The Rat Glucocorticoid Receptor Mutant K461A Differentiates between Two Different Mechanisms of Transrepression*

(Received for publication, March 11, 1997, and in revised form, May 30, 1997)

Thomas Meyer‡‡, D. Barry Starr‡†, and Jan Carlstedt-Duke‡‡
From the Departments of Medical Nutrition and Biosciences, Novum, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge, Sweden and Departments of Cellular and Molecular Pharmacology, and Biochemistry and Biophysics, PIBS Biochemistry and Molecular Biology Program, University of California San Francisco, San Francisco, California 94143-0450

The glucocorticoid receptor (GR) can both activate and repress transcription of target genes by interaction with specific genomic response elements, glucocorticoid response elements (GREs). Activation of transcription is usually the result of the direct interaction between GR and the GRE, whereas GR-mediated transcription repression is either the result of the indirect action of GR, mediated by a response element as a result of protein-protein interaction or by an occlusion mechanism in which GR displaces a general or regulatory transcription factor. A specific mutation of rat GR, K461A, has previously been described to transform the indirect protein-protein interaction-dependent transrepressive effect of GR into an activating function (Starr, D. B., Matsui, W., Thomas, J. R., and Yamamoto, K. R. (1996) Genes Dev. 10, 1271–1283). In HOS D4 and COS7 cells, this mutation was shown to transform the transrepressive effect of wild-type GR, acting on reporter constructs containing the composite GRE from the proliferin gene (plfG) or the negative tethering GRE from the collagenase A promoter (coA), into an activating function. In contrast, the K461A mutation had no effect on the transrepressive effect of GR on the human osteocalcin gene in which repression apparently occurs through the binding of GR to a negative GRE that overlaps the TATA box. The transrepressive function, typically 40% of the basal level in the absence of hormone, required only the isolated DNA-binding domain of wild type or mutant GR and was independent of the nature of transactivation domain. Thus, mutation of rat GR at position 461 differentiates between transrepressive functions of GR dependent on GR-DNA interaction (repression by occlusion) and GR-protein interaction (active repression).

Negative regulation of gene transcription by glucocorticoids and other ligands for nuclear receptor proteins appears to be carried out either by mechanisms involving interference with the DNA binding of upstream or general transcription factors or, alternatively, by repression mechanisms independent of DNA binding. In the first case, transcription repression involves a competition between transcriptionally active and inactive proteins for common or overlapping DNA-binding sites. Thus, transcription repression is achieved when the inactive factor displaces the more active one. For the latter mode, transcriptional repression is achieved by an as yet unidentified mechanism that is postulated to involve a physical interaction between the two proteins in a DNA-independent manner and, presumably, the formation of a transcriptionally inactive complex.

The signal transduction pathway of glucocorticoid hormones provides a number of well described examples for which possible mechanisms involved in negative gene regulation have been postulated. Signal transduction is mediated by an intracellular receptor protein, that, like many other members of the steroid receptor superfamily, functions as a ligand-activated nuclear transcriptional regulator (1, 2). The classical mode of gene regulation by glucocorticoids, which accounts for most cases of positive gene regulation, is known to be mediated by interaction of the ligand-activated GR with positive control elements (glucocorticoid response elements; GREs) which are present in single or multiple copies upstream of or within target genes (3).

Although not completely understood, the mechanism by which GR activates transcription in response to glucocorticoids is fairly simple in comparison to the variety of mechanisms employed by nuclear receptor proteins for the negative modulation of gene transcription (4–6). Despite recent advances, it has not been possible to formulate a simple, all inclusive model accounting for negative gene regulation mediated by GR (7, 8), although several physiologically relevant models have been suggested for the small number of genes studied mechanistically in a detailed manner. It has become apparent that simple DNA binding by the receptor may not be sufficient or, in some cases, even required for ligand-dependent repression for the majority of examples described for glucocorticoid-mediated transcription repression, which are related to their anti-inflammatory effects (9–11). One of the best characterized examples of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19). An example of this mode of repression is the interaction between the transcriptional activator AP1 and the activated GR. Under certain conditions, interference between the GR and the AP1 signal transduction pathways appears to occur on composite response elements that have the potential to bind both the receptor and the AP1 complex, whereas in other cases, the repression mechanism requires only an AP1-DNA interaction (18, 19).
ample of the first case is seen within the promoter region of the proliferin gene. A 25-bp composite GRE, termed pG1F, is responsible for mediating the negative GR effect. GR binds to pG1F in the absence of AP1, but regulates transcription only in the presence of AP1, activating if AP1 consists of c-Jun homodimers, and repressing if AP1 is comprised of c-Jun-c-Fos heterodimers (19, 20). An example of the alternative AP1-dependent mechanism is the glucocorticoid-dependent repression of the collagenase gene. This effect requires AP1 binding to its specific recognition element in the upstream promoter region of the collagenase gene, whereas direct GR-DNA contact does not seem to be a prerequisite for efficient ligand-dependent repression of the activated gene expression level. Although the GR does not bind to the AP1 site, a functional GR DBD is required for repression of AP1 activity. A direct protein-protein interaction between GR and AP1 has been demonstrated (18, 21).

There are some cases, however, where DNA binding by GR is both necessary and sufficient for transcription repression. For example, at the pro-opiomelanocortin gene, repression occurs without assistance or interference from other sequence-specific transcription factors (12, 13). Detailed analysis of the interaction between GR and the GRE indicated that three moieties of the receptor molecule form a unique complex with the pro-opiomelanocortin GRE, in contrast to a positive regulated GRE (14). Examples of repression due to transcriptional interference with other regulatory proteins at a response element have been postulated in several cases including the bovine prolactin and the c-fos genes (15–17). A final example of a gene negatively regulated by glucocorticoids through an interference mechanism is the human bone-specific gene osteocalcin (22–24). The overlap of a competitive nGRE with the basal TATA box element suggested that the hormone-activated GR can function as a negative regulator on osteocalcin gene activity by competing with a specific TFIID-induced complex at the DNA binding level and that this binding is mutually exclusive (25, 26).

GR action is strongly determined by its context within the unique architecture and requirements of each gene promoter (27, 28). As demonstrated by the above described examples of glucocorticoid-dependent transcriptional regulation, GREs can be classified into at least three independent subclasses: simple GREs capable of interacting with the hormone-induced receptor without the assistance of other sequence-specific regulators, resulting in transactivation or, in more specialized cases, repression; composite GREs having the ability to interact with the receptor protein and additional factors resulting in either transactivation or transrepression; and cases in which direct GR-DNA interaction is not required for GR-mediated gene regulation, called tethering GREs (18, 21). Starr et al. (29) defined a single amino acid change (K461A) within the rat GR capable of distinguishing between simple GREs and composite and tethering GREs. The receptor, containing a mutation within the DBD at position 461 (human GR 442), provides a powerful tool for comparing and defining mechanisms involved in transduction of negative regulation by GR.

To further characterize the mechanism proposed for the repression of the human osteocalcin gene by glucocorticoids, we compared the action of this specific mutated receptor, chimeric proteins containing the GR DBD as well as the isolated GR DBD on both the osteocalcin promoter and the two well defined AP1-dependent systems described above, coA and plfG. In this report we present evidence that the mechanism involved in the negative transcriptional effect mediated by glucocorticoids on the human osteocalcin promoter is strictly dependent on binding of GR to a composite functional GRE (competitive nGRE). DNA binding and cotransfection experiments suggest that the hormone activated GR and the specific GR mutant K461A repress osteocalcin gene activity in a similar fashion, primarily mediated by competitive binding to a dual binding site that disrupts an alternative protein-DNA contact.

**EXPERIMENTAL PROCEDURES**

**Preparation of Promoter Constructs and Plasmids**—The plasmid pOSCAT containing the promoter region of the target gene was cut with SstI and XhoI to obtain a fragment spanning nucleotides −344/+31 of the osteocalcin promoter (29). The fragment was ligated into the corresponding restriction sites of pGL2 Enh (Promega) to drive the firefly luciferase gene (pOS-344Luc). The constructs coA-Luc and plfG-Luc are as described previously (29). The rat GR expression vector 6RGR, 6RGR-K461A, 6RGR(407–525), and 6RGR-K461A(407–525) are as described elsewhere (29). The expression plasmids for 407–556VP16 and 407–556K461A-VP16 were constructed as follows. The HindIII-XhoI fragment from pG1-X556VP16 (kindly provided by J. A. Laesstini) was cloned into the HindIII-SpeI sites of KS + GR(407–525) (29). The resulting plasmid was cut with KpnI and PvuII, and the fragment containing the GR sequence was cloned into the KpnI-EcoRV sites of pSbR (30), yielding 6R-407–556VP16. The 6R-407–556K461A-VP16 construct was made by ligation of the KpnI-BstBI fragment from KS + GR-K461A(407–525) into the KpnI-BstBI sites of 6R-407–556VP16. These plasmids were verified by sequencing the relevant parts of the resulting constructs.

**Cell Culture**—HOS D4 osteosarcoma cells were cultured at 37 °C in a humidified atmosphere with 5% CO2 in Eagle’s medium buffered with bicarbonate and supplemented with 5% fetal calf serum, penicillin (100 IU/ml), and streptomycin (0.1 mg/ml). COS7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented as described above.

**Transient Transfection Assays and Luciferase Assay**—Cells were seeded in 6-cm plates 24 h before a transfection experiment and transfected at 50–60% confluence using the calcium phosphate coprecipitation technique. The precipitate contained 5 μg of supercoiled luciferase reporter plasmid DNA and varying amounts (0–2 μg) of different expression plasmids. The overall amount of DNA was kept constant by the addition of parent expression vector. After 12–14 h exposure to the calcium phosphate precipitate, medium was refreshed and cells treated for 24 h with 20 nM dexamethasone. Transfected cells were subsequently harvested for luciferase assay by scraping the cells into 1 ml of phosphate-buffered saline, centrifuging for 10 min in a microcentrifuge, and resuspending in 50 μl of lysis buffer (25 mM Tris acetate, pH 7.8, 2 mM dithiothreitol, 1.5 mM EDTA, 10% glycerol, and 1% Triton X-100). Luciferase activity was monitored according to the GenGlow luciferase assay kit (Bio Orbit) using an Anthos Lucifer 1 luminoimeter. The results are expressed as light units measured. All experiments were performed in triplicate on three separate occasions.

**Protein Expression**—Extracts from COS7 cells, transiently transfected with 15 μg of GR expression vector/15-cm cell culture plate, were prepared by homogenizing the cell pellets with a Dounce homogenizer in 500 μl of 10 mM sodium phosphate, pH 7.4, 1 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 400 mM KCl, and centrifugation at 100,000 × g for 1 h. The supernatant was aliquoted and stored at −70 °C.

**DNA-binding Assays**—GR binding activity was monitored by an electrophoretic gel mobility shift assay. A 32P-labeled, double-stranded oligonucleotide spanning the GRE sequence and TATA box of the human osteocalcin promoter (−41/−9) or mutated versions of this DNA stretch were used as specific probe (wt: AGCCCCAGAGGGTATAAACAGTCGCAGGGAGG, mutant: AGCCCCAGAGGGTATAAACAGTCGCAGGGAGG). The recombinant GR was incubated for 10 min on ice in a buffer containing 0.5 μg of poly(dIdC), 60 mM KCl, 10 mM Hepes, pH 7.9, 0.1 mM EDTA, 10% glycerol, 0.1 mM dithiothreitol, 20 μM dNTPs, and 32P-labeled oligonucleotides were incubated with the binding reactions for 10 min prior to addition of the 32P-labeled probe. After adding the specific DNA probe the mixture was incubated for 20 min at room temperature. The protein-DNA complexes were resolved on 5% native polyacrylamide gels.

**RESULTS**

GR can antagonize the function of c-Jun and c-Fos, which are both components of the phorbol ester-activated transcription factor AP1 (8). We employed two well defined examples for repression of AP1-activated gene activity by GR to further characterize the mechanism of glucocorticoid mediated repression of the human osteocalcin gene in comparison. In all three examples of gene promoter regions used in this study, the negative modulation of target gene activity seems to be medi-
mediated by interference of hormone activated GR protein with positive acting sequence-specific transcription factors. The experimental basis for this comparative study was based on a recently published GR mutant having the ability to activate genes independent of the class of GRE used (29). In this mutant, an amino acid change from Lys to Ala at position 461 within the DBD of the rat GR resulted in a phenotype characterized by hormone-dependent transactivation at simple GREs as well as at composite and tethering GREs. As reference systems in the present study, we used either a promoter construct containing three copies of a 25-bp DNA sequence (composite GRE) from the proliferin promoter (plfG) (19) known to mediate a negative GR effect or an nGRE (tethering GRE) from the the collagenase A promoter here denoted as colA (31). In contrast to the wt GR, which represses transcription from these two reporters, the GR mutant K461A activates transcription (25, 26, 29).

We were interested in studying the effect of this constitutively positive acting receptor variant on the osteocalcin promoter-controlled transcription rate where the interdigitation of a GR binding site and the TATA box suggest that repression of osteocalcin gene expression is mediated by interference of the GR with the basal transcription machinery. To this end, we constructed a reporter plasmid containing a fragment of the human osteocalcin promoter spanning nucleotides −344/+31 of the human osteocalcin promoter (pOS-344Luc) and incubated with (solid bars) or without (open bars) 20 nM dexamethasone (Dex). Luciferase activity was assayed in cell extracts from one 6-cm plate and related to the activity in cells transfected with wt GR unexposed to dexamethasone. Shown is the mean ± S.D. of three separate experiments, each carried out with three independent triplicate analyses.

The original characterization of the K461A mutant and its transformation from transrepression to transactivation of composite and tethering GREs was carried out in F9 mouse embryonic carcinoma cells and CV-1 cells (29). To exclude cell-specific differences in the function of the K461A mutant, the function of the composite plfG GRE and the tethering colA GRE was tested in COS7 cells. In contrast to the results obtained with the osteocalcin gene, the expression of the K461A GR mutant activated the gene activity in both reference promoters in COS7 cells (Fig. 2). In the presence of dexamethasone, wt GR repressed luciferase activity to 60% from a plfG GRE, whereas the K461A mutant induced luciferase activity about 25-fold (Fig. 2A). The effect on the tethering colA element was similar but to a much lesser degree (Fig. 2B) with dexamethasone-dependent repression of luciferase activity to about 60% with wt GR and dexamethasone-dependent stimulation of luciferase activity to about 160% with the K461A mutant.

We have previously shown that GR binds specifically to the negative response element thought to be responsible for the transrepressive effect on the human osteocalcin promoter (25, 26). The K461A mutant binds to the −41/−9 fragment of the human osteocalcin promoter, containing the nGRE, in a manner similar to that of wt GR (Fig. 3A, lanes 3 and 4). The GR-specific band with wt GR is competed for specifically by an unlabeled oligonucleotide containing a standard GRE sequence (tyrosine aminotransferase GRE; TAT) (Fig. 3A, lane 7). The use of a vitamin D-responsive element did not affect GR binding (Fig. 3A, lane 7). Mutation of the nGRE sequence diminishes the GR-specific complex (Fig. 3A, lane 10). Competitive DNA binding was estimated by titrating increasing amounts of the unlabeled probe into the binding reactions containing either wt GR or the K461A mutant. Measurement of the relative binding of the radiolabeled probe showed that there was no major difference in DNA-binding affinity between wt GR and the K461A mutant (Fig. 3B).

To further support the hypothesis that competitive GR binding is responsible for repression of the osteocalcin gene, we compared the effect of a GR chimera, containing the GR DBD K461A fused to the activation domain of the viral transcrip-
tional activator VP16, on osteocalcin, pflG and colA controlled reporter gene activity. As shown in Fig. 4, expression of the GR-VP16 chimera containing the mutant K461A DBD increased the luciferase activity measured in the case of both reference promoters used in this study (Fig. 4, B and C). However, the repression mediated by this constitutively active chimeric transcription factor on osteocalcin gene transcription was still comparable to the repressive effect mediated by the wt GR-VP16 protein (Fig. 4A). The magnitude of transactivation/transrepression with the GR K461A-VP16 chimera (Fig. 4A) was identical to the dexamethasone-dependent activity of the full-length GR variants on osteocalcin promoter activity (Fig. 1). Thus, the repression of the osteocalcin promoter by GR is dependent on the DNA binding function and is independent of the transactivation domain associated.

Finally, the function of the isolated DBD of the two GR variants was tested with regard to their transrepressive effect. Expression of the isolated DBD of either wt GR or the mutant K461A repressed the osteocalcin promoter to an equal degree, with virtually identical activity to the full-length variants (Fig. 5A). In contrast, the isolated wt DBD had no effect on the plfG reporter activity (Fig. 5B). The isolated DBD of the K461A GR mutant demonstrated a weak stimulatory activity on the plfG element (Fig. 5B). However, this was considerably reduced compared with the dexamethasone-dependent activity of the full-length K461A mutant (Fig. 2A).

DISCUSSION

The repression of gene transcription is of particular interest since the mechanism underlying these repressive effects are less well understood than those governing activation. It has previously been shown that administration of glucocorticoids leads to a transcripational repression of several target genes. These transrepressive effects of glucocorticoids are related to their clinically important anti-inflammatory effects (e.g. repression of collagenase A) or relevant side effects of pharmacological usage of glucocorticoids such as steroid-dependent osteoporosis (e.g. repression of osteocalcin) (23, 31, 33). In contrast to the unifying model proposed for gene activation by GR, a simple unique model has not been formulated to account
for receptor-dependent gene repression.

In the present study we further characterized the mechanism underlying the negative glucocorticoid-dependent regulation of the human osteocalcin gene in comparison with two well described reference systems for repression committed by GR on phorbol ester-activated gene transcription, the mouse prolactin gene, and the collagenase A gene. We took advantage of a recently described rat GR variant, GR K461A, that is able to distinguish between at least three functional subclasses of glucocorticoid responsive elements: simple GREs binding the activated GR molecule in a homodimeric fashion, composite GREs in which the exertion of receptor action requires the binding of additional sequence specific transcription factors to a common binding site, and tethering GREs in which GR mediates the transcriptional rate of target genes by interfering with stimulatory transcriptional activators already bound to DNA (Fig. 6) (27). The K461A mutation results in a reduced transactivating activity on simple GREs that corresponds to a reduced binding affinity for the GRE sequence (29). On tethering or composite GREs, the K461A mutation results in a switch from glucocorticoid-dependent transrepression, in the presence of both c-Jun and c-Fos, to transactivation. Identical results were obtained in this study, using COS7 cells or HOS D4 osteosarcoma cells. Thus, the effect of the K461A mutation on AP1-dependent GR transrepression is not cell-specific. However, the magnitude of AP1-dependent GR-dependent transactivation of the colA element induced by the K461A mutation was considerably decreased as compared with that obtained previously with either F9 or CV-1 cells. The magnitude of induction induced by K461A on the plfG element was considerably larger and of the same order of magnitude seen previously in F9 and CV-1 cells.

The osteocalcin gene is an osteoblast-specific gene expressed in late stages of differentiation (34). Although the exact function of osteocalcin remains unclear, osteocalcin production is related to bone density and increased osteoblast activity (35). Exposure to glucocorticoids results in the reduction of osteocalcin mRNA to about 50%. Analysis of the promoter region of the human osteocalcin gene identified one specific binding site for GR which completely overlapped the TATA box (26). Transient expression of reporter genes driven by constructs containing the minimal osteocalcin promoter resulted in a glucocorticoid-dependent reduction in reporter gene activity to 50% or less (23, 25). Mutations of the promoter that eliminated GR binding obliterated the glucocorticoid-dependent repression of reporter gene activity. GR homodimer and TBP bind competitively for overlapping DNA elements in vitro (25). Based on these results we proposed a mechanism for glucocorticoid-dependent repression of the osteocalcin gene in which binding of GR to a negative GRE (competitive nGRE, Fig. 6) reduces the availability of the promoter for the basal transcriptional complex. Glucocorticoid-dependent transrepression of target genes by competitive binding of GR and transcriptional activators to overlapping DNA elements has been proposed for the regulation of the type 1 vasoactive intestinal polypeptide receptor gene as well as for the prolactin gene (15, 17, 36).

In contrast to the switch of AP1-dependent GR transrepression to transactivation by the K461A mutation, no effect at all was seen on the GR-dependent transrepression of the osteocalcin promoter. Both wt GR and the K461A mutant induced repression of the osteocalcin promoter to about 40% of basal activity. Both GR variants bound equally well to the osteocalcin nGRE (Fig. 3B). This is in contrast to the decreased binding and function on a simple positive GRE and transactivation with the mutant as described previously (29). These results confirm the dependence of osteocalcin repression by glucocorticoids on GR-DNA interaction rather than by protein-protein interaction.

Detailed molecular studies have demonstrated the functional requirement of the GR DBD in the modulation of gene expression by GR. The DBD has been shown to be necessary for both the transactivation and the transrepression functions of the receptor (20, 28). The isolated DBD, either wt or the K461A mutant, were sufficient to induce transrepression of the osteocalcin promoter, which further strengthens the hypothesis of a direct competition in binding to overlapping DNA sequences between GR and TBP. In contrast, the isolated DBD was virtually inactive on the plfG element, even with the K461A mutation. Fusion of the DBD K461A to a heterologous transactivation domain from VP16 restored the function of the protein on the AP1-dependent elements, plfG and colA, resulting in constitutive transactivation with the K461A mutation. In contrast, the transrepression of the osteocalcin promoter remained unchanged, indicating that the glucocorticoid-dependent repression of the osteocalcin promoter is independent of the transactivation domain associated.

In conclusion, mutation of a single amino acid located at the

FIG. 5. Constitutive activity of the isolated GR DBD. COS7 cells were transfected with parent expression vector (Ctr) or expression vectors for GR DBD (wt or K461A mutant) together with a luciferase reporter gene. The reporter gene was A, driven by nucleotides −344/−31 of the human osteocalcin gene (OC) or B, driven by −33/−53 of the Drosophila alcohol dehydrogenase promoter with upstream elements consisting of three tandem copies of the proliferin GRE (plfG). Luciferase activity was assayed in cell extracts from one 6-cm plate and related to the activity in cells transfected with wt GR exposed to dexamethasone. The figure shows the mean ± S.D. of three separate experiments, each carried out with three independent triplicate analyses.

FIG. 6. Summary of wt GR and K461A mutant GR action. The action of GR on simple tethering or composite GREs as well as competitive nGREs is shown. The boxes represent upstream or basal activating transcription factors such as AP1 or TBP. The gray arrows show the action of the K461A mutant GR.
DNA-binding surface of rat GR, K461A, results in a receptor variant that can differentiate between two different mechanisms of glucocorticoid-dependent transrepression. The mutation switches AP1-dependent transrepression, involving protein-protein interaction, to transactivation, whereas DNA-dependent transrepression is unaffected. In the crystal structure of the rat GR DBD bound to a simple positive GRE (37, 38), the lysine side chain at position 461 makes a specific base contact with the DNA sequence. Loss of this contact following the lysine side chain at position 461 makes a specific base interaction with the DNA sequence. The K461A mutation does not affect the affinity for the GRE and thereby decreased transactivation, which is what was previously reported for this mutation. However, the exact contacts between GR and the osteocalcin nGRE have not been demonstrated. The K461A mutation does not result in any loss in transrepression function, which would indicate that this residue does not play as active a role in binding to the osteocalcin nGRE as it does in binding to a classical positive GRE. Another explanation may be that Lys-461 plays an active role in DNA sequence-dependent conformational change of GR required for transactivation. In the osteocalcin nGRE, GR appears to exert its role by competing away TBP from the promoter, thereby reducing the transcriptional affinity of GR required for transactivation. In the osteocalcin nGRE, TBP appears to exert its role by competing away TBP from the promoter, thereby reducing the transcriptional rate of the gene. Thus no further change or subsequent step in GR action would be required for the regulation of this gene.

Acknowledgments—We thank Tony Wright for the careful reading of the manuscript and Jeff Lefstin for providing the pG1-X556-VP16 plasmid.

REFERENCES
1. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans R. M. (1995) Cell 83, 815–839
2. Tsai, M. J., and O’Malley B. W. (1994) Annu. Rev. Biochem. 63, 451–486
3. Truss, M., and Beato, M. (1995) Endocr. Rev. 14, 459–479
4. Birnberg, N. C., Lissitzky, J. C., Hinman, M., and Herbert, E. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6982–6986
5. Akerblom, I. E., Slater, E. P., Beato, M., Baxter, J. D., and Mellon, P. L. (1988) Science 241, 350–353
6. Weiner, F. R., Czaja, M. J., Jefferson, D. M., Giambrone, M.-A., Tur-Kaspa, R., Reid, L. M., and Zern, M. A. (1987) J. Biol. Chem. 262, 6955–6968
7. Saatcioglu, F., Clare, F. X., and Karin, M. (1994) Semin. Cancer Biol. 5, 347–359
8. Karin, M., Yang-Yen, H. F., Chambard, J. C., Deng, T., and Saatcioglu, F. (1990) Eur. J. Clin. Pharmacol. 45, 843–44
9. Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.-Å., and Van der Saag, P. T. (1995) Mol. Endocrinol. 9, 401–412
10. Auppern, N., DiDonato, J. A., Rosette, C., Helnberg, A., and Karin, M. (1995) Science 270, 286–290
11. Scheinman, R. I., Gualberto, A., Jewell, C. M., Cidlowski, J. A., and Baldwin, A. S., Jr. (1995) Mol. Cell. Biol. 15, 943–953
12. Drouin, J., Sun, Y. L., Chamberland, M., Gauthier, Y., De Lean, A., Nemer, M., and Schmidt, T. J. (1993) EMBO J. 12, 145–156
13. Riegel, A. T., Lu, Y., Remenicik, J., Wolford, R. G., Berard, D. S., and Hager, G. L. (1991) Mol. Endocrinol. 5, 1973–1982
14. Dahlman-Wright, K., Wright, A., Gustafsson, J.-Å., and Carlstedt-Duke, J. (1991) J. Biol. Chem. 266, 3107–3112
15. Caira, M., Caira, W., and Okret, S. (1993) DNA Cell Biol. 12, 695–702
16. Karagianni, N., and Tsawdaroglou, N. (1994) Oncogene 9, 2237–2334
17. Sakai, D. D., Helms, S., Carlstedt-Duke, J., Gustafsson, J.-Å., Rettman, F. M., and Yamamoto, K. R. (1988) Genes Dev. 2, 1144–1154
18. König, H., Ponta, H., Rahmsdorf, H. J., and Herrlich, P. (1992) EMBO J. 11, 2241–2246
19. Diamond, M. I., Miner, J. N., Yoshinaga, S. K., and Yamamoto, K. R. (1990) Science 249, 1266–1272
20. Pearce, D., and Yamamoto, K. R. (1993) Science 259, 1161–1165
21. Heck, S., Kullmann, M., Gurt, A., Ponta, H., Rahmsdorf, H. J., Herrlich, P., and Cato, A. C. (1994) EMBO J. 13, 4087–4095
22. Celeste, A. J., Rosen, V., Buecker, J. L., Kripp, W. A., and Wozney, J. M. (1986) EMBO J. 5, 1885–1890
23. Morrison, N. A., Shine, J., Fragnas, J. C., Verkast, V., McMeneny, M. L., and Eisman, J. A. (1989) Science 246, 1158–1161
24. Hauschka, P. V., Lian, J. B., Cole, D. E., and Gundberg, C. M. (1989) Physiol. Rev. 69, 990–1047
25. Meyer, T., Gustafsson, J.-Å., and Carlstedt-Duke, J. (1997) DNA Cell Biol. 16, in press
26. Strimmett, P. E., Poellinger, L., Gustafsson, J.-Å., and Carlstedt-Duke, J. (1991) Mol. Cell. Biol. 11, 3379–3383
27. Miner, J. N., and Yamamoto, K. R. (1991) Trends Biochem. Sci. 16, 423–426
28. Lefstin, J. A., Thomas, J. R., and Yamamoto, K. R. (1994) Genes Dev. 8, 2842–2856
29. Starr, D. B., Matsui, W., Thomas, J. R., and Yamamoto, K. R. (1996) Genes Dev. 10, 1271–1283
30. Miner, J. N., Diamond, M. I., and Yamamoto, K. R. (1991) Cell Growth Differ. 2, 525–530
31. Jenat, C., Rahmsdorf, H. J., Park, K. K., Cato, A. C., Gebel, S., Ponta, H., and Herrlich, P. (1990) Cell 62, 1189–1204
32. Alissen, M., Barkhem, T., Strömstedt, P. E., Ahola, H., Kultoh, E., Gustafsson, J.-Å., Poellinger, L., and Nilsson, S. (1991) J. Biol. Chem. 266, 10578–10585
33. Heinrichs, A. A., Bortell, R., Rahman, S., Stein, J. L., Alnemri, E. S., Litwack, G., Lian, J. B., and Stein, G. S. (1993) Biochemistry 32, 11436–11444
34. Stein, G. S., Lian, J. B., Stein, J. L., Van Wijnen, A. J., and Montecino, M. (1996) Physiol. Rev. 76, 593–629
35. Ducy, P., Desbois, C., Boyce, B., Pinero, G., Story, B., Dunstan, C., Smith, E., Bonadio, J., Goldstein, S., Gundberg, C., Bradley, A., and Karsenty, G. (1996) Nature 382, 448–452
36. Pei, L. (1996) J. Biol. Chem. 271, 20879–20884
37. Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R., and Sigler, P. B. (1991) Nature 352, 497–505
38. Hard, T., Kellenbach, E., Boelens, R., Maier, B. A., Dahlman, K., Freedman, L. P., Carlstedt-Duke, J., Yamamoto, K. R., Gustafsson, J.-Å., and Kepstein, R. (1990) Science 249, 157–160
The Rat Glucocorticoid Receptor Mutant K461A Differentiates between Two Different Mechanisms of Transrepression
Thomas Meyer, D. Barry Starr and Jan Carlstedt-Duke

J. Biol. Chem. 1997, 272:21090-21095.
doi: 10.1074/jbc.272.34.21090

Access the most updated version of this article at http://www.jbc.org/content/272/34/21090

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 37 references, 16 of which can be accessed free at http://www.jbc.org/content/272/34/21090.full.html#ref-list-1