequence of the chemical modification was sufficiently different so as to modify the inhibiting properties of some derivatives. It has been reported previously that guanidyl LH is able to act as an inhibitor of LH action on Leydig cells (de la Llosa-Hermier et al. 1980). We tested the inhibitory properties of guanidyl ovine LH (G2) on LH action in pseudopregnant rats with the results shown in Fig. 2. No inhibition was observed.

The properties of guanidinated porcine LH are shown in Table 2 and in Figs 3 and 4. No difference was observed between the results obtained with the derivatives of ovine and porcine LH, underlining the importance of the modification of the \(\alpha\)-subunit.

**DISCUSSION**

A good general agreement was observed when binding-inhibition activities and steroidogenic potencies of most of the derivatives were compared. In the case of the methyl derivative, which was fully active, this agreement also included its potency in terms of adenylate cyclase stimulation. In the case of the other derivatives, however, the relative
potencies inferred from adenylate cyclase stimulation were much greater. Some common features between these results and those obtained previously with isolated Leydig cells or in vivo can nevertheless be seen: the elongation of the alkyl chain is accompanied by a decrease in biological activity while the methyl derivative is indistinguishable from the native hormone.

However, a comparison of the results obtained with ovaries from pseudopregnant rats and with isolated Leydig cells showed profound differences particularly with the guanidyl derivatives. The binding activity of guanidinated ovine LH (G2; 11 lysine residues modified out of 12) to receptors in female gonads (0-13 in terms of native LH) was much lower than to those in the male gonads (0-3). In the latter case the binding activity was relatively high (0:3) whereas the steroidogenic potency was very low (0:07). Since this derivative was able to bind to the receptors it may have prevented the occupation of testicular receptor sites by native LH. Consequently, in the presence of guanidinated LH, stimulation of testosterone biosynthesis in the Leydig cells induced by native LH was not significantly higher than that induced by the derivative, and was clearly lower than the stimulation induced by LH in the absence of the antagonist derivative. This derivative could therefore act on the rat Leydig cells as a poor agonist and as an antagonist at the same time (de la Llosa-Hermier et al. 1980). In the case of the pseudopregnant rat ovary, the basis for an antagonist action (high binding activity, low steroidogenic potency) did not exist and guanidinated ovine LH was unable to act as an inhibitor of LH stimulation of steroidogenesis. In fact, the ovary of the pseudopregnant rat was more discriminating as a target organ than the testis and the binding of derivatives to receptors from the pseudopregnant ovary occurred only at a very low level.

The results obtained with the guanidinated porcine LH were particularly interesting. As no lysine residues exist in the β-subunit of the porcine hormone (Maghuin-Register & Hennen, 1972), its guanidyl derivative differs from guanidyl ovine LH in that only the α-subunit is modified. Table 2 shows the values obtained for the relative potencies when the action of guanidyl porcine LH was assayed both on primed ovaries and on isolated Leydig cells. No significant difference was observed between the results obtained previously with guanidyl ovine LH (de la Llosa-Hermier et al. 1980) and in the present study with guanidyl porcine LH. Moreover, as shown in Fig. 3, guanidyl porcine LH was indeed able to antagonize the stimulating action of native LH on Leydig cells (as was guanidyl ovine LH) but it was without an inhibitory action when the ovary from the pseudopregnant rat was incubated with the native hormone (Fig. 4). It may be concluded, therefore, that the inhibitory properties found both for guanidyl ovine LH and for guanidyl porcine LH in Leydig cells are due to the chemical modification of the α-subunit since, as mentioned above, only this subunit can be modified in the porcine LH by guanidination.

The ability of the guanidyl derivatives to stimulate adenylate cyclase is relatively high in rat Leydig cells (relative potency: 0-53, de la Llosa-Hermier et al. 1980) as well as in the ovaries of pseudopregnant rats (0-3, Table 1). These values contrast with their poor steroidogenic potencies (0-07 and 0-13 respectively). We suggested previously (de la Llosa-Hermier et al. 1980) that different pools of adenylate cyclase may exist in a population of Leydig cells and that some of these pools may not be connected with the steroidogenic pathway even if they are stimulated by the action of the hormone on its receptors. Thus, the accumulation of cyclic AMP induced in these compartments would be inefficient. As guanidyl porcine LH might be able to discriminate between different pools of adenylate cyclase as does guanidyl ovine LH (stimulating mainly those pools of the enzyme not connected with the steroidogenic pathway), we think that the hormone–receptor complex reaches the membrane adenylate cyclase without dissociation of the α-subunit from the hormone–receptor complex during this process.

In addition to this discrepancy between the relatively high stimulating potency of some derivatives on adenylate cyclase and their low steroidogenic power, another discrepancy
Action of some LH derivatives on rat ovaries

concerns the activities of the binding inhibition and of adenylate cyclase in pseudopregnant rat ovaries. The concentration required to attain occupancy of half of the receptors compared with native LH was sevenfold higher for some derivatives (isopropyl, guanidyl LH) and fourfold higher when the ethyl derivative was employed. By contrast, half-maximal stimulation of adenylate cyclase in the case of the isopropyl or ethyl derivatives was reached at a concentration similar to that of the native hormone. Thus, binding-inhibition activity of the derivatives was very low and apparently only at a high concentration could the derivative molecules compete with the native hormone for the receptors. However, in spite of a low binding activity of these derivatives to the receptors, half-maximal stimulation of adenylate cyclase was reached at a concentration comparable to that of the native LH. If we assume that these two phenomena are closely related, it is necessary to admit that the receptors connected to the adenylate cyclase stimulated by any one of these derivatives (ethylated, isopropylated and guanidinated G2) and those examined in the binding experiments are not the same.

In fact, of course, the ovary of the pseudopregnant rat is a heterogenous tissue and it may be that the cells responsible for the relatively high accumulation of cyclic AMP are not necessarily those responsible for the biosynthesis of progesterone. In other words, it would be possible for the derivatives to stimulate mainly the adenylate cyclase of cells which are not capable of steroidogenesis without inducing a high accumulation of cyclic AMP in those which are capable of steroidogenesis. The accumulation of cyclic AMP in these cells could be negligible in comparison with the accumulation of cyclic AMP induced in the other type of cells. The relative activities for adenylate cyclase stimulation (mainly obtained in cells without steroidogenic activity) may then not agree with those obtained for the stimulation of steroidogenesis. If the stock of receptors connected to the steroidogenic pathway (i.e. those existing in the cells capable of steroidogenesis) is in an overwhelming proportion to those in cells which are not steroidogenic then the binding-inhibition activities (depending mainly on the steroidogenic cells) may agree quite well with those observed for the steroidogenesis activity and may not agree with those corresponding to the adenylate cyclase activities.

In this context, it must be pointed out that the amount of maximal activation of adenylate cyclase reached with the native hormone is, in the case of the primed ovary, greater than those reached with most of the derivatives. This difference suggests, therefore, a supplementary contribution to the maximal accumulation of cyclic AMP from cells which can only be stimulated by the native hormone. In other words, the pool of cells stimulated by native hormone is larger than that stimulated by the derivatives.

These facts clearly emphasize that it is necessary to be extremely cautious in comparing biological activities between a hormone and its derivatives, between structurally related hormones and between hormones of different species as well as in the interpretation of any results.

We acknowledge the help of Dr M. Jutisz who kindly provided the ovine LH and of Mr M. Poissonnier who prepared it. We are grateful to Mrs P. Lebouleux and to Mrs M. Roy for their excellent technical assistance. This work was supported in part by the Foundation of Hormone Research Grant no. 9181006 and by the Délégation Générale à la Recherche Scientifique et Technique (Contract no. 80.7.0335).

REFERENCES


