Glucocorticoid-Prolactin Interactions in Nb2 Lymphoma Cells: Antiproliferative versus Anticytolytic Effects (43545)

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Abstract. **The interaction between the immunosuppressive effects of glucocorticoids and the mitogenic effects of prolactin (PRL) were examined in Nb2 lymphoma cells, a pre-T cell line. The synthetic glucocorticoid, dexamethasone (Dex), caused a concentration-dependent (6.25–200 n*M*) inhibition of basal and ovine PRL (oPRL)-stimulated Nb2 cell proliferation. Although Dex was antiproliferative, the steroid had no effect on cell viability in the presence of PRL. However, when PRL was omitted from the medium, Dex increased the proportion of dead Nb2 cells by 24 hr in a concentration (25–200 n*M*)-dependent fashion without affecting total cell number. The antiproliferative and cytolytic effects of Dex were mimicked by other corticosteroids (cortisol, corticosterone, aldosterone, and deoxycorticosterone) in the expected order of glucocorticoid potency, but not by other steroids (17-β-estradiol, progesterone, testosterone, 5-α-dihydrotestosterone, and dehyhroepiandrosterone) or triiodothyronine. In addition, the antiproliferative and cytolytic effects of glucocorticoids were antagonized by the glucocorticoid receptor antagonist RU 486.**

Since corticosteroid-induced cytolysis was apparent only in the absence of mitogen, the anticytolytic effects of oPRL were tested. In the presence of Dex (100 n*M*), oPRL (25–1600 pg/ml) caused a concentration-dependent inhibition of cytolysis without changing cell number. Other lactogenic hormones (human growth hormone, human placental lactogen, rat PRL), but not trophic nonlactogenic hormones (rat growth hormone, human chorionic gonadotropin, ACTH), also inhibited Dex (100 n*M*)-induced cytolysis. Agarose gel electrophoresis of DNA extracted from Nb2 cells revealed that within 12 hr, 100 n*M* Dex induced DNA fragmentation, indicative of programmed cell death or apoptosis. Coincubation of cells with Dex and oPRL (1 ng/ml) inhibited Dex-induced fragmentation of Nb2 cell genomic DNA.

These studies reveal a complex interaction between glucocorticoids and PRL in Nb2 cells. Although a glucocorticoid receptor-mediated antiproliferative effect is evident, PRL (at concentrations that usually stimulate cell proliferation) has the capacity to protect the cell against glucocorticoid-receptor-mediated induction of apoptosis.

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The Nb2 lymphoma cell line was developed in an estrogen-treated male rat (1) and, on the basis of surface antigens expressed, determined to be of thymic origin and in an undifferentiated stage of development (2). Proliferation of the Nb2 cell line in culture occurs in the presence of lactogenic hormones (3). The sensitivity and specificity of the Nb2 cell line for lactogenic hormones make it a reliable *in vitro* bioassay system for the measurement of serum lactogens (4), as well as a model for the study of lactogenic hormone signal transduction (5–10).

Glucocorticoid hormones are known to have suppressive effects on normal and neoplastic lymphoid cells by such processes as inhibition of mitogenesis and initiation of programmed cell death or apoptosis (11–14). If the Nb2 cell line were glucocorticoid responsive, this cell could prove to be a useful model to study the

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interaction between two distinct hormone modalities, glucocorticoids and prolactin. However, lymphoid neoplasms can be either glucocorticoid responsive or glucocorticoid resistant (11).

Therefore, the purpose of this investigation was to determine whether Nb2 cells exhibited glucorticoid-receptor-mediated responses and, if so, to examine glucocorticoid-prolactin interactions. This paper not only demonstrates that the Nb2 cell is a target for glucocorticoid-receptor-mediated effects, but also describes a complex interaction between glucocorticoids and one if its mitogens, prolactin (PRL).

A preliminary report of these findings has appeared in abstract form (15).

Materials and Methods

Hormones. Several pituitary and placental protein/peptide hormones (ovine prolactin [oPRL S-15], human growth hormone [hGH-I-1], human placental lactogen [hPL-iodination grade], human chorionic gonadotropin [hCG CR-121], rat growth hormone [rGH-B-10], and rat PRL [rPRL-I-5]) were provided as a gift by the National Hormone and Pituitary Program. ACTH (corticotropin injection) was obtained from Parke-Davis (Detroit, MI). Dexamethasone (1,4-pregnadiene-9-fluor-16α-methyl-11β,17α,21-triol-3,20-dione), all other steroid hormones, and triiodothyronine (T_3) were obtained from Sigma Chemical Co., St. Louis, MO. RU 486 (11-β-(-4-dimethylaminophenyl)-17β-hydroxy-17α-(prop-1-ynyl)estra-4,9-dien-3-one) was provided by Roussel Uclaf (Paris, France).

Maintenance of Nb2 Lymphoma Cells. Nb2 lymphoma cells were obtained from two sources, Dr. Joseph Liberti, Department of Biochemistry and Molecular Biology, Medical College of Virginia, and Dr. Peter Gout, Cancer Control Agency of British Columbia. The first source (Nb2 clone 2), which was used in the majority of the experiments described herein, was provided to Dr. Liberti from Dr. Robert Corin, Sloan Kettering Institute. The source provided by Dr. Gout was the wild-type cell line and was used to verify key aspects of this study (i.e., those pertaining to glucocorticoid antiproliferative and glucocorticoid cytolytic actions as well as PRL anticytolytic effects).

Cells were maintained in Fischer's medium containing 10% horse serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 0.1 m*M* 2-mercaptoethanol, and supplemented with the mitogen, 10% fetal calf serum (FCS) in a water-saturated atmosphere of 5% CO_2 and 95% room air at 38°C, as reported previously (3, 4). Unless otherwise specified, all tissue culture medium reagents were purchased from Gibco BRL Life Technologies, Grand Island, NY.

Nb2 Cell Proliferation Assay. The Nb2 proliferation assay was performed as described previously with minor modifications using oPRL (1 ng/ml) as the standard mitogen (4). Before the assay, cells were washed twice by pelleting (centrifugation at 300*g*, 5 min, 10°C) and resuspension in stationary medium (maintenance medium devoid of FCS) and then incubated at 1×10^6 cells/ml overnight in stationary medium supplemented with 1% FCS. Cells were then pelleted, washed as above, resuspended in stationary medium, and then added with gentle mixing in volumes of 1.8 ml (6-well plates) or 0.9 ml (24-well plates) to a final cell concentration of 1×10^5 cells/ml to wells containing previously added hormone and/or vehicle.

Hormones dissolved in stationary medium (PRL, T_3) were added to the well before cell addition in a volume that was one tenth that of the final culture volume (0.1 ml for 24-well plates and 0.2 ml for 6-well plates). Steroid hormones and RU 486 (dissolved in 100% dimethylsulfoxide [DMSO]) were added such that the final concentration of DMSO was 0.25% of the culture volume. This final concentration of DMSO was also present in control wells devoid of steroid and/or RU 486. Preliminary experiments indicated that this concentration of DMSO affected neither viability nor PRL responsiveness of Nb2 cells. Cells that were incubated in maintenance medium served as a positive control of cell proliferation (i.e., to test for maximal proliferation) and cells incubated in stationary medium served as a negative control of cell proliferation (i.e., to test for proliferation in the absence of exogenous mitogen). All samples were assayed in duplicate.

The cell cultures were incubated for 72 hr in the same atmosphere as stated above and cell concentrations were determined either by an electronic counter or a hemacytometer (containing 0.04% trypan blue for estimation of cell viability). Preliminary experiments established that a minimum of three counts was both practical and necessary to obtain accurate estimates of cell number and viability by hemacytometry. For electronic cell counting, cells were suspended in Coulter Isoton II balanced electrolyte solution (0.5 ml of culture + 19.5 ml of Isoton), and total cell number was counted twice on a Coulter counter model ZM (Coulter Diagnostics, Hialeah, FL), with the lower threshold set at 0.3 and the upper threshold set at 55.2. At these settings, electronic cell counts were consistent with total cell counts obtained by hemacytometry.

Nb2 Cell Cytolytic Assay. Nb2 cells were maintained as described above and used when the cell concentration reached 1×10^6 cells/ml. At the time of the assay, cells washed as above were resuspended in stationary medium. Peptide hormone, T_3, steroid hormones, RU 486, or their respective vehicles were added to a 6-well tissue culture plate as stated above. The cell suspension was added to the wells after the hormones to a final concentration of 1×10^6 cells/ml and mixed gently by refluxing. A well with vehicle only (0.25% DMSO) served as the control for baseline cell viability.

The cell cultures were routinely incubated for 24 hr, based on preliminary experiments, for optimal

quantitation of cell viability. Cell viability was then estimated by trypan blue exclusion with the aid of a hemacytometer as described above. Each sample was counted three times and an average count of living and dead cells was calculated.

DNA Fragmentation Assay. Nb2 cells were grown to a concentration of 1×10^6 cells/ml in maintenance medium then pelleted by centrifugation at 300*g*, 10°C, for 5 min and washed twice by resuspension in stationary medium. Cells were resuspended in stationary medium at a final concentration of 1×10^6 cells/ml. A 30-ml culture was incubated with hormone(s) or vehicle in a 75-cm^2 tissue culture flask for the desired time. PRL or vehicle was added in a 3-ml volume (10% of the final culture volume). Steroid hormone(s) dissolved in DMSO or vehicle was added in a 75-μl volume (0.25% DMSO). The cells were pelleted by centrifugation (1000 rpm for 5 min at 10°C) after the desired incubation period and then lysed and processed for DNA analysis by agarose gel electrophoresis, as described previously (12). All reagents used in this procedure were purchased from Sigma and were of a molecular biology grade, except chloroform, which was purchased from Fisher Scientific, Springfield, NJ.

Statistics. Data from individual incubations (i.e., from individual experiments conducted on different days) were used to generate means and SE. Treatments were compared statistically by analysis of variance and Duncan's multiple range analysis (16, 17) using a statistical software package (SPSS Release 4.1).

Results

Antiproliferative Effect of Dexamethasone. Table I shows that dexamethasone (Dex) produced a concentration-dependent inhibition of basal and PRL-stimulated cell proliferation. A statistically significant decrement in cell proliferation in the absence of exogenous PRL was demonstrable at 100 n*M* and 200 n*M* dexamethasone. Cell proliferation observed in the absence of PRL has been attributed to lactogenic activity present in varying degrees in different batches of horse serum, an obligatory constituent of stationary medium (18). In PRL-stimulated cells, a statistically significant effect was demonstrable at 6.25 n*M* dexamethasone and a maximal effect was demonstrable at 100 n*M* and at 200 n*M* dexamethasone. In the presence of oPRL, statistically significant differences were also observed between the various concentrations of dexamethasone (e.g., 6.25 n*M* and 12.5 n*M* at $P < 0.05$; 12.5 n*M* and 25 n*M* at $P < 0.05$; and 25 n*M* and 200 n*M* at $P < 0.01$).

Glucocorticoid Specificity of the Antiproliferative Effect. As shown in Table II, all of the other corticosteroids tested (cortisol, corticosterone, aldosterone, and deoxycorticosterone [DOC]) were also capable of inhibiting mitogenesis produced by oPRL. Those with predominant glucocorticoid activity (Dex and cortisol) required lower doses to suppress mitogenesis or were more inhibitory than those with less glucocorticoid activity and/or higher mineralocorticoid activity (aldosterone and DOC). Steroids without glucocorticoid activity (17-β-estradiol, dehydroepiandrosterone, progesterone, testosterone, and 5-α-dihydrotestosterone) at concentrations of 1 μ*M* (Table II, Experiment 2), as well as T_3 at concentrations of 0.15–1.5 ng/ml (data not shown), had no inhibitory effect on PRL-induced Nb2 cell proliferation.

Table III shows that the glucocorticoid receptor antagonist, RU 486, inhibited the antiproliferative effect of 100 n*M* Dex in a concentration-dependent fashion, with a complete block of Dex effects being achieved with 500 n*M* RU 486 (Experiment 1). Table III also shows that 500 n*M* RU 486 blocked the antiproliferative effects of 1600 n*M* aldosterone without exhibiting inherent glucocorticoid agonist actions (Experiment 2). RU 486 at 500 n*M* also blocked the antiproliferative effects of cortisol and corticosterone (data not shown).

Cytolytic Effect of Dexamethasone. Although Dex inhibited PRL-induced proliferation, there was little evidence of glucocorticoid-induced cell death in the presence of the mitogen after 72 hr. In the experiment depicted in Table I, cell death averaged 13% for PRL-stimulated cells in the presence and absence of 100 n*M* dexamethasone. This suggested either that Nb2 cells were resistant to the cytolytic action of Dex or that the mitogen, PRL, produced a protective (or anticytolytic) effect.

Therefore, the cytolytic effect of Dex was tested by using an assay that involved incubating Nb2 cells with varying concentrations of steroid in Fischer's stationary

Table I. Dose-Related Effects of Dexamethasone on Proliferation of Nb2 Lymphoma Cells[a]

oPRL	Dexamethasone					
	0 n*M*	6.25 n*M*	12.5 n*M*	25 n*M*	100 n*M*	200 n*M*
0 pg/ml	0.54 ± 0.04	0.43 ± 0.02	0.37 ± 0.02	0.33 ± 0.01	0.30 ± 0.01[b]	0.28 ± 0.01[b]
1000 pg/ml	1.94 ± 0.13	1.70 ± 0.11[c]	1.43 ± 0.09[d]	1.21 ± 0.08[d]	1.02 ± 0.03[d]	0.96 ± 0.09[d]

[a] Data are mean ± SE of three individual incubations representing total cell number × 10^{-6}/ml after 72 hr of incubation.
[b] $P < 0.05$ vs 0 pg/ml of oPRL, 0 n*M* dexamethasone.
[c] $P < 0.05$ vs 1000 pg/ml of oPRL, 0 n*M* dexamethasone.
[d] $P < 0.01$ vs 1000 pg/ml of oPRL, 0 n*M* dexamethasone.

Table II. Effect of Steroids on PRL-Induced Proliferation of Nb2 Lymphoma Cells[a]

Experiment	Treatment		Total cell no. (10^{-6} cells/ml)
	oPRL (pg/ml)	Steroid (n*M*)	
1	0	0	0.56 ± 0.04[b]
	1000	0	2.25 ± 0.08
	1000	Dexamethasone (100)	0.74 ± 0.06[b]
	1000	Cortisol (400)	1.13
	1000	Corticosterone (1600)	1.02 ± 0.13[b,d]
	1000	Aldosterone (1600)	1.29 ± 0.11[b-d]
	1000	Deoxycorticosterone (1600)	1.72 ± 0.20[b,c]
2	0	0	0.26 ± 0.03[b]
	1000	0	1.88 ± 0.29
	1000	Dexamethasone (100)	0.43 ± 0.05[b]
	1000	Cortisol (1000)	0.44 ± 0.11[b]
	1000	Corticosterone (1000)	0.64 ± 0.06[b]
	1000	Aldosterone (1000)	1.36 ± 0.58
	1000	Progesterone (1000)	2.11 ± 0.10
	1000	Dehydroepiandrosterone (1000)	2.25 ± 0.07
	1000	Estradiol (1000)	2.54 ± 0.06
	1000	Testosterone (1000)	2.23 ± 0.23
	1000	5α-Dihydrotestosterone (1000)	2.43 ± 0.28

[a] Data are means ± SE obtained from three to five individual incubations. Values for cortisol in Experiment 1 are mean of two individual incubations.
[b] $P < 0.01$ vs o-PRL.
[c] $P < 0.01$ vs o-PRL + dexamethasone.
[d] $P < 0.05$ vs o-PRL + deoxycorticosterone.

Table III. Effect of RU 486 on Corticosteroid Inhibition of PRL-Induced Nb2 Lymphoma Cell Proliferation[a]

Experiment	oPRL (pg/ml)	Corticosteroid (n*M*)	RU 486 (n*M*)	Total cell no. ($\times 10^{-6}$/ml)
1	0	0	0	0.32 ± 0.05[b]
	1000	0	0	2.25 ± 0.29
	1000	Dexamethasone (100)	0	0.47 ± 0.13[b]
	1000	Dexamethasone (100)	31.25	0.95 ± 0.20[c]
	1000	Dexamethasone (100)	62.5	0.99 ± 0.04[c]
	1000	Dexamethasone (100)	125	1.58 ± 0.30[d]
	1000	Dexamethasone (100)	500	2.35 ± 0.68
2	0	0	0	0.56 ± 0.02[e,f]
	1000	0	0	2.35 ± 0.09
	1000	Aldosterone (1600)	0	1.35 ± 0.13[e]
	1000	Aldosterone (1600)	500	2.36 ± 0.17
	1000	0	500	2.35 ± 0.16

[a] Data are mean ± SE of three individual incubations.
[b] $P < 0.01$ vs o-PRL and o-PRL + dexamethasone + RU 486 (500 n*M*).
[c] $P < 0.05$ vs o-PRL and o-PRL + dexamethasone + RU 486 (500 n*M*).
[d] $P < 0.05$ vs o-PRL + dexamethasone.
[e] $P < 0.01$ vs o-PRL, o-PRL + aldosterone + RU 486, o-PRL + RU 486.
[f] $P < 0.01$ vs o-PRL + aldosterone.

medium (i.e., devoid of FCS or other mitogens) and measuring cell viability by trypan blue exclusion. As shown in Table IV, exposure of Nb2 cells to Dex under these incubation conditions was indeed cytolytic. Within 24 hr, Dex produced statistically significant, concentration (25–100 n*M*)-dependent increases in the percentage of dead cells, which is due to an increase in the number of dead cells, a corresponding decrease in the number of living cells without a change in total cell number. Preliminary studies revealed that the first detectable increase in cell death with 25 or 100 n*M* Dex was at 12 hr. The 24-hr time point was selected because there was minimal spontaneous cytolysis (i.e., in the absence of Dex) and maximal Dex-induced cytolysis.

Glucocorticoid Specificity of the Cytolytic Effect. Naturally occurring corticosteroids produced concentration-dependent cytolysis of Nb2 cells in accordance with their relative glucocorticoid activity. Dex increased dead cells 35.5%, 54.2%, and 59.5% above baseline at concentrations of 25, 100, and 400 n*M*, respectively.

Table IV. Effect of Dexamethasone on Viability of Nb2 Lymphoma Cells

Dexamethasone (n*M*)	Living cells[a]	Dead cells[a]	Total cells[a]	Percentage dead
0	1.12 ± 0.02	0.35 ± 0.04	1.47 ± 0.11	23.9 ± 2.3
25	0.63 ± 0.06[b,c]	0.81 ± 0.07[b,d]	1.44 ± 0.12	56.4 ± 2.0[b,e]
50	0.49 ± 0.12[b]	0.94 ± 0.06[b]	1.44 ± 0.17	67.3 ± 4.8[b]
100	0.37 ± 0.05[b]	1.10 ± 0.10[b]	1.49 ± 0.15	74.9 ± 0.9[b]
200	0.37 ± 0.06[b]	1.12 ± 0.13[b]	1.49 ± 0.18	75.4 ± 2.3[b]

[a] Data are means ± SE obtained from three to four individual incubations. Cell number × 10^{-6}/ml.
[b] $P < 0.01$ vs no dexamethasone.
[c] $P < 0.05$ vs 100 n*M* dexamethasone.
[d] $P < 0.05$ vs 100 and 200 n*M* dexamethasone.
[e] $P < 0.01$ vs 50, 100, and 200 n*M* dexamethasone.

Table V. Effect of RU 486 on Cytolytic Action of Corticosteroids in Nb2 Lymphoma Cells

Experiment	Treatment	Living cells[a]	Dead cells[a]	Total cells[a]	Percentage dead
1	Control	0.95 ± 0.11	0.33 ± 0.05	1.28 ± 0.15	25.8 ± 2.7
	Dexamethasone (100 n*M*)	0.17 ± 0.02[b]	0.81 ± 0.11[b]	0.99 ± 0.11	81.8 ± 2.4[b]
	Dexamethasone (100 n*M*) + RU 486 (500 n*M*)	0.88 ± 0.08	0.41 ± 0.06	1.29 ± 0.12	31.3 ± 2.3
	RU 486 (500 n*M*)	0.84 ± 0.04	0.43 ± 0.09	1.27 ± 0.14	32.3 ± 4.5
2	Control	1.45 ± 0.31	0.25 ± 0.04	1.70 ± 0.34	16.2 ± 2.7
	Aldosterone (1000 n*M*)	1.04 ± 0.31	0.55 ± 0.06[c]	1.59 ± 0.33	40.0 ± 8.3[d]
	Aldosterone (1000 n*M*) + RU 486 (500 n*M*)	1.38 ± 0.31	0.31 ± 0.05	1.69 ± 0.33	19.8 ± 4.4

[a] Data are means ± SE obtained from four to five individual incubations. Cell number × 10^{-6}/ml.
[b] $P < 0.01$ vs control, dexamethasone + RU 486, and RU 486.
[c] $P < 0.01$ vs control and aldosterone + RU 486.
[d] $P < 0.05$ vs control and aldosterone + RU 486.

The same concentrations of cortisol increased dead cells 6.4%, 42.9%, and 54.8%, whereas the corresponding increased death rates for corticosterone were 8.7%, 27.7%, and 45.1%. Aldosterone and DOC produced relatively small increases in cell death rate (2.7–9.2%) at 25 and 100 n*M* dose levels, whereas at 400 n*M*, the respective increases in death rate were 25.7% and 23.7%. Other ligands of the steroid receptor superfamily, such as 17-β-estradiol, dehydroepiandrosterone, progesterone, testosterone, and 5-α-dihydrotestosterone at 1 μM as well as T_3 (0.15–1.5 ng/ml), had no effect on Nb2 cell viability.

Table V, Experiment 1 shows that coincubation with 500 n*M* RU 486 blocked Dex (100 n*M*)-induced cytolysis without significantly affecting total cell number. In addition, Table V, Experiment 1 shows that at this dose, the antagonist alone had no effect on Nb2 cell viability or cell number. Table V, Experiment 2 shows that 500 n*M* RU 486 also inhibits the cytolytic effect of 1000 n*M* aldosterone. This dose of RU 486 also blocks cytolysis produced by cortisol (400 n*M*), corticosterone (400 n*M*), and DOC (1600 n*M*) (data not shown).

Anticytolytic Effect of PRL. Since removal of the mitogen from the culture medium unmasked glucocorticoid-induced cytolysis, the possibility that PRL would protect the cells against Dex (100 n*M*)-induced cytolysis was tested. As shown in Table VI, incubation of Nb2 cells with ovine PRL inhibited Dex-induced cytolysis in a concentration (25–1600 pg/ml)-dependent fashion, as reflected in an increase in living cell number and a decrease in dead cell number and percentage of dead cells, without a significant change in total cell number.

As shown in Table VII, other lactogenic hormones, hGH, hPL, and rPRL (all tested at 0.2–5 ng/ml), exhibited concentration-dependent inhibition of Dex (100 n*M*)-induced cytolysis of Nb2 cells. At a concentration of 1 ng/ml, the percentage of dead cells obtained with oPRL and hGH was significantly less than that of rPRL and hPL (at $P < 0.05$), whereas that of rPRL was significantly less than hPL (at $P < 0.05$). Other pituitary/placental nonlactogenic hormones, such as rGH and hCG at 20 ng/ml and ACTH at 500 mU/ml, failed to inhibit glucocorticoid-mediated cytolysis of Nb2 cells (Table VII).

Effects of Dexamethasone and PRL on DNA Fragmentation of Nb2 Cells. To further examine the mechanism of glucocorticoid-induced cytolysis of Nb2 cells, genomic DNA from cells treated with 100 n*M* Dex for various times (0, 4, 8, and 12 hr) was analyzed by agarose gel electrophoresis. The characteristic "ladder" pattern of DNA fragments, indicative of internucleosomal DNA cleavage, was evident within 12 hr of exposure to Dex (Fig. 1, Lane 4).

Table VI. Effect of oPRL on Cytolytic Action of Corticosteroids in Nb2 Lymphoma Cells

Treatment	Living cells[a]	Dead cells[a]	Total cells[a]	Percentage dead
Control	1.15 ± 0.13[b]	0.38 ± 0.05[b]	1.53 ± 0.15	24.8 ± 2.8[b]
Dex (100 n*M*)	0.21 ± 0.05	1.03 ± 0.20	1.24 ± 0.23	82.7 ± 3.0
Dex (100 n*M*) + PRL (25 pg/ml)	0.39 ± 0.11[c]	1.08 ± 0.19[c]	1.47 ± 0.29	74.1 ± 2.7[c]
Dex (100 n*M*) + PRL (100 pg/ml)	0.43 ± 0.06[c]	0.77 ± 0.12	1.20 ± 0.18	63.7 ± 2.2[b,c]
Dex (100 n*M*) + PRL (400 pg/ml)	0.65 ± 0.10[b]	0.60 ± 0.04[d]	1.24 ± 0.09	48.7 ± 4.7[b,c]
Dex (100 n*M*) + PRL (1.6 ng/ml)	0.84 ± 0.07[b]	0.45 ± 0.04[d]	1.29 ± 0.11	35.1 ± 1.1[b]

[a] Data are means ± SE obtained from three individual incubations. Cell number × 10^{-6}/ml.
[b] $P < 0.01$ vs dexamethasone.
[c] Significantly different (at $P < 0.05$ or 0.01) from dexamethasone + PRL, 1.6 ng/ml.
[d] $P < 0.05$ vs dexamethasone.

Table VII. Effect of Lactogenic and Nonlactogenic Hormones on Dexamethasone-Induced Cytolysis in Nb2 Lymphoma Cells

Treatment	Percentage dead[a]
Control	16.5 ± 1.6[b]
Dex (100 n*M*)	65.7 ± 2.4
Dex (100 n*M*) + oPRL (1 ng/ml)	24.3 ± 2.7[b]
Dex (100 n*M*) + rGH (20 ng/ml)	66.7 ± 3.9
Dex (100 n*M*) + hCG (20 ng/ml)	73.0 ± 3.5
Dex (100 n*M*) + ACTH (500 mU/ml)	72.0 ± 2.1
Dex (100 n*M*) + hGH (0.2 ng/ml)	29.7 ± 6.3[b]
Dex (100 n*M*) + hGH (1.0 ng/ml)	19.7 ± 4.8[b]
Dex (100 n*M*) + hGH (5.0 ng/ml)	19.3 ± 3.1[b]
Dex (100 n*M*) + hPL (0.2 ng/ml)	61.3 ± 6.3
Dex (100 n*M*) + hPL (1.0 ng/ml)	50.3 ± 8.5[b]
Dex (100 n*M*) + hPL (5.0 ng/ml)	32.0 ± 2.6[b,c]
Dex (100 n*M*) + rPRL (0.2 ng/ml)	57.7 ± 5.7
Dex (100 n*M*) + rPRL (1.0 ng/ml)	36.7 ± 5.5[b,d]
Dex (100 n*M*) + rPRL (5.0 ng/ml)	22.3 ± 4.9[b,d,e]

[a] Data are means ± SE obtained from three to eight individual incubations.
[b] $P < 0.01$ vs dexamethasone (100 n*M*).
[c] $P < 0.01$ vs dexamethasone (100 n*M*) + hPL (1.0 ng/ml) and dexamethasone (100 n*M*) + hPL (0.2 ng/ml).
[d] $P < 0.01$ vs dexamethasone (100 n*M*) + rPRL (0.2 ng/ml).
[e] $P < 0.05$ vs dexamethasone (100 n*M*) + rPRL (1.0 ng/ml).

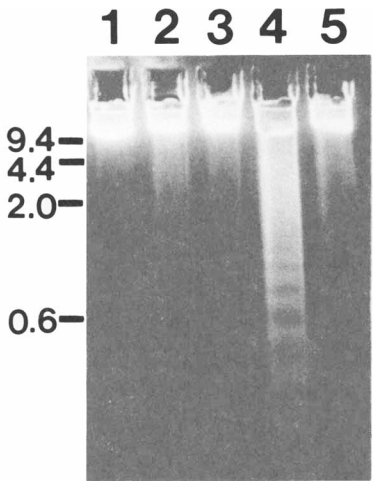

Figure 1. Ethidium bromide-stained, UV-irradiated agarose gel of DNA extracted from Nb2 cells showing effect of coincubation with oPRL (1 ng/ml) on Dex (100 n*M*)-induced DNA fragmentation. Lane 1, Dex for 0 hr; Lane 2, DMSO for 12 hr; Lane 3, PRL for 12 hr; Lane 4, Dex for 12 hr; Lane 5, Dex + PRL for 12 hr. Molecular weight markers expressed in kilobase pairs are indicated to the left of the Figure. This gel is representative of an experiment that was repeated three times.

Since PRL inhibited Dex-induced cytolysis, the effects of this mitogen on Dex-induced DNA fragmentation were examined. As shown in Figure 1, coincubation with PRL (1 ng/ml) inhibited DNA fragmentation induced by 100 n*M* Dex for 12 hr.

Discussion

Although the Nb2 lymphoma cell line has been employed as a model for studying the mechanism of PRL action (5–10), we initiated these studies of glucocorticoid responsiveness to determine whether this cell line would afford us the unique opportunity of studying the interaction between two distinct hormone modalities, lactogens and glucocorticoids, at the cellular level. Our studies not only revealed that the Nb2 cell was a target for glucocorticoids, but they also uncovered a provocative and seemingly complex interaction between this family of steroid hormones and the protein hormone, PRL, that is mitogenic for this cell line. While glucocorticoids are capable of inhibiting PRL-induced mitogenesis, an observation consistent with previous reports (18–20), the mitogen is simultaneously capable of protecting the cell against glucocorticoid-induced cytolysis.

Several observations herein indicate that the antiproliferative and cytolytic effects observed are mediated by glucocorticoid (Type II) receptors. Dex exhibits dose-dependent antiproliferative and cytolytic effects in the nanomolar concentration range, which is consistent with glucocorticoid receptor-mediated physiologic actions (21). Of the ligands of the steroid superfamily that were tested, only corticosteroids were antiproliferative and cytolytic, whereas androgens, estradiol, progesterone, and T_3 were not. Corticosteroids with predominant glucocorticoid activity were more potent than those with less glucocorticoid and/or more mineralocorticoid

activity. Corticosteroid-induced effects were inhibited by the synthetic glucorticoid receptor antagonist, RU 486. Our findings with RU 486 are consistent with its previously reported effective concentration range *in vitro* and its absence of glucocorticoid agonist activity (22, 23). Finally, Dex-induced DNA fragmentation, demonstrable as laddering on UV-irradiated ethidium bromide-stained agarose gels, was detectible at 12 hr. This is 12 hr before maximal levels of cytolysis were demonstrable by trypan blue exclusion. The ladders reflect multiples of 180 base pairs presumably generated by cleavage of DNA in the internucleosomal linker regions by endonuclease (24–26). Glucocorticoid-induced DNA fragmentation, regarded as a marker for programmed cell death or apoptosis, has been reported previously in normal and neoplastic lymphoid tissue (24–31) and appears to require the presence of the glucocorticoid-responsive element binding site of the glucocorticoid receptor (32).

Although both inhibition of mitogenesis and initiation of cytolysis appear to be mediated by glucocorticoid receptors, the latter occurred only when the cells were incubated in Fischer's stationary medium (i.e., when the mitogen was omitted). Other differences between the cytolytic and mitogenic assays are also worth noting. Mitogenic assays usually involved a 24-hr period of preincubation in Fischer's stationary medium containing 1% FCS and plating the cells at about 100,000–200,000/cells ml as suggested in the original protocol for this cell line (4), whereas cytolytic assays were usually conducted by plating cells at about 1,000,000 cells/ml and without the preincubation period. However, we found that the sensitivity to the cytolytic effects of Dex was not influenced either by the plating concentration (100,000 cells/ml or 1,000,000 cells/ml) or by the concentration of FCS (1% or 10%) during preincubation (unpublished observations).

The anticytolytic effect of PRL is likely to be mediated through the lactogenic hormone receptor, since other lactogens (hGH, rPRL, and hPL) inhibited Dex-induced cytolysis in what appears to be the expected order of mitogenic potency for Nb2 cells (oPRL and hGH > rPRL > hPL) (4), whereas nonlactogenic peptide/protein anterior pituitary or homologous hormones (including rat GH) did not. The anticytolytic effect of PRL is also manifest by inhibition of the key event of apoptosis, DNA fragmentation. The effective concentration range for the anticytolytic action of PRL corresponds to those that promote mitogenesis of Nb2 cells (4), even though, under these conditions, Dex can block the proliferative action of these mitogens. Furthermore, as shown in the first experiment in this paper, PRL (at 1000 pg/ml) is capable of blocking Dex-induced cytolysis for up to 72 hr, which indicates that the anticytolytic effect of PRL was sustained and not a transient event that merely delayed cytolysis.

The apparent inverse relationship between mitogenesis and susceptibility to cytolysis, reported herein, is consistent with the literature. Some reports have suggested that arrested proliferation is a condition for cytolysis (14, 33), whereas others have shown that mitogens or mitogenic signals, such as phorbol esters (34), concanavalin A (35), erythropoietin (36), and interleukin (IL) 2 (29), inhibit spontaneous and agent-induced apoptosis in cultured cells. Since IL-2 is a known mitogen of Nb2 cells (37), the interaction between this hormone and dexamethasone has been examined by us and found to be very similar to that of PRL. While IL-2-mediated Nb2 cell proliferation is inhibited by nanomolar concentrations of dexamethasone, mitogenic doses of IL-2 (10–1000 units/ml) block dexamethasone-induced cytolysis and DNA fragmentation (unpublished observations).

The simultaneity of dexamethasone-induced antiproliferation and prolactin-induced inhibition of apoptosis suggests a complex interaction between the two types of hormones. It is unlikely that the two effects can be explained solely on the basis of one hormone's interference with the other's receptor mechanisms (i.e., receptor levels or ligand-receptor binding). For example, dexamethasone-induced inhibition of PRL receptor mechanisms would be inconsistent with the PRL-induced anticytolytic effect, while PRL-induced inhibition of glucocorticoid receptors would be inconsistent with dexamethasone-induced antiproliferative effects. The interaction between PRL and dexamethasone reported herein suggests the involvement of either distinct subclasses of hormone receptors and/or distinct postreceptor mechanisms regulating proliferation and cell viability. For example, PRL-induced anticytolysis could be the result of alteration of postgenomic events associated with glucocorticoid-induced programmed cell death, such as Ca^{2+} influx into the cell or endonuclease activation (26, 28, 38, 39)

Studies are currently underway to gain mechanistic insights into the nature of the PRL-glucocorticoid interaction in Nb2 cells.

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