THE EFFECTS OF DESIALYLATION ON THE BIOLOGIC AND IMMUNOLOGIC ACTIVITY OF HUMAN PITUITARY LUTEINIZING HORMONE

Glenn D. Braunstein, Leo E. Reichert, Jr.*, Eylard V. Van Hall, Judith L. Vaitukaitis, and Griff T. Ross

Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014, and *Department of Biochemistry, Division of Basic Health Sciences, Emory University, Atlanta, Georgia 30322

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SUMMARY

A highly purified preparation of human pituitary luteinizing hormone was treated with neuraminidase resulting in 94% removal of the sialic acid moiety. This led to a marked loss of biologic activity as measured by the ventral prostate weight and ovarian ascorbic acid depletion assays, and enhancement of the radioimmunologic activity.

INTRODUCTION

The biologic activity of human luteinizing hormone (HLH) has been reported to be unaffected by removal of sialic acid (desialylation) (1,2), while the biologic activity of highly purified human chorionic gonadotropin (HCG) was reduced to less than 1% by complete desialylation with neuraminidase (3). We attempted to utilize this supposed differential effect of desialylation on the biologic activities of the two hormones to discriminate between HCG and HLH in urinary extracts thought to contain both. In preliminary experiments utilizing crude urinary and pituitary HLH preparations treated with a neuraminidase devoid of proteolytic activity, we observed an unexpected marked loss of biologic activity. Subsequently, a highly purified pituitary HLH preparation was treated with neuraminidase to remove sialic acid and the results of biologic and immunologic assays of the desialylated hormone are reported herein.

MATERIALS AND METHODS[1]

HLH Preparations: Partially purified HLH was derived from acetone-dried

[1]The purified HLH was prepared and desialylated in Atlanta, and the biologic and radioimmunoassays were performed in Bethesda.

human pituitary glands by ammonium sulfate extraction and fractionation, followed by ion-exchange chromatography on DEAE-cellulose at pH=8.0 (4). A fraction equivalent to F-1-2 of that reported was then rechromatographed on DEAE-cellulose in 0.1M glycine buffer at pH=9.5 (5), followed by gel filtration through Sephadex G-100 (6). The final product, LER 1486-2, was used as the starting material for these studies.

Neuraminidase Preparations: Purified HLH was desialylated with preparations of Clostridium perfringens neuraminidase (Sigma Chemical Company, St. Louis, Mo.) which was shown to be free of chymotrypsin activity when examined by the acetyl-L-tyrosine ethyl ester hydrolysis method of Schwert and Takenaka (7). HLH is known to be particularly susceptible to inactivation by this type of enzyme (8). The enzymic activity of the neuraminidase was checked prior to each hormone digestion using neuramin-lactose as a substrate. The commercial source of the neuraminidase did not influence the percentage of residual activity remaining after desialylation of several HLH preparations.

Desialylation Procedure: 30.05 mgm of LER 1486-2 were dissolved in 0.1M acetate buffer, pH=6.1, 0.93 mgm of C. perfringens neuraminidase (Sigma, Type V, 0.18 units/mgm) added to the hormone solution giving a ratio of mgm HLH:units enzyme of 179, and the mixture incubated at 37° for one hour. Two samples (LER 1486-2a) containing one milligram of protein were removed for N-acetylneuraminic acid (NANA) determination by the Warren method (9). The remaining solution was dialyzed and lyophilized (LER 1486-$2a_1$). The total recovery was 86% by weight.

Duplicate determinations of NANA were performed by the Warren procedure (9) following incubation of two aliquots containing one milligram of LER 1486-2 in 0.1 N sulfuric acid for one hour at 80°C. Similar samples of LER 1486-$2a_1$ were subjected to the same procedure.

A second enzyme digestion utilizing C. perfringens neuraminidase (Sigma, Type VI, 3.5 units/mgm) was performed on 23.38 mgm of LER 1486-$2a_1$ under the conditions described above, but with an HLH:units enzyme ratio of 30:1. Again,

two one milligram aliquots of the digested material were subjected to NANA determinations (LER 1486-2b) and the remaining solution dialyzed and lyophilized (LER 1486-$2b_1$) yielding 82% recovery by weight. The NANA content of the latter preparation was determined after sulfuric acid digestion as described above.

Bioassays: Biologic activity of the hormonal preparations were determined using the McArthur modification (10) of the ventral prostate weight assay (VPW) of Greep (11) and the ovarian ascorbic acid depletion assay (OAAD) of Parlow (12), as previously described (3). Five rats were used at each dose level in 2 x 2, 2 x 3, or 3 x 3 parallel line graded response assays. Assays were performed on aliquots of the same hormone solutions. LER 907, standardized against the Second International Reference Preparation of Human Menopausal Gonadotropin, was used as the reference preparation in all assays.

Radioimmunoassays: The double antibody radioimmunoassay for HLH as described by Odell, Ross, and Rayford (13) was used with LER 907 serving as the reference preparation.

Statistical Analysis: The methods of Brownlee (14) were applied to each individual assay for evaluation of parallelism of dose-response curves, calculation of relative potency, and 95% confidence limits. The method of Finney (15) was utilized to calculate the combined mean potencies and 95% confidence limits from valid assays.

RESULTS

NANA Analysis: Table I summarizes the results of NANA determinations made on the starting material, LER 1486-2, and the 66% and 94% desialylated preparations. It should be noted that the quantity of NANA present in the initial preparation, 2.3%, corresponds well with the values obtained by others (16-19).

Bioassays: Preliminary OAAD assays performed in Atlanta (LER) showed no significant differences in potency of the 66% and 94% desialylated preparations, both having residual activity of 15-20%. Table II gives the results of four VPW and three OAAD assays of LER 1486-2 and 1486-$2b_1$ performed at Bethesda. Aliquots of the same solutions were used in the OAAD and VPW assays.

TABLE I

Summary of NANA Analysis

Preparation	NANA[1] Released by Sulfuric Acid	% NANA[2]	% Desialylation	NANA[1] Released by Enzyme	Ratio mgm LH Units Enzyme
LER 1486-2 (control)	0.074	2.3	0	--	--
LER 1486-2a (1486-2 after first enzyme digestion)	--	--	--	0.084	179
LER 1486-$2a_1$ (1486-2a lyophilized and submitted to acid hydrolysis)	0.025	0.78	66%	--	--
LER 1486-2b (1486-2 after enzyme digestion, lyophilization and a second enzyme digestion)	--	--	--	0.034	30
LER 1486-$2b_1$ (1486-2b lyophilized)	0.005	0.14	94%	--	--

[1] Micromoles of NANA per mgm LH
[2] Assuming 100% release. Since apparently not all NANA is released, this is a minimum figure

The discrepancy in the potency estimates of HLH as measured in the OAAD and VPW assays has been previously noted, and occurs with several different luteinizing hormone preparations from both human and non-human sources (20,21). Immunoassay: The specific activities of 1.0 nanogram (ng) of LER 1486-2 and LER 1486-$2b_1$ were equivalent to 46.0 and 65.8 ng of LER 907 respectively, indicating that the immunoreactivity of HLH was significantly enhanced (143.7% residual activity) after 94% desialylation.

DISCUSSION

Several points in the present work warrant comments. First, significant

TABLE II

Results of OAAD and VPW Assays of Fully Sialylated and 94% Desialylated Human Pituitary Luteinizing Hormone

Preparation	Assay	Specific Activity (IU/mgm) (95% Confidence Limits)	# Assays	% Residual Activity
LER 1486-2 (control)	OAAD	3546 (2528-5525)	3	100.0%
LER 1486-2b_1 (1486-2 after enzyme digestion, lyophilized and second enzyme digestion)	OAAD	573 (434-797)	3	16.2%
LER 1486-2	VPW	1910 (1536-2374)	4	100.0%
LER 1486-2b_1	VPW	238 (190-293)	4	12.5%

loss of biologic activity occurs after desialylation of HLH by neuraminidase. This decreased bioreactivity may be due to increased metabolic clearance of the HLH after the removal of sialic acid, as has been shown to occur for HCG, follicle stimulating hormone, and other glycoproteins after desialylation (22). The reasons for the discrepancy in the amount of biologic activity remaining after desialylation reported herein and those previously reported (1,2) are not apparent. It is noteworthy that these earlier reports did not include measurement of the amount of sialic acid removed. Second, the immunoreactivity of HLH, tested by a double antibody method of radioimmunoassay using an antiserum made against HCG, is enhanced almost 1½ times by the removal of sialic acid. Mori, using complement-fixing techniques did not find similar augmentation by desialylation (2), but did find increased activity of chymotrypsin-treated HLH (23). Desialylation of other hormones has yielded variable results. The immunoreactivity of desialylated HCG does not change (3), while recent studies on human urinary FSH have shown augmentation of immuno-

reactivity and loss of biologic activity after desialylation with neuraminidase (24). The enhancement of immunoreactivity of pituitary HLH after the removal of sialic acid may be due to the uncovering of immunoreactive sites. Last, the divergent potency estimates between the immunologic and biologic assays of multiple purified HLH preparations (25) may be due to partial desialylation of some of these preparations during their purification.

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