Kinetic Pulse-Chase Labeling Study of the Glucocorticoid Receptor in Mouse Lymphoma Cells

EFFECT OF GLUCOCORTICOID AND ANTIGLUCOCORTICOID HORMONES ON INTRACELLULAR RECEPTOR HALF-LIFE*  

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A kinetic pulse-chase labeling technique was used to measure the intracellular half-life of the glucocorticoid receptor in S49 mouse lymphoma cells. Cells were pulse-labeled with [35S]methionine for 30 min and then cultured in the presence of unlabeled methionine (chase). Labeled receptors were quantitated at periodic time points during the chase by immunoadsorption to protein A-Sepharose using the BuGR2 monoclonal anti-receptor antibody. The decay of labeled receptors during the chase was linear on a semilog plot, consistent with first order kinetics. Receptor half-life was 9 h when cells were cultured in either phenol red-containing medium supplemented with fetal calf serum or in phenol red-free medium supplemented with charcoal extracted serum, indicating that endogenous steroids do not affect receptor half-life. Receptor half-life was also unchanged when cells were cultured in the presence of 0.1 μM dexamethasone, a glucocorticoid hormone, or 0.1 μM RU486 (11β-(4-dimethylamino-phenyl)-17β-hydroxy-17α-(propynyloestra-4,9-diene-3-one), an antiglucocorticoid hormone.

We conclude that the intracellular half-life of the glucocorticoid receptor in S49 mouse lymphoma cells is not regulated by either glucocorticoid or anti-glucocorticoid hormones.

The glucocorticosteroid hormone receptor is an important regulatory molecule that interacts with specific enhancer sequences on DNA and thereby regulates gene transcription (1). The role of the steroid hormone ligand appears to be that of an effector molecule that binds to the receptor and converts the receptor from an inactive, non-DNA binding form to an active, DNA binding form. The intracellular concentration of glucocorticoid receptors is an important determinant of the magnitude of the glucocorticoid response. This conclusion is based on two lines of evidence. First, there is a strong correlation between the intracellular concentration of glucocorticoid receptors and the cytolytic response of murine lymphoma cells to glucocorticoids (2–4). Second, the magnitude of transcriptional regulation by glucocorticoids in cells that have been transfected with the glucocorticoid receptor gene is proportional to the level of glucocorticoid receptor expression achieved (5). It is, therefore, possible that regulation of glucocorticoid receptor levels may be a physiological means of modulating cellular responses to glucocorticoids.

There is considerable evidence that glucocorticoid receptor levels in cells are regulated by glucocorticoid hormones. Glucocorticoid hormones have been found to down-regulate the level of their own receptor in a number of different cell lines (3, 6–11), in target tissues of intact rats (12, 13), and in lymphocytes of steroid-treated human volunteers (14). More recently, glucocorticoid receptor levels have been shown to be upregulated in a human leukemia cell line, suggesting that the pattern of glucocorticoid receptor autoregulation may be tissue-specific (15). Anti-glucocorticoid hormones, such as RU486, do not appear to regulate the level of glucocorticoid receptors, despite their ability to bind with high affinity to the glucocorticoid receptor (16).

Glucocorticoids could down-regulate glucocorticoid receptors by decreasing the rate of receptor synthesis or by increasing the rate of receptor degradation. To investigate the latter possibility, we have employed a novel pulse-chase labeling technique to measure the intracellular half-life of the glucocorticoid receptor, both in the presence and absence of hormone. Our results, which are described here, indicate that neither glucocorticoid nor anti-glucocorticoid hormones have a significant effect on the intracellular half-life of the glucocorticoid receptor in mouse lymphoma cells.

EXPERIMENTAL PROCEDURES

RESULTS*

Measurement of Receptor Half-life—A kinetic pulse-chase labeling technique was used to measure the intracellular half-life of the glucocorticoid receptor in wild-type, glucocorticoid-responsive S49.1 mouse lymphoma cells. Cells were pulse-labeled with [35S]methionine for 30 min and then cultured in

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1 The abbreviations and trivial names used are: RU486, 11β-(4-dimethylamino-phenyl)-17β-hydroxy-17α-(propynyloestra-4,9-diene-3-one); F125s, phosphate-buffered saline; dexamethasone, SKF-16a-methyl-11β, 17α, 21-trihydroxypregna-1,4-diene-3,20-dione; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HSP90, heat shock protein 90; DMEM, Dulbecco’s modified Eagle’s medium.

2 Portions of this paper (including “Experimental Procedures” and Figs. 2–7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
medium supplemented with 1 mg/ml unlabeled methionine for a 24-h period referred to as the chase. Cell viability, determined by measuring trypan blue dye exclusion, was greater than 90% throughout the chase. To insure that [35S]methionine incorporation did not continue during the chase, cytosol prepared from cells at the beginning of the chase (0 h) and at different time points during the chase was subjected to SDS-PAGE (Fig. 1A). The amount of labeled protein did not increase during the chase, indicating that incorporation of [35S]methionine into newly synthesized protein was effectively blocked by culturing cells in excess unlabeled methionine.

At different time points during the chase, cytosol was prepared from cells and labeled receptors were immunoadsorbed to protein A-Sepharose using the BuGR-2 monoclonal antireceptor antibody. Immunoadsorbed proteins were subjected to SDS-PAGE and analyzed by autoradiography (Fig. 1B). The band opposite the 97.4-kDa standard corresponds to intact glucocorticoid receptor. Lower molecular weight receptor fragments were not detected in this or subsequent experiments. To quantitate the amount of labeled receptor recovered at each time point in the chase, the density of the receptor band on autoradiograms was measured by scanning densitometry (Fig. 1C). The density of the receptor band decreased with time, indicating that the amount of labeled receptor that was recovered from cytosol decreased during the chase.

The validity of scanning densitometry for measuring relative amounts of labeled protein on autoradiograms is documented in Fig. 2. In this experiment, serial dilutions of an immunoadsorbed [35S]methionine-labeled protein (the 90-kDa heat shock protein, HSP90) were subjected to gel electrophoresis and duplicate gels were analyzed by two different methods. In one method, the gel was analyzed by autoradiography and the absorbance of bands on the autoradiogram was measured by scanning densitometry. In the other method, the wet gel was sliced and the amount of radioactivity in gel slices was measured using a liquid scintillation counter. As shown in Fig. 2, there is a linear relationship between absorbance and radioactivity over the range of laser densitometry slit widths employed in this study.

Multiple separate pulse-chase labeling experiments were performed to test the reproducibility of the observation shown in Fig. 1. In one experiment, the amount of labeled receptor was quantitated at frequent intervals throughout the chase (Fig. 3A). The decay of labeled receptor appears to be linear (correlation coefficient = 0.93), consistent with the kinetics of a first order reaction, although more complex kinetics cannot be completely excluded. Recovery of labeled receptors at 4, 6, 8, and 22 h into the chase was quantitated in 10 separate experiments (Fig. 3B). Based on these data, the intracellular half-life of the glucocorticoid receptor is 9 h.

Although the decay of labeled receptors described in the preceding experiments is likely to represent intracellular receptor degradation, an alternative explanation might be that labeled receptors gradually shift to the nucleus during the chase and are therefore not recovered in cytosol extracts. To exclude this possibility, the same pulse-chase labeling method was applied to a glucocorticoid-unresponsive variant of the S49 mouse lymphoma cell line, S49.22r. The receptors in this variant are defective in terms of nuclear translocation and DNA binding due to a single amino acid substitution in the DNA-binding region (28, 29). We found the rate of decay of labeled receptors is the same in S49.22r cells and in wild type S49 cells (data not shown), indicating that nuclear translocation is not responsible for the decrease in recovery of labeled receptors from cells during the chase.

Effect of Glucocorticoid Hormones on Receptor Half-life—In the preceding experiments, cells were cultured in medium supplemented with 10% fetal calf serum. The cortisol concentration in this medium was 8 nM. To determine receptor half-life under cortisol-free conditions, cells were cultured in phenol red-free medium supplemented with charcoal-extracted serum. As shown in Fig. 4, glucocorticoid receptor half-life is the same in the presence of cortisol (fetal calf serum) as in the absence of cortisol (charcoal-extracted serum). Thus, it does not appear that cortisol at the low concentration present in fetal calf serum regulates glucocorticoid receptor half-life.

**FIG. 1. Pulse-chase labeling and analysis.** S49.1 cells were pulse-labeled with [35S]methionine and then cultured in medium supplemented with 1 mg/ml unlabeled methionine (chase) for 22 h. At different times during the chase, cells were removed from culture and cytosol was prepared. A, cytosol samples corresponding to different times in the chase were subjected to SDS-PAGE. An autoradiogram of the gel is shown. B, labeled receptors were immunoadsorbed from cytosol samples corresponding to different times in the chase. Immunoadsorbed proteins were subjected to SDS-PAGE and an autoradiogram of the gel is shown. ab, antireceptor antibody; c, no antibody control. The band corresponding to the glucocorticoid receptor is located opposite the 97.4-kDa standard and is detected in ab lanes but not in c lanes. C, the absorbance of each receptor band on the autoradiogram in B was measured by scanning densitometry and normalized with respect to the absorbance of the receptor band at the beginning of the chase (0 h time point).
The pulse-chase labeling technique was used to determine the effect of pharmacologic concentrations of the glucocorticoid hormone dexamethasone on receptor half-life. Addition of dexamethasone to pulse-labeled cells at the beginning of the chase induced a concentration dependent decrease in the recovery of labeled receptors from cytosol at 4 h into the chase (Fig. 5). The effect of dexamethasone appeared to be specific for the glucocorticoid receptor since dexamethasone did not decrease the recovery of a control protein (the 90-kDa heat shock protein HSP90) from the same cells. Two points are worthy of note in regard to experimental design. First, dexamethasone was added to cells after completion of the pulse-labeling period. Thus, dexamethasone did not affect the incorporation of $^{[35]}$S-methionine into protein and the amount of labeled protein present in cytosol extracts of dexamethasone-treated and control cells was the same (data not shown). Second, both liganded and unliganded receptors are immunoabsorbed by the BuGR-2 antibody with equal efficiency (data not shown). For the following reasons, we conclude that the dexamethasone-induced decrease in recovery of labeled receptors is due to nuclear translocation and tight nuclear binding such that labeled receptors are not recovered in the cytosol fraction. First, the dexamethasone-induced decrease in recovery of labeled receptors from wild type S49 cells was detected as early as 30 min into the chase (data not shown), consistent with earlier evidence that dexamethasone-induced nuclear localization occurs within minutes of adding dexamethasone to cells (30). Second, dexamethasone did not decrease the recovery of labeled receptors from nuclear transfer defective S49.22r cells (data not shown).

Following the dexamethasone-induced shift of labeled receptors into the nucleus, the recovery of labeled receptors from dexamethasone-treated and control cells decreased at the same rate, indicating that the intracellular half-life of the glucocorticoid receptor is not altered by dexamethasone (Fig. 6). In these experiments, control and dexamethasone-treated cells were cultured under cortisol-free conditions (phenol red-free medium and charcoal-extracted serum). Dexamethasone also failed to change receptor half-life in cells that were cultured in the presence of unextracted fetal calf serum (data not shown).

Effect of RU486 on Receptor Dynamics—The same pulse-chase labeling technique was used to determine the effect of the antagliocorticoid hormone RU486 on glucocorticoid receptor half-life in cells that were cultured under cortisol-free conditions (phenol red-free medium supplemented with charcoal-extracted fetal calf serum). Cells were pulse-labeled with $^{[35]}$S-methionine and RU486 was added to cultures at the beginning of the chase to give a final concentration of 0.1 μM. Based on earlier studies, this concentration of RU486 is sufficient to achieve receptor saturation. The recovery of labeled receptors from cells during the chase decreased at the same rate in the presence and absence of RU486, indicating that RU486 does not affect the intracellular half-life of the glucocorticoid receptor (Fig. 7). In contrast to dexamethasone, RU486 did not induce a rapid decrease in the recovery of labeled receptors from cells. Thus, unlike dexamethasone-receptor complexes, RU486-receptor complexes do not appear to associate tightly with the nucleus and are recovered in the cytosol fraction.

DISCUSSION

The mechanism of glucocorticoid receptor autoregulation has been the subject of investigation in a number of different laboratories. Several laboratories have reported that the level of glucocorticoid receptor mRNA in a variety of types of cells is decreased in response to glucocorticoids (31–33). It appears from these studies that glucocorticoids directly regulate transcription of the glucocorticoid receptor gene. On the basis of this information, one would predict that glucocorticoids decrease the rate of glucocorticoid receptor synthesis in cells. However, other investigators have recently reported that glucocorticoids upregulate the level of glucocorticoid receptor mRNA in human leukemia cells, suggesting that patterns of glucocorticoid receptor autoregulation may be different in different types of cells (15).

Other investigators have sought to understand glucocorticoid receptor autoregulation by investigating the effect of glucocorticoids on the rate of glucocorticoid receptor turnover in cells. Svec and Rudis (6) found that glucocorticoid receptors in AtT-20 cells are depleted more rapidly when cells are incubated with both cycloheximide and dexamethasone than when cells are incubated with cycloheximide alone, suggesting that the half-life of the glucocorticoid receptor is shorter in the presence of hormonal ligand than in the absence of hormonal ligand. However, cycloheximide may disrupt normal metabolic processes in the cell and may itself alter the rate of intracellular receptor degradation (34). The effect of glucocorticoid hormones on intracellular receptor half-life has been extensively investigated by Samuels and coworkers using the technique of dense amino acid labeling (8, 11). Their results indicate that glucocorticoid receptor half-life in GH3 pituitary tumor cells is significantly shorter when the cells are cultured in the presence of glucocorticoid hormone than when the cells are cultured in the absence of glucocorticoid hormone. A similar conclusion regarding the effect of estrogen on estrogen receptor half-life has been reached by Katznellenbogen and coworkers (35) using similar techniques. However, one potential difficulty associated with measuring receptor half-life by the dense amino acid labeling technique is that radiolabeled hormonal ligands are required to detect and quantitate receptors. Thus, it may be difficult to discriminate between changes in the amount of receptor present in cells and alterations in the ligand binding properties of receptors. This is an important consideration since glucocorticoid receptors are able to reversibly convert from an “active” form that binds hormone to an “inactive” form that does not bind hormone (36–39).

Because of potential limitations associated with present techniques for measuring glucocorticoid receptor half-life in cells, the present study was undertaken to measure intracellular glucocorticoid receptor half-life by means of a kinetic pulse-chase labeling method. This method has two advantages over previous methods. First, it does not involve use of protein synthesis inhibitors. Second, glucocorticoid receptors are synthetically labeled with $^{[35]}$S-methionine and quantitated by reaction with monoclonal antireceptor antibody. Thus, receptor quantitation does not require binding of radiolabeled hormonal ligand to the receptor, enabling detection of both active and inactive forms of the receptor as well as comparison of the effects of different hormonal ligands on receptor half-life.

The results of the present study indicate that the intracellular half-life of the glucocorticoid receptor in S49 mouse lymphoma cells is 9 h. Receptor half-life appears to be the same in both wild type, glucocorticoid responsive cells (S49.1), and a glucocorticoid resistant variant in which glucocorticoid receptors are unable to undergo nuclear translocation (S49.22r). Glucocorticoid receptor half-life was unaffected by culture conditions. Thus, receptor half-life was the same when cells were cultured in the presence of fetal calf serum that contains cortisol and when cells were cultured in the presence...
of fetal calf serum that had been extracted to remove cortisol. In addition, the presence or absence of phenol red in the culture medium did not affect receptor half-life.

Our studies also show that pharmacologic concentrations of a glucocorticoid hormone, dexamethasone, and an antiglucocorticoid hormone, RU486, do not affect receptor half-life in S49 mouse lymphoma cells. This conclusion is different from that of dense amino acid labeling studies discussed above in which glucocorticoid hormones were found to shorten the half-life of the glucocorticoid receptor. The discrepancy between the findings of the present study and earlier studies in which glucocorticoid hormones were found to shorten the early stages of this work.

half-life in cells. Using this technique, we conclude that is an effective method for measuring glucocorticoid receptor half-life or may represent a difference in receptor regulation in different types of cells.

In summary, we find that the pulse-chase labeling technique is an effective method for measuring glucocorticoid receptor half-life in cells. Using this technique, we conclude that receptor half-life is not regulated by glucocorticoid or anti-glucocorticoid hormones in S49 mouse lymphoma cells.

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Glucocorticoid Receptor Half-life

EXPERIMENTAL PROCEDURES

Chemicals—Acrylamide and other chemicals for gel electrophoresis were from Bio-Rad. The low molecular weight standards for gel electrophoresis, from Pharmacia LKB Biotechnology Inc., were phosphorylase b (97.4 kDa), albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and a-lactalbumin (14.4 kDa). Protein A-Sepharose, Triton X-100, and other chemicals were from Sigma. Tween 20 was from Bio-Rad. [3H]Mephenytoin (3H) was from New England Nuclear. Buffer A—phosphate buffered saline (PBS), 1 mg/mL BSA, 10 mM MgCl2, 150 mM NaCl, pH 8.0. Buffer B—contained 250 mM Tris, pH 9.0 (20 mM EDTA, 500 mM NaCl, 100 mM glycerol, 0.2% Triton X-100). The sample buffer for gel electrophoresis contained 0.8% Tris, pH 8.3, 2% sodium dodecyl sulfate (SDS), 10% (v/v) glycerol, 0.05% bromophenol blue. Cell culture—The 3451 mouse lymphoma cell line was obtained from American Type Culture Collection. The glucocorticoid unresponsive variant, 3451v, was from Dr. Keith Yamamoto, University of California, San Francisco. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum, 0.05 mM dexamethasone, penicillin, and streptomycin. DMEM and serum were purchased from Biologicals. Cells were maintained at 37°C in a 5% CO2/95% air atmosphere and were used for experiments during the exponential phase of growth. In certain experiments, as described in the text, cells were cultured in phenol red-free DMEM (prepared by Biologicals) supplemented with serum that had been extracted with activated charcoal to remove endogenous cortisol (17).

Antibodies—Cultured tissue culture supernatant from the 3458 hybridoma clone was a gift of Dr. Robert Harison, University of Arkansas for Medical Sciences, Little Rock, AR. The 3458-2 clone produces IgG1 monoclonal antibodies that react with an epitope located in or near the DNA-binding site of mouse and rat glucocorticoid receptors (18-21). Reticine fluid containing the ACSR monoclonal antibody was a gift of Dr. David Teo, Mayo Clinic, Rochester, NY. ACSR is an IgG1 monoclonal antibody prepared against the 98-kDa protein purified from the testis fluid of the test randomly mated male mouse (22). ACSR has a broad spectrum of species cross-reactivity and recognizes the 90-kDa heat shock protein (HSP90) (23).

Fusion-chase labeling—Cells were labeled using a modification of a previously described method (24). Cells were gently pelleted, resuspended at a concentration of 5 million cells/mL in methionine-free culture medium supplemented with 10% serum and then incubated for 1 h at 37°C in a 5% CO2, 95% air atmosphere. The cells were then gently pelleted and resuspended at a concentration of 50 million cells/mL in the same methionine-deficient medium. [35S]Methionine (1150 Ci/mmol) was added to give a final concentration of 50 µCi/mL, and the cells were incubated for 30 min at 37°C in a 5% CO2, 95% air atmosphere. The cells were then gently pelleted and resuspended at a concentration of 8-10 million cells/mL in culture medium supplemented with 1 mg/mL unlabeled methionine plus 10% serum, 0.4 mM glutamine, penicillin, and streptomycin. The cell suspension was divided into equal aliquots in tissue culture flasks and incubated at 37°C in a 5% CO2/95% air atmosphere for up to 24 h. This period of time is referred to as the chase. At the beginning of the chase and at each time point during the chase, all of the cells were removed from a single flask and cytosol was prepared for the immunodetection assay described below.

Immunodetection—Deoxycholate (5% deoxycholate was dissolved in absolute ethanol to give a stock solution of 4.1% deoxycholate (26). Provided by Dr. R. Deveraux at the Centre de Recherches Roussel-Uclaf, Romainville, France) was dissolved in absolute ethanol to give a stock solution with a concentration of 4.1%. In experiments involving the addition of one of these hormones to the cells, the solutions were diluted in sterile culture medium and added to cultures at the beginning of the chase. An equivalent amount of absolute ethanol diluted in culture medium was added to control cultures.

Cultural conditions—The method of cytosol preparation was modified after that of Menders et al. (25). Cells were washed twice with PBS at 4°C and after the final wash pelleted by centrifugation at 1,000 g for 5 min. The cells were then gently homogenized in an RBC lysis buffer containing 250 mM sucrose and 500 mM NaCl and stored at 4°C in the dark. The samples were centrifuged for 10 min at 12,000 g for 20 min in an Eppendorf microcentrifuge. The clear supernatant was referred to as cytosol and was used immediately in the immunodetection assay described in the following section.

Immunodetection Assay—Cytosol, prepared from 40 to 50 million cells, was mixed with an equal volume of buffer to inhibit the crude tissue culture supernatant containing anti-glucocorticoid receptor antibody (Rho-33) or a 1:20 dilution of the same solution containing anti-GRP30 antibody (ACR5). Tween-20 was added to give a final concentration of 0.1% and the antibody-cytoplasm extract mixture was continuously mixed by gentle rotation at 4°C for 1 h prior to addition of protein A-Sepharose. Protein A-Sepharose was suspended in 0.2 g/ml buffer A and added in a volume equal to one-third of the volume of the antibody-cytoplasm extract mixture. The mixture was continuously mixed by gentle rotation for 10 min at 4°C. The protein A-Sepharose was then pelleted by centrifugation at 14,000 g for 1 min at 4°C. The pellets were washed 5 times with buffer B, centrifuging each time in an Eppendorf microcentrifuge for 5 min at 4°C. The protein A-Sepharose pellets were suspended in 5 µl of the sample buffer for gel electrophoresis and heated at 100°C for 10 min. The samples were clarified by centrifugation in an Eppendorf microcentrifuge for 5 min at 25°C and subjected to gel electrophoresis.

Gel electrophoresis—The method of gel electrophoresis (SDS-PAGE) was modified after that of Laemmli (26) and has been described in detail (27). Gels were stained with Coomassie Brilliant Blue and destained as described previously (27). Gels were rinsed with water for 30 min and then soaked in 1 M sodium sulfate for 30 min prior to drying onto filter paper under vacuum. The position of size standards was marked on the dried gel using 1% containing [35S]methionine. Gels were exposed to Kodak XAR-5 film at -60°C for 24-30 hr. The absence of bands on autoradiograms was measured using an ABC II Ultrasonics A.L. Laser densitometer. The absorbance of receptor bands corresponding to different time points in the chase were normalized to the absorbance of the receptor band corresponding to the beginning of the chase (0 h time point, as defined above). In certain experiments, gel slices were dried for 1 h at 105°C and radioactivity in the gel slices was counted in a liquid scintillation counter as described previously (27).
FIG. 4. Effect of culture condition on receptor half-life. Pulse-chase labeling and analysis was performed as described in Figure 1 using 849.1 cells cultured either in phenol red containing medium supplemented with fetal calf serum (FCS) or in phenol red free medium supplemented with charcoal extracted fetal calf serum (CES). The amount of labeled receptor recovered at each time point in the chase was measured by densitometric scanning of receptor bands on autoradiograms of gels and normalized with respect to the amount of labeled receptor recovered at the beginning of the chase (0 h time point). Results represent the mean +/- standard error of the mean for six separate experiments. Each experiment was performed in duplicate.

FIG. 5. Effect of dexamethasone on receptor recovery. 849.1 cells, cultured in the presence of fetal calf serum, were pulse-labeled with [3H]glucocorticoid and cultured during the chase in the presence or absence of dexamethasone at the indicated concentrations. At 4 h into the chase, labeled proteins were immunoprecipitated with protein A-Sepharose using either anti-receptor antibody (R202-21) or anti-RPSP3 antibody (AC19). Immunoprecipitated proteins were then subjected to SDS-PAGE. Panel A. Autoradiogram of gel showing immunoprecipitated receptors. ah, anti-receptor antibody; c, no antibody control. Panel B. Autoradiogram of gel showing immunoprecipitated RPSP3. ab, anti-RPSP3 antibody. Panel C. Scanning densitometry of receptor and RPSP3 bands shown in panels A and B. The abundances of receptor and RPSP3 bands were normalized with respect to control levels (no dexamethasone) for each at 4 h into the chase.

FIG. 6. Effect of dexamethasone on receptor half-life. Pulse-chase labeling and analysis was performed as described in Figure 1 using 849.1 cells cultured in phenol red free medium supplemented with charcoal extracted fetal calf serum. During the chase, cells were cultured in the absence of dexamethasone (CES) or in the presence of 0.1 μM dexamethasone (CES + DEX). The amount of labeled receptor recovered at each time point in the chase was measured by densitometric scanning of receptor bands on autoradiograms of gels and normalized with respect to the amount of labeled receptor recovered at the beginning of the chase (0 h time point). Results represent the mean +/- standard error of the mean for five separate experiments. Each experiment was performed in duplicate.

FIG. 7. Effect of RU486 on receptor half-life. Pulse-chase labeling and analysis was performed as described in Figure 1 using 849.1 cells cultured in phenol red free medium supplemented with charcoal extracted fetal calf serum. During the chase, cells were cultured in the absence of RU486 (CES) or in the presence of 0.1 μM RU486 (CES + RU486). The amount of labeled receptor recovered at each time point in the chase was measured by densitometric scanning of receptor bands on autoradiograms of gels and normalized with respect to the amount of labeled receptor recovered at the beginning of the chase (0 h time point). Results represent the mean +/- standard error of the mean for four separate experiments. Each experiment was performed in duplicate.