Androgen and Glucocorticoid Receptor Heterodimer Formation

A POSSIBLE MECHANISM FOR MUTUAL INHIBITION OF TRANSCRIPTIONAL ACTIVITY*

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The androgen and glucocorticoid hormones elicit divergent and often opposing effects in cells, tissues, and animals. A wide range of physiological and molecular biological evidence suggests that the receptors that mediate these effects, the androgen and glucocorticoid receptors (AR and GR, respectively), influence each other's transcriptional activity. We now show that coexpressed AR and GR indeed do interact at the transcriptional level and that this interaction is correlated with their ability to form heterodimers at a common DNA site, in vitro and in vivo. Furthermore, mutants that cannot heterodimerize do not inhibit each other's activity. These observations provide the first evidence that the opposing physiological effects of the androgen and glucocorticoid hormones are due to the direct physical interaction between their receptors at the transcriptional level.

The androgen and glucocorticoid hormones have profound effects on metabolism, animal behavior, and cellular proliferation. They act through intracellular receptors, members of the nuclear receptor superfamily, that exert their effects by binding to specific DNA sites and modulating the transcriptional activity of linked genes (for review see Ref. 1). Several mammalian tissues coexpress the receptors for these two hormones, and in a number of these tissues they have opposing effects (2–9). For example, the androgens act through the androgen receptor (AR) to stimulate protein synthesis in skeletal muscle (2–9). For example, the androgens act through the androgen receptor (AR) to stimulate protein synthesis in skeletal muscle (2–9).

Additional experiments are needed to verify interaction at some level in their signaling pathways (2). These opposing effects of androgens and glucocorticoids appear directly in triggering aggressive dominant male behavior (2, 5, 7). In contrast, glucocorticoids act through the glucocorticoid receptor (GR) to cause skeletal muscle protein degradation and to inhibit smooth muscle and prostate proliferation and are correlated with suppressive behavior in male rats (2, 5, 6, 8, 9). These opposing effects of androgens and glucocorticoids appear to reflect interaction at some level in their signaling pathways since glucocorticoids inhibit both the proliferative and anabolic effects of androgens (7, 9).

AR and GR display a high degree of sequence homology, particularly in their DNA-binding domains (DBDs), and bind to a common DNA site termed a hormone response element (HRE) (10). Receptor specificity does not appear to be determined primarily by protein-DNA interaction, since their transcriptional activities differ markedly in some contexts (Fig. 1 and Refs. 11–13). It appears that factors other than DNA affinity control GR and AR activity at different types of HREs, as has been described previously for GR and its close relative, the mineralocorticoid receptor (MR) (14–17).

HREs contain imperfect palindromes composed of two half-sites that AR, GR, and MR bind as head-to-head homodimers, and recent evidence has suggested that, in addition to homodimer formation, MR and GR heterodimerize (16, 18). In view of these observations, it seemed plausible that AR and GR might also interact directly through heterodimer formation to inhibit each other's transcriptional activities, thus providing a molecular basis for the observation of an inhibitory interaction at the cell and tissue level. To examine this possibility, we performed co-transfection experiments with various AR-specific and GR-specific HREs in cultured cells and tested for direct physical interactions in gel shift assays. We also used a genetic approach to demonstrate that heterodimer formation was essential for their inhibitory effects at the transcriptional level.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—60–80% confluent monolayers of monkey CV-1b cells (Cell Culture Facility, University of California, San Francisco) were grown in 5% CO₂, 95% air (relative humidity, 90%) at 37 °C in Dulbecco's modified Eagle's medium H-16 (Mediatech) supplemented with 5% fetal calf serum (Life Technologies, Inc.). Cells were transfected by the LipofectAMINE method as described (Life Technologies, Inc.), with 100 ng of GR expression plasmid (6RGR), 100 ng of AR expression plasmid (6RAR), and 100 ng of each expression plasmid together, along with 50 ng of reporter plasmid TAT1-TATA and 100 ng of reporter plasmid C9 (kind gift of Diane M. Robins, University of Michigan, Ann Arbor, MI). 20 ng of RSV-β-gal plasmid was included as an internal standard, and the BlueScript KS vector plasmid was used as carrier to bring the total amount of DNA to 1 μg. The reporter TAT1-TATA contains one HRE with sequence TGTACAGGATGTTCT of the Drosophila alcohol dehydrogenase minimal promoter (C9) fused to the Drosophila alcohol dehydrogenase minimal promoter (~33/4) driving luciferase expression. The reporter C9 contains Slp enhancer sequences upstream of a thymidine kinase chloramphenicol acetyltransferase (CAT) reporter gene (13). The effect of cotransfection of GR and AR on multimerized HREs was also determined by using 50 ng of reporter plasmid TAT3-TATA, which contains a trimerized HRE fused to the same Drosophila alcohol dehydrogenase minimal promoter driving luciferase expression. Fresh medium containing 5% stripped serum (charcoal treated to remove endogenous steroids) and penicillin G at 100 units/ml and streptomycin sulfate at 100 μg/ml was added 18 h before transfection. Lipid-DNA complexes were prepared according to the manufacturer's manual. Cells were incubated in the medium containing DNA-lipid complexes for 5 h, after which an equal volume of medium with twice the serum was added and cells were incubated for 16 h. Cells were then washed twice in 37 °C phosphate-buffered saline and refed with fresh medium. Corticosterone (100 nm) and dihydrotestosterone (100 nm) were added to one of two identical transfactions; 24 h later, cells were harvested and extracts were prepared as described previously (14). The extracts were assayed for luciferase and CAT activity. Both luciferase and CAT values were normalized to

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1 AR, androgen receptor; GR, glucocorticoid receptor; DBD, DNA-binding domain; HRE, hormone response element; MR, mineralocorticoid receptor; CAT, chloramphenicol acetyltransferase; HA, hemagglutinin; PCR, polymerase chain reaction.

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tein content and to β-galactosidase activity (expression driven by the Rous sarcoma virus promoter) without any significant difference.

Receptor Protein Expression—For in vitro measurements, receptor DBDs lacking the ligand-binding domains and N termini were expressed in bacteria. Sequences encoding the rat AR DBD were cloned into the plasmid pET-14b (Novagen) and expressed in Escherichia coli strain BL21(DE3)pLysS as a recombinant protein with the sequence AR residues 535-623 (89 residues, 11.0 kDa). An influenza hemagglutinin (HA)/epitope-tagged version of this recombinant protein was also obtained by introducing a three HA-epitope sequence fragment in front of the DBD region. Purification was as described (19), except that the AR was precipitated by addition of 45% ammonium sulfate, re-dissolved with and dialyzed in HEGDZ50 (20 mM Hepes, pH 7.5, 0.5 mM EDTA, 10% (v/v) glycerol, 5 mM dithiothreitol, 50 μM ZnSO₄, 50 mM NaCl). Rat GR recombinant protein T7X556 (179 residues; 19.0 kDa) was expressed and purified as described (16).

Electrophoretic Mobility Shift Assay—The TAT0.5 (containing a single HRE half-site) and TAT1.0 (containing one full HRE site) probes were excised from the plasmids possessing the HRE sequences; TAT0.5 (TATCCAGGTGTCTCT) and TAT1.0 (TGTACAGGTGTCTCT) (the half- and full sites are underlined). The DNA fragments were gel-purified and ethanol-precipitated. Probe was 32P-end-labeled by filling in with Klenow (Boehringer Mannheim). Binding was quantified by an electrophoretic mobility shift assay. GR or AR DNA-binding domains were mixed with the 32P-labeled HRE in reaction buffer (50 mM NaCl, 20 mM Tris-HCl, pH 7.9, 1 mM EDTA, 10% glycerol, 0.1% Nonidet P-40, 1 mM dithiothreitol, 200 ng of poly(dI-dC), 100 ng of bovine serum albumin) in a total volume of 20 μl. Either the anti-HA (influenza hemagglutinin) monoclonal antibody or an anti-GR monoclonal antibody, BuGR (20), directed against amino acids 407–420, was included in the reaction mixtures where indicated. After incubation for 15 min at room temperature, reaction mixtures were applied to a 5% non-denaturing polyacrylamide gel (37:5:1, acrylamide/bisacrylamide; National Diagnostics) buffered with 0.5 × TBE (44.5 mM Tris-HCl, 44.5 mM boric acid, 1 mM EDTA, pH 8), and resolved by electrophoresis (250 V) at room temperature, followed by autoradiography.

In Vivo Interaction of Complementary Mutants—The mammalian expression vector 6RAR was made by inserting a 2.8-kilobase pair XhoI fragment, a rat AR fragment from the vector pCMV5 (a generous gift of Diane M. Robins) into the vector 6R. The AR point mutation (Arg-581 → Asp) was introduced into the plasmid 6RAR using PCR to generate AR/R581D mutant as described previously. The N-terminal deletion derivatives of AR wild type (ARAN) and AR/R581D mutant (ARAN/R581D) were obtained by the following steps. First, the DBD region was obtained by PCR with the introduction of a BglII site in front of the 6RAR or 6RAR/R581D plasmid fragments, which had been deleted of the entire N-terminal and DBD regions. GR point mutants and GR N-terminal deletion mutants have been described previously (16). The in vivo interaction of AR and GR was determined by cotransfection with AR and GR complementary mutants. Cells were transfected with full-length receptor expression plasmids following the procedures described above. For the N-terminal deletion receptors, cells were transfected as described above except with 20 ng of GR expression plasmid, 20 ng of AR expression plasmid, or 10 ng of each expression plasmid together, along with 50 ng of reporter plasmid TAT3-TATA. Corticosterone (100 nM) and dihydrotestosterone (1000 nM) were used. Luciferase values were also normalized to both protein content and to β-galactosidase activity without any significant difference.

RESULTS

GR and AR Mutually Inhibit Each Other’s Transcriptional Activity—We were initially interested in asking if AR and GR could influence each other’s transcriptional activities at HREs to which they are both able to bind. We began by transfecting CV-1b cells with the androgen-specific HRE C’Δ9 (driving CAT expression) and the GR-responsive HRE TAT1-TATA (driving luciferase expression) with AR and GR either alone or the two receptors together. CV-1b cells lack endogenous GR (14) and AR (13) activity and therefore provide a null background for expression of transfected wild type or mutant receptors. As shown in Fig. 1A, C’Δ9 reporter activity was strongly induced by AR, but responded only marginally to GR in transfected CV-1b cells, consistent with the prior observations of Adler et al. (13). In contrast, AR had marginal activity on TAT1-TATA while GR induced a robust transcriptional response (Fig. 1B). When the receptors were coexpressed, GR inhibited AR-induced activity at C’Δ9 while AR inhibited GR at TAT1-TATA. In a series of titration experiments, we found that inhibition occurred at all levels of transfected receptors, suggesting that it was not due to squelching or occlusion (13), and was consistent with the formation of an inhibitory heterodimer. AR also inhibited GR transcriptional activity at TAT3-TATA, a reporter that contains three HREs driving a minimal promoter. As shown in Fig. 1C, AR and GR activities were not as disparate on TAT3 as on TAT1 and C’Δ9, although, like TAT1, GR had considerably more activity than AR. Again, AR inhibited GR activity, albeit more modestly than at TAT1 (Fig. 1C, AR + GR). Thus, AR and GR activities differ markedly at a variety of
We next sought to determine whether the receptors physically interact in vivo. We developed to demonstrate heterodimer formation between MR and GR through introduction of complementary salt bridge mutations (16, 17). AR, GR, and MR (as well as progesterone receptor) share a high degree of sequence homology in their DBD regions (21–23). Within the DBD, a conserved Arg-Asp motif was shown by x-ray crystallographic analysis of GR to participate in homodimer formation by forming a reciprocal pair of salt bridges (21, 22). Furthermore, it has been demonstrated that this same salt-bridge pair is a point of direct interaction between MR and GR (16). Thus, it seemed likely that these same residues would mediate AR-GR interaction in vivo as well.

Initially we used N-terminal deletion derivatives of AR and GR for introduction of point mutations (as shown in Fig. 2) to avoid the confounding effects of the dimer mutations on full-length receptor activity (see Ref. 16 and below). Previous evidence demonstrated that salt bridge mutations decrease the transcriptional activity of GRΔN on either a TAT1- or TAT3-containing reporter gene (16, 17). Thus, as shown in Fig. 3, both GRΔN/R479D and GRΔN/D481R had much lower activity than wild type GRΔN when cotransfected with TAT3-TATA. Similarly, the homologous Arg-Asp mutant of ARΔN, ARΔN/R581D, had lower activity than ARΔN as well (Fig. 3). If AR and GR heterodimerized using the same salt bridge, then coexpression of complementary mutants should be able to reestablish the ionic interaction and restore heterodimer formation, and consequently, wild type transcriptional activity. Coexpression of noncomplementary mutants, on the other hand, would not reestablish the ionic interaction and little or no transcriptional activity should result. In keeping with this prediction, cotransfection of the complementary mutants, ARΔN/R581D and GRΔN/D481R, fully restored transcriptional activity to that obtained by wild type receptors (Fig. 3, compare ARΔN + GRΔN with ARΔN/R581D + GRΔN/D481R) while coexpression of non-complementary mutants did not (Fig. 3, ARΔN/R581D + GRΔN/R479D). These data strongly support the idea that ARΔN and GRΔN heterodimerize at TAT3-TATA and this interaction is mediated via the DBD region.

To look directly at heterodimer formation, we performed gel shift assays using E. coli-expressed proteins encompassing the receptor DBDs. AR and GR truncated proteins of differing size (AR, 11 kDa; GR, 19 kDa) containing their DBDs were expressed in E. coli and were partially purified (see "Experimental Procedures"). To unequivocally identify the positions of monomer and dimer bands for each receptor, their HRE binding activities were initially analyzed with oligonucleotides and the relative mobility of bands using single half-sites versus full HRE demonstrated the gel locations of monomer and homodimer (compare lanes 3 and 4). Specificity of binding was demonstrated by competition using cold specific and non-specific oligonucleotides (data not shown). GR demonstrated a β-galactosidase activities and represent the mean ± S.E. of three independent experiments.

To test whether AR and GR interacted in vivo, we cotransfected with wild type receptors or salt bridge mutants as shown. 100 nM corticosterone and 1000 nM dihydrotestosterone were added for 24 h. Luciferase values were normalized with β-galactosidase activities and represent the mean ± S.E. of three independent experiments.

To look for heterodimer formation, various amounts of AR and GR were mixed with the single full-site HRE probe, keep-
ing the total amount of protein constant, as shown in Fig. 4B, lanes 2–12. A band of intermediate size appears in reaction mixtures containing both receptors, strongly supporting heterodimer formation. Furthermore, in experiments in which a highly specific monoclonal anti-GR antibody (BuGR, see “Experimental Procedures”) was included, the upper GR homodimer band, the intermediate AR-GR heterodimer band and the GR monomer band were complete supershifted (Fig. 4C, lanes 5–7). Although the AR homodimer and AR monomer bands were not supershifted by BuGR in the absence of GR, their intensity was reduced in the presence of GR (Fig. 4C, compare lanes 5 and 6). This might be due to stabilization by BuGR of the AR-GR heterodimer consequently favoring heterodimer formation and thus depleting AR homodimer and monomer bands, as has been described for other DNA-binding proteins. Similar supershift results were obtained with an HA/epitope-tagged derivative of AR DBD that both the AR homodimer and AR-GR heterodimer could be supershifted by the anti-HA antibody (data not shown). Thus, from these results, we conclude that AR and GR heterodimerize directly via DBD regions in vitro.

Evidence for an Inhibitory Function of the AR and GR DBD Dimer Interfaces—We last tested heterodimer formation of full-length receptors. Recently, the role of the DBD dimer interface in steroid receptor function has come into question. Liu et al. demonstrated that disruption of the DBD dimer interface in the context of full-length MR or GR markedly increased receptor activity on multimerized HREs (17) in striking contrast with its effect on receptor N-terminal deletion derivatives (Ref. 16 and Fig. 3). Restoration of the contacts through coexpression of complementary mutants decreased activity to near wild type levels. These observations suggested the surprising conclusion that the DBD dimer interface restrains receptor activity by interfering with its capacity for transcriptional synergy. To examine if this same principle “constrained self-synergy” applied to AR, the Arg-581 → Asp mutation was introduced into full-length AR and the synergistic activity of the mutant receptor (AR/R581D) was examined.

As shown in Fig. 5, the activity of AR/R581D was 4-fold greater than that of wild type AR at TAT3-TATA. The activity of GR was also potentiated by the salt bridge mutations as shown previously. In a parallel set of experiments, AR/R581D activity on TAT1-TATA was found to be lower than wild type (data not shown). Thus, AR self-synergy, like that of GR and MR, is increased by disruption of the DBD dimer interfaces, further supporting the idea that the dimer interface is inhibitory. Coexpression of complementary salt-bridge mutants returned the elevated transcriptional activity back to the wild type level, while coexpression of noncomplementary mutants did not. It is notable that the transcriptional activity elicited by AR/R581D + GR/D481R was lower than that of wild type GR alone consistent with an inhibitory effect transmitted through half (TAT0.5) or full (TAT1.0) HRE sites. Exposure time: 24 h. Lanes 1 and 2, probe only; lanes 3 and 4, AR-DBD was added; lanes 5 and 6, GR-DBD was added. Lanes 1, 3, and 5, TAT0.5 was used; lanes 2, 4, and 6, TAT1.0 was used. Conditions of vast probe excess were used to favor monomer formation so that both monomer and dimer bands could be well visualized on the TAT1 element. B, various amounts of purified AR and GR DBDs incubated with the radioactive probe TAT1.0. Lane 1, no protein; lane 2, AR but no GR; lanes 3–11, increasing amounts of AR and decreasing amounts of GR were added, keeping the total protein constant; lane 12, GR but no AR. C, anti-GR antibody (BuGR) was included in the reaction mixture (lanes 5–7). Note that the intermediate band representing AR-GR heterodimer, upper GR homodimer band, and lower GR monomer band were supershifted by the antibody. Lane 1, probe and antibody only, no protein; lanes 2 and 5, AR-DBD was added; lanes 3 and 6, AR-DBD and GR-DBD was added at 1 to 1 ratio, keeping the total protein constant; lanes 4 and 7, GR-DBD was added.
and cultured cells suggest that AR and GR not only mediate opposite effects on cellular metabolism and proliferation but that their signaling pathways are mutually inhibitory (2, 6–9). We have demonstrated that AR and GR inhibit each other’s transcriptional activities in cultured cells, that they heterodimerize in cells and in vitro, and that re-establishing heterodimer formation by coexpression of complementary salt-bridge mutants restores AR inhibition of GR at TAT3-TATA. Taken together, these data strongly suggest that heterodimer formation underlies their inhibitory interaction at the transcriptional level. We propose that the opposing effects of androgens and glucocorticoids are due, at least in part, to mutual inhibition of AR and GR through heterodimer formation. This might have important implications for balancing the anabolic, pro-aggressive actions of the androgens on the one hand with the catabolic homeostatic actions of the glucocorticoids on the other. For example, as glucocorticoid levels rise to predominate over testosterone in a stressed animal, heterodimer formation will ensure that the energy-consuming anabolic effects mediated by AR homodimers are curtailed at the same time that GR homodimers begin to activate transcription of genes involved in catabolism. In a similar fashion, the behavioral effects of androgens (those mediated by AR rather than estrogen receptor) also might be blocked by GR-AR heterodimers resulting in dramatic changes in behavior. When AR activity predominates (high androgen/glucocorticoid rates), a reciprocal effect might be observed. It is important to note that these inhibitory interactions at a subset of HREs do not rule out the possibility that at other HREs AR and GR do not interact, have similar effects, or perhaps even synergize. Indeed, AR and GR both repress AP-1 activity at either a composite GRE (25, 29) or a simple AP-1 site (30). Moreover, repression does not require an intact DBD dimer interface (27) and AR and GR do not influence each other’s activities.

Heterodimer formation is widely recognized as a key component of transcriptional regulation by the nuclear receptors. Retinoid X receptor, the ubiquitously expressed receptor for 9-cis-retinoic acid, interacts with several members of the nuclear receptor superfamily (notably, retinoic acid, thyroid, and vitamin D receptors) (22, 33). The resulting heterodimers bind primarily to direct repeats whose spacing is determined by the DBD dimer interface (34, 35). Recently, heterodimer formation between MR and GR has been described (16, 18), challenging the idea that heterodimerization is limited to the nuclear receptors. Our present observations suggest that this important regulatory interaction extends to AR and may extend to the entire family. However, the nuclear receptor and steroid receptor heterodimers differ in several important respects. Notably, in the context of our present observations, there is no evidence that the DBD dimer interface differentially controls receptor half-site spacing selection as it does for the nuclear receptors. Furthermore, there is little evidence that direct repeat elements exist and it appears that steroid receptor homo- and heterodimers bind in a head-to-head fashion. The present data also add further support to the novel idea that the primary role of the DBD dimer interface is not to stabilize receptor occupancy on the DNA but rather to curtail the energy-consuming anabolic effects mediated by AR homodimers. This is consistent with the idea that heterodimerization is limited to the nuclear receptors. Our present observations suggest that this important regulatory interaction extends to AR and may extend to the entire family. However, the nuclear receptor and steroid receptor heterodimers differ in several important respects. Notably, in the context of our present observations, there is no evidence that the DBD dimer interface differentially controls receptor half-site spacing selection as it does for the nuclear receptors. Furthermore, there is little evidence that direct repeat elements exist and it appears that steroid receptor homo- and heterodimers bind in a head-to-head fashion. The present data also add further support to the novel idea that the primary role of the DBD dimer interface is not to stabilize receptor occupancy on the DNA but rather to curtail receptor self-synergy (17). Indeed this site of direct contact between subunits appears to convey inhibitory information where receptor multimers are bound to clusters of HREs. In

**FIG. 5. Inhibitory function of the AR and GR DBD dimer interfaces.** CV-1b cells were cotransfected with complementary and non-complementary salt bridge mutants of full-length AR and GR. TAT3-TATA was cotransfected with wild type receptors or salt bridge mutants as shown. Luciferase values were normalized to β-galactosidase activities and represent the mean ± S.E. of three independent experiments.

The DBD dimer interface. These results strengthen our hypothesis that AR and GR mutual inhibition proceeds through heterodimerization and further support the idea that the role of the DBD dimer interface in this receptor subfamily is to limit transcriptional self-synergy, not to stabilize receptor occupancy at the HRE, i.e. this protein-protein contact appears to transmit inhibitory information from one subunit to the other in the context of a heterodimer.

It is interesting to note that a mutation within the DBD dimer interface of AR has been implicated in the human disease, Reifenstein’s syndrome (24). In transfection experiments, Kaspar et al. found that the mutant AR was surprisingly unaffected by the mutation at paired HREs while its activity was decreased at a simple HRE. We also find that the effect of the dimer mutations on receptor activity is context-dependent; receptor activity is increased at multiple simple HREs (Fig. 5 and Ref. 17), decreased at a single HRE (17), and unaffected at a complex HRE with interspersed receptor binding sites and sites for non-receptor factors. For example, we tested the activity of wild type and mutant GR and AR to repress AP-1 activity at a composite GRE containing reporter, the plgF (25). In cotransfection experiments into F9 cells, while the receptors showed qualitatively the same results on TAT3-TATA as in CV-1b cells, we found that activation by AP-1 was repressed by both AR (26, 27) and GR (14) in a ligand-dependent manner as reported previously (data not shown). Interestingly, we also found that both AR and GR salt-bridge mutants could repress the AP-1 activation of transcription at a level similar to wild type. Furthermore, in cotransfection experiments with complementary mutants (AR/R581D + GR/D481R) or non-complementary mutants (AR/R581D + GR/R479D), we found that repression was comparable to wild type, suggesting that the two receptors do not heterodimerize. These results support the hypothesis that GR and AR act as monomers when repressing AP-1 activation as suggested previously (28).

**DISCUSSION**

A variety of studies in whole animals, isolated tissues, and cultured cells suggest that AR and GR not only mediate opposite effects on cellular metabolism and proliferation but that their signaling pathways are mutually inhibitory (2, 6–9). We have demonstrated that AR and GR inhibit each other’s transcriptional activities in cultured cells, that they heterodimerize in cells and in vitro, and that re-establishing heterodimer formation by coexpression of complementary salt-bridge mutants restores AR inhibition of GR at TAT3-TATA. Taken together, these data strongly suggest that heterodimer formation underlies their inhibitory interaction at the transcriptional level. We propose that the opposing effects of androgens and glucocorticoids are due, at least in part, to mutual inhibition of AR and GR through heterodimer formation. This might have important implications for balancing the anabolic, pro-aggressive actions of the androgens on the one hand with the catabolic homeostatic actions of the glucocorticoids on the other. For example, as glucocorticoid levels rise to predominate over testosterone in a stressed animal, heterodimer formation will ensure that the energy-consuming anabolic effects mediated by AR homodimers are curtailed at the same time that GR homodimers begin to activate transcription of genes involved in catabolism. In a similar fashion, the behavioral effects of androgens (those mediated by AR rather than estrogen receptor) also might be blocked by GR-AR heterodimers resulting in dramatic changes in behavior. When AR activity predominates (high androgen/glucocorticoid rates), a reciprocal effect might be observed. It is important to note that these inhibitory interactions at a subset of HREs do not rule out the possibility that at other HREs AR and GR do not interact, have similar effects, or perhaps even synergize. Indeed, AR and GR both repress AP-1 activity at either a composite GRE (25, 29) or a simple AP-1 site (30). Moreover, repression does not require an intact DBD dimer interface (27) and AR and GR do not influence each other’s activities.

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$^2$ S. Chen, unpublished data.
light of these observations, it is interesting to note that receptor binding to multiple HREs is highly cooperative and moreover that this cooperativity requires the receptor N terminus (36, 37), a domain that is also required for the augmented activity of the dimer mutants (compare Fig. 3 and 5, and see Ref. 17). While the physiological implications and mechanistic basis of these observations remain to be explored, they clearly challenge the paradigm of a static interaction that merely stabilizes receptor occupancy at a palindromic HRE. Indeed, these data suggest the intriguing possibility that the inhibitory effect of the DBD dimer interface might be modulated by DNA sequence, half-site spacing, or protein factors and thus play a crucial regulatory role in receptor transcriptional activity.

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