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Characterization of Glucocorticoid Receptor in HeLa-S3 Cells†

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ABSTRACT: Glucocorticoid receptor of the human cell line HeLa-S3 has been characterized and has been compared to rat and to mouse glucocorticoid receptors. If HeLa cells were lysed in the absence of glucocorticoid, glucocorticoid receptor was isolated in a nonactivated form, which did not bind to DNA–cellulose. If HeLa cells were preincubated with glucocorticoid, glucocorticoid receptor was isolated in an activated, DNA-binding form. HeLa cell glucocorticoid receptor bound [^{3}H]triamcinolone acetonide with a dissociation constant (K_D = 1.3 nM at 0 °C) that was similar to those of mouse and rat glucocorticoid receptors. Similarly, the relative binding affinities for steroid hormones decreased in the order of triamcinolone acetonide > dexamethasone > promegestone > methyltrienolone > aldosterone ≥ moxestrol. Nonactivated and activated receptors were characterized by high-resolution anion-exchange chromatography (FPLC), DNA–cellulose chromatography, and sucrose gradient centrifugation. Human, mouse, and rat nonactivated glucocorticoid receptors had very similar ionic and sedimentation properties. Activated glucocorticoid receptors were eluted at similar salt concentrations from DNA–cellulose columns but at different salt concentrations from the FPLC column. A monoclonal mouse anti-rat liver glucocorticoid receptor antibody [Westphal, H. M., Mugele, K., Beato, M., & Gehring, U. (1984) *EMBO J. 3*, 1493-1498] did not cross-react with HeLa cell glucocorticoid receptor. Glucocorticoid receptors of HeLa, HTC, and S49.1 cells were affinity labeled with [^{3}H]dexamethasone and with [^{3}H]dexamethasone 21-mesylate. The molecular weights of [^{3}H]dexamethasone 21-mesylate labeled glucocorticoid receptors (M_T 96 000 ± 1000) were undistinguishable by polyacrylamide gel electrophoresis. Protease digestions of [^{3}H]dexamethasone 21-mesylate labeled HeLa cell and HTC cell glucocorticoid receptor by α-chymotrypsin and by *Staphylococcus aureus* V8 protease revealed structural differences between human and rat glucocorticoid receptor. Since the steroid-binding and the DNA-binding domains were apparently similar in human and rat glucocorticoid receptor, the differences in FPLC, antibody binding, and protease digestion patterns probably reflect structural differences in the immunogenic domains of human and rat glucocorticoid receptor.

Glucocorticoid receptors (GR) of various tissues and species, especially of rat liver, have extensively been characterized [for recent reviews, see Carlstedt-Duke et al. (1983), Schmidt & Litwack (1982), and Gustafsson et al. (1984)]. GRs are apparently the mediator of glucocorticoid action, which eventually results in the selective activation/repression of the expression of various genes. The extensive studies of isolating and purifying GRs have resulted in a complex, sometimes even confusing, picture of GR properties (Koblinsky et al., 1972; Yamamoto et al., 1976; Eisen & Glinsman, 1978; Litwack et al., 1978; Govindan & Gronemeyer, 1984; Wrange et al., 1979; Sherman et al., 1983). GRs have been isolated in the form of several isotypes, which were characterized by their abilities to interact with hormone or with DNA, respectively (Higgins et al., 1973; Grippo et al., 1983). These studies were usually interpreted in support of the common paradigm of steroid action (Gorski et al., 1968; Jensen et al., 1968), which postulated an interaction of GR with glucocorticoid in the cytosol prior to its possible interaction with chromatin in the nucleus. It was attempted to assign these isotypes to "cytosolic" and to "nuclear" variants of GR. Accordingly, GR has been localized immunocytochemically in the nuclei and cytoplasm of target cells (Antakly & Eisen, 1984). However, recent papers (Welshons et al., 1984; King & Greene, 1984, Gasc et al., 1984) about the cellular locations of estrogen and progesterone receptors have cast some doubt on the general significance of receptor isotypes produced by elaborate purification schemes in vitro.

Albeit the plethora of literature on GR of rat liver, little is known about the properties of human GR (Currie & Cidlowski, 1980; Kontula et al., 1981; Harmon et al., 1984; Eliard & Rousseau, 1984). Monoclonal antibodies, which were raised against rat liver GR, generally failed to cross-react with GR of human cells (Westpahl et al., 1982; Okret et al., 1984). One study with an immunoglobulin fraction of serum from a rabbit immunized with rat liver GR reported a cross-reaction to GR of human tissue (Okret et al., 1981). We have investigated the properties of GR from HeLa cell lysates. In this paper we report the general molecular properties of human GR. High-resolution anion-exchange chromatography (FPLC) as well as DNA–cellulose column chromatography were used to characterize various forms of human GR generated in HeLa cell lysates. The molecular properties of the steroid hormone

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binding moiety were further characterized by affinity labeling with dexamethasone as well as with dexamethasone 21-mesylate. The labeled human GR was compared to labeled mouse and rat GR. Pronounced differences between the structures of rat and human GR became apparent by proteolytic degradation studies when labeled GRs had been digested with *Staphylococcus aureus* V8 protease or with chymotrypsin.

Materials and Methods

Chemicals. [^{3}H]Triamcinolone acetonide (37 Ci/mmol) ([^{3}H]TA), [^{3}H]dexamethasone (46 Ci/mmol), [^{3}H]dexamethasone 21-mesylate (48 Ci/mmol), moxestrol, progesterone, and methyltrienolone were from New England Nuclear. Aldosterone, triamcinolone acetonide, dexamethasone, leupeptin, dextran T 500, calf thymus DNA immobilized on cellulose, and molecular weight standards were from Sigma. Charcoal and DEAE-cellulose DE-52 were from Serva (Heidelberg, FRG). The prepacked Mono Q ion-exchange column was from Pharmacia. Phosphocellulose P11 was from Whatman. Sodium molybdate and soybean trypsin inhibitor were from Merck. *Staphylococcus aureus* V8 protease was from Miles. α-Chymotrypsin was from Worthington. X-ray film was from Fuji. Quickszint was from Zinsser (Frankfurt, FRG). Cell culture media and newborn calf serum were from Gibco. All other chemicals, including reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), were from Merck, Sigma, or Serva in the highest quality available.

Buffers and Solutions. All glassware was freshly siliconized before use. Buffers were prepared freshly and were degassed before the addition of 5 mM 2-mercaptoethanol. pH was adjusted at 0 °C with the use of temperature-corrected standard buffers.

Dextran-coated charcoal was prepared freshly before use. 5% charcoal was coated with 0.5% dextran. Fines were carefully removed, and the coated charcoal was resuspended in buffer B (described later). Salt concentrations in buffers and in fractions eluted from FPLC columns or DNA–cellulose columns were determined by conductivity measurements.

Radioactivity Measurement. Radioactivity was measured in duplicate samples in an LS9000 Beckman liquid scintillation spectrometer. A total of 5 mL of Quickszint was added per sample. Correction for quenching was done by using the external standard technique.

Cell Culture. HeLa-S3 and rat HTC cells were grown as spinner cultures in modified Eagle's medium (MEM)–Jokliks medium supplemented with 4.5 g/L glucose, 4.7 g/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 5% newborn calf serum. Cells were grown at densities of (4–8) $\times 10^5$ cells/mL. Generation times were 18–20 h. (Serum was treated before use with 2.5% dextran-coated charcoal for 30 min at 0 °C in order to minimize the concentrations of corticosteroids in the serum.) Mouse S49.1 cells were cultured in suspension culture in DMEM medium supplemented with 5% newborn calf serum. Generation times were 16 h. Cells were grown at densities of (1–3) $\times 10^5$ cells/mL.

Preparation of Cytosols. Cells were harvested at a density of (5–7) $\times 10^5$ cells/mL. They were washed twice with serum-free tissue culture medium. They were resuspended at a density of 10^8 cells/mL culture medium containing 10^{-8} M [^{3}H]TA. The suspension was incubated 60 min at 37 °C. After the cells were washed in ice-cold phosphate-buffered saline, they were resuspended in 4 °C in 3 volumes of 50 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM 2-mercaptoethanol, 2 mM leupeptin, and 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0) (buffer A), which contained 25 μg/mL soybean trypsin inhibitor, and were homogenized in a douncer with 10 strokes (pestle B). Lysates were cleared by differential centrifugation at 800*g* for 5 min and at 17000*g* for 15 min followed by 30 min at 200000*g*. The 200000*g* supernatant, or cytosol, was used immediately. Alternatively, cells were harvested and were washed twice with ice-cold phosphate-buffered saline. Cells were resuspended at 4 °C in 3 volumes of 50 mM NaCl, 10 mM Na_2MoO_4, 1 mM EDTA, 2 mM leupeptin, and 10 mM Tris-HCl (pH 8.0) (buffer B), which contained 25 μg/mL soybean trypsin inhibitor, and were homogenized. The lysate was cleared by differential centrifugation as described. After lipids had been removed from the cleared lysate by aspiration, the 200000*g* supernatant (cytosol) was used immediately or quick frozen in liquid nitrogen. Cytosol frozen in this manner and stored at −70 °C retained its original receptor binding capacity for at least 6 months. Protein concentrations in cytosols were determined according to Bradford (1976).

Preparation of Nuclear Extracts. Cells were harvested, in vivo labeled with [^{3}H]TA, and lysed as described in the preceding paragraph. The 800*g* pellets of the cell homogenates contained the crude nuclei. They were lysed in 20 volumes of water. The resultant chromatin was washed twice with 20 volumes of buffer A. Then it was extracted with 250 mM NaCl, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 10 mM Tris-HCl (pH 8.0) buffer with 10 strokes (pestle B) in a douncer. The slurry was centrifuged 30 min at 200000*g*. The 200000*g* supernatant (nuclear extract) was used immediately or quick frozen in liquid nitrogen and stored at −70 °C.

Hormone Binding. Cytosol was adjusted with buffer B to a protein concentration of 20 mg/mL. Equilibrium measurements were done after a 2-h incubation of [^{3}H]TA or of [^{3}H]dexamethasone with HeLa cell lysates. All points were done in duplicate. Unbound hormone was removed with 2.5% dextran-coated charcoal (Beato & Feigelson, 1972). Background binding was determined in parallel experiments with a 100-fold excess of unlabeled hormone over [^{3}H]TA or [^{3}H]dexamethasone, respectively. Receptor concentration and equilibrium binding constants were calculated by plotting bound over free hormone (B/F) vs. bound hormone according to Scatchard (1949). K_D values were determined from B/F ratios varying between 0.05 and 0.5.

Competitive binding curves for various steroid hormones (Table II) were obtained as follows: 100-μL aliquots of cytosol were incubated 2 h at 4 °C with 2×10^{-9} M [^{3}H]TA in the presence of 10^{-9}–10^{-5} M unlabeled hormone. Free hormone was removed by treatment with 2.5% dextran-coated charcoal. Radioactivity was assayed as above. Specifically bound [^{3}H]TA was plotted vs. the corresponding concentration of added unlabeled hormone. Each competitive binding curve was constructed from 10 points done in duplicate. Relative binding affinities, which are tabulated in Table II, were estimated from the midpoints of these curves (Ojasoo & Raynaud, 1978).

Dissociation and association constants were determined according to Eliard & Rousseau (1984). Briefly, this determination is based on the assumption that steroid binding to glucocorticoid receptor in cytosol involves a noncooperative and reversible bimolecular reaction and that neither steroid nor receptor is degraded during the incubations. A fixed concentration of receptor was incubated with different fixed concentrations of [^{3}H]TA. The [^{3}H]TA–HeLa GR complex was assayed at various time points until equilibrium was reached. If the concentration of glucocorticoid receptor

complex was plotted as a function of time, the rate constant k_1 was obtained from eq 5 of Schrader (1975). For determining the dissociation constant, labeled receptor was incubated in the presence of a 100-fold excess of unlabeled hormone. The concentration of [^{3}H]TA–GR complex was assayed at different time points. The rate constant k_{-1} was obtained from these measurements by eq 7 of Schrader (1975).

High-Resolution Ion-Exchange Chromatography. The FPLC system from Pharmacia was equipped with a 1-mL Mono Q anion-exchange column. Labeled cytosol, nuclear extract, or fractions from various steps of the partial purification procedure (as will be discussed) containing 5–20 mg of protein were adjusted to 25 mM NaCl and recentrifuged 15 min at 200000*g* before loading onto the column. [^{3}H]-TA–GR complexes were eluted at 4 °C with a linear 25–700 mM NaCl gradient in 1 mM EDTA, 10% glycerol, and 10 mM Tris-HCl (pH 8) (buffer C). Proteins were eluted at 28 mM NaCl/min. Chromatography time was 1 h. Fractions (750 μL) were collected and assayed for radioactivity and actual salt concentrations by conductivity measurements. Usually, >80% of [^{3}H]radioactivity was recovered from the Mono Q column.

Covalent Labeling of Cytosols with [^{3}H]Dexamethasone 21-Mesylate. Glucocorticoid receptor was $(NH_4)_2SO_4$ precipitated (Eisen et al., 1981). Precipitates were centrifuged (0 °C, 10 min at 20000*g*) and were resuspended in $^1/_4$ volume of 2 mM EDTA, 25 mM Na_2MoO_4, and 20 mM HEPES–NaOH (pH 8.5) (buffer D), quick frozen in liquid nitrogen, and stored at −70 °C. Fresh or thawed material was incubated 2 h at 0 °C with 10^{-8} M [^{3}H]dexamethasone 21-mesylate. Reactions were terminated by adding 2 mM 2-mercaptoethanol. Unreacted, free [^{3}H]dexamethasone 21-mesylate was removed with 5% dextran-coated charcoal.

Photoaffinity Labeling of HeLa Glucocorticoid Receptor. [^{3}H]Dexamethasone-labeled GR was partially purified by anion-exchange chromatography to a specific activity of 0.6 pmol of GR/mg of protein. It was irradiated 3 min at wavelengths ≥ 310 nm with a 500-W high-pressure mercury lamp in an apparatus as previously described (Gronemeyer & Pongs, 1980). Precipitation of protein with 5% trichloroacetic acid was used to calculate the yield of photoaffinity labeling HeLa GR with [^{3}H]dexamethasone, since this only precipitates [^{3}H]dexamethasone, which is covalently attached to protein. The trichloroacetic acid precipitated material was collected by filtration through glass-fiber filters (GFC; Whatman). After filters had been washed twice with trichloroacetic acid and once with 70% ethanol, filters were air-dried, transferred into scintillation vials, and counted. Background values were obtained with cytosol irradiated after incubating with 10^{-8} M [^{3}H]dexamethasone and a 100-fold excess of unlabeled dexamethasone. The difference between [^{3}H]dexamethasone counts detected on the GFC filters and those bound to receptor before irradiation was used to calculate the yield of the photoaffinity-labeling reaction.

Procedures for Partial Purification of Covalently Labeled Receptors. Affinity-labeled glucocorticoid receptors were partially purified according to Wrange et al. (1979). Labeled receptor extracts (300–500 mg of protein) were desalted on Sephadex G-75 columns equilibrated with 10 mM Na_2MoO_4–buffer A. The flow-through volume was passed immediately over a phosphocellulose column (2.5 cm × 10 cm) and, subsequently, over a DNA–cellulose column (2.5 cm × 5 cm) equilibrated with 10 mM Na_2MoO_4–buffer A. The final flow through (10–20 mL) was loaded onto a DEAE-cellulose (DE-52) column (2.5 cm × 5 cm) equilibrated with 10 mM Na_2MoO_4–buffer A. Labeled receptors were step eluted with 10 mM Na_2MoO_4–buffer A (175–225 mM NaCl step). The collected material was precipitated in 1 M LiCl with 70% ethanol. Precipitates were centrifuged at 0 °C for 10 min at 20000*g*, quick frozen in liquid nitrogen, and stored at −70 °C until further use.

DNA–Cellulose Chromatography of Activated Receptors. [^{3}H]TA-labeled receptors of HeLa cell, HTC cell, or S49.1 cell lysates were partially purified by phosphocellulose and DNA–cellulose chromatography as described above for affinity-labeled receptors. The flow through of the DNA–cellulose column was loaded onto a DEAE-cellulose (DE-52) column (2.5 cm × 5 cm) equilibrated with buffer A. Receptors were eluted with 175–225 mM NaCl–buffer A. The pooled fractions were incubated 30 min at 20 °C. After a 5-fold dilution with 1 mM EDTA, 5 mM 2-mercaptoethanol, and 10 mM Tris-HCl (pH 8.0) buffer, partially purified receptors were loaded onto a second DNA–cellulose column (1 cm × 4 cm) equilibrated with buffer A. The column was washed with 4 volumes of buffer A containing 10% glycerol. Receptors were eluted with a 50–200 mM NaCl gradient in 50 mL of buffer A containing 10% glycerol or with 25 mM $MgCl_2$ in buffer A.

Polyacrylamide Gel Electrophoresis. Ethanol-precipitated, partially purified, affinity-labeled receptors were taken up in sample buffer, boiled for 2 min, and then loaded on top of a polyacrylamide gel according to Laemmli (1970). Constant percentage acrylamide gels were run at room temperature at 30 mA/gel [7.5–12.5% acrylamide (T); 2, 7% *N,N'*-methylenebis(acrylamide) (C)]. Molecular weight markers were myoglobin (M_T 17 000), bovine serum albumin (M_T 66 300), ^{14}C-methylated *Escherichia coli* RNA polymerase (M_T 165 000, 155 000, 90 000, and 39 000), and ^{14}C-methylated BrCN–myoglobin fragments (M_T 10600 and 6200). Fluorography of gels was carried out according to Bonner & Laskey (1974). Gels were dried by using a Bio-Rad gel drier and were fluorographed at −70 °C for 5–40 days with preflashed Fuji X-ray film.

Precipitation of Glucocorticoid Receptors with Antibodies. Monoclonal mouse anti-rat liver GR antibody S49 was a gift of M. Beato (University Marburg, FRG). The antibody binding assay of Westphal et al. (1982) was slightly modified. Antibodies were preincubated 30 min at 0 °C with 2 mM leupeptin before use. A total of 250 μL of labeled cytosol (0.5 pmol of GR), of HeLa S3 cells, or of rat HTC cells was incubated with 10-fold excess of monoclonal antibody for 1 h at 0 °C. After treatment with 2.5% dextran-coated charcoal to remove unbound [^{3}H]TA, the incubation mixtures were loaded on top of a linear 5–20% sucrose gradient in buffer B. Centrifugation was for 18 h at 40 000 rpm in a SW41 rotor at 4 °C. Lysozyme and γ-globulin were used as internal standards for estimating sedimentation values of [^{3}H]TA–GR–antibody complexes. Absorbancy was measured at 280 nm. Control incubations were done with a monoclonal mouse anti-ecdysterone antibody previously described (Dworniczak et al., 1982).

Limited Proteolysis of [^{3}H]Dexamethasone 21-Mesylate Labeled Receptors. Partially purified, activated [^{3}H]dexamethasone 21-mesylate labeled HeLa cell and HTC cell receptors were dissolved in 1 mM EDTA, 0.2% SDS, 20% glycerol, and 125 mM Tris-HCl (pH 6.8) buffer at a concentration of 8–40 pmol of labeled receptor/mL. Protein concentrations varied between 80 and 400 μg/mL and were adjusted to 1 mg/mL with conalbumin. Digestions with *Staphylococcus aureus* V8 protease or with α-chymotrypsin

Table I: Kinetic Constants of Binding [^{3}H]Triamcinolone Acetonide to Human Receptors[a]

temp °C	K_D (nM)	k_1 (10^3 M^{-1} s^{-1})	k_{-1} (μs^{-1})	k_{-1}/k_1 (nM)
0	1.3 ± 0.3	54 ± 5	9 ± 1	0.17 ± 0.04
20	4.0 ± 0.6	42 ± 4	66 ± 7	1.6 ± 0.3

[a] Apparent dissociation constants K_D were derived from Scatchard (1949) plots as described under Materials and Methods. The rate constants k_1 and k_{-1} were obtained according to Schrader (1975) (see Materials and Methods).

were conducted at 37 °C for 5–60 min as indicated in the legends to Figures 5 and 6. Protease concentrations were 0.1 μg/25 μL of assay. Reactions were stopped by addition of soybean trypsin inhibitor or by 5% 2-mercaptoethanol followed by boiling for 2 min. Two times sample buffer was added, and the proteolytic peptides were analyzed by SDS–PAGE. Twofold concentrated SDS sample buffer for PAGE contained 0.6M Tris (pH 8.8), 2% SDS, 0.2 M dithiothreitol, 20% glycerol, and bromophenol blue as the tracking dye.

Results

Kinetic Properties of Binding Glucocorticoid to Human GR. Hormone binding experiments were routinely performed by incubating cytosol with [^{3}H]triamcinolone acetonide (TA) for 2 h at 0 °C (steady-state conditions). Unbound hormone was removed by dextran-coated charcoal (Beato & Feigelson, 1972). Nonspecific binding was determined by incubating in parallel with a 1000-fold excess of unlabeled hormone. The S200 extracts from HeLa cells contain about 100–200 fmol of receptor/mg of protein; i.e., the concentration of GR per milligram of soluble HeLa protein is 10–50 fold lower than in comparable rat lysates (Higgins et al., 1973). The 2-fold variation in HeLa receptor concentration depended on the conditions of cell growth and on the use of charcoal-treated sera in the tissue culture media. Generally, cells growing at densities of 5×10^5/mL of tissue culture medium supplemented with charcoal-treated serum yielded lysates with higher amounts of GR per milligram of protein. Salt extracts of the nuclei in our HeLa cell lysates contained less than 5% of the amount of GR that was detected in the cytosol fraction. This indicated that our lysate conditions extracted nearly all cellular GR with the cytosol fraction, which also contained material binding glucocorticoid with low affinity ($k_{-1} = 2.0 \times 10^{-3}$ M^{-1}; data not shown). Receptor concentrations for other hormones, e.g., estrogen, progesterone, and androgen were, if any, <1 fmol/mg of protein. The equilibrium binding constants of the [^{3}H]TA–GR interaction were determined by Scatchard (1949) analysis as well as by association and dissociation kinetics (Schrader, 1975; Eliard & Rousseau, 1984). The data are summarized in Table I. GR in lysates of HeLa cells had at 0 °C a faster on-rate for binding [^{3}H]TA than GR in lysates of IM-9 cells for binding dexamethasone (Eliard & Rousseau, 1984). Vice versa, HeLa cell GR had at 0 °C a slower off-rate for binding [^{3}H]TA than IM-9–GR for binding dexamethasone. This is consistent with the observation that the rate of association is higher for steroids with high affinity than for steroids with low affinity to the receptor. Steroids that bind with high affinity do so in general because of their much slower rate of dissociation (Pratt et al., 1975). There was a discrepancy by almost 1 order of magnitude between the K_D value of 1.3 (±0.3) nM determined by equilibrium measurements and the K_D value of 0.17 nM calculated by dividing the off-rate value by the on-rate value. Similarly to previous data, a rise in ambient temperature from 0 to 20 °C caused a 7-fold increase in the off-rate for binding [^{3}H]TA to HeLa cell GR while k_1 was almost not affected. The K_D value for binding [^{3}H]TA to HeLa GR was at 20 °C 3-fold higher than at 4 °C. The specificity of steroid hormone–GR interaction was estimated from the midpoints of competitive binding curves (Ojasoo & Raynaud, 1978). The Relative binding affinities (RBAs) were similar to those for rat liver GR except the RBA of binding moxestrol (Ojasoo & Raynaud, 1978). RBA of moxestrol binding to rat liver GR is about 7 times lower than the RBA of binding TA. RBA of binding moxestrol to HeLa cell GR was 25 times lower than the RBA of binding TA. RBA of binding dexamethasone to HeLa cell–GR was half of binding TA. Relative binding affinities (RBA) for steroid hormones decreased in the order TA > dexamethasone > promegestone > methyltrienolone > aldosterone ≥ moxestrol.

High-Resolution Ion-Exchange Chromatography of Human GR. Ionic properties of human GR were studied by FPLC. [^{3}H]TA-labeled HeLa cell lysates were treated 2 times with dextran-coated charcoal with a time interval of 1 h. This procedure eliminated contaminating low-affinity binders. Bound hormone did not significantly dissociate during FPLC. More than 80% of bound [^{3}H]TA was eluted in the peak fractions. FPLC studies were directly performed with crude lysates, ensuring a rapid analysis of GR. Analogous to chromatographic studies with DEAE-cellulose, two receptor forms could be discriminated by FPLC (Figure 1). The activated form eluted at 250 mM NaCl and the nonactivated at 400 mM NaCl. It should be noted that addition of molybdate to elution buffers had no effect on the chromatographic properties of GR in FPLC in contrast to DEAE-cellulose chromatography (Weisz et al., 1984).

Activated GR was directly obtained either with cytosolic lysates of HeLa cells, which had been [^{3}H]TA labeled in vivo (Figure 1C), or with salt extracts of nuclei (Figure 1D). Obviously, treatment of cells with [^{3}H]TA prior to lysis stabilized activated GR. Figure 1C shows that a small amount (10–20%) of [^{3}H]-TA–GR in these lysates was eluted at high salt. These data may suggest that the appearance of a high-salt, nonactivated form of HeLa cell GR, which does not bind to DNA, was an extraction artifact.

The chromatographic properties of HeLa cell GR were compared to those of mouse and rat GR. For comparison, we have used a rat cell line (HTC) and three murine cell lines (S49.1wt, S49.1nt$^-$, and S49.1nt^i). Extracts were prepared from these cell lines by the same protocol which was used for the S200 extracts from HeLa cells. In accordance with previous chromatographic studies (Gustafsson et al., 1984) FPLC discriminated two receptor forms, which were eluted either at high salt or after heat activation at low salt (Table II). Nonactivated GRs were eluted at 400 mM NaCl, i.e., at the same position (Table II) within the separation capabilities of the FPLC system. The accuracy of determining the mean salt concentration in eluted receptor fractions was ±10 mM. Activated human, mouse, and rat receptors were eluted at different salt concentrations. HeLa cell GR was eluted at 250 mM (Figure 1B and Table II), whereas rat and mouse GRs were eluted at lower salt concentrations as summarized in Table II. This indicated that activated HeLa cell GR has a surface charge which is different from those of rat and mouse GRs. This does apparently not affect general DNA binding properties. The heat-activated forms of human, mouse, and rat GR were eluted from DNA–cellulose at 175–180 mM salt (Table II). For comparison, we have included the analyses of mouse GRs of mutant S49.1nt$^-$ and of S49.1nt^i cells (Yamamoto et al., 1976). In agreement with previously published data (Yamamoto et al., 1976, Gehring, 1980), the

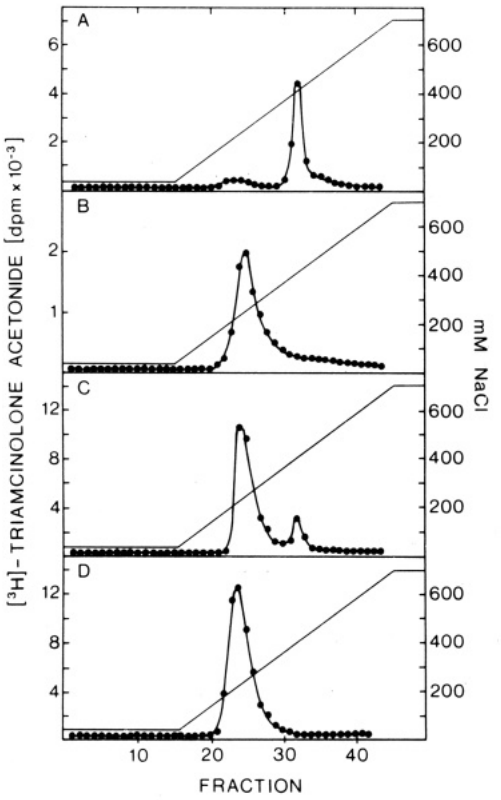

FIGURE 1: Analysis of human glucocorticoid receptor by high-resolution anion-exchange chromatography. Extracts of HeLa cells were prepared and were labeled with [^{3}H]triamcinolone acetonide as described under Materials and Methods. They were adjusted to 25 mM NaCl in buffer B and loaded on top of an FPLC–Mono Q column. The FPLC–Mono Q column was eluted with a linear 25–700 mM NaCl gradient in buffer B as described under Materials and Methods. A 750-μL fraction was collected and assayed for radioactivity, protein content, and conductivity. (A) FPLC of cytosol (5 mg of protein/250 μL of buffer B labeled with 10^{-8} M [^{3}H]triamcinolone acetonide. [^{3}H]Triamcinolone acetonide–GR complex in (A) was pooled (fractions 31 and 32), heated for 30 min at 20 °C, diluted to 25 mM NaCl, and rechromatographed. (C) FPLC of cytosol prepared from HeLa cells, which had been incubated in vivo with 10^{-8} M [^{3}H]-triamcinolone acetonide before cell lysis and extract preparation (see Materials and Methods). (D) FPLC of nuclear extract of in vivo labeled HeLa cells.

mutant S49.1 cell GRs were eluted from DNA–cellulose at salt concentrations different from wild-type GR.

Affinity Labeling of Human Glucocorticoid Receptor. Rat and mouse GR proteins have been previously characterized by affinity-labeling methods. Two methods have widely been used, chemical affinity labeling with dexamethasone 21-mesylate (Simons et al., 1983; Harmon et al., 1984; Wrange et al., 1984) or photoaffinity labeling by direct irradiation of the dexamethasone–GR complex (Nordeen et al., 1981; Dellweg et al., 1982). The chemical method is much more efficient than the latter. Nevertheless, we have employed both methods for characterizing HeLa cell GR. $(NH_4)_2SO_4$-precipitated, molybdate-stabilized [^{3}H]TA–GR complex was irradiated as described under Materials and Methods. The yield of [^{3}H]TA binding to GR was approximately 2%. This is rather low when compared to other systems, including photoaffinity labeling of mouse GR, where 5 times higher yields have been obtained (Dellweg et al., 1982). The product of the photoaffinity labeling reaction was analyzed by SDS–PAGE. Fluorography of the gel revealed one labeled peptide, which had an apparent molecular weight of 96 000 ± 2500 (Figure 2a). Covalent labeling of rat GR with dexamethasone 21-mesylate is usually greater than 90% (Simons et al., 1983). HeLa cell GR was labeled with a comparable efficiency. For comparison, we have

Table II: Analysis of Glucocorticoid Receptor from Different Cell Types by High-Resolution Anion-Exchange and by DNA–Cellulose Chromatography[a]

cell line	FPLC (mM NaCl required for elution)	DNA–cellulose chromatography (mM NaCl required for elution)
human, HeLa-S3	400, 250[b]	180
rat, HTC	400, 200[b]	180
mouse, S49.1 wt	400, 170[b]	175
S49.1 nt$^-$	400, ND[c]	86
S49.1 nt^i	400, 150[b]	225

[a] Crude cell lysates were labeled with [^{3}H]triamcinolone acetonide and were chromatographed on a Mono Q column as described in the legend to Figure 1. Radioactivity and salt concentrations in the fraction were assayed as described under Materials and Methods. Salt concentrations are given as mean values of those in the top peak fractions. As shown in Figure 1, the peak width spanned up to 120 mM NaCl; that is, nonactivated glucocorticoid receptor was eluted from the Mono Q columns with 380–450 mM NaCl–buffer B. Heat-activated HeLa cell glucocorticoid receptor was eluted with 170–300 mM NaCl–buffer B. Peak widths were similar for the chromatography of the other glucocorticoid receptors. [b] [^{3}H]Triamcinolone acetonide–receptor complex, eluted from the FPLC–Mono Q columns at 400 mM NaCl, were pooled. The pooled fractions were heat-activated (Atger & Milgrom, 1976), adjusted to 25 mM NaCl–buffer B, and rechromatographed on a Mono Q column (FPLC). All experiments were done in duplicate. [^{3}H]Triamcinolone acetonide labeled receptors in cell extracts were partially purified by phosphocellulose, calf thymus DNA–cellulose, and DEAE-cellulose chromatography (see Materials and Methods). Finally, the labeled receptors were heat-activated by incubating for 30 min at 20 °C. They were applied on top of a calf thymus DNA–cellulose column. Elution was with 50–200 mM NaCl–buffer A gradient. Salt concentrations and radioactivity in the fractions were assayed as in Figure 1. [c] ND, not determined.

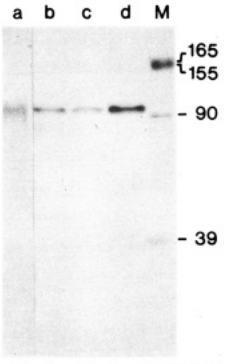

FIGURE 2: Fluorograph of affinity-labeled human glucocorticoid receptor. (Lane a) glucocorticoid receptor in HeLa cell extracts was labeled with [^{3}H]dexamethasone and was irradiated as described under Materials and Methods. The irradiation products were applied to a 10% SDS–polyacrylamide gel. After electrophoresis, the gel was dried and fluorographed. Exposure time was 40 days. (Lane b) HeLa cell glucocorticoid receptor was reacted with 10^{-8} M [^{3}H]dexamethasone 21-mesylate for 2 h at 0 °C in buffer C (see Materials and Methods). Reactions were terminated by the addition of 2 mM 2-mercaptoethanol. The reaction mixture was applied to an FPLC–Mono Q column. Labeled glucocorticoid receptor was isolated as in Figure 1. SDS–PAGE and fluorography was as in lane a. Exposure time was 5 days. (Lane c) HeLa cell cytosol was reacted with 10^{-8} M [^{3}H]dexamethasone 21-mesylate as in lane b and then was incubated 30 min at 20 °C. After the reaction had been terminated, labeled glucocorticoid receptor was isolated by DNA–cellulose chromatography. SDS–PAGE and fluorography was as in lane b. (Lane d) A mixture of the labeled glucocorticoid receptors in lanes b and c was applied. (Lane M) ^{14}C-methylated *E. coli* RNA polymerase was used as molecular weight marker. Molecular weights ($\times 10^3$) of RNA polymerase subunits are given at the right.

labeled HeLa cell GR with dexamethasone 21-mesylate and have subsequently purified nonactivated GR by FPLC. Similarly, heat-activated HeLa cell GR was affinity labeled and was subsequently purified by DNA–cellulose chromatography

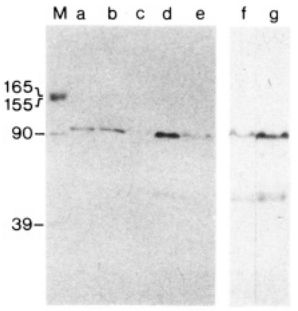

FIGURE 3: Fluorograph of dexamethasone 21-mesylate labeled glucocorticoid receptors from human, mouse, and rat cell lines. Glucocorticoid receptor cytosols of HeLa, HTC, and S49.1 cells were reacted with [^{3}H]dexamethasone 21-mesylate for 2 h at 0 °C as in Figure 2b. Labeled glucocorticoid receptors were isolated by FPLC (see Figure 1). SDS–PAGE in fluorography was as in Figure 2. (Lane M) ^{14}C-Methylated *E. coli* RNA polymerase as molecular weight marker. Molecular weights ($\times 10^3$) of RNA polymerase subunits are given on the left. The following amounts of labeled glucocorticoid receptor were applied on the 10% SDS–polyacrylamide gel: lane a, 0.15 pmol of HeLa cell glucocorticoid receptor; lane b, 0.15 pmol of HTC cell glucocorticoid receptor; lane c, 0.07 pmol of S49.1 cell glucocorticoid receptor; lane d, 0.15 pmol of HeLa cell together with 0.15 pmol of HTC cell glucocorticoid receptor; lane e, 0.15 pmol of HeLa cell together with 0.07 pmol of S49.1 cell glucocorticoid receptor. Exposure time was 8 days. A 20-day exposure of lanes c and e is shown in lanes f and g.

as described under Materials and Methods. The products of both affinity labeling reactions were analyzed by SDS–PAGE (Figure 2b,c). The apparent molecular weights of the denatured and reduced steroid hormone labeled peptides were in all cases 96 000 ± 1000. This value was quite similar to the published M_T 98 000 of dexamethasone 21-mesylate labeled rat GR (Reichmann et al., 1984) but was different from the published M_T 90 000 of dexamethasone 21-mesylate labeled human leukemic GR (Harmon et al., 1984). Analysis of a mixture of labeled nonactivated with heat-activated GR (Figure 2d) showed that the apparent molecular weights of both receptor forms were identical within the limits of detection. We have compared the dexamethasone 21-mesylate labeled HeLa cell GR with similarly labeled rat and mouse GR, respectively (Figure 3). Mixing human GR with rat GR (Figure 3d) or with mouse GR (Figure 3e,g) showed that the molecular weights of denatured affinity-labeled human, mouse, and rat GRs were indistinguishable. Preparations of dexamethasone 21-mesylate labeled GRs contained two more, weakly labeled peptides of M_T 51 000 and 48 000, respectively, (Figure 3d–g). We have not investigated further the nature of these side products of the affinity-labeling reactions. These peptides might be proteolytic degradation products of GR. They also could be completely unrelated to GR.

Reaction of Human GR with Anti-Rat Liver GR Antibody. Differences in the structural properties of human and rat GR became more apparent when we studied GR with a monoclonal anti-rat liver GR antibody (Westphal et al., 1984). This antibody cross-reacted with rodent as well as with avian GRs. However, as shown in Figure 4, it did not cross-react with human GR. The sedimentation constant of 5 S of activated HeLa cell GR was not altered by incubation with antibody (Figure 4B). HTC cell GR had a similar sedimentation constant; in the presence of antibody, however, it was shifted to 9 S, probably due to the formation of anti-rat GR antibody–[^{3}H]TA–GR complex (Figure 4A). Since the monoclonal anti-rat GR antibody cross-reacted with mouse, rabbit, guinea pig, and hen GRs (Westphal et al., 1982), these GRs apparently have a common anttigenic determinant, which is not shared by HeLa cell GR.

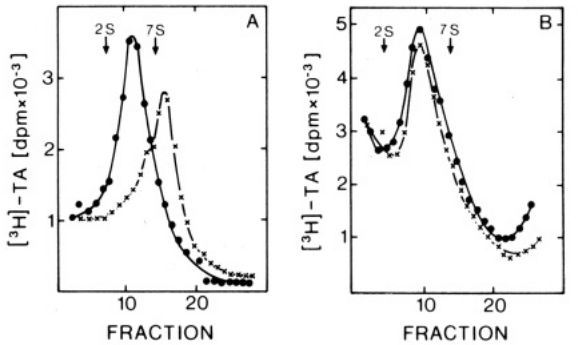

FIGURE 4: Density gradient centrifugation of [^{3}H]triamcinolone acetonide–glucocorticoid receptor complex incubated with monoclonal anti-glucocorticoid receptor antibody. [^{3}H]Triamcinolone acetonide (TA) labeled cytosols from HTC (A) or HeLa cells (B) were incubated 30 min at 0 °C with a 1:40 dilution of either a specific monoclonal antibody (×) (Westphal et al., 1982) or an unspecific antibody (●) (Dworniczak et al., 1983) as described under Materials and Methods. Centrifugation was in a linear 5–20% sucrose gradient in buffer B at 4 °C for 18 h. Arrows indicate the sedimentation position of lysozyme (2 S) and of γ-globulin (7 S), which were cocentrifuged.

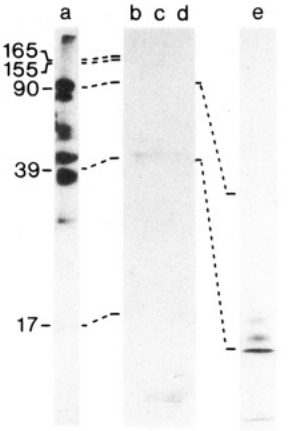

FIGURE 5: Fluorograph of [^{3}H]dexamethasone 21-mesylate labeled rat HTC and human HeLa glucocorticoid receptor digested by α-chymotrypsin. [^{3}H]Dexamethasone 21-mesylate labeled receptors were partially purified as described under Materials and Methods. (Lane a) A total of 0.45 pmol of labeled HTC cell receptor was digested 10 min by 0.1 μg of α-chymotrypsin at 37 °C. (Lanes b–d) A total of 0.15 pmol of labeled HeLa cell receptor was digested 10, 20, and 40 min, respectively, by 0.1 μg of α-chymotrypsin at 37 °C. (Lane e) An equimolar mixture (0.3 pmol) of labeled HeLa cell receptor digested 1, 5, 10, 30, and 60 min was applied. Termination of the digestions, SDS–PAGE, and fluorography were as described under Materials and Methods. Exposure times were in lane a 28 days and in lanes b–e 10 days. Lanes a–d are fluorographs of 12.5% SDS–polyacrylamide gels and lane e is a fluorograph of 7.5% SDS–polyacrylamide gel. Molecular weight markers (dashed lines) were *E. coli* RNA polymerase subunits as in Figure 2 and myoglobin. Corresponding molecular weights ($\times 10^3$) are given on the left of lane a.

Limited Proteolysis. Previously, rat as well as mouse GRs have been intensively studied by proteolytic digestions (Gehring & Hotz, 1983; Sherman et al., 1983; Reichmann et al., 1984; Wrange et al., 1984). These studies in conjunction with DNA-binding and antibody-binding studies have suggested that GRs have a domain structure that is subdivided into a DNA-binding, a steroid-binding, and an antibody-binding domain (Carlstedt-Duke et al., 1982; Dellweg et al., 1982). When dexamethasone 21-mesylate labeled rat HTC cell GR was treated with α-chymotrypsin and analyzed by SDS–PAGE, the molecular weights of the major proteolytic GR fragments obtained were basically similar to those obtained previously (Reichmann et al., 1984) (Figure 5a). The α-chymotrypsin digestion pattern of molybdate-stabilized HTC

Table III: Molecular Weight of Proteolytic Fragments of Rat and Human Glucocorticoid Receptors[a]

protease	[^{3}H]dexamethasone 21-mesylate labeled glucocorticoid receptor			purified rat liver glucocorticoid receptor
	HTC cells			
	Reichmann[b]		HeLa cells	
α-chymotrypsin	64 400	85 000		
		66 000		
		60 000		50 000[c]
		55 000		
	49 000	51 000		
	46 200		45 000	45 000[c]
	42 000	43 000	40 500	
	32 100	37 000	38 500	39 000[c]
	30 500			
	27 700	28 000		
	17 200		17 000	
V8 protease		85 000		
	77 900	70 000		
	62 500	66 000	66 000	ND[d]
	58 800	61 000	61 000	
	56 100			
	51 100	51 000	51 000	
	43 400	49 000	49 000	
		45 000	45 000	
		41 000		
		37 000		
		34 000		
	31 200	32 500		
		29 000	29 000	
			29 000	
		23 500	23 500	
		18 000	19 000	
		17 000		
		15 500	15 500	
		13 500	14 000	
		10 000	10 000	

[a] Molecular weights were determined from the fluorographs of gels such as shown in Figures 5 and 6. Average molecular weight values were calculated from semilog plots of the molecular weights of the protein standards vs. R_f. These plots were nonlinear; i.e., below M_T 40 000 the accuracy of molecular weight values is ±1000. Those bands that are underlined represent the most intense bands that are seen at various stages of proteolysis. [b] These molecular weights of proteolytic fragments of [^{3}H]dexamethasone 21-mesylate labeled native HTC cell glucocorticoid receptor were taken from Reichmann et al. (1984) for comparison. [c] Molecular weight values of proteolytic fragments of purified rat liver glucocorticoid receptor were taken from Wrange et al. (1984). [d] ND, not determined.

cell GR showed three main fragments of M_T 85 000, 42 000, and 37 000. In addition, we have observed faint bands in the digestion patterns of rat GR corresponding to fragments of M_T 66 000, 60 000, 55 000, 51 000, 30 000, and 28 000 (Table III). Though we have not done an extensive analysis of the digestion of HTC cell GR by chymotrypsin, a comparison to the data of Reichman et al. (1984) in Table III showed that they did not observe a major proteolytic fragment of HTC cell GR with M_T 37 000. Since we have used a partially purified HTC cell GR in the digestion studies, the differences in GR preparation between the work of Reichman et al. (1984) and ours might be responsible for this discrepancy.

Dexamethasone 21-mesylate labeled HeLa cell GR was digested by α-chymotrypsin and was analyzed by SDS–PAGE. Molecular weights of the major proteolytic HeLa cell GR fragments obtained were compared with those of HTC cell GR (Figure 5b–d; Table III). The α-chymotrypsin digestion pattern of HeLa cell GR showed two main fragments of M_T 38 500 and of M_T 17 000, respectively. We have also detected in the digestion patterns two fainter bands corresponding to fragments of M_T 40 500 and 45 000 (Figure 5e). More extensive digestion with α-chymotrypsin followed by SDS–PAGE

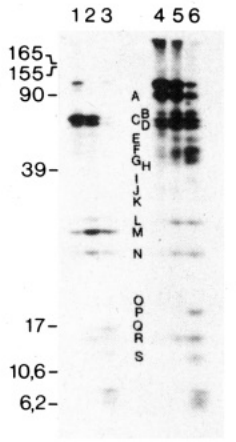

FIGURE 6: Fluorograph of [^{3}H]dexamethasone 21-mesylate labeled rat and human glucocorticoid receptors digested by *Staphylococcus aureus* V8 protease. [^{3}H]Dexamethasone 21-mesylate labeled receptors were partially purified as described under Materials and Methods. (Lanes 1–3) A total of 0.3 pmol of labeled HeLa cell glucocorticoid receptor was digested by 0.1 μg of V8 protease at 37 °C for 5, 15, and 45 min, respectively. (Lanes 4–6) A total of 0.45 pmol of labeled HTC cell glucocorticoid receptor was digested by 0.1 μg of V8 protease at 37 °C for 5, 15, and 45 min, respectively. Digestions were terminated with the addition of 10 mM 2-mercaptoethanol. Samples were applied to 12.5% SDS–polyacrylamide gels, electrophoresed, and fluorographed as described under Materials and Methods. Exposure time was 28 days. Molecular weight markers were *E. coli* RNA polymerase subunits as in Figure 2 as well as myoglobin and two BrCN fragments of myoglobin (M_T 10 600 and 6200). Molecular weights (×10^3) are given on the left. Proteolytic fragments are numbered in alphabetical order for discussion.

analysis increased only the amount of radioactivity that migrated with the tracking dye in the front of the gel (Figure 5d). We have not identified the size of these small, labeled fragments which were $M_T \geq 10000$. The main differences were observed in the upper molecular weight range. The differences in digestion patterns of HTC cell GR and of HeLa cell GR might reflect different structures of the two denatured glucocorticoid receptors. Alternatively, the differences might be caused by different rates of digestion, which would be due to the unequal ratios of HTC cell and HeLa cell receptors per milligram of protein in the receptor preparations.

Since the α-chymotrypsin digestion patterns appeared to show some structural differences between rat and human GR, we have used a second protease, which might yield more pronounced differences. [^{3}H]Dexamethasone 21-mesylate labeled HTC cell GR and HeLa cell GR were digested by *Staphylococcus aureus* V8 protease essentially similar to the conditions of digestion by α-chymotrypsin. V8 protease digestion of HeLa cell GR yielded a higher proportion of large molecular weight fragments than did α-chymotrypsin (Figure 6). This is similar to recent results, which have been published for the digestions of dexamethasone 21-mesylate labeled HTC cell GR by α-chymotrypsin, trypsin, and V8 protease (Reichmann et al., 1984). When the V8 protease digests of rat and human GR were analyzed by SDS–PAGE, qualitative differences were readily apparent in the digestion patterns. The major fragments A, B, H, and P in Figure 6 were not present in digests of HeLa cell GR; vice versa, fragments M and Q were not detected in digests of HTC cell GR. This reciprocal lack of proteolytic fragments would be expected if human and rat GR have different structures, which are attacked by V8 protease. Since it has been reported that digestions of native or denatured rat GR by various proteases yielded qualitatively the same digestion patterns (Reichmann et al., 1984), the qualitative differences in the digestion patterns of Figure 6 probably reflect differences in the amino acid

sequences of rat and human GR. Quantitative differences were also observed; e.g., fragments E–G in Figure 6 were apparently produced at different rates. This might be again due to the unequal ratios of picomoles of receptor per milligram of protein in the HTC cell GR and HeLa cell GR preparations.

Table III lists the molecular weights of the observed proteolytic fragments in Figures 5 and 6. For comparison, molecular weights of proteolytic fragments are encluded, which have been published for the α-chymotrypsin and V8 protease digests of purified rat liver GR (Wrange et al., 1984) and of crude, [^{3}H]dexamethasone 21-mesylate labeled HTC cell GR (Reichman et al., 1984). As gradient and linear gels do not yield identical molecular weights, most, if not all, discrepancies between the data in the literature and ours would be expected to be due to analyses by different SDS–PAGE. Thus, we believe that differences of 2000–3000 daltons in apparent molecular weights are not real but are PAGE artifacts. The most noteworthy difference to the data of Reichman et al. (1984) was that we have detected more intermediate proteolytic fragments with partially purified [^{3}H]dexamethasone 21-mesylate labeled HTC cell glucocorticoid receptor.

Discussion

The molecular weights and the general properties of human GR were similar to rat and mouse GR (Figures 1–3). Steroid hormone was bound by GR with kinetic parameters and specificities (Table I which are comparable to the data published for rat and mouse GR (Ojasoo & Raynaud, 1978; Schmidt & Litwack, 1982). The reactivity of the steroid hormone binding domain toward photoactivated dexamethasone as well as to dexamethasone 21-mesylate was similar to that of rat GR. The latter GR gave yields of 2.5% covalent binding after photoirradiation (Nordeen et al., 1981; Wrange et al., 1984) and 70–90% after reaction with dexamethasone 21-mesylate (Simons et al., 1983; Reichman et al., 1984). Human GR gave very similar yields: 2% for covalent binding of dexamethasone after irradiation and 70% for covalent binding of dexamethasone 21-mesylate.

General DNA-binding characteristics of human GR were qualitatively similar to those of rat and mouse GR. The DNA-binding form of human GR had a different bulk ionic charge as compared to those of rat and mouse GR, since it was eluted from the FPLC–ion-exchange column at a different salt concentration than that of rat or mouse GR (Figure 1; Table II). The differences of net charge between the activated receptors do not alter the general DNA–receptor interaction. Whether this influences DNA-sequence recognition has yet to be investigated.

A more detailed analysis revealed that the human glucocorticoid receptor complex characteristically differed from rat and mouse GR. Monoclonal anti-rat GR antibodies did not cross-react with HeLa cell GR (Figure 4). As the hormone binding sites as well as the DNA-binding domain did not exhibit bulk differences, the differences in the antibody-binding domain of GRs probably reflect the bulk difference of net charges seen by FPLC (Table II). α-Chymotrypsin digests of HeLa cell GR generated a major M_T 38 500 proteolytic fragment, which might be similar to the M_T 39 000–42 000 proteolytic fragment of rat GR, which contains the steroid- and DNA-binding domain (Wrange & Gustafsson, 1978; Gehring & Hotz, 1983). A different set of proteolytic fragments was produced in α-chymotrypsin digests of HeLa cell GR was compared to HTC cell GR (Figure 5; Table III). Since HeLa cell lysates only contained 100–200 fmol of receptor/mg of protein, it is not entirely clear whether the observed differences in the patterns of digestion by α-chymotrypsin indicate differences in amino acid sequences or are due to qualitative differences generated by different rates of proteolysis.

Two major proteolytic fragments (C and D in Figure 6) were produced by V8 protease digestion of HTC cell as well as HeLa cell GR. More extensive digestion produced major fragments (H and P in Figure 6), which were not detected in V8 protease digests of HeLa cell GR. On the other hand, two proteolytic fragments of HeLa cell GR digests (fragments M and Q in Figure 6) were not detected in HTC cell GR digests. Also, the digestion patterns in the low molecular weight range differed between rat and human GR. Thus, the V8 protease digestions most clearly reflect the structural differences that were evident in FPLC and in antibody-binding experiments. These differences are most likely based on differences in the amino acid sequences of human and rat GR.

Glucocorticoid receptor literature is abound of studies about the conversion of a cytoplasmic receptor form into an activated DNA-binding form. These studies were thought to support the classical model of hormone action [for review, see Clark (1984)]. According to this model, the steroid hormone enters the target cell, binds to a specific cytoplasmic receptor, and then is translocated to the nucleus via an "activation" process, mimicked in vitro, for example, by heat (Milgrom et al., 1973). In this context, it is interesting that we have found in lysates of HeLa cells preincubated with [^{3}H]TA in vivo predominantly the DNA-binding form of GR (Figure 1) contrary to lysates that were prepared by standard procedures and were incubated with [^{3}H]TA in vitro. There are two possibilities to understand this result. Nonactive GR is rapidly converted in vivo to active GR by binding steroid hormone. In fact, equilibrium was reached in vitro within 10 min between the activated and the nonactivated form of GR (Leach et al., 1979). Alternatively, binding steroid hormone prevents the cellular active GR from being converted into a nonactivated species during cell lysis.

Acknowledgments

We thank M. Beato for kindly providing the monoclonal anti-rat GR antibody.

Registry No. Triamcinolone acetonide, 76-25-5; dexamethasone, 50-02-2; promegestone, 34184-77-5; methyltrienolone, 965-93-5; aldosterone, 52-39-1; moxestrol, 34816-55-2; dexamethasone 21-mesylate, 2265-22-7.

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