ANAHORMONE OF SHEEP LUTEINIZING HORMONE INDUCING ANTIBODIES AGAINST HUMAN GONADOTROPIN

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UDC 612.433'62 : 577.17.014

Actylation of sheep luteinizing hormone inactivates its hormonal activity but leaves its antigenic specificity intact. The resulting anahormone retains its common antigenic properties with human gonadotropins.

The possibility of separating the properties of a protein is of great interest from the theoretical point of view, for it broadens our knowledge of the structure of active centers. In this respect hormonal proteins form a convenient object for investigation for they possess as a rule several types of biological activity, and they can thus be used to study the relationship between structure and function of the protein molecule.

The object of the present investigation was to study the possibility of separating the hormonal and antigenic activity of sheep luteinizing hormone (LH).

EXPERIMENTAL METHOD

Gonadotropic hormones were obtained from sheep pituitary glands [7]. Sheep LH was purified by ion-exchange chromatography on CM-cellulose [9] with selection of the LH_2 fraction. The hormonal activity of the LH was determined by Parlow's test [8], and the minimal active dose was 0.4-0.5 μg. The hormonal effect was abolished by acetylation with acetic anhydride in the following manner: 50 mg of hormone was dissolved in 50 ml sodium phosphate buffer, pH 8, and 0.05 ml of 10% solution of detergents (a wetting agent of the OP type based on nonylphenol, containing 13 ethylene oxide residues and sodium dodecylsulfate) was added. The solution of the hormone with the detergents was kept at 4°C for 1 h. Acetylation was carried out at 0°C under alkaline conditions by gradual addition drop by drop of acetic anhydride (1 ml) and 1 N NaOH solution to pH 8-9. The excess of acetic anhydride was removed by dialysis and the hormone was lyophilized.

EXPERIMENTAL RESULTS

As Table 1 shows, acetylation of sheep LH completely abolished its hormonal effect in the test based on reduction of the ascorbic acid content in rat ovaries [8], even when the hormone was given in a dose 80 times greater than the minimal active dose.

Antisera were prepared in rabbits against the LH anahormone and also against the active preparation. Immunization was carried out with the use of Freund's complete adjuvant by subcutaneous injection of 5 mg of each hormone at intervals of 1 week. After 6 weeks the titer of the antiserum against active hormone was 1 : 24,000, and against the anahorme 1 : 16,000. Preservation of the original immunological characteristics by the anahormone of sheep LH was demonstrated by testing this preparation for its ability

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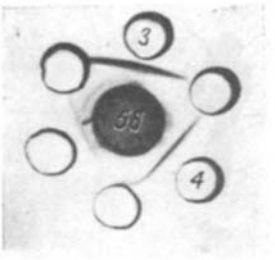
(Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Il'in.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 9, pp. 69-72, September, 1971. Original article submitted November 20, 1970.

TABLE 1. Effect of Acetylation on Hormonal Activity of Sheep LH

Preparation	Dose (in μg)	Number of rats	Concentration of ascorbic acid in rat ovaries (in μg/100 mg weight)	P
Physiological saline	–	6	100.0	
Native hormone	4	5	62.5	0.002
	16	5	31.8	0.002
Acetylated hormone	350	10	95.5	> 0.535



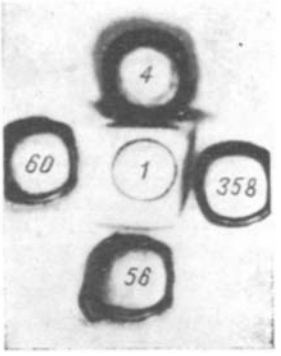


Fig. 1 Fig. 2

Fig. 1. Ouchterlony's gel diffusion test: 3) sheep LH; 4) human pituitary gonadotropin; 56) serum against acetylated sheep LH.

Fig. 2. Ouchterlony's gel diffusion test: 1) human LH; 4) antiserum against sheep LH; 60) antiserum against sheep follicle-stimulating hormone; 358) antiserum against human LH; 56) antiserum against acetylated sheep LH.

to inhibit hemagglutination. The original sheep LH inhibited the hemagglutination reaction in a dilution of 1 : 320, and the acetylated LH in a dilution of 1 : 240. In other words, the acetylated derivative, deprived of its gonadotropic action, retained not less than 75% of its original antigenicity.

In Ouchterlony's gel-diffusion test, antiserum against the acetylated hormone gave a distinct precipitation band with the active sheep LH (Fig. 1).

In addition, the antiserum obtained against inactivated sheep LH reacted with human luteinizing gonadotropin. Antiserum against the anahormone reacted in the hemagglutination test in the same dilutions (1 : 16,000) both with the original sheep LH and with the preparation of human LH. This indicates, first, the antigenic similarity between human and sheep LH, and second, that the inactivated hormone, against which the antiserum was prepared, still preserved its initial immunological characteristics. In the precipitation test, antisera against the active and inactivated sheep hormone as well as antiserum against human gonadotropin formed confluent precipitation bands with human LH, indicating the possession of common antigenic properties by all three preparations: the active sheep LH, the inactivated sheep LH, and human LH (Fig. 2).

To confirm more fully the specificity of the immunological reaction, experiments were carried out to study neutralization of the hormonal action of sheep and human LH by antiserum prepared against inactivated sheep LH. The results of these tests, are given in Table 2, demonstrate that the action of human LH can be completely neutralized by antiserum prepared against inactivated LH of animal origin.

TABLE 2. Biological Neutralization of Human Pituitary Gonadotropins by Antiserum Against Acetylated Sheep LH in Parlow's Test [8] (M±m)

Experimental conditions	Number of rats	Concentration of ascorbic acid in rat ovaries (μg/100 mg weight)	P
Physiological saline	6	68.9±5.0	–
40 μg human LH +0.7 ml normal rabbit serum	5	29.0±7.5	< 0.002
40 μg human LH + 0.7 ml serum against acetylated sheep LH	6	71.3±7.6	> 0.1
Physiological saline	8	82.7±5.8	–
40 μg human LH + 0.7 ml normal rabbit serum	7	34.6±2.4	< 0.001
40 μg human LH + 0.7 ml serum against acetylated sheep LH	6	76.6±6.2	> 0.1

TABLE 3. Suppression of Activity of Human Pituitary Gonadotropins by Antiserum Against Sheep Pituitary LH in the Mouse Uterus Test (M±m)

Experimental conditions	Number of animals	Weight of uterus (in mg)	P
3 μg human LH + 0.5 ml normal rabbit serum	12	30.2±3.2	–
3 μg human LH + 0.5 ml antiserum against active sheep LH	10	6.8±0.6	< 0.001
3 μg human LH + 0.5 ml antiserum against acetylated sheep LH	9	9.3±0.9	< 0.001

To confirm this important conclusion, experiments were carried out to study biological neutralization, using yet another method of evaluating hormonal activity: the test based on the increase in weight of the uterus (Table 3). Crossed interaction also was observed between human gonadotropins and antiserum against inactivated LH, and it was manifested as inhibition of the gonadotropic action of human LH.

The results of this investigation are evidence of the structural separation of the centers responsible for hormonal and immunological specificity in the hormone protein molecule. Acetylation, which blocks free amino-groups and, to some extent also, hydroxyl groups abolished the hormonal effect without disturbing the initial antigenic properties. Considering that the procedures were carried out in the presence of ionic and nonionic detergents, it also seems likely that the antigenic properties likewise are not determined by the configuration of the molecule. Theoretically, it follows from the separation of the properties of the hormonal protein obtained in this investigation that active immunization is possible against the native hormone, using inactivated hormone as the antigen. This conclusion is confirmed by the results of biological neutralization of the hormonal effect of LH by antiserum against acetylated LH. Because of this property, acetylated LH can be classed as an anahormone, i.e., as a derivative of the protein hormone in which loss of hormonal action does not lead to loss of the original antigenicity in this case [1-6]. Moreover, the original antigenic properties were preserved not only against homologous LH, but also against human LH. As a result of this, the antiserum prepared against the anahormone of sheep LH reacted in immunological tests with native human LH and was able to inhibit its hormonal effect. These crossed interactions between anahormone of animal origin and human hormone may be of significant practical importance.

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