Molecular Determinants of Glucocorticoid Receptor Function and Tissue Sensitivity to Glucocorticoids

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I. Introduction

Steroid hormones are essential constituents of the intercellular communication system that maintains homeostasis in higher organisms. Glucocorticoids, a major subclass of steroid hormones, modulate a large number of metabolic, cardiovascular, immune, and behavioral functions (for a review see Refs. 1 and 2). Glucocorticoids are produced by the adrenal cortex under the regulatory influence of ACTH. The latter is produced by corticotrophs of the anterior pituitary, in turn, under the regulatory influence of hypothalamic CRH and arginine vasopressin (AVP). The hypothalamic-pituitary-adrenal (HPA) axis is kept in balance by the negative feedback effects of cortisol on the secretion of ACTH, CRH, and usually, to a lesser extent, AVP. In the resting state, basal levels of CRH, AVP, ACTH, and cortisol are released in a pulsatile and circadian fashion. At these baseline levels, the main function of cortisol is to sustain normoglycemia and to prevent arterial hypotension. Whether and to what extent the immunosuppressive effects of cortisol are relevant at resting state levels in humans is still a matter of dispute (3). Activation of the HPA axis during physical or emotional stress and the resulting increase in plasma cortisol levels are probably required for central nervous system (CNS) activation, higher blood glucose concentrations, and an elevated mean blood pressure in the stress state. Cortisol also restrains a potential concurrent inflammatory/immune reaction, which might otherwise lead to tissue damage.

II. The Glucocorticoid Receptor (GR)

At the cellular level, most known effects of glucocorticoids are mediated by a ~94-kDa intracellular protein, the GR. GR belongs to the phylogenetically conserved superfamily of nuclear hormone receptors, which includes receptors for mineralocorticoids, androgens, progestins, estrogens, vitamin D, thyroid hormone, retinoic acid, and a growing number of so-called orphan receptors for which no specific ligand has yet been identified (4, 5). In the hormone-bound state, these receptors specifically bind to and modulate the activity of target gene promoters and are, therefore, also known as ligand-dependent transcription factors (4, 5).

A. Structure of the GR

The overall concept of steroid hormone receptor action was laid down by Jensen and co-workers in the 1960s (6). Over the last 10 yr, cloning, mutagenesis, and in vitro analysis of various steroid hormone receptors have unequivocally proven the correctness of this concept and have further added to our understanding of the structure and function of these proteins (4, 7-9). All members of the nuclear hormone receptor family share a characteristic three-domain structure,
which was first described for the GR (Fig. 1): The N-terminal domain contains sequences responsible for activation of target genes and presumably interacts with components of the basal transcription machinery and/or with other transcription factors (10-12). Two highly conserved “zinc fingers” in the central part of the receptor molecule constitute the DNA-binding domain (10, 13, 14), which also participates in receptor dimerization (15), nuclear translocation (16), and transactivation (11, 17). The function of the C-terminal or ligand-binding domain is even more complex. In addition to specifically binding the hormonal ligand (10, 18), it contains sequences important for heat shock protein (hsp) binding (19-21), nuclear translocation (16), dimerization (22), and transactivation (23-26), as well as for silencing of the receptor in the absence of hormone (11, 27).

B. GR-mediated transcriptional regulation

1. The unliganded GR. The following model represents our current understanding of how the GR is transformed from a silent to an active transcription factor in response to glucocorticoids (7-9, 28) (Fig. 2). The unliganded GR is part of a multiprotein complex that consists of the receptor, two molecules of hsp90, and one molecule each of hsp70, and hsp56, an immunophilin of the FK506- and rapamycin-binding class (20, 21, 29-32). In addition, and depending on the stringency of the extraction conditions, other, less well characterized proteins have occasionally been found to participate in this complex (29). In the absence of hormone, this complex most likely undergoes constant cycles of dissociation and ATP- and hsp70-dependent reassociation (31, 33).

2. GR activation. As lipophilic substances, glucocorticoids are able to cross the cell membrane readily to interact with the intracellular GR. The physiological significance of additional glucocorticoid-binding sites in the cell membrane is still a matter of dispute and will not be discussed in this paper (for a review see Ref. 45). Ligand binding induces an as yet undetermined conformational change in the GR molecule that has a number of functional consequences: the hormone-bound GR dissociates from the hsp complex and is no longer able to reassociate with it (7, 8, 20, 33). Furthermore, the partially phosphorylated receptor protein becomes hyperphosphorylated (33, 46, 47). Finally, nuclear localization signals within the ligand-binding domain of the receptor (16)
may be unmasked after hormone binding and may cause nuclear translocation of cytoplasmic GR molecules (40).

3. **Type 1 mechanism of GR action.** Within the nucleus, the hormone-activated GR can act in two ways, referred to as type 1 and type 2 mechanism of action throughout this review. The type 1 mechanism is characterized by the GR interacting with specific DNA sequences, whereas the type 2 mechanism involves interaction of the GR with other transcription factors in the absence of specific DNA binding. The type 1 mechanism represents the classic model of GR action. In this model, a receptor homodimer binds to short, palindromically arranged DNA sequences termed glucocorticoid response elements (GREs) in the promoter region of glucocorticoid-responsive genes. When bound to the GRE, the GR homodimer interacts with components of the basic transcription machinery, either directly, *i.e.* by physical contact between the GR transactivation domains and basic transcription factors such as TFIIB, or indirectly, *i.e.* via “bridging” factors (7-9, 48-50), such as the recently identified steroid receptor coactivator 1 (SRC-1) (51). This interaction is sufficient to stabilize the preinitiation complex on the promoter and, thus, to enhance transcription by RNA polymerase II (8). In addition, binding of the GR homodimer to the GRE can induce a rearrangement of the chromatin structure in the respective promoter region, thus allowing other transcription factors to bind to the previously inacessible DNA (52-55). In some promoters, binding of the activated GR to so-called negative glucocorticoid response elements (nGREs) causes inhibition rather than enhancement of transcription. The prototype of a nGRE is located in the POMC promoter and only slightly resembles the classic GRE (56). Instead of binding a GR homodimer, the nGRE accommodates three GR molecules (56). For reasons that are not well understood, this GR/nGRE complex represses transcription of the POMC gene.

4. **Type 2 mechanism of GR action.** Many effects of glucocorticoids are achieved by inhibition rather than by activation of target genes. This is especially true for the antiinflammatory/immunosuppressive effects of glucocorticoids that involve negative transcriptional regulation of immune genes, such as the collagenase and the interleukin-2 genes. Surprisingly, the promoters of these genes do not contain nGRE sequences or any other GR-binding sites, yet they are repressed by glucocorticoids (57-60). It is now well established that these genes are regulated by a different mechanism of GR action (61-66), referred to as the type 2 mechanism. The above mentioned genes are positively regulated by activating protein-1 (AP-1), a transcription factor composed of dimers of Jun and Fos family proteins (67, 68), the activity of which is modulated by growth factors and cytokines via mitogen-activated protein kinases (69). AP-1 binds to specific target sequences within responsive promoters (70). Deletion of these AP-1-binding sites abrogates both AP-1-mediated stimulation and GR-mediated repression of gene transcription, even though GR itself does not bind to AP-1 sites (57, 71). It was therefore concluded that GR inhibits AP-1 in the absence of DNA binding, possibly by direct protein/protein interaction (57, 71-73). This interaction most likely takes place with AP-1 bound to the target DNA, since genomic footprinting experiments have demonstrated that glucocorticoid...
coids do not alter the occupancy of AP-1 sites in the collagenase promoter (74).

Although AP-1 has been studied most extensively, it is not the only transcription factor to be modulated by GR. A similar pattern of GR-mediated transrepression has been reported for RelA, the p65 subunit of the transcription factor NF-κB (75-77), also an activator of many immune system genes (78, 79). In addition to physically interacting with the p65 subunit, GR suppresses NF-κB activity by induction of the IkB inhibitory protein, which traps NF-κB in inactive cytoplasmic complexes (80, 81).

Other transcription factors with activity that can be altered by GR are the octamer transcription factors Oct-1 and Oct-2 (82, 83), the Spi-1/Pu.1 oncoprotein (84), and other steroid receptors, such as the estrogen receptor (85) and the thyroid hormone receptor (86).

III. Glucocorticoid Responsiveness vs. Glucocorticoid Sensitivity

The response of a single cell exposed to glucocorticoids is the result of the interplay between the following three parameters: the concentration of free hormone, the relative potency of the hormone, and the ability of the cell to receive and transduce the hormonal signal. As described above, the regulation of the plasma and tissue cortisol concentration by the hypothalamic-hypophyseal system has been extensively studied and is relatively well understood, as are the pathophysiological implications of abnormal cortisol secretion (1, 2, 87). The concentration of free hormone is also influenced by the plasma and tissue levels of corticosteroid-binding globulin (CBG), which are themselves under a complex regulatory control (87, 88). The relative potency of any endogenous or synthetic glucocorticoid is influenced by its bioavailability, affinity for the GR, and ability to retain the GR in the nucleus (87, 89). Although the third parameter, i.e., the ability of cells to respond to a given concentration of a defined glucocorticoid, has been recognized as a variable, we are far from understanding how frequently and to what extent it is involved in pathophysiological processes. It is believed, however, that virtually every step in the GR activation cascade can be interfered with by endogenous or exogenous factors (90).

At this point, it may be useful to introduce the two distinct concepts of glucocorticoid responsiveness and glucocorticoid sensitivity, terms that have been used interchangeably by most authors in the past. We define glucocorticoid responsiveness as the ability of a system defined by cell type and target gene to exhibit measurable changes in response to glucocorticoids. In practical terms, this definition refers to the ability of cells to respond to a given concentration of a defined glucocorticoid. Each factor is categorized according to the step with which it appears to interfere with glucocorticoid-induced transcription as described above. Each factor is categorized with respect to the step with which it appears to interfere most strongly (Table 1).

A. Intracellular hormone availability

Glucocorticoids that have diffused through the cell membrane must gain access to the GR to exert their effects. As mentioned above, this step is interrupted in kidney cells by means of 11β-HSD, rendering these cells unresponsive to physiological concentrations of glucocorticoids (91-93). However, 11β-HSD exists in at least two isoforms, 11β-HSD 1 and 11β-HSD 2, with 11β-HSD 1 being expressed also in nonmineralocorticoid target tissues (94). Its expression level and/or activity do not seem to be high enough to completely abrogate glucocorticoid effects (95, 96). In tissues other than the kidney, this enzyme may, therefore, modulate glucocorticoid sensitivity. A second mechanism by which cells could regulate the intracellular level of glucocorticoids was recently reported by Kralli et al. (97). These authors identified a yeast transporter protein [ligand effect modulator (LEM1)] that actively and specifically exports glucocorticoids from the
Table 1. Factors and conditions that can alter glucocorticoid sensitivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Factor/determinant</th>
<th>Effect</th>
<th>Glucocorticoid sensitivity</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular hormone availability</td>
<td>11β-HSD1, 11β-HSD2 LEM1 (yeast)</td>
<td>Inactivates cortisol</td>
<td>↓</td>
<td>91-96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exports glucocorticoids</td>
<td>↓</td>
<td>97</td>
</tr>
<tr>
<td>GR expression level</td>
<td>Microdeletion of one GR allele Glucocorticoids</td>
<td>Reduces GR expr. level by 50%</td>
<td>↓</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce GR expression level</td>
<td>↓</td>
<td>102-106, 108, 111</td>
</tr>
<tr>
<td>Hormone binding affinity</td>
<td>FK506</td>
<td>Increases binding affinity</td>
<td>↑</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>Mutations in GR coding region ATP depletion</td>
<td>Can enhance or reduce affinity</td>
<td>↑ ↓</td>
<td>18, 114-116</td>
</tr>
<tr>
<td></td>
<td>hsp90 depletion (yeast) hsp90 mutations</td>
<td>Inhibition of GR/hsp complex formation?</td>
<td>↓</td>
<td>33, 46, 117</td>
</tr>
<tr>
<td></td>
<td>IL-2 + IL-4</td>
<td>Keeps GR in a low affinity state</td>
<td>↓</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of GR/hsp complex formation</td>
<td>↓</td>
<td>44, 121, 122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces hormone binding affinity of the nuclear GR fraction</td>
<td>↓</td>
<td>124, 125</td>
</tr>
<tr>
<td>Hormone-induced dissociation from hsp complex</td>
<td>&quot;Stimulator&quot;</td>
<td>Promotes GR/hsp dissociation</td>
<td>↑</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>&quot;Modulator&quot;</td>
<td>Stabilizes GR/hsp complex</td>
<td>↓</td>
<td>90, 131-133</td>
</tr>
<tr>
<td>GR phosphorylation</td>
<td>Neoplastic transformation Kinases/phosphatase inhibitors</td>
<td>Hyperphosphorylation of GR?</td>
<td>↑</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyperphosphorylation of GR?</td>
<td>↑ ?</td>
<td>46, 142</td>
</tr>
<tr>
<td>Nuclear translocation</td>
<td>Heat shock FK 506, cyclosporin A</td>
<td>Promotes nuclear translocation</td>
<td>↑</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May promote nuclear translocation</td>
<td>↑</td>
<td>146-148</td>
</tr>
<tr>
<td>DNA/GRE binding</td>
<td>ASTP</td>
<td>Increases GR binding to chromatin</td>
<td>↑</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>&quot;0.7-3 kDa, 5 kDa, and 72 kDa nuclear factors&quot; SWI/SNF family members</td>
<td>Increase GR binding to DNA</td>
<td>↑</td>
<td>150, 153, 154</td>
</tr>
<tr>
<td></td>
<td>Protein kinase A activation</td>
<td>May enhance accessibility of chromatin for GR</td>
<td>↑</td>
<td>156, 158</td>
</tr>
<tr>
<td></td>
<td>37 kDa &quot;translocation inhibitor&quot; Pyridoxal phosphate (vit. B6)</td>
<td>Increases DNA binding affinity of GR</td>
<td>↑</td>
<td>159, 161</td>
</tr>
<tr>
<td>Interaction with the basal transcription machinery and/or other transcription factors</td>
<td>&quot;Synergistic&quot; transcription factors (AP-1, NF-1, CCAAT and CAACC box binding factors, SP-1, Oct-1, CREB, HNFs, COUP-TF, HIV-1 vpr, thyroid hormone receptor, GR, &quot;GME binding factor&quot;)</td>
<td>Enhance GR-mediated transcription</td>
<td>↑</td>
<td>64, 167-181, 202</td>
</tr>
<tr>
<td></td>
<td>Dominant negative inhibitors of the GR (AP-1, RelA/p65, calsertulin, Spi-1/Pu.1, HIV-1 vpr, other steroid receptors, progesterone receptor-A, GRβ RU486</td>
<td>Inhibit GR-mediated transcription</td>
<td>↓</td>
<td>57, 61-66, 71-77, 84, 85, 182, 183, 185, 186, 188, 189</td>
</tr>
<tr>
<td></td>
<td>RU486-bound GR does not interact properly with basal transcription machinery; inhibits GR-induced transactivation; inhibits or stimulates GR-induced transrepression</td>
<td></td>
<td>↓</td>
<td>57, 63, 76, 77, 126-129</td>
</tr>
</tbody>
</table>

All factors are listed with respect to the parameter that they are most likely to interfere with.

cell. They speculated that potential mammalian homologs of this protein might have an important role in regulating intracellular hormone availability and, thus, glucocorticoid sensitivity.

B. GR expression level

It is well established that hormone-binding capacity, i.e. the level of cellular GR expression, is closely correlated with the magnitude of the GR-mediated response (98). This has been
demonstrated in vivo in transgenic mice expressing GR antisense RNA. In tissues where the transgene is expressed, the level of GR is reduced, leading to signs of glucocorticoid resistance (see below) (99). Our laboratory previously described a human kindred with a microdeletion and nonexpression of one of the two GR alleles. Affected members of this family had 50% of normal receptors and compensated glucocorticoid resistance (100).

The expression level of the GR varies in a tissue-specific manner, with the thymus probably expressing one of the highest numbers of receptors per cell (101). It is not known whether this tissue-specific expression pattern is subject to major regulatory variations. Among the factors that do alter the level of GR expression, glucocorticoids themselves appear to be the most potent regulators and have been shown to cause down-regulation of the receptor in many cell lines and in tissues or cells from intact animals and healthy human subjects (102-105). It is conceivable that the negative effect of glucocorticoids on GR expression represents a short-loop feedback mechanism protecting tissues from excessive glucocorticoid levels in hypercorticalemic states. At least three mechanisms may contribute to glucocorticoid-mediated down-regulation of the GR. At the transcriptional level, glucocorticoids seem to inhibit GR mRNA generation by interfering with AP-1- and/or AP-2-mediated transcriptional activation of the GR gene (106, 107). It was also shown that the activated GR can bind to sites within the coding DNA and/or mRNA (104, 108) rather than within the GR promoter, which lacks consensus GREs (107, 109, 110). This led to down-regulation of GR mRNA, either by inhibition of transcription and/or by reduction of mRNA stability and translatability (104, 108). Finally, the half-life of the GR protein may be decreased in the presence of glucocorticoids (111). In addition to glucocorticoids, the effects of other steroids (112) and neurotransmitters (113) add to the complexity of GR transcription and expression. This may be exemplified by the described inhibitory effects of estrogens on GR expression in the pituitary (112).

C. Hormone-binding affinity

The potency of the GR as a transcriptional regulator also correlates with its hormone-binding affinity, which is determined by a number of different factors. Point mutations within the region coding for the GR ligand-binding domain causing substitutions of amino acids can lead to altered hormone-binding affinity of the receptor. Almost all such modifications of the GR ligand-binding domain are known to cause reduction of either glucocorticoid-binding affinity (114) or ligand/receptor complex stability (115) and are associated with various clinical syndromes of glucocorticoid hyposensitivity (see below). GRs with increased steroid affinity and activity have been artificially constructed (18, 116) but have not yet been shown to occur in vivo.

One important function of the GR/hsp complex is to maintain the receptor in a ligand-friendly high affinity conformation (20, 21, 29-32, 43, 44). Proper assembly and folding of this complex are, therefore, essential for normal hormone/receptor interaction. First, the unliganded GR must associate with the preformed hsp complex. Since this is an energy-dependent process, artificially ATP-depleted cells contain only so-called “null” receptors that appear to be unable to associate with the hsp complex and to bind hormone (33, 46). These results are consistent with previous findings in rat thymus cells showing that hormone-binding affinity falls and rises with cellular ATP levels (117). Second, the expression level of hsp90 can influence GR function. In support of this notion, Picard (118) demonstrated severely impaired GR function in yeast mutants expressing low levels of hsp90. High levels of hsp90 are found in target tissues that are particularly glucocorticoid sensitive, e.g. in the thymus (119). More recently, increased hsp90 mRNA levels were demonstrated in fibroblasts from patients with GR defects (120). This may indicate the organism’s effort to compensate for the impaired receptor function. However, a direct link between increased hsp90 levels and GR function has not been established as yet. Finally, the structural integrity of the hsp90 molecule may also be important in establishing a proper conformation of the GR ligand-binding domain. Thus, artificial hsp90 deletion mutants that can still associate with the GR fail to maintain the steroid-binding properties of the receptor (121, 122). More recently, hsp90 mutants were shown to reduce the transcriptional activity of GR in yeast (44). Despite the potential pathophysiological significance of naturally occurring hsp90 mutants, such abnormalities have not yet been demonstrated in vivo. The roles of hsp70 and hsp56 in GR function are even less well understood. However, it was recently demonstrated that FK506, an immunosuppressant and a ligand for hsp56, could stabilize GR/hsp complex in vitro, thus increasing hormone-binding affinity ~3-fold (123).

Factors that do not normally participate in the GR/hsp complex may also affect its conformation and thereby interfere with hormone-binding affinity. Kam and co-workers (124, 125) recently demonstrated a ~6-fold reduction of steroid-binding affinity in human lymphocytes treated with a combination of interleukin-2 and interleukin-4. Since only the nuclear fraction of GR was affected by this change in affinity, the interleukins most likely induced a nuclear factor which then interacted with the activated GR, or, alternatively, altered some enzymatic activity, influencing the receptor’s affinity for the ligand. The important role of the glucocorticoid-signaling pathway in the control of the immune response make the identification and characterization of such a factor a desirable goal of future research.

D. Hormone-induced conformational change and dissociation from the hsp complex

The induction of a specific conformational change in the GR molecule is believed to be the most important consequence of ligand binding (8). The exact nature of this event is still awaiting experimental clarification by magnetic resonance analysis or x-ray crystallography. The concept, however, became widely accepted when the glucocorticoid antagonist RU 486 was introduced and shown to bind the receptor without evoking a glucocorticoid-like effect (for a review see Refs. 126-128). Binding of RU 486 to the GR obviously induces a conformational change which is distinct from that induced by the agonist. The RU 486/GR complex is still able
to dissociate from the hsp heterooligomer and bind to GRE sequences, yet it fails to interact properly with the basal transcription machinery and/or with other transactivators (127). Interestingly, the RU 486-bound receptor can mediate transrepression of AP-1 and NF-kB-p65 in some cases (63, 77, 129), whereas it has antagonistic effects in others (57, 76). These seemingly contradictory results indicate cell type- and/or promoter-specific effects of RU 486-GR downstream from the hsp dissociation step.

The ligand-induced conformational change causes the GR to dissociate from the hsp complex. Numerous laboratories have reported the existence of endogenous regulators that either stimulate or inhibit this process. Schmidt et al. (130) described a heat-stable protein referred to as “stimulator” that can enhance the efficiency of glucocorticoid-induced GR/hsp dissociation (130). The cDNA and/or amino acid sequence of this protein has not yet been determined. It is also not known whether its expression varies intra- or interindividually. Modulator, a 1500-kDa phosphoglyceride, is the inhibitory counterpart to the “stimulator” protein (131, 132). The authors proposed a model in which the modulator binds both GR and hsp90, stabilizes the inactive complex, and, thus, negatively interferes with GR-mediated transactivation (90, 133).

E. GR phosphorylation

Concomitantly with or shortly after dissociation from the hsp complex, the basally phosphorylated GR becomes hyperphosphorylated, mostly on serine residues (33, 46, 47). While phosphorylation may be important for progesterone hsp complex, the basally phosphorylated GR becomes hyperphosphorylated, mostly on serine residues (33, 46, 47). From the normal to the neoplastic stage. The intracellular GR inhibition could not be established when diverse phosphatases, phase of the cell cycle (138, 139), when hormone treatment from the hsp dissociation step.

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Concomitantly with or shortly after dissociation from the hsp complex, the basally phosphorylated GR becomes hyperphosphorylated, mostly on serine residues (33, 46, 47). While phosphorylation may be important for progesterone receptor function (134) and may even lead to its activation (90, 133). Furthermore, a consistent pattern of enhancement or inhibition (90, 133).

F. Nuclear translocation

Depending on the predominant direction of GR trafficking, the receptor may appear either as a nuclear or as a cytoplasmic protein (37-42). Differences between cell types in the subcellular distribution of GR may be due to a different hsp content and/or status of receptor phosphorylation (21, 35, 36, 143, 144). Nuclear translocation seems to be accelerated in response to hormone, which may unmask a nuclear localization signal (40). It has been demonstrated that heat shock also enhances GR-mediated transcription by further stimulating the hormone-induced nuclear transfer of the receptor (145). Treatment of cells with the hsp56-binding drug FK506 not only increased the hormone-binding affinity of the GR (see above) but also stimulated its nuclear translocation, resulting in enhancement of glucocorticoid-mediated transcription at low, but not at maximum, hormone concentrations (146, 147). Similar results were obtained with the cyclophilin-binding agent cyclosporin A (148). The immunosuppressive activity of FK506/immunophilin and cyclosporin A/cyclophilin complexes was previously shown to be due to inhibition of the Ca"+/calmodulin-dependent phosphatase calcineurin, which plays an important role in the activation of interleukin-2 gene expression (149). The presence of two strongly immunosuppressive mediators, GR and immunophilin, within the same protein complex is intriguing and may have other as yet unidentified consequences.

G. DNA/GRE binding

Within the nucleus, the activated GR becomes associated with DNA/chromatin and, finally, with specific GREs or other DNA sequences.

Nuclear extracts from various rat and human cell lines can enhance the binding of activated GR complexes to naked DNA and/or chromatin in vitro (150, 151). The search for distinct factors present in these extracts led to the purification of several proteins that assist in DNA binding of the GR. The most well characterized is a low molecular radius (700-3000 Da) factor originally isolated from rat hepatoma cells. This factor is also present in nuclear extracts from human HeLa and MCF-7 cells and is required for approximately 40% of the activated GR complexes to bind to calf thymus DNA in vitro (150). Why some receptors do and others do not require this factor to bind to DNA is not known. A second factor termed ASTP (ATP-stimulated glucocorticoid-receptor translocation promoter) is present in rat liver cells and has now been cloned and sequenced (152). ASTP is a 93-kDa histone-binding protein that increases GR binding to nuclei or chromatin in the presence of ATP but does not affect GR binding to naked DNA. Other, less well characterized proteins involved in DNA binding of GR include a 72-kDa protein and various species of approximately 5 kDa (153,154). None of the above proteins has yet been analyzed in terms of their expression pattern and functional relevance in vivo.

More recently, several human homologs of yeast SWI/SNF and brm proteins have been cloned (155-157) and shown to potentiate GR-mediated transactivation (156). These proteins are helicases and may therefore also facilitate binding of the GR to nucleosomal DNA. Overexpression of the human brm homolog enhanced GR-mediated transactivation only in cell lines that did not contain endogenous brm and had no effect in cell lines expressing this gene (156). BRG1,
another human protein belonging to this family, was shown to be expressed in multiple human tissues with an exclusively nuclear distribution (155). The members of this family appear to be highly conserved throughout evolution and may, therefore, play an important role in steroid receptor function. Interestingly, the enhancing effects of SWI/SNF proteins on GR-mediated transactivation depend on the presence of the retinoblastoma gene product (158).

Stimulation of the cAMP/protein kinase A (PKA) pathway has been reported to augment transcription in response to glucocorticoids (159, 160). This effect was still demonstrated in F9 embryonal carcinoma cells (which lack endogenous cAMP response element-binding protein) and was associated with a dramatic increase in GR DNA-binding affinity (159). Furthermore, footprinting studies in hepatoma cells revealed that binding of GR to the glucocorticoid-responsive unit within the rat tyrosine aminotransferase promoter required PKA activation (161). The effect of PKA does not involve changes in the phosphorylation pattern of GR and is probably mediated by phosphorylation of factors interacting with the receptor (162).

It appears that the ability of the hormone-activated GR to bind to DNA can also be negatively regulated. In support of this concept, Dahmer et al. (163) reported the purification of a 37-kDa “translocation inhibitor” from rat liver that inhibited the binding of activated GR to rat nuclei. No further reports have since appeared concerning this inhibitor. Pyridoxal phosphate, the active form of vitamin B6, has also been shown to inhibit DNA binding of the activated GR leading to ~50% inhibition of glucocorticoid-induced transactivation (164, 165). Conversely, treatment of cells with 4-deoxypyridoxin, an inhibitor of pyridoxal phosphate synthesis, enhances the transcriptional effects of glucocorticoids (164, 165). Intracellular pyridoxal phosphate levels vary profoundly among different tissues and cell types and may thus account for tissue-specific differences in glucocorticoid sensitivity (165).

H. Interaction with other nuclear factors

The last step of GR-mediated transactivation involves complex interactions of the receptor with components of the basal transcription machinery and/or with other transcriptional activators or repressors (50). Depending on the exact arrangement of the promoter under investigation, the presence of “third” factors, and the arrangement of the chromatin (64, 166), the same transactivators may interact in a synergistic or antagonistic manner with the GR (50). In simplified terms, four different promoter settings can be envisioned, which may be exemplified for the prototypical interaction between GR and AP-1. On promoters containing AP-1-binding sites but no GREs, AP-1-mediated activation is inhibited by GR in most cases (type 2 mechanism of glucocorticoid action, see above) (57, 61-66, 71-73). In contrast, GRE-regulated promoters that lack AP-1 sites are generally inhibited by AP-1 (57, 61, 62, 64, 65, 71-73). A promoter containing both GREs and AP-1-binding sites can be activated synergistically by GR and AP-1 (64, 167). These generalizations are by no means absolute, since cell type-specific expression of different combinations of AP-1 subunits (c-Jun, Jun-B, Jun-D, c-Fos, FosB, FosB2, Fra-1, Fra-2) and “third” factors may also determine the outcome of AP-1/GR interaction (64, 168). This has been most clearly demonstrated for the fourth possible promoter arrangement, the so-called “composite element,” which was described in the mouse proliferin gene promoter. In this case, a GRE and an AP-1-binding site overlap, forming a single element with new properties. On this element, the hormone-bound GR is inactive in the absence of c-Jun, stimulatory in the presence of c-Jun, and inhibitory in the presence of c-Jun and c-Fos (169).

The interaction of GR with other nuclear factors, even though less well characterized, is undoubtedly no less complex. The following distinction between coactivators and antagonists of the GR is, therefore, a simplification that reflects our poor understanding of these interactions.

Apart from AP-1, synergism with the GR has been demonstrated for the following DNA-binding proteins: NF-1 (170-172), CCAAT box-binding factor (171), CAACC box-binding factor (171, 173), SP-1 (171), Oct-1/OTF-1 (173, 174), CREB (175), the liver-specific transcription factors HNF3 and HNF4 (176, 177), the “orphan” nuclear receptor COUP-TF (176), the activated thyroid hormone receptor (178), and the GR homodimer itself, which can facilitate binding of another GR homodimer to adjacent GRE sites in a cooperative manner (Ref. 179, and references therein). In addition, Simons and co-workers (180, 181) reported the existence of a so-called glucocorticoid modulatory element (GME) upstream of the rat tyrosine aminotransferase gene which, in the presence of a putative transacting factor, enhances GR/GRE-mediated transcription.

Nuclear factors that interfere negatively with GR-mediated transactivation are referred to as dominant negative inhibitors. These molecules probably represent the most important endogenous regulators of glucocorticoid sensitivity and can, theoretically, act through the following mechanisms (182): steric hindrance of the GR by binding to DNA sequences overlapping a GRE, formation of inactive complexes with the GR in solution or on the DNA, competition with the GR for GRE-binding sites, or titration (“squelching”) of accessory factors necessary for the interaction of the GR with the basic transcription complex. AP-1 is the most prominent and best characterized dominant negative inhibitor of GR (see above). RelA, the p65 subunit of the composite transcription factor NF-kB, is also not only inhibited by GR in stimulating NF-kB-responsive genes (see above) but also antagonizes GR action on GRE-regulated promoters, probably through direct physical interaction (75-77). Calreticulin represents another example of a protein that establishes physical contact with the GR and, thus, inhibits its effects on gene transcription (183). Calreticulin is not a transcription factor but acts as a major Ca2+-binding (storage) protein in the lumen of the endoplasmic reticulum (184). Its unexplained presence in the cell nucleus and its previously reported ability to bind to a synthetic peptide resembling the DNA-binding domain of nuclear hormone receptors led Burns et al. (183) to investigate possible interactions with the GR. In experiments similar to those described above, they demonstrated inhibition of GR-mediated gene transcription in mouse fibroblasts cotransfected with a plasmid coding for calreticulin and a GRE-driven reporter plasmid (183).
Other factors that can act as dominant negative inhibitors of the hormone-activated GR on certain promoters include the Spi-1/Pu.1 oncoprotein (84), the human deficiency virus type 1 (HIV-1) vpr gene product (185), and other steroid receptors (85), with the progesterone receptor-A isoform being the most potent inhibitor (186). The exact mechanism of action has not been established for these factors.

No study mentioned above distinguished between the two different GR isoforms, termed GRα and GRβ, that are generated by alternative splicing of the human GR pre-mRNA (110, 187) (Fig. 1). Recent results obtained in our laboratory indicate, however, that the distinction between GRα and GRβ may be more relevant than previously thought (188). These two protein isoforms have the first 727 amino acids in common, and, thus, both contain the transactivation and the DNA-binding domains. GRβ differs from GRα only in its C terminus with replacement of the last 50 amino acids of the latter with a unique 15-amino acid sequence (110, 187). This difference renders GRβ unable to bind glucocorticoid hormones (187) and to be transcriptionally active (10, 114). In a recent study, we demonstrated that overexpression of GRβ could antagonize the effects of hormone-activated GRα on a glucocorticoid-responsive reporter gene (188). This dominant negative effect of GRβ on GRα-mediated gene transcription was dose-dependent with overexpression of GRβ leading to up to 90% reduction of reporter gene activity.

In contrast to the GR inhibitors mentioned above, GRβ bound specifically to GRE sequences in vitro (188). GRβ did not reduce total radiolabeled GRE-binding activity when coincubated with GRα, suggesting occupation of GRE target sites by suboptimally transactivating GRβ/GRβ homodimers or GRα/GRβ heterodimers to be the underlying mechanism of its inhibitory effect. The concept of GRα/GRβ heterodimerization is supported by more recent results from our laboratory, showing coimmunoprecipitation of GRα and GRβ by isoform-specific antibodies (189) (Fig. 3). GRβ mRNA (188) and protein (189) were shown to be expressed in many human tissues, suggesting that this isoform might be physiologically relevant. The existence of at least two human GR isoforms that apparently exert opposite effects renders the picture of GR-mediated transcription more complex. It demands careful reevaluation of previous, mostly nonligand-binding studies, that did not distinguish between the two receptor isoforms. It will be of particular interest to determine whether the other inhibitors of GR function also interact with GRβ and/or with each other. To further understand the mechanisms regulating glucocorticoid sensitivity in target tissues, it will be necessary to determine the relative amounts of all these molecules and whether their level of expression is subject to regulatory processes. This will also increase our understanding of many pathophysiological states that are potentially associated with tissue-specific and/or acquired glucocorticoid resistance or hypersensitivity.

V. Pathophysiological Consequences of Impaired Glucocorticoid Sensitivity

As discussed in the previous sections, each step in the chain of events leading to activation of GR can be subject to modulating influences that either decrease or increase the glucocorticoid sensitivity of the respective tissue. In the last section of this review, we will refer to animal and
human models with impaired glucocorticoid sensitivity and briefly discuss the (possible) underlying molecular defects.

A. Animal models

1. Transgenic mice. Several laboratories have now reported the generation of mice with generalized nonexpression or tissue-specific underexpression of GR. These studies have both confirmed previous assumptions and revealed new aspects about the physiological role of GR, especially during development.

Cole et al. (190) recently reported the effects of targeted disruption of the GR gene (GR−/− mice). As would be expected, these mice die within the first few hours after birth because of respiratory failure due to severe lung atelectasis (neonatal respiratory distress syndrome). Other symptoms of generalized nonresponsiveness to glucocorticoids are the lack of induction of key liver glucoconeogenic enzymes and elevated ACTH- and corticosterone levels indicative of an impaired negative feedback regulation of the HPA axis. The adrenal medulla of GR−/− mice lacked adrenergic chromaffin cells, whereas noradrenergic cells developed normally, suggesting that glucocorticoids are necessary for proliferation and/or survival of the adrenergic cell population.

Two groups have reported the effects of tissue-specific GR underexpression in mice bearing a GR antisense transgene. Partial inhibition of GR expression results in glucocorticoid hyposensitivity rather than nonresponsiveness in these animals. Mice expressing a GR antisense RNA under the control of the neurofilament gene promoter show reduced GR levels in the CNS and the pituitary and are particularly useful to study the feedback regulation within the HPA axis (99). Due to hypothalamic and pituitary glucocorticoid hyposensitivity, these mice have elevated ACTH and corticosterone levels, which cause cushingoid symptoms in the normally sensitive peripheral tissues. More recently, King et al. (191) described transgenic mice that expressed antisense transcripts to GR specifically in thymocytes. Unexpectedly, mice bearing this transgene showed a reduction in thymic size, primarily owing to a decrease in the number of mature T cells. While glucocorticoids were previously shown to cause partial inhibition of GR expression results in glucocorticoid hyposensitivity, these mice have elevated ACTH and corticosterone levels, which cause cushingoid symptoms in the normally sensitive peripheral tissues. More recently, King et al. (191) described transgenic mice that expressed antisense transcripts to GR specifically in thymocytes. Unexpectedly, mice bearing this transgene showed a reduction in thymic size, primarily owing to a decrease in the number of mature T cells. While glucocorticoids were previously shown to cause partial inhibition of GR expression in thymocytes, the predicted amino acid sequence of their GR is very similar to the human GRa and GRδ isoforms (see above) to analyze potential differences in the expression pattern of these isoforms in New World monkeys. Our results indicate that both isoforms are expressed in these animals, with the inhibitory β isoform being ~10-fold overexpressed compared with humans (199). An altered splicing pattern of the GR pre-mRNA may, therefore, contribute to the steroid resistance in these animals.

B. Human disease models

Impaired glucocorticoid sensitivity has also been described as the cause of, or as a contributing factor to, various pathological conditions in humans. We will briefly summarize the clinical conditions that have been shown or are theorized to involve abnormalities of the glucocorticoid/GR transduction system (Table 2).

1. Glucocorticoid hyposensitivity (glucocorticoid resistance). Clinical syndromes of glucocorticoid hyposensitivity (glucocorticoid resistance) are due to generalized or tissue-specific defects of the GR transduction system. Generalized glucocorticoid resistance is defined as hyposensitivity to cortisol in all tissues, including the hypothalamus and the pituitary (200). Due to the impaired negative feedback, ACTH and cortisol levels are elevated in this syndrome, compensating for the peripheral resistance. Our laboratory has identified a point mutation leading to the homozygous substitution of a single amino acid in the GR hormone-binding domain as the cause of familial glucocorticoid resistance in the first family reported with this condition (114). This mutation lowered the affinity of the GR for its cognate hormone approximately three times. In another family, we described a heterozygous microdeletion in an exon/intron splice site in one GR allele which led to a 50% reduction of GR expression in affected family members (100).

Acquired generalized glucocorticoid resistance is observed in a subgroup of patients with acquired immunodeficiency syndrome (201). In these patients, the concomitantly elevated cortisol levels are usually not sufficient to overcome the peripheral resistance, and symptoms of adrenal insufficiency are frequently observed. The molecular mechanisms leading to this type of glucocorticoid resistance are not...
Table 2. Clinical conditions associated with alterations in glucocorticoid sensitivity

<table>
<thead>
<tr>
<th>Type of glucocorticoid resistance/hypersensitivity</th>
<th>Disease/clinical syndrome</th>
<th>Underlying defect</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Generalized glucocorticoid resistance</strong></td>
<td>Familial or sporadic glucocorticoid resistance</td>
<td>Point mutations or microdeletions in GR gene, leading to reduced hormone binding activity or receptor expression; hap90 mutations? defective co-activators? excessive co-repressors?</td>
<td>44, 100, 114, 121, 122, 167-181, 200, 210</td>
</tr>
<tr>
<td></td>
<td>AIDS</td>
<td>Reduced hormone binding affinity of GR (cytokine-induced? viral protein-induced?)</td>
<td>124, 185, 201, 202</td>
</tr>
<tr>
<td><strong>Tissue-specific glucocorticoid resistance</strong></td>
<td>Steroid-resistant asthma type I (lymphocytes)</td>
<td>Reduced hormone binding affinity of GR (cytokine-induced?)</td>
<td>124, 125, 205</td>
</tr>
<tr>
<td></td>
<td>Steroid-resistant asthma type II (lymphocytes)</td>
<td>Altered direction of GR pre-mRNA splicing (underexpression of GRα/overexpression of GRβ)</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Rheumatoid arthritis (lymphocytes) and osteoarthritis (synovial chondrocytes)</td>
<td>Reduced expression of normal affinity GR (underexpression of GRα/overexpression of GRβ?)</td>
<td>203, 204</td>
</tr>
<tr>
<td></td>
<td>Glucocorticoid-resistant lymphoid tumors</td>
<td>Reduction of GR expression and/or somatic mutations generating an &quot;activation labile&quot; receptor; altered direction of GR pre-mRNA splicing?</td>
<td>115, 207</td>
</tr>
<tr>
<td><strong>Generalized glucocorticoid hypersensitivity</strong></td>
<td>&quot;Full scale&quot; generalized glucocorticoid hypersensitivity</td>
<td>Point mutations, leading to enhanced hormone binding and/or transactivational activity? defective dominant negative inhibitors? altered coactivators/corepressors?</td>
<td>18, 116, 182, 188, 189, 211</td>
</tr>
<tr>
<td><strong>Tissue-specific glucocorticoid hypersensitivity</strong></td>
<td>Cardiovascular system</td>
<td>Primary hypertension</td>
<td>RFLPs (abnormal GR gene structure?)</td>
</tr>
<tr>
<td></td>
<td>Visceral fat</td>
<td>Visceral obesity</td>
<td>RFLPs (abnormal GR gene structure?)</td>
</tr>
</tbody>
</table>

known. However, the vpr gene product of HIV-1 was recently shown to physically associate with GR (202) and to inhibit GR-mediated transactivation in HeLa cells (185).

Tissue-specific glucocorticoid resistance usually becomes clinically apparent, since it is not compensated for by increased cortisol levels. This type of resistance may result in pathophysiological, e.g. inflammatory, processes and/or may contribute to such processes by not allowing glucocorticoids to exert their physiological, e.g. antiinflammatory, effects; these processes include glucocorticoid-resistant asthma, rheumatoid arthritis, and osteoarthritis (124, 125, 203-206). Tissue-specific glucocorticoid resistance has also been observed in lymphoid tumor cells, which fail to respond to the lytic effects of glucocorticoids as the disease progresses (115, 207). Finally, glucocorticoid resistance can also affect ACTH-producing cells. This includes "central" glucocorticoid resistance, characterized by impaired negative feedback regulation and, thus, uninhibited ACTH production by pituitary adenomas (Cushing's disease) and ectopic ACTH-secreting tumors (208-210). The possible underlying causes for the different types of tissue-specific glucocorticoid resistance are listed in Table 2.

2. Glucocorticoid hypersensitivity. Clinical syndromes of glucocorticoid hypersensitivity have been less well studied. Full-scale generalized hypersensitivity is extremely rare. Iida et al. (211) described a patient affected with this syndrome who displayed cushingoid symptoms despite low plasma glucocorticoid levels. The molecular mechanisms underlying this defect have not been elucidated. Point mutations leading to a "super" GR have been artificially created and shown to enhance glucocorticoid-mediated transactivation in vitro (18, 116). Whether such point mutations are also responsible for in vivo glucocorticoid hypersensitivity remains to be shown. Alternatively, abnormally low expression of inhibitors of the GR pathway, such as the β-isofrom of the human GR, could cause tissues to become hypersensitive to the effects of glucocorticoids (188, 189).

A mild form of generalized glucocorticoid hypersensitivity may lead to abnormalities in systems that are more sensitive to glucocorticoids even under physiological conditions. The regulation of blood pressure and adipose tissue distribution represent such a system (212, 213). The higher frequency of particular restriction fragment length polymorphisms of the GR gene in patients with familial hypertension...
(214) or central obesity (215) may reflect an abnormality of the GR transduction system in these diseases. Finally, a third system that might be affected by tissue-specific glucocorticoid hypersensitivity is the CNS. Up to 70% of patients with chronic hypercortisolism present with depression of the atypical type, the most common cause of depression in the general population (216). Similarly, if the CNS targets were sensing excessive glucocorticoid effects, depression would be the expected result.

VI. Summary

Glucocorticoids play an essential role in maintaining basal and stress-related homeostasis, and lack of glucocorticoid action is incompatible with life in primates. Most known effects of glucocorticoids are mediated by the intracellular GR. The magnitude of a cell’s response to glucocorticoids depends both on the hormone level it is exposed to and on its glucocorticoid sensitivity, i.e. the efficiency of GR-mediated signal transduction. In this review, we have summarized the multiple endogenous and exogenous factors that have been shown to be involved in this signaling cascade and, thus, to alter glucocorticoid sensitivity.

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