INACTIVATION OF BIOLOGICAL ACTION OF ESTROGENS AND GROWTH HORMONE BY ANTIBODIES AGAINST A HORMONALLY INACTIVE CONJUGATION PRODUCT OF HUMAN STH AND ESTRONE

L. M. Bershtein, B. N. Sofronov,
A. L. Remizov, I. G. Kovaleva,
and V. M. Dil'man

UDC 612.432.018

A hormonally inactive conjugation product of estrone with human STH (STE), preserving the antigenic properties of the original hormones, was obtained for the first time with the aid of water-soluble carbodiimide. Immunization with this preparation, which the writers call an anahormone-chimera, enables action to be taken simultaneously against the endocrine glands and homeostatic systems of the body (energy metabolism, reproductive system), mainly because of the ability of the antiserum against STE to block the hormonal action of STH and estrone.

The production of a conjugation product of bovine ACTH and human growth hormone by linking them together with water-soluble carbodiimide (CBM), was demonstrated previously [1]. Compounds of this class, produced by peptide-type condensation of heterogeneous hormones, are described by the writers as anahormone-chimeras [1, 2].

This paper describes a similar attempt to combine the molecule of a protein hormone (human STH) with a steroid estrogen (estrone). The object was to increase the immunogenicity of the steroid hapten and to obtain a preparation suitable for simultaneous immunization with two anahormones produced by conjugation and capable of activity simultaneously on homeostasis of energy metabolism and reproductive activity.

EXPERIMENTAL METHOD

Estrone (Roussel, France) was converted into an azo-dye containing a carboxyl group by azo-coupling with diazotized para-aminobenzoic acid [11]. STH was isolated from human pituitary glands by Raben's method [13]. To obtain the conjugation product of STH and p-carboxyphenyl-azoestrone (STE) the compound methyl-p-toluenesulfonate 1-cyclohexyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide (CDM; Bonnasies, France) was used. The azoestrone (5 mg) was dissolved in 2 ml of a 0.2 M solution of Na_2CO_3, treated with constant stirring with 1.5 ml of each of two solutions containing 16 mg STH and 20 mg CDM respectively and allowed to stand at room temperature. After 30 min the mixture was transferred to a dialysis chamber and dialyzed at first against 0.2 M Na_2CO_3 and then against distilled water for a total of 6 days at 4°C. The degree of homogeneity of the product was studied by thin-layer and column chromatography on Sephadex, by electrophoresis on paper, and by ultracentrifugation. The STE preparation was also tested for growth (by the tibial test [3]), for lipid-mobilizing (by the change in concentration of nonesterified fatty acids – NEFA – in the blood [7]), and estrogenic (in the rat uterus test [4, 12]) activity. The immunological properties of the conjugation product were studied in the passive hemagglutination and hemagglutination inhibition tests [6, 14]. The quantity of the conjugation product taken for each test was calculated on the basis of the weights of the

Laboratory of Endocrinology, N. N. Petrov Cancer Research Institute, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Serebrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 85-88, February, 1973. Original article submitted May 24, 1972.

TABLE 1. Results of Study of Growth Activity of Preparations by Tibial Test (M ± m)

Group of animals	Experimental conditions	No. of rabbits	Width of tibial cartilage (in μ)
1	Control	23	274,8±1,7
2	Hydrocortisone (1.5 mg × 3)	25	156,7±2,4
3	Hydrocortisone + STH (0.3 mg × 4)	10	208,1±3,0
4	Hydrocortisone + STE (0.8 mg as STG × 4)	10	143,6±1,5
5	Hydrocortisone + (STH + normal rabbit serum)	13	215,4±1,9
6	Hydrocortisone + (STH + immune serum against STE)	14	167,6±1,2

Note. STH was incubated with the sera for 20 h at 4°C at the rate 0.45 ml serum to 0.3 mg hormone. P_{3-4} and $P_{5-6} < 0.001$.

TABLE 2. Results of Study of Lipid-Mobilizing Activity of Preparations (M ± m)

Preparation	No. of rabbits	Content of NEFA (in μeq/liter)		
		fasting	after 30 min	after 60 min
STH (2 mg)	4	539±43	1639±196 (From + 130 To +328%)	1220±154 (From + 26 To +278%)
STE (2.5 mg as STH).	4	383±65	379±43 (From − 15 To +14%)	385±21 (From − 30 To +25%)
STH + immune serum against STH) (1 ml/2 mg STH)	4	509±32	523±59 (From − 9 To +15%)	500±32 (From − 9 +7%)

Note. Preparations injected intravenously into rabbits deprived of food for 14-16 h. Immune serum incubated with hormones for 20 h at 4°C. Limits of deviation of individual values from original level in percent given in parentheses.

hormonal components contained in it. To obtain antiserum against STE, the latter (in doses corresponding to 1-1.5 mg estrone and 3-4.5 mg STH) was injected intravenously into female rabbits at intervals of 10-14 days. Blood for titration of the antibodies was taken on the 10th-11th day after the 6th-9th injection of the antigen.

EXPERIMENTAL RESULTS

Analysis of the STE absorption spectra revealed two characteristic absorption maxima: at 280 nm, belonging to the protein part of the molecule, and 345 nm, belonging to the azo-group. During column chromatography on Sephadex G-75 and G-100 and ultracentrifugation with a maximal velocity of 50,000 rpm and the resulting STE preparation gave two peaks of which the predominant component had the higher molecular weight. Electrophoretic investigation of the preparation (0.05 M veronal-medinal buffer, pH 8.2), however, revealed only one zone with considerably lower mobility than that of growth hormone.

Biological testing of the STE showed that it possessed neither growth, lipid-mobilizing, nor estrogenic activity (Tables 1-3).

Despite the absence of hormonal activity in the conjugation product of STH and estrone, immunological analysis showed that STE preserves the antigenic characteristics of the original hormones. In particular, the antiserum produced against STE agglutinated erythrocytes loaded both with STH and with STE in the same dilution to 1:3200. After exhaustion of the antiserum with STH it still was capable of agglutinating erythrocytes loaded with STE in dilutions up to 1:100, which indicated that the serum contained antibodies against estrone also. The presence of antibodies against both components of the conjugation product also was confirmed by the ability of the antiserum against STE to inhibit the hormonal action of both STH and

TABLE 3. Results of Study of Estrogenic Activity of Preparations in Rat Uterus Test (M ± m)

Group of animals	Experimental conditions	No. of rats	Wt. of uterus (in mg)	Rel. wt. of uterus/body weight
1	Control	20	21,1±0,6	0,49±0,01
2	Esterone (1 μg)	20	57,8±1,3	1,37±0,03
3	STE (1 μg as estrone)	10	19,7±0,6	0,47±0,01
4	STE (10 μg as estrone)	10	19,0±0,7	0,45±0,02
5	STE (100 μg as estrone)	5	19,2±1,7	0,44±0,02
6	Control	10	18,9±0,8	0,45±0,02
7	Estrone (0.2 μg)	10	47,4±1,5	1,10±0,03
8	Estrone (0.2 μg)+0.8 ml normal rabbit serum	10	45,2±1,2	1,09±0,02
9	Estrone (0.2 μg)+0.8 ml immune against STE	10	27,5±0,8	0,66±0,03

Note. In groups 1-5 these total doses of the preparations were injected in equal fractions subcutaneously during 2 days, and the animals were autopsied 48 h after the beginning of the experiment. In groups 6-9 the preparations were injected by the same method once only and the animals were autopsied 6 h after the injection. The sera were incubated with estrone for 20 h at 4°C. P_{2-5} and $P_{8-9} < 0.001$.

estrone. It must be remembered that although the strongest neutralization of estrogenic action was found in the Eastwood 6-hour test, a tendency toward that effect was also found both in the 48-hour rat uterus test and in the attempt to block endogeneous estrogen production activated by chorionic gonadotropin (CGT) in vivo. In particular, after injection of 0.5 unit CGT subcutaneously on two successive days into sexually immature rats 2 h after intraperitoneal injection of 0.5 ml normal rabbit serum or of immune serum against STE the following values were obtained for the weight of the uterus and the ratio between the weight of the uterus and the body weight (ten animals in each group): 1) control, 20.0 ± 1.3 ml and 0.54 ± 0.01 respectively; 2) CGT+normal serum, 62.7 ± 4.8 mg and 1.58 ± 0.1; 3) CGT+immune serum to STE, 46.2 ± 5.0 mg and 1.18 ± 0.12 ($P_{2-3} < 0.05$).

Conjugation of steroid estrogens with proteins, particularly with bovine or human serum albumin, converts them into complete antigens [5, 8, 9] and at the same time deprives them of their hormonal action [10]. In the present investigation a hormonally inactive conjugation product of estrone with a hormonal carrier protein (human STH) was obtained for the first time. Although the results of the investigation showed that STE is not homogeneous during chromatography on Sephadex or during ultracentrifugation, the fact that the end product possesses no hormonal activity although it preserves the immunological features of STH and estrone, is evidence in the writers' opinion that conjugation took place successfully. Besides the increase in the antigenic properties of the steroid, immunization with this preparation (the anahormone-chimera) enables action to be taken simultaneously against various endocrine glands and the homeostatic systems (energy metabolism and reproductive system) of the body. This factor, combined with the hypotheses expressed previously [1, 2], affords wide prospects for the conjugation of unlike and like hormones, as combinations both with each other and with certain tissue (including tumor) antigens, a result which could have considerable theoretical and practical importance.

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