

Influence of some chemical modifications on the steroidogenic activity of luteinizing hormone (LH)

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Summary. We report the influence of different chemical modifications of luteinizing hormone (LH) lysine residues on progesterone synthesis, induced *in vitro* by this hormone, in the pseudo-pregnant rat ovary :

- *Carbamylation* of half of the lysines, with abolition of the positive charges, sufficed to suppress 90 p. 100 of LH biological activity,
- *Guanidination* of 65 p. 100 of the lysines caused a 55 p. 100 loss of the activity,
- *Methylation* of 75 p. 100 of the lysines did not modify the biological potency of the hormone.

The influence of some chemical modifications of the lysine residues of luteinizing hormone (LH) was studied recently in our laboratory (De La Llosa *et al.*, 1974) by means of the ovarian ascorbic acid depletion test (Parlow, 1961) : this *in vivo* bioassay is very sensitive to any changes in the clearance rate of the hormone produced by the chemical modifications. We therefore thought that it would be interesting to examine the biological potencies of these derivatives using *in vitro* bioassays. The *in vitro* biosynthesis of progesterone by pseudopregnant rat ovaries can be employed to measure the potency of LH (Hermier and Jutisz, 1968 ; Watson 1971). In this method, a global response of *in vitro* steroidogenesis to stimulation by LH is obtained, proportional to the LH concentration. In the present work, we have tested several LH derivatives. One of them (methylated LH) is of particular interest because it is employed in our laboratory as a labelled ligand in the study of LH binding to receptors (De la Llosa-Hermier *et al.*, 1976).

Materials and methods.

Highly purified ovine LH was obtained in our laboratory (Justisz and Courte, 1968). The relative potency was $2 \times$ LH-NIH-SI. Derivatives of LH (methylated, carbamylated and guanidinated LH) were prepared as previously reported. The degree of chemical modifications was determined by aminoacid analysis (De la Llosa *et al.*, 1974). The relative potencies of the derivatives in the Parlow test are : 1.43 for the

methylated LH (75 p. 100 modified lysine), 0.55 for the guanidinated LH (65 p. 100 lysine modified), and 0.06 for the carbamylated LH (53 p. 100 modified lysine), in terms of native luteinizing hormone (De La Llosa *et al.*, 1974).

Pseudopregnant rat ovaries were obtained by treatment with PMSG (50 UI/rat at 28 days) and HCG (50 UI/rat at 31 days). The rats were killed at 37 days. The ovaries were removed, minced, and pre-incubated 15 minutes in Krebs-Ringer medium (pH 7.4), containing 0.1 p. 100 bovine serum albumin and saturated by $O_2 + CO_2$ (93 : 7 p. 100). The incubation was performed under constant conditions for 3 hrs (1 g of wet ovaries/10 ml of medium). After incubation, the ovaries were homogenized in the medium and the progesterone concentrations were determined by extractions with diethyl ether, chloroform (4:1) followed by thin layer chromatography (Merck Silica Sel G) and spectrophotometric analysis. Small amounts of progesterone ^{14}C -4 were added to enable correction of the losses of the steroid during extraction and purification (Hermier and Justisz, 1968).

Results.

Steroidogenic activity of methylated LH.

Stimulation by methylated LH and native LH of progesterone production in incubated slices of pseudopregnant rat ovaries gave dose response curves between 4×10^{-10} M and 5×10^{-9} M as shown in figure 1. The maximal activation is not different for the native or the methylated LH.

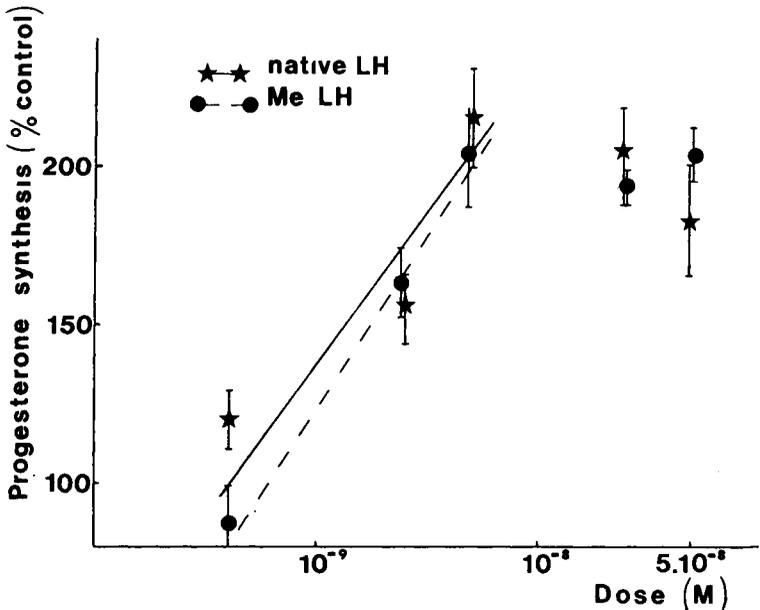


FIG. 1. — Progesterone biosynthesis, by pseudopregnant rat ovaries, *in vitro*, during incubation for 3 hrs, induced by native LH or methylated LH. Each point shows the mean \pm confidence limits, $p < 0.05$ (1 incubation). Progesterone control : 69.08 ± 4.91 μ g/g.

The relative potency expressed in terms of native LH and calculated by comparison of two doses of standard LH and two doses of methylated LH is shown in table 1. The potencies of methylated LH and native LH are not significantly different.

TABLE 1

Steroidogenic activity of three derivatives of luteinizing hormone (LH)	
Derivatives	Relative potencies in terms of native luteinizing hormone with 95 p. 100 confidence limits
Methylated	1.00 (0.88-1.14)
	0.73 (0.54-1.00)
Guanidinated	0.51 (0.39-0.67)
	0.38 (0.32-0.45)
Carbamylated	0.15 (0.05-0.43)
	0.055 (0.04-0.07)

Two bioassays are performed for each derivative. The relative potencies are calculated by comparison between the responses (biosynthesis of progesterone *in vitro*) induced by two different doses of LH and by two doses of LH derivatives, in the same ratio.

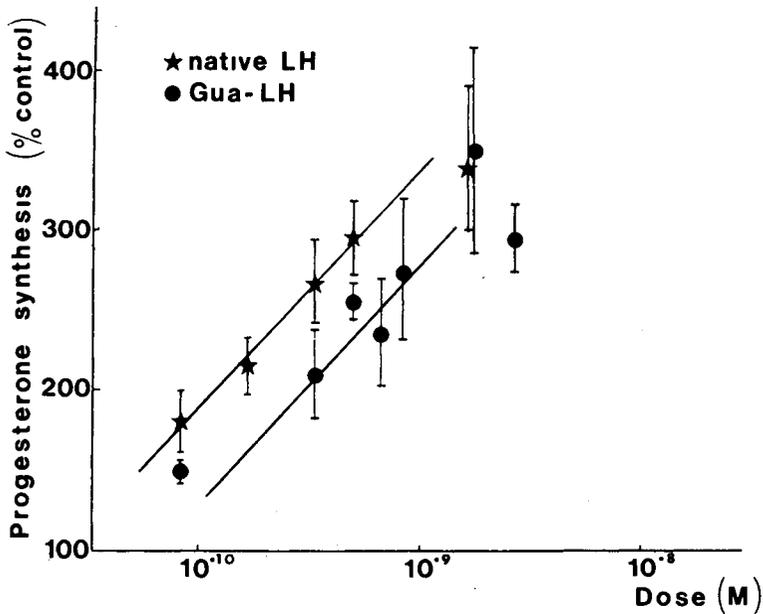


FIG. 2. — Progesterone biosynthesis by pseudopregnant rat ovaries, *in vitro*, during incubation for 3 hrs, induced by native LH or guanidinated LH. Each point shows the mean \pm SEM (3 incubations). Progesterone control : 35.69 ± 3.83 μ g/g.

Steroidogenic activity of guanidinated LH.

Fig. 2 shows the stimulation by guanidinated LH and by native LH of progesterone production, in slices of pseudopregnant rat ovaries incubated for three hours. No differences are observed between the maximal activation produced by the two hormones (native and modified).

A marked decrease of biological activity is observed as a consequence of this modification (table 1). The residual activity is about 45 p. 100.

Steroidogenic activity of carbamylated LH.

The stimulation by carbamylated LH (53 p. 100 of modified lysine) and native LH, of progesterone biosynthesis in incubated slices of pseudopregnant rat ovaries is shown in figure 3. The maximal stimulation attained by untreated LH and carbamylated LH is similar.

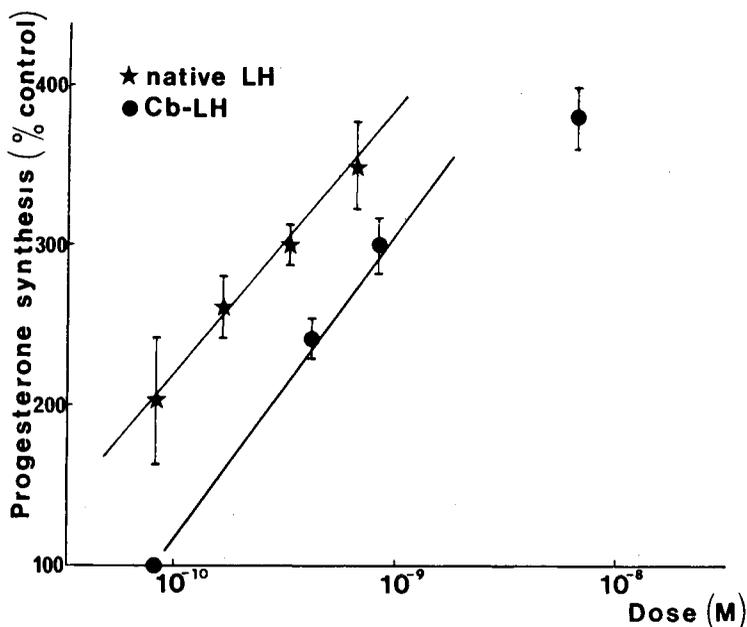


FIG. 3. — Progesterone biosynthesis by pseudopregnant rat ovaries *in vitro* during incubation for 3 hrs, induced by native LH or carbamylated LH. Each point shows the mean \pm SEM (2 incubations). Progesterone control : 30.07 ± 0.75 μ g/g.

The relative potency calculated with respect to native LH (table 1) shows that a significant loss of biological activity results from carbamylation (about 90 p. 100). The very low residual activity may be explained by a contamination by native LH.

Discussion.

Good agreement is observed between the biological potencies reported in this paper using the biosynthesis of progesterone as a criterium and those previously

reported using the ovarian ascorbic acid depletion test. This confirms that the positive charges of one or several residues of lysine are essential for the biological activity (table 2).

TABLE 2

Comparative biological activities of some derivatives of luteinizing hormone (LH)
(Relative potencies in terms of native LH)

Derivatives	Progesterone biosynthesis *	Binding activity with $^3\text{H-LH}$ **	Ovarian ascorbic acid depletion test
		De La Llosa-Hermier <i>et al.</i> 1977	De La Llosa <i>et al.</i> 1974
Methylated	1.00 (0.88-1.14)	0.97 \pm 0.025	1.43 (1.01-2.02)
Guanidinated . .	0.51 (0.39-0.67)	0.315 \pm 0.065	0.55 (0.29-1.04)
Carbamylated	0.15 (0.05-0.43)	0.005 \pm 0.007	0.06 (0.01-0.30)

* See table 1.

** Calculated by comparison of the hormone concentration needed for 50 p. 100 inhibition of specific binding in bovine corpora lutea homogenate (means \pm SEM).

Carbamylated derivatives (abolition of the positive charges) are inactive in all bioassays employed to date, including radioligand receptor assays (Liu *et al.*, 1974, De La Llosa-Hermier *et al.*, 1977) and stimulation of rat ovarian adenylate cyclase (Tertrin-Clary and De La Llosa, 1975).

The guanidinated derivative exhibits diminished activity. The derivative used in this paper was incompletely guanidinated (65 p. 100 of modified lysine) to permit comparison with the carbamyl derivative. In the radioligand receptor assay using $^3\text{H-LH}$, we have obtained about 35 p. 100 of activity (in terms of native LH) for the same guanidinated LH (De La Llosa-Hermier *et al.*, 1977). According to Liu *et al.* (1975) a complete guanidination (98 p. 100) leads to an almost total loss of biological activity both in the OAAD bioassay and in the radioligand receptor assay. The decreased activity of these derivatives may be due to steric hindrance resulting from the larger guanidyl groups.

Methylated luteinizing hormone, a derivative with the amino group substituted, but without significant change of the pK of this basic group, exhibits the same biological activities as native LH (progesterone biosynthesis and radioligand receptor assay with $^3\text{H-LH}$ see table 2). *In vivo* (ovarian ascorbic acid depletion) methylated LH is more active than native LH. It must be pointed out first, however, that the clearance rate may be different for the native LH and the derivative, and second that between the ovarian ascorbic acid depletion and the steroidogenesis there may not be a strict linear correlation.

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Résumé. L'effet de différentes modifications chimiques des résidus lysines de la LH sur la synthèse de progestérone stimulée *in vitro* par cette hormone dans l'ovaire de la ratte pseudo-gestante a été étudié :

- la *carbamylation* de 50 p. 100 des lysines (avec perte des charges positives) est suffisante pour provoquer 90 p. 100 de perte d'activité biologique.
- la *guanidylation* de 65 p. 100 des lysines entraîne une perte d'activité d'environ 55 p. 100.
- la *méthylation* de 75 p. 100 des lysines ne modifie pas l'activité biologique.

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