LIPID-MOBILIZING, AND LACTOGENIC EFFECTS OF SOMATOTROPIC HORMONE BY ANTISERUM AGAINST THE ANAHORMONE OF HUMAN PLACENTAL LACTOGEN

V. M. Dil'man, L. M. Bershtein, V. N. Golubev, and I. G. Kovaleva

UDC 612.433'65.014.46:615.373.3

The azo-derivative of human placental lactogen (HPL) loses its specific hormonal action but preserves its original antigenic characteristics, so that it can be classed as an anahormone-antigen. Antiserum against the anahormone can block the growth, lipid-mobilizing, and lactogenic effects of human somatotropic hormone. It is thus possible, in principle, to use active immunization with the azo-derivative of HPL in order to neutralize the biological action of endogenous somatotropic hormone in a number of pathological conditions and, in particular, in cancer, senile diabetes, and atherosclerosis.

A disturbance of the regulation of somatotropic hormone (SH) and its production in excess is known to lie at the basis of several pathological conditions and to contribute to the development of certain malignant neoplasms [3, 4, 6]. Immunological intervention is an important measure aimed at neutralizing the pathogenic effects of SH [5, 8, 9, 17]. It was shown previously that there is the greatest justification from this point of view for the use of SH anahormone-antigens for active immunization. These are preparations which, through chemical modification, have lost the hormonal properties of SH and have acquired additional antigenic determinants essential for overcoming the natural tolerance to SH [7].

Taking into account the antigenic, physicochemical, and biological similarity between SH and human placental lactogen (HPL) [16, 20], and the readier availability of placentas (compared with pituitary glands), in the investigation described below an attempt was made to obtain anahormone-antigen on the basis of HPL with a view to neutralizing the chief hormonal effects of SH.

EXPERIMENTAL METHOD

HPL was obtained by the method described previously [1], and SH was isolated from pituitary glands by the method of Raben [18]. Azo-coupling of HPL to 25% saturation with diazotized novocainamide was carried out by the method of Golubev et al. [2]. The growth and lipid-mobilizing activity of SH was studied by the tibial test [10] and by changes in the concentration of free fatty acids (FFA) in the blood [13] respectively, and the lactogenic activity of SH and HPL was studied by the intraductal test on the mammary glands of pseudopregnant rabbits [12]. The rabbits were immunized with azo-HPL by subcutaneous injection of 15 mg of the preparation into the region of the axillary and inguinal lymph glands, mixed with Freund's complete adjuvant, at intervals of 10-14 days. The titer of antibodies against SH and against a standard preparation of HPL ("placental lactogen, human"; National Institute for Medical Research, London) was determined by Boyden's passive hemagglutination test [11]. Antiserum obtained 7-10 days after the 8th-9th injection of antigen was used for the biological neutralization test.

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. 73, No. 4, pp. 68-71, April, 1972. Original article submitted October 6, 1971.

TABLE 1. Effect of Antiserum Against Anahormone of Human Placental Lactogen (azo-25%-HLP) on Growth Activity of SH in the Tibial Test

Group	No. of rats	Width of tibial cartilage (in μ)	Change in percent
I. Control	9	293,3±7,8	—
II. Hydrocortisone (1.5 mg × 3)	9	164,5±4,1	—44,0 (compared with I)
III. Hydrocortisone + [SH (0.2 mg × 3) + normal rabbit serum]	10	208,0±4,4	+26,8 (compared with II)
IV. Hydrocortisone + [SH (0.2 mg × 3) + antiserum against azo-25%-HPL]	12	166,8±2,4	+1,4 (compared with II)

Notes. 1) SH was incubated with sera for 20 h at 4°C at the rate of 0.25 ml serum to 0.2 mg hormone. 2) titer of antibodies against SH in antiserum, produced to azo-25%-HPL, was 1:8000.

TABLE 2. Effect of Antiserum Against Anahormone of Human Placental Lactogen (azo-25%-HPL) on Lipid-Mobilizing Activity of SH in Rabbits

Preparation	Concentration of FFA (in μeq/liter)			
	fasting state	after 30 min	after 60 min	after 120 min
SH [6]	291,7±31,1	1004,5±138,4	802,1±135,5	519,0±121,3
SH + antiserum				
SH + antiserum [8]	287,1±22,8	351,9±45,4	311,5±13,6	293,9±31,1
P	>0,1	<0,01	<0,01	>0,05

Notes. 1) Number of experimental animals given in parentheses. 2) SH injected intravenously in dose of 1.5-2 mg. 3) SH incubated with antiserum against azo-25%-HPL for 20 h at 4° and product injected 2-3 days after experiment with native SH at the rate of 0.5-0.6 ml serum to 1 mg SH. 4) Titer of antibodies against SH in antiserum formed against azo-25%-HPL was 1:4000=1:8000.

EXPERIMENTAL RESULTS

Preliminary experiments showed that 50-100% saturation of HPL with diazotized novocainamide leads to partial loss of its initial antigenic properties. On the other hand, at 25% saturation the original antigen characteristics of the HPL were preserved, while at the same time the preparation had lost its lactogenic activity. On this basis, the 25% azo-derivative of HPL was classed as an anahormone-antigen and used in the subsequent work. The antiserum obtained as the result of immunization of rabbits with this anahormone had the ability to neutralize the growth, lipid-mobilizing, and lactogenic activities of SH and also the lactogenic activity of HPL (Tables 1 and 2; Fig. 1).

Despite the purely relative immunochemical similarity between SH and HPL [16, 19], antiserum against the azo-derivative of HPL thus inhibits the chief biological effects of SH. On this basis, and because the azo-derivative possesses no hormonal action of its own, the HPL anahormone can be recommended for the use in active immunization aimed at neutralizing the action of SH in man in several pathological conditions and, in particular, in cancer, in senile diabetes mellitus, and in atherosclerosis

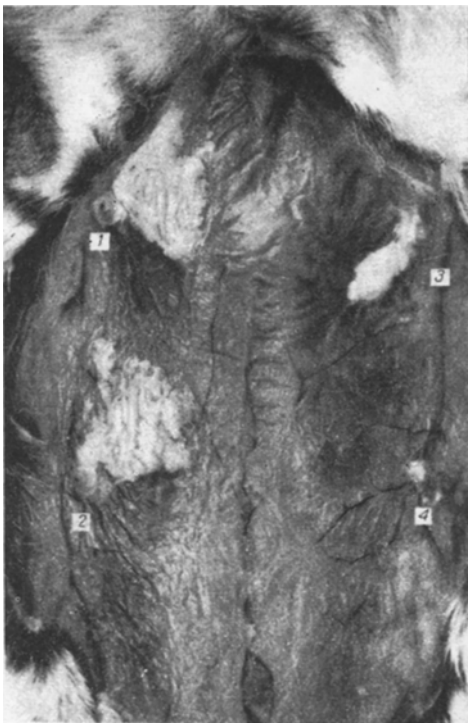

Fig. 1. Effect of antiserum against azo-derivatives of human placental lactogen (azo-25%-HPL) on lactogenic activity of SH and HPL. Hormones incubated with sera for 20 h at 4°C. Incubated material injected intraductally into mammary glands of pseudopregnant (treated with chorionic gonadotropin) rabbits. After 7 days the animals were sacrificed and the effect was assessed by the presence and intensity of formation of milk at the sites of injection of the preparations: 1) 200 μg SH + 0.4 ml normal rabbit serum; 2) 100 μg HPL + 0.4 ml normal rabbit serum; 3) 200 μg SH + 0.4 ml antiserum against azo-25%-HPL (titer against SH 13,200); 4) 100 μg HPL + 0.3 ml antiserum against azo-25%-HPL (titer against SH 13,200).

[6]. In addition, in future it may prove useful to investigate this preparation experimentally, and later clinically, in certain metabolic disturbances observed in pregnancy.*

LITERATURE CITED

1. L. M. Bershtein, I. G. Kovaleva, and V. N. Golubev, Lab. Delo, No. 12 (1971).
2. V. M. Golubev, V. M. Dil'man, I. G. Kovaleva, et al., Dokl. Akad. Nauk SSSR, 184, 966 (1969).
3. V. M. Dil'man, Vopr. Onkol., No. 6, 45 (1970).
4. V. M. Dil'man, Kazansk. Med. Zh., No. 4, 3 (1970).
5. V. M. Dil'man, Aging, The Menopause, and Cancer [in Russian], Leningrad (1968).
6. V. M. Dil'man, Lancet, 1, 1211 (1971).
7. V. M. Dil'man, L. N. Bulovskaya, I. A. Vasil'eva, et al., in: Proceedings of a Conference to Review the Work of the Cancer Research Institute [in Russian], Leningrad (1970), p. 48.
8. V. M. Dil'man and B. N. Sofronov, Vopr. Onkol., 11, 109 (1966).
9. A. F. Lazarev, Vestn. Akad. Med. Nauk SSSR, No. 12, 82 (1969).
10. I. A. Éskin and N. V. Mikhailova, Byull. Éksperim, Biol. i Med., No. 3, 103 (1959).
11. S. V. Boyden, J. Exp. Med., 93, 107 (1951).
12. A. Chadwick, J. Endocrinol., 27, 253 (1963).
13. V. Dole, Proc. Soc. Exp. Biol. (New York), 93, 532 (1956).
14. A. El Tomi, L. Boots, and V. Stevens, Endocrinology, 87, 1181 (1970).
15. A. El Tomi and L. Boots, Am. J. Obstet. Gynec., 109, 74 (1971).
16. D. B. Grant and S. L. Kaplan, Acta Endocrinol. (Copenhagen), 63, 736 (1970).
17. T. Hajashida, Endocrinology, 69, 1036 (1961).
18. M. Raben, Recent Progress in Hormone Research, 15, 71 (1959).
19. H. A. Selenkow, B. N. Saxena, and K. Emerson, in: A. Pecile and C. Finzi (editors), International Symposium on Foeto-Placental Unit, Amsterdam (1969), p. 340.
20. L. M. Sherwood, Proc. Nat. Acad. Sci. (Washington), 58, 2307 (1967).

*Bearing in mind the fact that atrophy of the uterus and ovaries has been observed after active immunization of rabbits and rats with native HPL [14, 15], it seems likely that the anahormone consisting of the azo-derivative of HPL should be able to influence not only the homeostasis of energy metabolism, but also reproductive homeostasis.