Proliferative Response of Cultured Nb2 Rat Lymphoma Cells to Human Serum Prolactin and Growth Hormone

A. A. Bulatov, T. A. Osipova, and G. V. Raevskaya

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Human prolactin and growth hormone induce specific proliferative responses in a culture of Nb2 rat lymphoma cells. These responses can be employed for characterization of the activity of serum immunoreactive prolactin and growth hormone in patients with endocrine disorders.

Key Words: *prolactin; growth hormone; blood serum; Nb2 lymphoma cells; proliferation*

Prolactin (PL) and growth hormone (GH) are structurally, evolutionarily, and functionally related protein hormones secreted by hypophysis. They are the major endocrine regulators of processes associated with reproduction and growth. Both hormones are species-specific. The receptors for PL, GH, and cytokines belong to the superfamily of molecules with specific conserved elements [9,10]. Only human GH is capable of binding to PL receptors (lactogenic receptors) and inducing some biological effects typical of PL.

Immunochemical methods have found a wide application in experimental and clinical endocrinology for quantitative and qualitative characterization of PL and GH. However, immunochemical analysis does not always provide adequate information regarding physiological activity of circulating hormones [11,13]. This may be due to molecular polymorphism of PL and GH. Since several isoforms of PL and GH differing in immunological and biological activities may be involved in endocrine regulations and its disorders, not only immunochemical but also biological methods should be used for the characterization of PL and GH circulating in the blood and other biological fluids [1,2,12].

Due to low specificity and sensitivity of conventional methods they cannot be used for the detection of GH and PL in blood serum. Therefore, Nb2 rat lymphoma cells bearing specific lactogenic receptors and responding to physiological concentrations of PL or other lactogenic hormones by increased proliferation [8,14] can be employed as a test system for the investigation of these hormones. It was shown that PL stimulates the transcription of genes responsible for cell growth in this culture [15].

The aim of this study was to explore the possibility of using the proliferative responses of Nb2 cells for evaluating the functional activity of serum PL and GH from patients with endocrine disorders and comparing it with the immunoreactivity of these hormones.

MATERIALS AND METHODS

Rat lymphoma cell line Nb2 was cultured as described elsewhere [8,13,14]. Suspension of Nb2-11C cells sensitive to lactogenic hormones was cultured for 2-3 passages in 50-ml plastic flasks (Nuclon) in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum (FCS, Flow), 10% horse serum (Flow), 0.1 mM 2-mercaptoethanol (Sigma), 10 mM MOPS (Sigma), 50 U/ml penicillin (Flow), and 20 mg/ml streptomycin (Flow). Mycoplasmic contamination was controlled with the use of fluorescent dye [7]. For synchronization of proliferation, 24 h before experiment the cells were washed with FCS-free

Laboratory of Protein Hormones, Endocrinology Research Center, Russian Academy of Medical Sciences, Moscow

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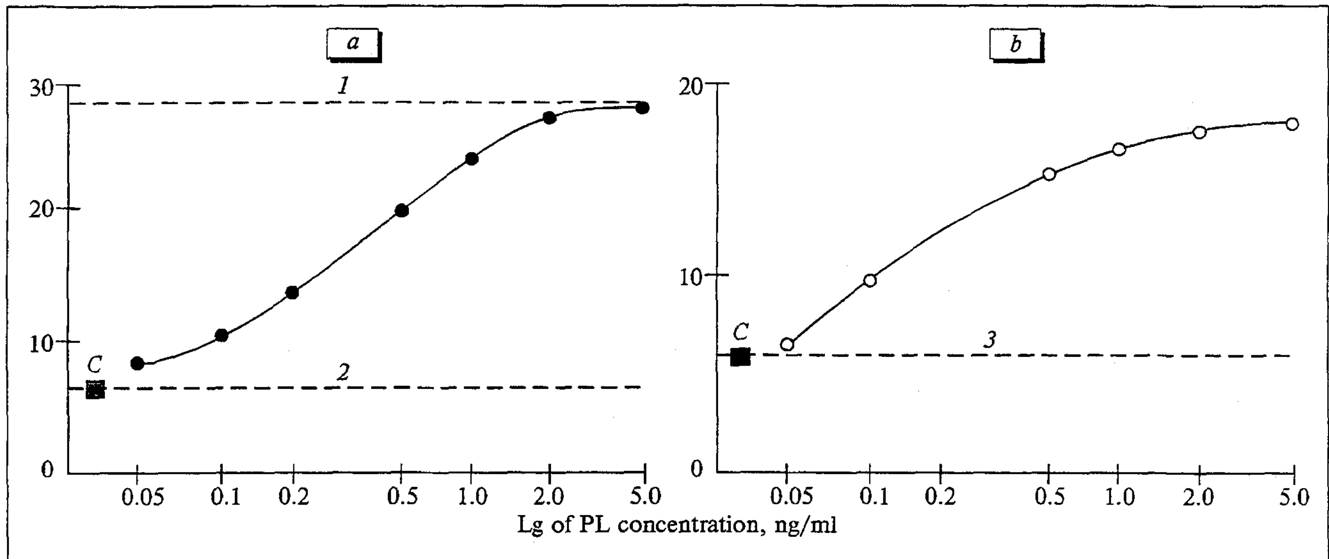

Fig. 1. Proliferation rate of Nb2 rat lymphoma cells as a function of the concentration of highly purified human prolactin (PL, *a*) or growth hormone (GH, *b*). *1*) 10% fetal calf serum; *2*) PL+anti-PL monoclonal antibody; *3*) GH+anti-GH monoclonal antibody. *C*) control. Here and in Fig. 2: ordinate: number of cells/ml culture medium, $*10^{-4}$ after 64 h of incubation with hormone. Each point represents the mean of three determinations.

growth medium and seeded in 24-well plates at a density 6×10^4 cells/well in a final volume of 1 ml. The volume of test sample was not higher than 50 µl. Final concentration of human serum in a well was lower than 5%, since the growth of Nb2 lymphoma cells is suppressed by higher serum concentrations [14]. Determinations were performed in three parallel samples. Wells with an equal volume of RPMI-1640 served as a negative control. Wells with growth medium containing 10% FCS served as a positive control. After a 64-h incubation, the intensity of proliferation was assessed by counting cells in the Goryaev chamber.

Highly purified laboratory preparations of human and bovine GH and PL were used in the study. Immunological activity of PL and GH was 30 and 2 U/mg, respectively. Sera were obtained from the Department of Neuroendocrinology and from the Pediatric Department of the Endocrinology Research Center. Serum contents of immunoreactive PL (iPL) and GH (iGH) were determined by radioimmunoassay using test systems based on highly purified hormones and monospecific antisera (Laboratory of Protein Hormones). In some experiments, proliferative activity of PL and GH was assayed in the presence of monoclonal antibodies (MAB, 1:1500) to these hormones [3-5].

RESULTS

The effects of human and bovine PL and GH purified from the pituitary gland were studied on Nb2 rat

TABLE 1. Content of Biologically Active Prolactin (bio-PL) in Sera of Women with Hyperprolactinemia of Various Severity Determined by Proliferative Response of Serum Immunoreactive Prolactin (iPL) in a Culture of Nb2 Rat Lymphoma Cells

Patient No.	Serum iPL		Added to wells		Cell number/ml medium, $\times10^{-4}$ after 64 h of incubation	Serum bio-PL, ng/ml
	µU/ml	ng/ml	serum, µl	iPL, ng/ml		
1	2983	99.4	8.00	0.8	21.0	93.7
2	6210	207.0	3.90	0.8	21.8	217.8
3	10065	335.5	2.40	0.8	21.6	333.0
4	53354	1778.0	0.45	0.8	21.8	1820.0
5	54680	1822.7	0.42	0.8	21.8	1920.0
6	2213	73.8	16.30	1.2	22.0	61.3
7	3000	100.0	12.00	1.2	22.0	91.7
8	3044	101.5	11.80	1.2	22.0	93.2

TABLE 2. Content of Immunoreactive (iGH) and Biologically Active Growth Hormone (bio-GH) in Sera of Children with Idiopathic Dwarfism Determined by Proliferative Response of Serum Immunoreactive Prolactin (iPL) in a Culture of Nb2 Rat Lymphoma Cells Before and 15 and 90 min After Administration of the GH-Stimulating Peptide (GHRP-2)

Patient No.	Time after peptide administration, min	iGH, ng/ml	Volume of serum added to well, μl	Cell number/ml medium, $\times 10^{-4}$ after 64 h of incubation	Bio-GH, ng/ml
1	0	1.9	25	8.4	1.8
	15	94.3	25	21.4	85.8
	90	19.8	25	10.4	8.0
2	0	1.4	50	8.6	2.0
	15	30.0	50	15.5	23.5
	90	0.9	50	7.0	0.2
3	0	<0.2	50	6.0	<0.1
	15	25.4	50	14.5	22.8

lymphoma cells. The hormones were added to FCS-free culture medium to a final concentration of 0.01-10 ng/ml. The number of cells remained unchanged in synchronized cell cultures (a 64-h incubation in FCS-free medium with horse serum) and increased in the presence of 0.05 ng/ml human PL (Fig. 1, *a*). The proliferation rate reached the maximum (close to that upon culturing in the presence of FCS) at 2-5 ng/ml PL and remained at this level at 10 ng/ml PL. The response of Nb2 cells to human PL was dose-dependent in the 0.05-2 ng/ml concentration range. The proliferation rate in the presence of 0.05-5 ng/ml bovine PL was similar to that in the presence of the same concentrations of human PL. Human GH, which exhibits lactogenic activity, stimulated the proliferation of cultured Nb2 cells in a dose-dependent manner (Fig. 1, *b*). The stimulatory effect was observed at 0.05 ng/ml and reached the maximum at 5 ng/ml human GH. Experiments with bovine GH, which does not display lactogenic activity, confirmed the fact that cultured Nb2 cells proliferate only in the presence of hormones with lactogenic activity [8,14]. Bovine GH had no effect on the proliferation of these cells even at the highest concentration (10 ng/ml).

The proliferative effect of human PL and GH was blocked by anti-GH and anti-PL MAB in the whole concentration range (Fig. 1, *a*, *b*). However, hormonal effect was not abolished by a simultaneous addition of human PL with anti-GH MAB but not with anti-PL MAB (and vice versa). The monoclonal antibodies (1:1500) had no appreciable effect on cell growth.

The fact that the proliferative response of Nb2 cells to maximally effective concentrations of human PL and GH is abolished by the corresponding MAB implies a possibility of individual identification of these hormones in a mixture.

The proliferative response of Nb2 cells to serum PL was studied with the use of sera from women with the classic hyperprolactinemic syndrome (n=8) manifesting itself as galactorrhea, menstrual disorders, and infertility. The content of iPL in these sera varied in a wide range (Table 1). Gel-filtration [2] showed that in all these sera the monomeric isoform (Mr=23 kD), which predominates in the hypophysis, comprised 70-90% of the total iPL content. Since the content of GH in these sera was low (<2 ng/ml) relative to that of PL, GH did not modify the proliferative response of Nb2 cells to PL.

Two profiles of the proliferative activity of serum iPL (0.2, 0.4, and 0.8 ng/ml culture medium) are shown in Fig. 2. Sera were diluted with FCS-free culture medium. The dose-dependence of the proliferative response to iPL was similar to that of the response to purified hypophyseal human PL.

In all studied sera, the content of biologically active PL, which stimulates proliferation of Nb2, is consistent with that of iPL (Table 1).

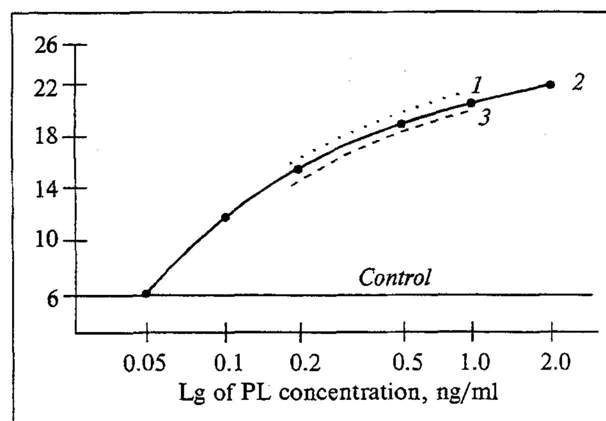

Fig. 2. Proliferative effects of immunoreactive prolactin (PL) in two blood sera added in concentrations 0.2, 0.4, and 0.8 ng/ml to culture medium (*1*, *3*) and highly purified human PL (*2*) in a culture of Nb2 rat lymphoma cells.

The relationship between proliferative activity of serum GH in a culture of Nb2 cells and its immunoreactivity was studied with the use of sera from children with idiopathic dwarfism (n=3) treated with the growth-stimulating peptide GHRP-2 [6]. This peptide induces rapid release of GH from the hypophysis without any effect on the secretion of PL and other hypophyseal hormones. The peptide was administered intravenously in a dose of 1 μg/kg body weight in order to evaluate the somatotropic reserve of the hypophysis (Table 2).

In all patients, serum content of biologically active GH correlated with that of iGH. A considerable increase in the contents of iGH and biologically active GH was observed 15 min after administration of GHRP-2. The contents of both immunoreactive and biologically active GH decreased 90 min after administration of GHRP-2.

Proliferative activity of serum PL and GH was completely suppressed by the corresponding MAB.

Thus, our results indicate that human PL and GH induce specific proliferative responses in Nb2 rat lymphoma cells sensitive to lactogenic hormones. This response can be employed as a parameter of functional activity of immunologically reactive forms of these hormones circulating in the serum. The use of this model in combination with various physical and chemical factors opens new prospects in the investigation of molecular polymorphism of human GH and PL as well as the physiological, pathogenic, and diagnostic significance of these hormones.

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