The Function of Steroid Hormone Receptors Is Inhibited by the hsp90-specific Compound Geldanamycin*

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Steroid hormone receptors are members of the ever growing family of nuclear receptors (for reviews, see Refs. 1 and 2). They are characterized by sequence homologies that are most prominent within the DNA binding domains. In contrast to most other members of the family, steroid hormone receptors are known to be complexed with heat shock proteins, most notably hsp90,1 and heat shock proteins are involved in steroid signal transduction (for reviews, see Refs. 3 and 4). The association with heat shock proteins thus prevents these receptors from interacting with specific DNA recognition sequences while they are not yet complexed with the respective steroid ligands. On the other hand, hsp90 in association with receptor polypeptide appears to significantly contribute to the receptors’ hormone binding ability, as has been established for several steroid hormone receptors (5–9).

In recent years, a group of streptomyces antibiotics called benzochinone ansamycins were shown to affect the biological activity of several tyrosine-specific protein kinases (10–17) that are known to similarly associate with hsp90. Interestingly, geldanamycin and herbimycin A, major representatives of ansamycin antibiotics, were found to directly interact with hsp90 (18). This observation prompted a series of investigations into the effects of these drugs on steroid hormone receptors. In the present study we show that geldanamycin at non-toxic concentrations inhibits hormonal inducibility in several responsive cell systems. Moreover, this drug affects the binding of glucocorticoid, progesterin, androgen, and estrogen to their specific receptors but does not per se interfere with the interaction of hsp90 with the receptor polypeptide, thus leaving the overall heteromeric receptor structure intact.

EXPERIMENTAL PROCEDURES

Chemicals—[6,7-3H]ORG 2058 (1.8 TBq/mmol), [2,4,6,7-3H]estradiol (3.3 TBq/mmol), and [1,2,4-3H]triamcinolone acetonide (1.0 TBq/mmol) were obtained from Amersham Corp., [1,2-3H]testosterone (2.0 TBq/mmol) from NEN Life Science Products, and [3S]Methionine (45 TBq/mmol) from ICN. EGS was purchased from Pierce, lactacystin from Biomol, and cycloheximide from Sigma. Other chemicals were of reagent grade or better.

Cell Cultures—Mouse thymic lymphoma WEHI-7 cells, rat hepatoma HTC cells, human mammary carcinoma T-47D cells, COS-7 cells, Rat1 fibroblasts transfected with the cDNA for the human androgen receptor, and NIH 3T3 cells stably transfected with a mouse mammary tumor virus-chloramphenicol acetyltransferase construct were grown at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (50 units/ml), and streptomycin (50 μg/ml), as well as Geneticin sulfate (450 μg/ml) in the case of transfected Rat1A cells. Human mammary carcinoma MCF-7 cells were grown in the same medium except that it was devoid of phenol red and the serum was stripped with charcoal. E36ts20 Chinese hamster cells were grown at 32 °C in normal medium with serum. Attached cells were released by treatment with 1 mM EDTA in saline, and cells were washed twice with phosphate-buffered isotonic sucrose. Cell pellets were kept at –80 °C until needed.

COS-7 cells were transiently transfected by use of the cationic liposome-mediated transfection reagent DOTAP (Boehringer Mannheim) with plasmids pSV2Wrec (19) and pGMCS (20) encoding the mouse glucocorticoid receptor and hormone-responsive chloramphenicol acetyltransferase, respectively. 2.5 μg of DNA of each were used per Petri dish. 24 h post-transfection, cells were used for hormone induction experiments.

Cell Extracts and Incubations—Cells were homogenized as before (21) in 20 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and the high speed supernatant was obtained. When cell extracts were incubated at 37 °C, we added 20 mM sodium molybdate to stabilize receptors. Cross-linking with EGS was carried out as before for 45 min (22, 23).

Enzyme Assays—Assays for chloramphenicol acetyltransferase (24) and for tyrosine aminotransferase (25) were carried out according to standard protocols.

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1 The abbreviations and trivial names used are: hsp90 and hsp70, heat shock proteins of Mr 90,000 and 70,000, respectively; EGS, ethyl-ene glycol bis(succinimidyl succinate), ORG 2058, 16α-ethyl-21-hydroxy-19-norpregn-4-ene-3,20-dione; PAGE, polyacrylamide gel electrophoresis; trimcinolone acetonide, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-ketal with acetone.
Hormone Binding—Scatchard analysis was as before (26) with $^3$H-labeled steroids routinely at four different concentrations between 1 and 40 nM. In parallel incubations, a roughly 1000-fold excess of unlabeled hormone was added to assess unspecific binding. All incubations were in duplicate. Cell extracts were used at 3–5 mg/ml protein, and incubation was for 2 h at 0 °C. Scatchard experiments with whole cell fractions were for 45 min at 37 °C using 5 × 10^6 cells/ml. $^3$H]Triamcinolone acetonide was used as ligand for the glucocorticoid receptor, $^3$H]estradiol for the estrogen receptor, $^3$H]testosterone for the androgen receptor, and $^3$H]HORJ2058 for the progestin receptor. Extracts of T-47D cells also contained 1 μg cortisol to saturate endogenous glucocorticoid receptors. For the binding assays of Fig. 6, B and C (in duplicate or triplicate), with extracts of WEHI-7 and E36ts20 cells we used 30 nM $^3$H]triamcinolone acetonide with or without 10 μM unlabeled steroid.

Immunopurification of Glucocorticoid Receptors—The monoclonal antibody mab49 (27) coupled to Sepharose was used as before (22). Most importantly, the immunomatrix was extensively washed with buffer containing 600 mM KCl and 0.2% Triton X-100. Elution was with 3.5 M NaSCN. Bovine insulin (50 μg/ml) was added as carrier, and proteins were precipitated with trichloroacetic acid. Pellets were dissolved in 0.4 M Tris base.

SDS-PAGE and Immunoblotting—EGS cross-linked receptors after immunopurification and acid precipitation were reprecipitated with cold acetone (8-fold volume) and submitted to continuous SDS-PAGE as before with incompletely cross-linked phosphorylase a as markers (22). Elution from gels was by maceration in 50 mM Tris buffer, pH 7.2 (containing 0.1% SDS), followed by cleavage of cross-links with 1 mg hydroxylamine at 37 °C for 3 h (22, 23). Standard discontinuous SDS-PAGE was in 10% gels with rabbit muscle phosphorylase (97.4 kDa), bovine serum albumin (66.4 kDa), and bovine liver catalase (59 kDa) as molecular size markers.

Proteins were transferred to Immobilon-P membranes (Millipore Corp.) in 25 mM Tris, 48 mM glycine, containing 10% methanol, overnight at 50 V in the cold. Membranes were incubated with mab49 (27) or BuGR2 (28), specific for glucocorticoid receptors; antiserum against hsp90 (29); 1.500 diluted antiserum against murine p59 (30); or 1.000 diluted J3J monoclonal antibody specific for p23 (31) as described (22, 29). Incubation with appropriate peroxidase-conjugated second antibodies (Sigma) and chemiluminescence (ECL, Amersham Corp.) were used for detection; evaluation was by scanning densitometry.

Gel Filtration—Chromatography on Sephadryl S-300 (Pharmacia) was in 20 mM potassium phosphate buffer, pH 7.4, 150 mM KCl, 20 mM sodium molybdate, 1 mM EDTA, 10% glycerol. Columns (bed volume, 200–210 ml) were calibrated in separate runs with blue dextran 2000, bovine serum albumin (66.4 kDa), and bovine liver catalase (59 kDa) as molecular size markers.

In Vitro Receptor Synthesis—The rat glucocorticoid receptor (20) was synthesized by combined transcription/translation (TNT; Promega) in 50-μl samples for 90 min at 30 °C using 1 μg of the cDNA construct that contains the SPS promoter. Preincubation with geldanamycin was for 30 min at 30 °C. Samples were incubated with $^3$H]triamcinolone acetonide (60 nM) for 2 h at 0 °C and subsequently passed through Sephadex G-50 (bed volume, 1.3 ml). Effluent fractions (150 μl) were treated with alkaline H$_2$O$_2$ (20%) for 15 min at room temperature, and excess H$_2$O$_2$ was removed at 90 °C before scintillation counting. Some synthesis reactions were in the presence of [35S]methionine.

RESULTS

Effect of Geldanamycin on Intact Cells—In preliminary experiments with several cell lines in culture we established the concentrations of geldanamycin that are tolerated by these cells. We observed that incubation with 0.1 μM/g/ml for up to 2 days had only a slight effect on the doubling time of the cells used in this study, except for the WEHI-7 line, which appeared to be particularly sensitive and was affected even by low concentrations of geldanamycin. However, treatment at 0.3–1.0 μM/g/ml for several hours and subsequent washout was found to have no deleterious effect on further growth of WEHI-7 cells. We therefore routinely used this concentration range in our experiments with intact cells.

To check whether geldanamycin affects hormonally controlled gene expression, we first turned to one of the classical systems: induction of hepatic tyrosine aminotransferase in response to glucocorticoid. As shown in Fig. 1A, the induction of enzymatic activity in rat hepatoma cells of line HTC was significantly reduced upon administration of geldanamycin together with the steroid. In another experiment we employed 3T3 fibroblasts that have been stably transfected with steroid-inducible chloramphenical acetyltransferase (32) and exposed them to geldanamycin. Glucocorticoid induction of enzyme activity was greatly repressed (Fig. 1B). Quantitative evaluation by scanning showed an 8.7-fold increase in activity by triamcinolone acetonide (Fig. 1B, lane 2 versus lane 1), which was reduced to 1.2-fold by the drug (lane 4 versus lane 1). We also used COS-7 cells and transiently transfected them with a corresponding chloramphenical acetyltransferase reporter construct as well as the mouse glucocorticoid receptor (Fig. 1C). In this experiment, treatment with geldanamycin and triamcinolone acetonide was overnight. We observed a 28-fold induction of chloramphenical acetyltransferase by hormone, which was inhibited by geldanamycin to roughly half.

These data clearly show that geldanamycin interferes with
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Effect of Geldanamycin on Hormone Binding—For hormone binding studies we initially used intact WEHI-7 mouse lymphoma cells and the glucocorticoid \(^{3}H\)triamcinolone acetone. The Scatchard analyses of Fig. 2 were carried out at 37 \(^{\circ}\)C in the presence of increasing concentrations of geldanamycin. Within 3 h of treatment with 0.3 \(\mu\)g/ml, hormone binding capacity decreased to about a third of that of untreated cells; some effect was also seen on the affinity for the ligand. Inhibition of hormone binding is readily reproduced in cell-free experiments using extracts of cells preincubated with the drug (Table I). Several such binding studies yielded a 80–90% reduction in the number of glucocorticoid binding sites after preincubation of cells with 0.3 \(\mu\)g/ml geldanamycin.

Interestingly, the addition of geldanamycin to receptor containing extracts of untreated WEHI-7 cells did not produce any effect. No inhibition was seen whether the cell extract was preincubated with geldanamycin for 2 h in the cold or for 30 min at 37 \(^{\circ}\)C (Table I). Similarly, the inhibitory effect of geldanamycin was not seen when cells were kept in the cold during exposure to the drug (data not shown). These observations may suggest that active cellular processes are required for inhibition to occur.

To substantiate this view, we used glucocorticoid receptors in vitro synthesized in the reticulocyte lysate system. While the control sample showed ample binding of \(^{3}H\)triamcinolone acetone, there was almost no hormone binding when receptor synthesis was carried out in the presence of geldanamycin (Fig. 3A). To check whether the drug might affect the in vitro synthesis system itself, we carried out parallel incubations in the presence of \(^{35}S\)methionine. We detected equal amounts of receptor polypeptide in the treated sample and the control (Fig. 3A, inset).

To find out about the specificity of inhibition by geldanamycin, we also investigated the progesterin receptor of T-47D mammary carcinoma cells, the estrogen receptor of MCF-7 mammary carcinoma cells, and the androgen receptor overexpressed in fibroblasts. In these studies we used binding assays with cell extracts rather than intact cells, since they are less error-prone according to our experience. As summarized in Table I, inhibition of hormone binding was observed in each case upon pre-treatment of intact cells with geldanamycin, although the drug affected the respective receptors to different extents. Interestingly, the androgen receptor was significantly less sensitive than the other receptors. This corresponds to the previous observation that there is no absolute requirement of hsp90 association for hormone binding to the androgen receptor (33). Also, with progesterin and estrogen receptors, pretreatment of intact cells with geldanamycin was required for the inhibitory effect to show up, and there was no inhibition of hormone binding if extracts were incubated with the drug (Table I).

Receptor Levels in Geldanamycin-treated Cells—The above binding studies made it necessary to directly investigate the receptors of geldanamycin-treated cells. For example, hormone binding might be affected if the drug disrupted the association of hsp90 with receptor polypeptides. In fact, the glucocorticoid receptor stripped of heat shock proteins has been reported to possess significantly decreased ligand binding activity (5–7). Moreover, receptor devoid of hsp90 might have a much faster intracellular turnover than the complexed form; consequently, cells would accumulate much lower receptor levels. We therefore checked by immunoblotting for the glucocorticoid receptor polypeptide in extracts of geldanamycin-treated WEHI-7 cells. In all cell samples we detected significant levels of immunoreactive receptor; however, the intensity of the receptor signal possessed significantly decreased ligand binding activity (5–7). Furthermore, receptor devoid of hsp90 might have a much faster intracellular turnover than the complexed form; consequently, cells would accumulate much lower receptor levels. We therefore checked by immunoblotting for the glucocorticoid receptor polypeptide in extracts of geldanamycin-treated WEHI-7 cells. In all cell samples we detected significant levels of immunoreactive receptor; however, the intensity of the receptor signal possessed significantly decreased ligand binding activity (5–7).
We next investigated the size of glucocorticoid receptors by gel permeation chromatography. We had previously established that the receptor complexed with heat shock proteins elutes from Sephacryl S-300 columns with a Stokes' radius of roughly 8 nm, while the receptor stripped of hsp90 has a radius of 6 nm (21, 29). In the experiment of Fig. 4B, we therefore pooled the effluents corresponding to the void volume and the 8- and 6-nm regions, respectively, and analyzed them by immunoblotting for receptor polypeptide. The receptor was exclusively recovered in the 8-nm region independent of pretreatment of the cells with geldanamycin. Interestingly, there was no receptor material recovered in the 6-nm region, demonstrating that geldanamycin-treated cells do not contain the naked receptor polypeptide. In another experiment we pooled 2-ml effluent fractions and individually analyzed them by immunoblotting. We did not obtain any evidence for a change in receptor size by pretreating cells with the drug (data not shown).

To independently assess the size of glucocorticoid receptors contained in geldanamycin-treated WEHI-7 cells, we used chemical cross-linking with EGS (22, 23). This stabilization of heteromeric structures allows their analysis by continuous SDS-PAGE and immunoblotting. High molecular weight receptor material of 340 ± 15 kDa was detected as judged from cross-linked phosphorylase marker bands (Fig. 4C). A comparison of lanes 1 and 2 again shows less receptor material in the sample from cells exposed to geldanamycin. However, by far the major portion of immunodetectable receptors is of the same size whether extracted from untreated or treated cells.

To investigate the components of hormone binding-incompe-
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Regeneration of Active Receptors after Geldanamycin Treatment—We also wanted to find out whether the effect of geldanamycin on hormone binding is reversible. This is indeed the case as shown in Fig. 6A. After washout of the drug, it takes several hours in culture before glucocorticoid binding activity is regenerated in WEHI-7 cells. Within 4 h we had attained roughly half of the original activity, and after 1 day binding activity had recovered completely.

To learn whether de novo receptor synthesis is required for restoration of hormone binding ability, we inhibited protein synthesis by adding cycloheximide (20 μg/ml) to cell cultures. Control experiments with [35S]methionine showed that within 5 h of exposure to the inhibitor, general protein synthesis as well as de novo synthesis of receptor polypeptides was reduced by at least 95% (data not shown). In the experiment of Fig. 6B we also made use of lactacystin, a highly specific and irreversible inhibitor of intracellular protein degradation by proteasomes (34). Indeed, treatment of WEHI-7 cells with lactacystin significantly diminished the loss of immunodetectable receptor brought about by geldanamycin (lane 3 versus lane 2). As shown in Fig. 6B (experiment 4), the simultaneous presence of cycloheximide for 5 h allowed regeneration of steroid binding ability. Such a regain of intermediate binding activity with new protein synthesis inhibited was reproducibly seen. Unfortunately, however, prolonged exposure of WEHI-7 cells to cycloheximide affected cellular integrity so that we could not carry these experiments for much longer incubation times.

In another set of experiments we used E36la20 hamster cells that harbor a defect in protein degradation by the ubiquitin-proteasome pathway (35). These cells contain glucocorticoid receptors at 5–10-fold lower levels than WEHI-7 cells, which nevertheless are detectable by immunoblotting and can be used for binding studies, albeit with somewhat lower accuracy. We also observed that E36la20 cells can withstand cycloheximide for extended periods of time before deteriorating. Geldanamycin treatment for 2 h again decreased hormone binding ability (Fig. 6C, column 2 versus column 1), even more drastically than in WEHI-7 cells. Moreover, receptor degradation was less prevalent in these cells (lane 2 versus lane 1). The degree of receptor loss again varied among experiments with 68 ± 22% (S.D.) of control levels in four independent experiments. Fig. 6C (column 4) shows that cycloheximide treatment after wash out of geldanamycin allowed recovery of almost 50% of the original hormone binding activity within 12 h. Perhaps not surprisingly, full restoration of hormone binding was only attained if...
protein synthesis was allowed to proceed (Fig. 6, panel C, column 3, and panel A).

**DISCUSSION**

The heat shock protein hsp90 is one of the most abundant cytosolic proteins in eukaryotes, even under normal growth conditions. It reaches up to the percentage level of the total soluble protein pool. Collectively, hsp90 is a mixture of two proteins of similar size that are the products of two related genes (for a review, see Ref. 36). Studies in yeast have shown that only one of these genes can be inactivated without producing deleterious cell effects (37). Although this points to some vital function(s), it is not clear at present which cellular event involving hsp90 is essential for cell growth and survival. Nevertheless, it has become evident over the past years that hsp90 interacts with a host of intracellular proteins. These include receptors for steroid hormones and aryl hydrocarbons, a variety of protein kinases, and some transcription factors (for a review, see Ref. 4). Recently, association with the reverse transcriptase of hepatitis B virus has also been described (38).

With respect to steroid hormone receptors, hsp90 appears to play a dual role, as pointed out in the Introduction. The present investigation made use of the antibiotic geldanamycin, which specifically interacts with hsp90 (18) and interferes with at least some of its functions. We found that geldanamycin indeed inhibits steroid hormone action in several target systems. Both the expression of an endogenous hormone-responsive gene and of appropriate reporter constructs were affected in qualitatively similar ways (cf. Fig. 1). Also, a recent paper (39) that appeared in print while this work was under consideration describes inhibition of glucocorticoid inducibility in transfected cells upon exposure to geldanamycin. These effects nicely correlate with inhibition of hormone binding ability in intact cells at 37 °C. We observed that binding of glucocorticoid, progestin, androgen, and estrogen to the respective receptors in cell extracts is only affected if the drug is administered to the respective target cells under cell growth conditions. There is no effect on hormone binding activity if presynthesized receptors are exposed to geldanamycin. In accordance with this, the drug prevents the generation of hormone binding ability if it is present during *in vitro* receptor synthesis and assembly, while *in vitro* transcription or translation *per se* are not impaired. Clearly, the receptor-hsp90 complex or its assembly is affected in some way. This conclusion is in support of recent data from others (40, 41) who observed an effect of geldanamycin on an hsp90-containing multichaperone complex in reticulocyte lysate that is essential for the assembly of receptor heterocomplexes.

When Whitesell *et al.* (18) studied the effect of geldanamycin on the pp60src tyrosine-specific protein kinase, they observed disruption of the hsp90 heterocomplex, while *in vitro* measured kinase activity decreased only modestly by pretreatment of the virally transformed cells with geldanamycin. These observations made us wonder whether the drug might similarly interfere with the association of hsp90 with steroid hormone receptors polypeptides. However, when we carefully analyzed the inactive glucocorticoid receptor of geldanamycin-treated lymphoma cells we found that the receptor complex was perfectly normal in size. Moreover, the receptor complex that was stabilized by chemical cross-linking and extensively purified by immunoaffinity chromatography and SDS-PAGE was found to contain normal amounts of both hsp90 and the FK506-binding immunophilin p56, as compared with the functionally intact receptor of untreated cells. Therefore, the heterotetrameric structure consisting of one receptor polypeptide, two molecules of hsp90, and one p59 subunit that had previously been established (22) appears to be maintained upon drug treatment.

When we analyzed the extensively purified glucocorticoid receptor for p23, no specific immunosignal was obtained. This is of interest because p23 had been detected by copurification with the avian progestin receptor (31) and was repeatedly found in association with *in vitro* reconstituted receptor complexes (4, 40, 41). It is perhaps not surprising that standard coprecipitation techniques as often used result in copurification of proteins like p23 (31, 39), which we do not find as components of cross-linked and extensively purified receptor heteromers. Our purification procedure would certainly remove such
loosely bound proteins. Nevertheless, p23 is well known to participate in the process of receptor heterocomplex assembly (40-42), and the hsp90-p23 complex is critically affected by geldanamycin (40, 41).

The above observations on the subunit constitution then lead to the question of how hormone binding ability is affected within the heteromeric receptor of at least similar overall structure. We suspect that geldanamycin produces conformational changes in receptor heteromers that may be rather subtle but nevertheless functionally important. Observations on the effect of geldanamycin on wild-type and mutated forms of p53 (43) in fact support the view that this drug is either able to distinguish between protein conformations or perhaps produces altered conformational states, possibly through the action of heat shock proteins. In any event, hsp90 is known to have chaperoning activity (36), and geldanamycin may well affect its folding properties on receptors. The fact that geldanamycin inhibits the receptors for glucocorticoid, progestin, androgen, and estrogen (cf. Table I) supports the view that hsp90 plays the very same role for hormone binding throughout the entire class of steroid hormone receptors.

The view that the presence of geldanamycin during receptor complex assembly produces conformational alterations that interfere with the recognition of steroidal ligands is indirectly supported by observations by others (39-41) who have described increased levels of heat shock protein hsp70 in association with receptors. In fact, hsp70 is a major molecular chaperone that participates in protein folding events and is known to preferentially bind to partially unfolded or malfolded proteins (for a review, see Ref. 44). Increased binding of hsp70 then appears quite logical if, for example, the hormone binding domain of receptors, which also contains the areas of contact with hsp90 (for a review, see Ref. 45), is incorrectly folded due to geldanamycin interacting with hsp90.

The glucocorticoid receptor polypeptide either in vitro translated by reticulocyte lysate or presynthesized and subsequently incubated with this system in the presence of ATP is known to associate with hsp90 (for a review, see Ref. 4). However, when we analyzed such in vitro synthesized and assembled receptor material, we realized that not all receptor polypeptides were in the high molecular weight state; roughly 30% remained in the monomeric form with a 6-nm Stokes’ radius. Presumably, this material is malfolded and thus bait for hsp70, as discussed above. Interestingly, geldanamycin did not significantly influence the relative amount of monomeric receptor produced in vitro. The shift in elution profile of heteromeric receptors from an 8.1- to 7.2-nm Stokes’ radius caused by geldanamycin (cf. Fig. 3B) suggests that some receptor-associating component, most likely p59, is missing from in vitro produced receptors. In this context, it is of interest that a minimal receptor assembly system has recently been reconstituted from components of reticulocyte lysate (42) in which the p59 component is not required but hormone binding ability is nevertheless generated. Furthermore, the chicken progestin receptor reconstituted by reticulocyte lysate in the presence of geldanamycin was found to be devoid of p59 and other immunophilins (40, 41). Taken together, these data show that the in vitro synthesized and assembled receptor in the presence of geldanamycin is not equivalent to that contained in cells although hormone binding is impaired in both cases. Still another difference between glucocorticoid receptors in cells or produced by in vitro synthesis and assembly in the presence of geldanamycin concerns receptor stability. In contrast to the effects seen in intact cells (see below), no destabilization of receptors was observed in the reticulocyte lysate system although this cell extract is well known for containing the ubiquitin-proteasome pathway of protein degradation (for reviews, see Refs. 46 and 47).

When we analyzed geldanamycin-treated mouse lymphoma cells for immunoreactive glucocorticoid receptor polypeptide, we consistently observed a reduction in receptor levels although the cells had only been in contact with the drug for 2 h. Receptor loss in cells exposed to geldanamycin turned out to be rather variable in our hands. Also, in transfected HeLa cells a decrease in the stability of glucocorticoid receptors was recently described upon exposure to geldanamycin (39). A receptor half-life of approximately 1 h was reported (39), in contrast to about 4 h in untreated control cells. In other experiments, the progestin receptor was transiently transacted into COS-1 cells (40). Upon treatment with geldanamycin, the hormone binding ability decreased, but there was no loss of immunoreactive receptor although the concentration of the drug was at least 10-fold higher than that used in our cell studies.

Enhanced glucocorticoid receptor degradation in geldanamycin-treated cells appears to involve the ubiquitin-proteasome pathway of intracellular protein breakdown. Support for this view comes from two independent lines of evidence. 1) The selective and covalent protease inhibitor lactacystin of streptomyces origin (34) significantly diminished the loss of immunodetectable glucocorticoid receptors in our experiments with WEHI-7 cells (cf. Fig. 6B) as well as in 3T3 cells (39). 2) Moreover, we observed that in E36ts20 Chinese hamster cells the glucocorticoid receptor polypeptide was considerably more stable upon geldanamycin treatment as compared with murine WEHI-7 cells (cf. Fig. 6C). E36ts20 cells are known to carry a defect in the ubiquitin-activating enzyme E1 (35). Interestingly, tyrosine kinase p185erbB2 is in vivo associated with the glucose-regulated protein GRP94, which has homology to hsp90 (48). Like hsp90, this protein binds geldanamycin, resulting in disruption of the complex (48). Such binding of geldanamycin or the analog herbimycin A to hsp90 or GRP94 further leads to degradation of the respective tyrosine kinase molecules in which the ubiquitin-proteasome pathway was again found to be involved (49, 50).

In experiments in which we first exposed cells to geldanamycin and then removed it by wash out, we found reversal to hormone binding ability. In principle, this restoration of activity may either be due to reactivation of receptor molecules preexposed to the drug or depend on new synthesis of receptor components. To distinguish between these alternatives, we inhibited protein synthesis. We still observed significant regain of hormone binding under such conditions (Fig. 6, panels B and C), in particular in the E36ts20 hamster cell line. This then leads to the conclusion that receptors are not locked into an inactive complex upon exposure of living cells to geldanamycin. In intact cells, binding-competent receptor complexes can rather be restored (at least in part) from preexisting components after dissociation of the drug. However, steroid hormone receptors within living cells are certainly not static, but rather dynamic. By continuously cycling between the free receptor form and states bound to heat shock proteins, they are thought to maintain the ability to bind hormone (8, 40). Therefore, it does not matter much, in principle, for the cellular pool of binding-competent and hence biologically active receptors whether new receptor polypeptides enter the cycle at one point or presynthesized molecules join at another.

A comparison of the above described and discussed ansamycin effects on steroid hormone receptors and on tyrosine-specific protein kinases brings up some interesting issues. While the hsp90 heterocomplex with pp60-src is being disrupted (18), association with the receptor polypeptide is maintained (cf. Fig. 5). On the other hand, the protein kinase may remain enzymatically active (18) while the receptors’ hormone binding abil-
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