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Steroid Hormone Action 41-54

Steroid Hormone Antagonists at the Receptor Level: A Role for the Heat-Shock Protein MW 90,000 (hsp 90)

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Antisteroid hormones compete for hormone binding at the receptor level and prevent the hormonal response. A new concept is proposed for explaining the antiglucocorticosteroid activity of RU 486 in the chick oviduct system. It is based on the ability of the antisteroid to stabilize the hetero-oligomeric 8S-form of the glucocorticosteroid receptor (GR), which involves the interaction of the 94k-receptor and heat-shock protein MW 90,000 (hsp 90). It is proposed that hsp 90 caps the DNA binding site of the receptor, and this prevents it from binding to the DNA of hormone regulatory elements (HRE) and increasing transcription of regulated genes. This paper reviews other antiglucocorticosteroid and antiestrogen systems with reference to this hypothesis and also describes a four-step analysis of the molecular mechanism of antisteroid hormone action at the receptor level.

Key words: steroid hormone antagonists, receptor antisteroids, antihormones, heat-shock protein MW 90,000 (hsp 90), RU 486, hormone regulatory elements (HRE), antiglucocorticosteroid, antiestrogen, tamoxifen

To suppress selectively and safely the effect(s) of a given hormone can be of medical usefulness. Antiprogesterone (to interrupt the luteal phase and early pregnancy), antiglucocorticosteroid (to decrease deleterious effects of corticosteroids produced in excess in some tumoral processes), antiestrogens (active in "receptor +" breast cancers), antialdosterone (used in many cases of high blood pressure), and antiandrogens (for treating hypersexualisms) have been successfully utilized in human beings.

By definition, antihormones are not drugs decreasing steroid production or availability to target cells. In other words, their targets are neither the biosynthetic mechanisms for steroids, nor their metabolism, nor their transport in the blood circulation. They also are not addressed to postreceptor events, since in this case they could probably not be specific enough, most cellular activities being under multihormonal controls. At the receptor level, presently we only consider analogues which

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bind to the same site as corresponding endogenous steroids, which then cannot exert their effects any more. Though not definitively proven at the molecular level, the prevailing binding pattern of these antihormones seems of a competitive nature. No solid data currently indicate distinct receptor sites engaged in antihormonal activity (perhaps, in the future, it will become possible to conceive new antihormones which will be chemically very different from steroids, when we know more about the tridimensional structure of receptors).

Two preliminary remarks have to be made when studying antihormone action at the molecular level: (1) From the list of antihormones (Table I), it appears that their binding affinity is not correlated to antisteroid effect. A low-affinity agonist may, by occupying the site for a shorter time than the endogenous hormone, give operationally an antihormonal effect in pharmacological experiments. However, the same ligand administered continuously can be a full agonist, as is estriol—an impeded estrogen [1] of low affinity for estrogen receptor and rapidly metabolised—which was erroneously believed to be an antiestrogen. Tamoxifen, which has very low affinity for the estrogen receptor, is a very effective antiestrogen because its low metabolic clearance rate makes it available in large concentration at the target level; 4-hydroxy-tamoxifen is of higher affinity, and a better antiestrogen in in vitro systems but not in the intact animal because of its rapid metabolism. To be an agonist or antagonist depends on the properties of the (ant)agonist-receptor complexes, but the binding affinity of the ligand is not a predictive index, and the potency of (ant)agonist is largely dependent on its overall metabolism. (2) The binding of antihormone to receptor, instead of the endogenous hormone, does not result in the formation of active complexes. This results from the different structures of hormones and antihormones and therefore of their binding modalities to the receptor, provoking distinct protein receptor transconformations. In fact, when one examines the structure of natural and synthetic agonists vs antagonists such as tamoxifen and RU 486 (Fig. 1), it appears that the chemical group substituted at the C11β position (or to the corresponding carbon in the triphenylethylene antiestrogen) probably plays the decisive role with respect to "abnormal" interaction with the receptor. An antihormone, in order to fit the receptor binding site, shares homologous structural features with the

TABLE I. Binding Affinity and Clinical Efficacy and Usefulness of Antisteroids

Antisteroids	Affinity[a]	Clinical efficacy and usefulness
Antiandrogens		
Flutamid-anandron	±	Good
Cyproterone acetate	+	Good
Antiestrogens		
Tamoxifen	+	Excellent
4-hydroxytamoxifen	+++	Not used
Antimineralocorticosteroid		
Spironolactone	+	Good
Antiglucocorticosteroid		
RU 486	++++	Excellent
Antiprogesterone		
RU 486	+++	Excellent

[a]Three pluses refer to approximative affinity of the corresponding natural agonist. ± means very weak affinity.

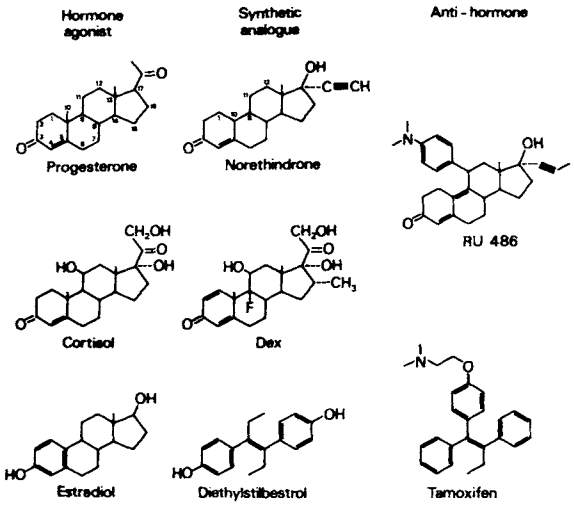

Fig. 1. Progestins, glucocorticosteroids, and antisteroid RU 486. Estrogens and antiestrogen tamoxifen. Norethindrone, a 19-norsteroid, is a synthetic progestin. Note the main structural characteristics of RU 486, ie, the presence of 11β-extra cycle, and the third cycle of tamoxifen, which are both situated at a position corresponding to the β-side of the steroid overall plane, just off the C11 carbon. "RU 486" is a short for RU 38486, the number used internally in Roussel Uclaf. Diethylstilbestrol is a synthetic agonist of estradiol.

corresponding agonists, physiological as well as pharmacological (see the structures in the antiestrogen, antiprogesterone, and antiglucocorticosteroid compounds of Fig. 1). This may explain the frequent (maybe constant) mixed agonist/antagonist activities observed with antihormonal compounds, to which we come back later in mechanistic terms. Tamoxifen gives an interesting example of the complexity of problems in this field: it is a pure nonagonist, antiestrogen, in the chick oviduct system [2]. However, in progesterone or dexamethasone-treated animals, tamoxifen becomes estrogenic, as assessed by the increase of egg-white-specific protein synthesis, of tissue differentiation in young animals, and of receptor interaction in chromatin [3–7]. This change of properties does not occur in chick liver, where tamoxifen is also a pure antiestrogen, but insensitive to the presence of dexamethasone as judged by the lack of increase of estrogen-regulated specific protein gene transcription after simultaneous administration of tamoxifen and glucocorticosteroid (C. Lazier, personal communication). Therefore there is a tissue-specific mechanism involved in the estrogenic response to tamoxifen. There is also a species-specific component in antiestrogen action, since it is well known that tamoxifen is very estrogenic in mice, and a mixed agonist-antagonist in rat and in human. Recently we have injected minced chick oviduct tissue into nude mice peritoneum, and it was observed that the response to tamoxifen injection in the mice was as to that of an estrogen, as observed immunohistologically by ovalbumin detection [8]. We will offer an hypothesis to explain these puzzling data, after we have summarized our current working hypothesis for relating receptor structure and hormone/antihormone activity.

STEROID RECEPTOR: A HETERO-OLIGOMERIC STRUCTURE INCLUDING hsp 90 WHICH IS RELEASED DURING HORMONE-INDUCED TRANSFORMATION (Fig. 2)

In the absence of steroid hormone, all or a very large fraction of any steroid receptor is found in the cytosol (ie, supernatant) fraction of target tissue homogenates in low ionic strength buffer. All receptors thus obtained sediment as an 8–10S hormone-binding entity (designated as 8S-R), of apparent MW ~300k [9,10]. If the extraction is performed with high ionic strength buffer (usually > 0.25 M KCl), or if secondarily the 8S-R is exposed to such a salt-containing medium, the hormone-binding peak sediments with an ~4S sedimentation coefficient (4S-R). Hormone affinity chromatography purification has been performed on this premise, and thanks to the stabilizing effect of molybdate ions [11–13], the 8S-R form of the progesterone receptor, not binding to DNA-cellulose, was isolated [14], while the purified hormone-binding, DNA-binding, 4S-R was also obtained in relatively good yield [15]. A monoclonal antibody to 8S-PR [16] was found to detect a nonhormone-binding, non-DNA-binding, MW ~90,000 protein in the 8S-PR [17], and this protein was characterized as hsp 90 by biochemical and cloning experiments [18,19], in accord with the results which were then obtained by D. Toft and his collaborators [20]. In fact, we [21] and others [22] showed that 8S-R forms of all steroid hormone receptors, whatever the hormone, the organ, or the species, contain the same hsp 90 molecule (recently M.E. Oblin and M. Lombes demonstrated it for the mineralocorticosteroid receptor of the chick colon, unpublished results). Data from cDNA sequence data of chick hsp 90 (N. Binart et al, in preparation) and the corresponding protein in *E coli*, yeast, *Drosophila*, and human indicate not only overall evolutionary conservation, but also one (possibly two) remarkably charged sequences(s) of amino acids able to form an α-helix with alignment of negative residues (M.G. Catelli, N. Binart, J. Garnier, and E.E. Baulieu, unpublished results). It was therefore postulated that this segment could interact with the positively charged, potentially two finger-shaped, putative DNA-binding site of the steroid receptors [23–25], which shows the highest degree of homology between proteins product of the erb-A-related superfamily [26,27]. This homology is > 50% between different steroid hormone receptors and > 90% between receptors for the same steroid hormone in different species. The postulated finger structure (Fig. 3), as in TFIIIA and similar DNA-binding metalloproteins [23,28], probably contains a divalent metal (likely Zn^{2+}). Using 1,10-phenantrolin and EDTA, two metal chelators, we have observed that the 5S-ER (dimer of the 65k-estrogen receptor which binds to DNA and likely is its active form [29]) is irreversibly prevented from binding DNA-cellulose after metal removal [30].

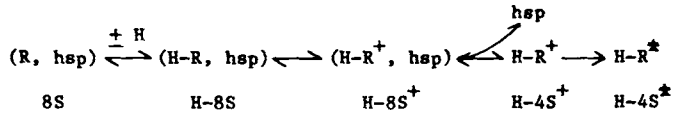

Fig. 2. Series of reactions leading from hetero-oligomeric 8S receptor (inactive) to active hormone receptor complexes. The stochiometry of R and hsp in the 8S-R is not indicated (see text). The $^+$ indicates that there is some activation of the receptor within the 8S complex (see text). The * indicates that the receptor is active. We have shown, by drawing a one-way arrow, that a practically irreversible transconformation takes place after release of HR^+ from hsp, which might explain the difficulty to recombine H-R* with hsp.

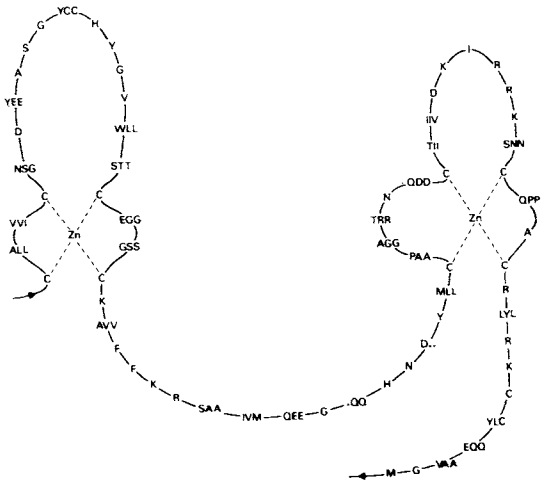

Fig. 3. Putative DNA-binding site of estradiol, glucocorticosteroid, and progesterone receptors, as deduced from cloned cDNA sequences. Sixty-seven amino acid positions are represented. When the amino acid is the same for all three receptors, only one letter is indicated. If not, letters correspond to ER, GR, and PR in this order, respectively. In three instances, the dot represents one missing amino acid. This scheme has been arbitrarily drawn on the basis of Klug's model, taking the eight first cysteines (of nine) as the basis for two Zn^{2+}-coordination systems. Note that within the left finger, as well as before the right finger, there is one histidine which could serve as bond donor to the metal.

In reassociation experiments [30,31], we found that treatment by phenanthroline precluded the ~40% recombination of 5S-ER with hsp 90 [30]. Further (unpublished) experiments of G. Redeuilh and M. Sabbah have improved the reassociation conditions (obtaining ~100%), and yet the phenanthroline inhibition effect was observed. Therefore we propose that there is a common stabilizing effect of Zn^{2+} on the receptor structure, permitting DNA and hsp 90 binding.

hsp 90 is constitutively present in most if not all cells, whether target or not for steroid hormones. Contrary to other heat-shock proteins, its concentration, in the absence of heat-shock or stress, is in the 0.1–2% range of cytosoluble proteins. hsp 90 is essentially a cytoplasmic protein, and, upon purification, is obtained as a dimer, not dissociated by KCl, and does not bind hormone or DNA [32] (unpublished data of P. Aranyi, M.G. Catelli, C. Radanyi, G. Redeuilh, and M. Renoir). A small fraction of hsp 90 appears to be located in the nucleus (unpublished immunogold electron microscopic detection of J.M. Gasc), and this small percentage may account for the nuclear localization of steroid hormone receptors in their nontransformed 8S-form in absence of hormones [17, 33–38].

It is therefore proposed that hsp 90 is a cap for the receptors, binding to their DNA-binding site (Fig. 4). Complexes would form just after receptor biosynthesis, therefore precluding interaction of the receptor with the DNA under physiological cellular conditions, until the incoming hormone intervenes. This could explain the finding of 8S-receptors in the cytosol. Preliminary stoichiometric data indicate two hsp molecules and one receptor subunit for the chick 8S-progesterone receptor [39] and the rat 8S-GR (J.A. Gustafsson, personal communication). However, in the 8S-estrogen receptor of the calf uterus, we found two 65k-hormone-binding units for two hsp molecules [40]. It is notable that neither the vitamin D (more exactly calcitriol)

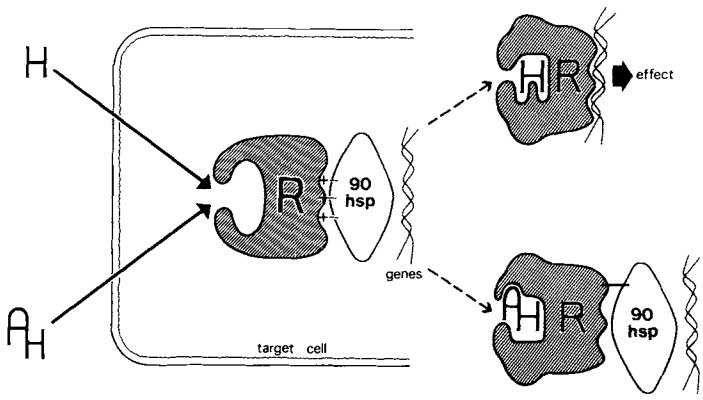

Fig. 4. Schematic representation of the receptor system and its transformation upon binding of agonist (hormone H) or antagonist (antihormone AH). In absence of hormones, the receptor is under hetero-oligomeric 8S form, containing R and hsp 90. hsp 90 caps the receptor in binding to the DNA-binding site (ionic bonds), as discussed in the text. The stoichiometry of the complex is not represented. No interaction of the 8S-receptor with DNA takes place. In case of hormone binding, hsp 90 is released; the receptor interacts with DNA and triggers the response at the DNA level. Note that we have indicated a change in the hormone-binding site, with higher affinity for H in transformed than in nontransformed receptor. In case of binding of AH, we have indicated that hsp 90 binds to R very tightly (——); thus no interaction with DNA can occur.

[41] nor the thyroid hormone receptor [42,43] has been yet found in 8S-forms. Their structure as that of v-erb-A products, shows a short N-terminal region preceding the DNA-binding site (regions A and B in Chambon's nomenclature). However, the nt^- mutant of the GR also clearly is truncated at the N-terminal and forms an 8S-R structure [44].

A number of cell-free experiments indicate that binding of the agonist steroid drives the dissociation of 8S-R [review in 45; and see also 46,47], releasing hsp 90 and the hormone-binding, DNA-binding 4S-R (or 5S-R in the particular case of estrogen receptor; Figs. 2,4). The reaction is accelerated by temperature increase and/or by high ionic strength [48,49], and in fact is obtainable in vitro in absence of hormone, but more slowly [50,51]. Whole cell experiments favor the physiological significance of the 8S structure, which is certainly not a molybdate artefact [17], and appears to be present in intact cells [52], the reversible equilibrium 8S→4S being shifted to the right by hormone [53].

It is proposed that, upon hormone binding, the hormone-binding unit of the receptor is physiologically trans(con)formed. This change of structure is responsible for hsp release, and the putative DNA binding site may then interact with either nonspecific DNA or specific hormone-regulatory elements (HRE) of DNA (Fig. 5). It is not known if hormone binding, besides release of hsp 90, does or does not lead to a change of the structure and then of the properties of the DNA-binding site of the receptor. For instance, the latter could acquire higher affinity for nonspecific and/or HRE DNA. It is now known that the receptor, separated from hsp 90, has affinity for both nonspecific and HRE DNA, even in the absence of hormone [50 and see later]. The triple-binding equilibrium of the receptor with hsp 90, available nonspecific DNA and HRE DNA is likely to be important to hormone action. The 8S-steroid receptors are not identical in terms of stability. For instance, in the chick oviduct, GR

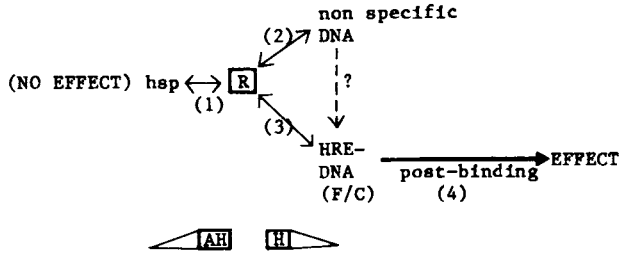

Fig. 5. Triple interaction of receptor R (so-called 4S-R) with hsp 90, nonspecific DNA, and DNA of hormone-regulatory element (HRE). In addition, on this figure, the fourth step in hormone action, "post-binding," also is represented, and leads to effect. F and C means factors and chromatin features implicated, besides HRE-DNA binding, in the hormonal response. At the bottom, "AH" and "H" suggests that, in the presence of antihormone AH, the system is driven to the left, while the binding of hormone H drives it to the right.

is more stable than the progesterone receptor, while in the rabbit the progesterone receptor is very unstable. No systematic study has been performed, but differences may come from the fine structure of the receptor segments interacting with hsp 90, thus differing according to hormone and species. They may also depend on the phosphorylation state of both receptor and hsp 90, and probably of many other factors proper to each cell type and pathophysiological circumstances. In fact, kinetic differences in the transformation of 8S-R to 4S-R even may have to be considered when envisaging quantitatively the effect of a given hormone in different target cells; particularly one should integrate these kinetics within the time-dependent reaction involved in the hormonal response. Whether or not the receptor, after triggering the response, is recycled back to the 8S-R form [53], associated or not to an energy-dependent step [52], is unknown. Indeed, we do not know to which macromolecular structure the hormone-free receptor is loosely bound in the nucleus, and whether activated hormone-receptor complexes (4S-R) only bind to HRE-DNA. Besides precluding DNA binding of the receptor, hsp 90 may play a role in positioning the 8S-R in the cell (hsp 90 binds to actin [54]) and/or protecting the receptor against denaturation (unpublished experiments of T. Buchou and J. Mester indicate protection against thermal inactivation).

A ROLE FOR hsp 90 IN THE ANTIGLUCOCORTICOSTEROID EFFECT OF RU 486

RU 486 (a 19-norsteroid, see Fig. 1 [reviews in 55]) binds with high affinity to the chick oviduct GR [56] and antagonizes ovalbumin and conalbumin synthesis induced by glucocorticosteroids [57].

In explants incubated at 37°C in the presence of the active synthetic glucocorticosteroid triamcinolone acetonide (TA) or RU 486 (20 nM), the saturable binding of GR to nuclei was very dependent of the nature of the entire (anti)hormonal ligand. Almost no RU 486-GR complexes were found in the nuclear fraction after incubation of the tissue with the antihormone, contrasting to the nuclear localization of ~80% of the total intracellular TA-GR complexes after TA exposure. Since there is high affinity for nuclei of transformed GR complexes [48,49] and since the hsp-containing

8S-form of GR has low affinity for either DNA or nuclei, we studied the possible influence of RU 486 transformation of 8S-GR to 4S-GR [58].

In the oviduct cytosol, the effect of TA and RU 486 on the size of the receptor was first analyzed by liquid chromatography (HPLC). When binding TA the untransformed GR had a Stokes radius of ~6.7 nm, while it was 4.9 nm after temperature jump (1 hr at 25°C) or ionic strength treatment (0.3 M KCl, 1 hr at 0°C). Complexes of GR-binding RU 486 had a Stokes radius of ~6.7 nm, whether the receptor had or had not been exposed to transforming conditions. Glycerol gradient analysis confirmed that the size of RU 486-GR complexes was unchanged under the same transformation conditions, and actually it was the same as that of TA-GR sedimentation coefficient (~8.6S) in low salt, irrespective of the presence or absence of sodium molybdate or of 0.4 M KCl in 50 mM sodium molybdate. In all cases, 8.6S-complexes were shifted to ~11S after interaction with the BF_4 monoclonal antibody [16], confirming the presence of hsp 90 [21]. TA-GR complexes, in the absence of molybdate, shifted to 4.4S after exposure to transforming ionic strength or temperature. Experiments in which 8S-GR was exposed for 1 hr to different concentrations of KCl at 0°C or to various temperatures (Fig. 6) confirmed the stabilization provoked by RU 486 binding as compared to TA. For instance, the 50% transformation of TA-8S-R to TA-4S-R was obtained with 0.3 M KCl, while less than 10% of the 8S-R to 4S-R transition occurred with RU 486-GR or in the absence of steroidal ligand (Fig. 5). Complementary experiments using oviduct explants incubated with either TA or

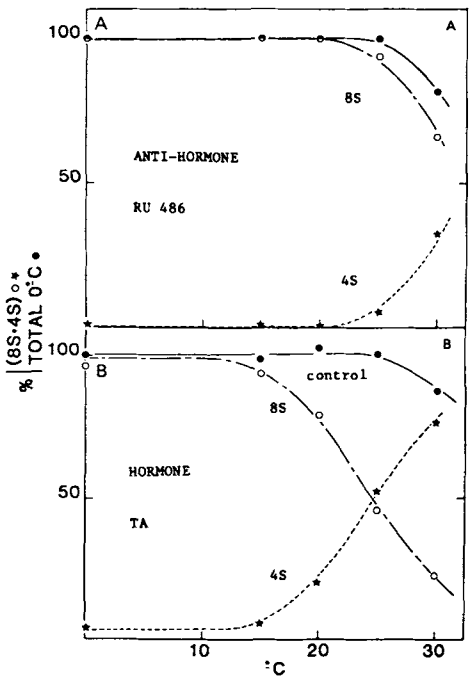

Fig. 6. Chick oviduct glucocorticosteroid receptor transformation, when binding hormone of antihormone. Transformation is driven by temperature increase for 30 min. These unpublished results of Groyer et al [see also 58] indicate that when the agonist triamcinolone acetonide (TA) is binding to the receptor, 50% is transformed into 4S-R at 25°C, while only 10% is transformed if RU 486 is the ligand.

RU 486 (20 nM) for 1 hr at 37°C confirmed that the same agents produced the same effects as in cell-free cytosol. When RU 486 was used, 94% of total receptor was in the cytosol, and 60% of this was in 8S-form. Conversely, of the 20% of total receptor remaining in the cytosol after TA, almost all (~90%) was 4S. We do not know if the presence of some 4S-GR in the cytosol in the case of TA reflects either simply a redistribution between subcellular compartments under our experimental conditions or the formation of a variety of TA 4S-GR of lower affinity for nuclear components. We do not know, either, if the 4S-GR complexes found in the cytosol after exposure to RU 486 are due to experimental conditions leading to secondary transformation or to formation of 4S-complexes dissociated from the 8S and of low affinity for nuclear constituents.

The DNA-binding activities of the TA- and RU 486-GR complexes were tested in DNA-cellulose-binding experiments. Exposure of the cytosol to 25°C for 1 hr did not provoke any binding to the resin whether TA or RU 486 was the ligand if there was 50 mM sodium molybdate in the medium. In the absence of molybdate ions, binding of TA-GR was five times larger than that of RU 486-GR. This value refers to binding as a function of total receptor concentration. However, these results should take into account two distinct steps: transformation of the 8S-form of ligand receptor complexes to 4S complexes, and the binding of each of these forms to DNA. When we replotted the binding data as a function of the 4S-R concentration, we found that the two 4S-GR complexes, with either TA or RU 486, bind identically to DNA [58]. Complementary experiments verified that all 4S-R had the same DNA-binding properties. They were isolated after ultracentrifugation and assayed separately for DNA-cellulose binding, which was identical whatever the ligand. The elution pattern from DNA-cellulose was studied by using increasing concentrations of KCl and was found identical whether TA- or RU 486-GR complexes were studied. These results are perfectly compatible with recent findings [59,60] indicating that GR and progesterone receptor bind with a similar affinity to HRE-DNA of MMTV-LTR and uteroglobin gene, respectively, whether occupied by agonist, antagonist, or in the absence of ligand. Recent experiments in our laboratory show identical binding of TA- and RU 486-GR to a ^{32}P-MMTV-LTR fragment (bp −220 to +102, generous gift of H. Richard-Foy and G. Hager) and in vitro ovalbumin and conalbumin gene transcription was enhanced when adding purified preparations of GR complexes with TA or RU 486 to chick oviduct nuclei [61].

Finally, contrary to the agonist TA which promotes receptor transformation and release of the active 94k-GR unit, RU 486 in the chick oviduct system stabilizes the 8S non-DNA-binding, nontransformed form of the receptor, preventing its interaction with the hormone-responsive machinery (curiously, molybdate ions have a similar effect, of which the mechanism is also unknown). It follows that hsp 90 may then be considered as a protein acting as a transcription regulator, which itself does not bind to DNA but precludes the DNA-binding receptor to do so. The steroid receptor systems will involve several proteins, as is the case for other regulatory protein systems. For example, the cAMP-dependent protein kinases are also composed of two proteins, but here the ligand binding unit is not the effector moiety, and upon cAMP binding to the R-unit, the catalytic part is released in active form [62].

A number of publications [63–67] also have indicated the decrease of "transfer to the nucleus" of GR when binding RU 486, as compared to TA. In the study of liver GR interaction with DNA-cellulose, a decrease of the number of activated

receptor complexes binding to DNA was observed when RU 486 was the ligand, as compared to what occurred with agonist glucocorticosteroid [66], a result directly compatible with the postulated capping effect of hsp 90. Quantitative differences among published results are difficult to rationalize, since operationally these experiments implicate the separation of nuclei and cytosol fractions in an arbitrary manner, and results may depend on tissue and species differences, and on a number of experimental factors such as temperature, volume of buffers, salts, metal chelator, reducing agents, and pH of the medium centrifugation technique. P. Formstecher, also with RU 486, finds stabilization of the 8S-form of rat liver GR [67] and obtains the same result with the carboxamide class of antiglucocorticosteroids [68]. A Schütz publication [69] relates that, in whole hepatoma cells, there is no guanine protection from artificial methylation in the HRE of the tyrosine aminotransferase promoter in the absence of hormone, in contrast to what occurs in the presence of glucocorticosteroids. An obvious possibility is that the hormone is necessary to separate the DNA-binding unit of the receptor from hsp 90.

A ROLE FOR hsp 90 IN STEROID/ANTISTEROID HORMONE ACTION

The preceding examples deal with the glucocorticosteroid receptor. With the estrogen/antiestrogen system, the situation is not as clear. Again, some data obtained with various antiestrogens are compatible with a decreased transformation of the receptor due to the antihormone binding, whether the transformation is assessed by sedimentation coefficient and/or DNA-binding studies or by partition coefficient [70–72]. However, in the context of pure antiestrogenic effect of tamoxifen in the chick, we found ~80% as much nuclear estrogen receptor after tamoxifen as compared to after estradiol [2]. We have not yet performed specific experiments to compare properly the physicochemical characteristics of the nuclear estrogen- and antiestrogen-receptor complexes (see later): however, the administration of antiestrogenic ligand apparently provokes a tighter association of the receptor to a nuclear structure, and we know that the estradiol- and tamoxifen 5S-receptor complexes differ by several criteria (partition coefficient [72], interaction with monoclonal antibody [73], size and rate of ligand dissociation [74,75].

Very little is known with respect to other receptors. In the rabbit progesterone receptor system RU 486 administration seems to be associated with 8S to 4S transformation of the receptor [76]. However, unpublished in vitro experiments of M. Renoir show stabilization of the rabbit 8S-progesterone receptor form when binding RU 486. Working with spironolactone, Edelman and his group [77] found decreased transfer of the receptor to the nucleus as compared to the effect of aldosterone.

The basic alternative to the model proposing 8S-R stabilization as the mechanism of action for antihormonal effect proposes that antihormone-4S-receptor complexes are formed, but that they are inappropriate to trigger hormone action. As just stated in the case of antiestrogens, there are data supporting this classical concept, which simply implies that ligand-binding-induced transconformation of the hormone-binding unit is dependent on the property of the ligand. In fact, we have been able to detect a decreased rate of ligand dissociation from charcoal-treated 8S-receptor complexes with estradiol, but not with antiestrogen [78]: this suggests that an antihormone, when binding to an 8S-receptor, may produce not only a different effect on the

release of hsp 90 as compared to the hormone, but also other changes in the receptor properties.

MULTISTEP MECHANISM FOR ANTIHORMONE ACTION AT THE RECEPTOR LEVEL

An antihormone may act at one or several of the different steps involved in the receptor functioning. Since we do not know much about the detailed molecular events mediating hormone action at the receptor level, in particular the binding to DNA and the possible (probable) intervention of chromatin elements (eg, transcription factors, chromatin structure), we only discuss here four possibilities (Fig. 5), based on current concepts that are suggested by recent experimental data. (1) Antihormones may stabilize the receptor-hsp 90 hetero-oligomer, preventing the release of the active receptor. Acknowledging many uncertainties in the understanding of the 8S-receptor structure, we believe that enough data suggest strongly such a mechanism in several cases of antiglucocorticosteroid effect. Stabilization does not insure that 100% of the nonactive form of the receptor will remain as such, and one has to integrate this effect within the kinetic context of receptor turnover. Very little is known of the temporal dimension of the (anti)hormone action at the cellular level. (2) The binding of antihormones may be followed by release of antihormone-4S receptor complexes with high affinity for nonspecific DNA. This may decrease the availability of the receptor for binding to available HRE at the level at which is triggered the hormone response. There is no direct evidence for such a mechanism, but nobody has really studied concurrent affinity of (anti)hormone-receptor complexes for nonspecific DNA. (3) Conversely, the antihormone-4S-receptor complexes may have decreased or insufficient affinity for HRE, as compared to agonist-receptor complexes. Indeed, besides some consensus sequence, HRE of genes regulated by the same hormone differ, and the (anti)hormonal effects may then also differ for this reason. The net result, based on the same considerations as for hypothesis 2, is also an decreased hormonal response. (4) The antihormone-4S-receptor complexes released from 8S-complexes interact identically to agonist-4S-receptor complexes with both specific and nonspecific DNA, but these complexes either cannot approach DNA for chromatin constraint and/or are unable to promote yet-undefined postbinding to DNA events which are necessary for transcription. Such a possibility could accommodate the cases where the 8S-R dissociates upon antisteroid binding and the antagonist-receptor complexes have high affinity for HRE-DNA.

This analysis is thus characterized by a cascade of potential consequences of antihormone binding, each of them being possibly operational in antihormone activity. According to the nature of ligand and of receptor molecules, they may or may not all occur. Naturally if the stabilization of the hetero-oligomer were efficient at 100%, the other mechanisms would not play any role. However, stabilization is only limited. If the fraction of released antihormone-receptor complexes binds well to DNA and has agonist activity, the model is able to predict the mixed agonist/antagonist activity of the antihormone ligand, depending on the equilibrium 8S/4S reached after antisteroid binding. Indeed, such an equilibrium may also differ according to the hormonal environment, the species, and the antihormonal ligand,[1] explaining some of the

[1]Recently, Wakeling and Bowler [79] have presented an antiestrogen devoid of agonist property in the uterus system where tamoxifen is a partial agonist.

complex responses to tamoxifen described above, or some agonist activity of the powerful antagonist RU 486 [80,81]. In addition or altenatively to the 8S-stabilizing property ("1" on Fig. 5) increased binding to nonspecific DNA ("2"), decreased binding to HRE ("3"), and/or deficient postbinding to DNA effects ("4") may lead also to efficient antihormonal activity. Theoretically, a compound which induces several of the above-cited mechanisms could be more efficient than another able to provoke only one of them. Some of these possibilities are experimentally testable, offering a novel molecular approach to testing steroid hormone antagonists.

In summary, the main point here discussed is a new concept involving the stabilization of the nonactive hetero-oligomeric 8S-form of steroid receptor, in which the DNA-binding site is capped by the heat-shock protein hsp 90. While the hormone releases the active form of the receptor from this hetero-oligomer, therefore triggering the hormonal response, the antihormone RU 486 (at least in the chick oviduct glucocoritcosteroid receptor system) stabilizes the 8S-complex, and it is proposed that this stabilization is involved in its anticorticosteroid activity. Stabilization of the hetero-oligomer non-DNA binding complexes gives to hsp 90 the property of a transcription modulator which itself does not bind to DNA but acts by preventing a DNA-binding protein interacting with hormone regulatory elements of the DNA. Several such and other mechanisms proposed for antihormone action are already testable experimentally.

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