Interaction of the Glucocorticoid Receptor and the Chicken Ovalbumin Upstream Promoter–Transcription Factor II (COUP-TFII)

Implications for the Actions of Glucocorticoids on Glucose, Lipoprotein, and Xenobiotic Metabolism

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ABSTRACT: Glucocorticoids exert their extremely diverse effects on numerous biologic activities of humans via only one protein module, the glucocorticoid receptor (GR). The GR binds to the glucocorticoid response elements located in the promoter region of target genes and regulates their transcriptional activity. In addition, GR associates with other transcription factors through direct protein–protein interactions and mutually represses or stimulates each other's transcriptional activities. The latter activity of GR may be more important than the former one, granted that mice harboring a mutant GR, which is active in terms of protein–protein interactions but inactive in terms of transactivation via DNA, survive and procreate, in contrast to mice with a deletion of the entire GR gene that die immediately after birth. We recently found that GR physically interacts with the chicken ovalbumin upstream promoter–transcription factor II (COUP-TFII), which plays a critical role in the metabolism of glucose, cholesterol, and xenobiotics, as well as in the development of the central nervous system in fetus. GR stimulates COUP-TFII–induced transactivation by attracting cofactors via its activation function-1, while COUP-TFII represses the GR-governed transcriptional activity by tethering corepressors, such as the silencing mediator for retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressors (NCoRs) via its C-terminal domain. Their mutual interaction may play an important role in gluconeogenesis, lipoprotein metabolism, and enzymatic clearance of clinically important compounds and bioactive chemicals, by regulating their rate-limiting enzymes and molecules, including the phosphoenolpyruvate carboxykinase (PEPCK), the cytochrome P450 CYP3A and CYP7A, and several apolipoproteins. It appears that glucocorticoids exert their intermediary effects partly via physical interaction with COUP-TFII.

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INTRODUCTION

Glucocorticoids are steroid hormones secreted by the adrenal glands, important for maintenance of basal and stress-related homeostasis.[1] They regulate a variety of biologic processes and exert a profound influence on many physiologic functions, such as the energy catabolism, by regulating the metabolic rate of glucose, fatty acids, and cholesterol, and the clearance of bioactive compounds in the liver.[2,3] Glucocorticoids are also used as potent immunosuppressive agents in the management of many inflammatory, autoimmune, and lymphoproliferative diseases, while they produce many adverse effects, such as glucose intolerance/overt diabetes mellitus, and dyslipidemia, due to their effects on the corresponding metabolic pathways.[4]

Glucocorticoids exert their diverse effects through the glucocorticoid receptor (GR).[1] GR belongs to the nuclear receptor superfamily and functions as a ligand-inducible transcription factor.[5] Ligand-activated GR binds the glucocorticoid response elements (GREs) located in the enhancer region of the glucocorticoid-responsive genes and alters their transcriptional activity.[6] More importantly, GR affects other signal transduction cascades through mutual protein–protein interactions with other transcription factors, which act downstream of these signaling events.[7] Ligand-activated GR physically interacts with such transcription factors and suppresses/enhances their transcriptional activity with several mechanisms.[8–10] This activity of GR may be more important than the GRE-mediated one, granted that mice harboring a mutant GRα, which is active in terms of protein–protein interactions but inactive in terms of transactivation via DNA, survive and procreate, in contrast to mice with a deletion of the entire GR gene that die immediately after birth from severe respiratory distress syndrome.[11,12] Thus, protein–protein interaction and subsequent modulation of other signaling pathways may be critical to sustain extremely diverse effects of glucocorticoids on broad arrays of tissues/organs via the single receptor molecule, GR. Furthermore, this activity may be particularly important in suppressing the immune function and inflammation by glucocorticoids.[11,13] A substantial part of the effects of glucocorticoids on the immune system may be explained by the interaction between GR and nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and probably signal transducers and activators of transcription (STATs).[8,13–15] GR is also known to influence the transcriptional activity of other transcription factors, such as CREB, CAAT/enhancer-binding protein (C/EBP), Nur77, p53, hepatocyte nuclear factor (HNF)-6, GATA-1, Oct-1 and-2, and nuclear factor (NF)-1.[16–23]

We have recently found that GR interacts with one of the orphan nuclear receptors, the chicken ovalbumin upstream promoter–transcription factor II, and mutually affects each other's transcriptional activity.[24] This orphan nuclear receptor plays an important role in glucose, fatty acid, cholesterol, and xenobiotic metabolism, as well as embryonic development.[25] In this review, we describe details of our recent results of physical/functional interaction between GR and COUP-TFII. We speculate that their interaction may play a critical role in the glucocorticoid action on the intermediary metabolism.

STRUCTURE AND ACTION OF GR

The human GR (hGR), a single polypeptide chain of 777 amino acid residues, is a member of the steroid/sterol/thyroid/retinoid/orphan receptor superfamily of nuclear transactivating factors, with over 150 members currently cloned and characterized across species.[26] Together with the mineralocorticoid, progesterone, estrogen, and androgen receptors, GR forms the steroid receptor subfamily. Steroid receptors display a modular structure comprising five to six regions (A–F), with the N-terminal A/B region, also called the immunogenic domain, and the C and E regions corresponding to the DNA-binding (DBD) and ligand-binding (LBD) domains, respectively (FIG. 1).[27] The hGR cDNA was isolated by expression cloning in 1985.[28] The gene of the hGR consists of 9 exons and is located on chromosome 5.[27] It encodes two 3′ splicing variants, GRα and β, from alternative use of a different

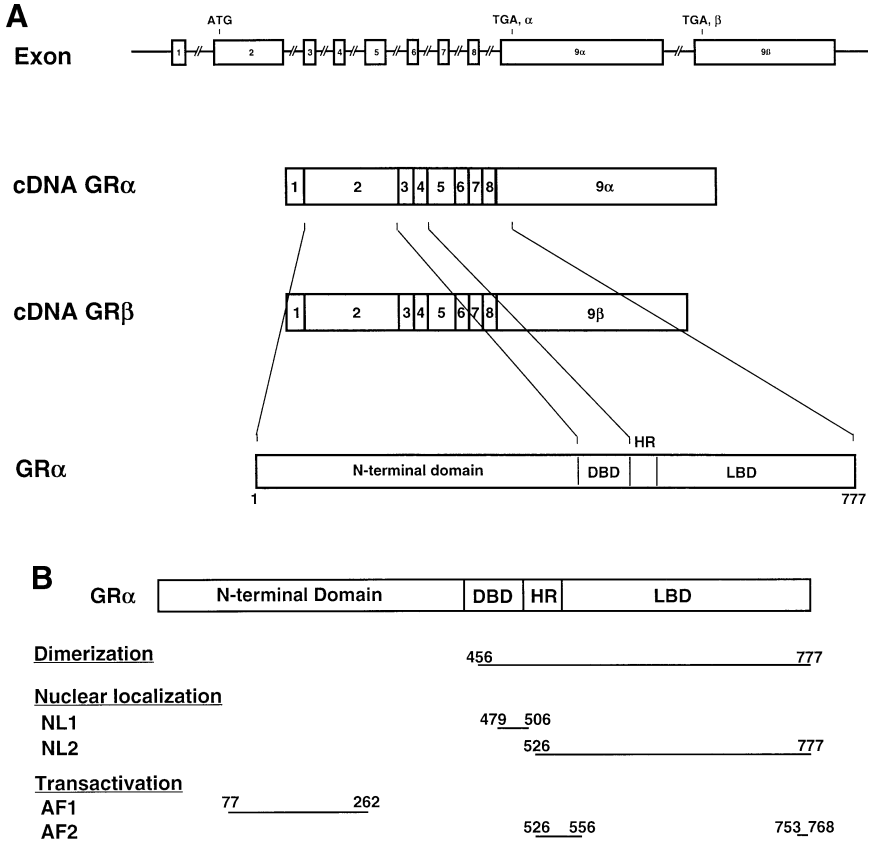

FIGURE 1. (**A** and **B**) Genomic structure and domains of linearized GRα and GRβ molecules. GR, glucocorticoid receptor; HR, hinge region; DBD, DNA-binding domain; LBD, ligand-binding domain; NL, nuclear localization signal; AF-1 and -2, activation function 1 and 2. Unique portions of GRα and GRβ are indicated in grey color.

terminal exon 9α and β, respectively. The hGRα encodes a 777 amino acid protein, while the hGRβ contains 742 amino acids. The first 727 amino acids from the N-terminus are identical in both isoforms. hGRα possesses an additional 50 amino acids, while hGRβ encodes an additional 15 nonhomologous amino acids in its C-terminus. The molecular weights of each receptor isoforms are 97 and 94 kDa, respectively. hGRα is the classic GR that binds to glucocorticoids and transactivates or transrepresses glucocorticoid-responsive promoters. On the other hand, hGRβ does not bind to glucocorticoids and functions as a weak transdominant inhibitor of GRα transactivation; however, its physiologic and pathologic roles are not well known.[29]

The N-terminal domain of GR contains one of the transactivation domains of the GR, activation function (AF) 1, located at amino acids 77–262.[30,31] The activity of this transactivation domain is ligand-independent. Its core activation domain is localized at amino acids 187–244.[31] The DBD of the hGR corresponds to amino acids 420–480, and contains two zinc finger motifs through which it binds to specific sequences of DNA, the glucocorticoid response elements (GREs).[32] The LBD of the GR is comprises 12 helical structures and changes its tertiary configuration in following LBD, thus creating the AF2 surface, which acts as a lignad-induced transactivation domain of the GR.[33,34]

The GR is located primarily in the cytoplasm of cells, as part of a heterooligomeric complex with heat-shock proteins (hsp) 90, 70, and 50 and, possibly, other proteins through interaction via the LBD.[35–38] Upon ligand binding, the GR dissociates from the hsp, homodimerizes, and translocates into the nucleus, where it binds to the hormone response elements in the promoter regions of target genes and/or to other transcription factors.[7] The GR contains two nuclear translocation signals (NL), NL1 and NL2; NL1 contains a classic basic-type nuclear localization signal (NLS) structure that overlaps with and extends along the C-terminal from the DNA binding domain of the GR.[39] The function of NL1 is dependent on the importin α, a component of a nuclear translocation system that is energy-dependent protein import machinery through the nuclear pore. NL2, with an uncharacterized motif, overlaps with almost the entire LBD.

GR exerts its transcriptional activity on its responsive promoters via binding to its recognition sequence, GREs.[6] The GRE-bound GR stimulates the transcription rates of responsive genes by facilitating the formation of the transcription initiation complex, including the RNA polymerase II and its ancillary factors.[6] In addition to these molecules, GR, via its AF 1 and 2 domains, first attracts several proteins and protein complexes, which may bridge the DNA-bound GR and the transcription initiation complex as well as modulate the tightly assembled chromatin structure surrounding the promoter region.[40]

INTERACTION OF GR WITH COUP-TFII

COUP-TFII

The chicken ovalbumin upstream promoter–transcription factor II (COUP-TFII) is a protein of 414 amino acids, and its gene is located on chromosome 15.[41] It is an "orphan receptor" (i.e., its native ligand is unknown) and a member of the nuclear steroid/thyroid hormone receptor superfamily.[25] COUP-TFII, along with its closely

related protein COUP-TFI in humans, has been characterized as a family of negative coregulators of gene transcription.[25] COUP-TFII binds to the responsive elements with several different sequences, including AGGTCA direct repeats with different spacings. COUP-TFII most strongly interacts with a motif with one base-pair spacing between AGGTCA sequences (called DR1).[25,42,43] Since several other receptors, such as the vitamin D receptor (VDR), thyroid hormone receptor (TR), retinoic acid receptor (RAR), the retinoid X receptor (RXR), the peroxisome proliferators–activated receptors (PPARs), and the orphan nuclear receptor, hepatocyte nuclear factor-4 (HNF-4), bind direct repeats of AGGTCA and use these sequences as their responsive elements, COUP-TFs exert their negative regulatory function by competing for the common response element.[25] COUP-TFs can also actively silence the basal transcription machinery of their target promoters by attracting corepressor molecules, such as the silencing mediator for retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressors (NCoRs), and histone diacetylases through direct interaction with its C-terminal 35 amino acids.[25,44] In addition, COUP-TFII, through the same responsive sequences, can stimulate the transcriptional activity of several target genes that catalyze the glucose and cholesterol metabolism.[25,45–47]

Physical Interaction between GR and COUP-TFII

To identify molecules that interact with GR, we performed a LexA-based yeast two-hybrid screening assay using GRα and β LBDs as baits in the human Jarkat cDNA library.[24] We found that both GRα and β LBDs interact with two independent clones that contain the human COUP-TFII coding sequence. This interaction was confirmed by yeast mating and GST pull-down assays. In the latter, we used bacterially produced and purified GST-fused COUP-TFII, together with *in vitro* translated and [^{35}S]-labeled GRα and β. Both GRα and β bound to GST-COUP-TFII in a ligand-independent fashion. Using a set of GST-COUP-TFII fusion fragments, we found that GRα and β bound to COUP-TFII in a region enclosed between amino acids 75 and 163, a portion corresponding to the DBD of this transcription factor[24] (FIG. 2). Similarly, using bacterially produced and purified GRα and β with [^{35}S]-labeled COUP-TFII, we found that COUP-TFII bound to both GRα and β in a region enclosed by amino acids 490 to 502 that is located in the "hinge region" of the receptors[24] (FIG. 2). To further characterize this interaction, we used several GRα mutants, with point mutations in the region 490–502, in the same GST pull-down assay. GRαQ501A and Q502A, in which aspartic acid was replaced with alanine at positions 501 or 502, respectively, were unable to bind COUP-TFII. GRαA458T, which has a threonine instead of an alanine at position 458 and, therefore, is unable to dimerize, was also able to interact with COUP-TFII, indicating that GRα interacts with COUP-TFII as a monomer.[24]

We next performed chromatin immunoprecipitation assays to study the *in vivo* association of GR and COUP-TFII in the context of a natural chromatin-bound promoter. The rat cholesterol 7α-hydroxylase (CYP7A) promoter, which is known to respond to COUP-TFII through two sets of the COUP-TFII–responsive elements, was used in this experiment.[45,46] In H4IIE rat hepatoma cells, GRα was successfully coprecipitated with this promoter in a dexamethasone (DEX)-dependent fashion.[24] In contrast, GRβ was not coprecipitated with this promoter, despite the *in vitro* bind-

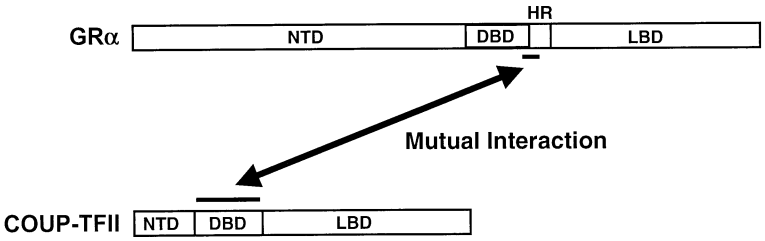

FIGURE 2. Linearized GRα and COUP-TFII and their mutual interaction domains (indicated with *bold line*). NTD, N-terminal domain; DBD, DNA-binding domain; HR, hinge region; LBD, ligand-binding domain.

ing to COUP-TFII. GR and COUP-TFII also formed complexes on the endogenous GREs of the tyrosine aminotransferase promoter. The results were also confirmed using regular immunoprecipitation assays. These results clearly indicate that GR and COUP-TFII form complexes on their responsive promoters *in vivo*.

Reciprocal Transcriptional Modulation of GR and COUP-TFII

The functional aspects of the physical interaction between GR and COUP-TFII were studied in a transient transfection assay using their respective responsive promoters, the MMTV promoter, containing four GREs, and a short fragment of the rat CYP7A promoter that contains two COUP-TFII responsive elements.[45,46] The CYP7A is known as the cholesterol 7α-hydroxylase gene and its product catalyzes the first and rate-limiting step of cholesterol to bile acid in the liver, thus playing a pivotal role in the elimination of cholesterol from the body and maintenance of the cholesterol homeostasis.[48] COUP-TFII suppressed the DEX-stimulated, GR-induced transactivation of the MMTV promoter in a dose-dependent fashion.[24] In contrast, overexpression of GRα enhanced COUP-TFII–stimulated CYP7A promoter activity in a dose-dependent manner. GRβ did not influence COUP-TFII–induced transactivation on the same promoter.

We next examined the mechanisms of the functional interaction between GRα and COUP-TFII. GRα contains two transactivation domains, AF-1 and AF-2, whose activities are supported by a specific interaction with several coactivators and chromatin modulators.[40] We examined the contribution of each of these domains on GR-induced enhancement of COUP-TFII transactivation. GRα(Δ77–261), which is devoid of the AF-1 transactivation domain, completely lost its enhancing effect on the COUP-TFII–induced transcriptional activity of the CYP7A promoter in a glucocorticoid-dependent fashion, while GRα(1–550), which has the AF-1 but not the AF-2 domain, still enhanced COUP-TFII–induced transactivation in a glucocorticoid-independent fashion.[24] These results suggest that the AF-1 domain of GR plays an important role in GR-induced enhancement of COUP-TFII transactivation (FIG. 3).

COUP-TFII is known to actively suppress the transcriptional activity of several promoters by attracting the corepressor SMRT through direct binding via its last 35 amino acids.[44] We thus examined SMRT on COUP-TFII–induced suppression of GR-induced transactivation. Coexpression of SMRT synergistically enhanced the

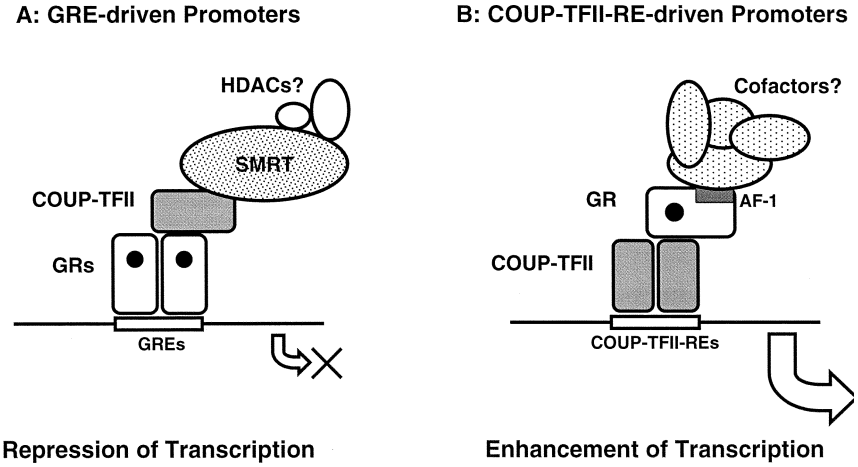

FIGURE 3. Functional interaction of GR and COUP-TFII on their responsive promoters. GR enhances COUP-TFII–induced transactivation through its AF-1 domain probably by attracting AF-1–associating cofactors, while COUP-TFII suppresses GR-induced transactivation in part by attracting SMRT and other corepressors. GRs, glucocorticoid receptors; COUP-TFII, chicken ovalbumin upstream promoter–transcription factor II; GRE, glucocorticoid-responsive element; COUP-TFII-RE, COUP-TFII–responsive element; AF-1, activation function-1; SMRT, silencing mediator for retinoid and thyroid hormone receptors; HDACs, histone deacetylases.

suppressive effect of COUP-TFII on GR transactivation. COUP-TFII(1–380), which is devoid of the SMRT-binding domain, significantly lost the suppressive effect and SMRT did not effectively cooperate with this mutant COUP-TFII.[24] Therefore, COUP-TFII, at least in part, suppressed the GR-induced transcriptional activity by attracting SMRT to the glucocorticoid-responsive promoters. Simplified models of functional interaction of GR and COUP-TFII are shown in FIGURE 3.

Glucose Metabolism: Modulation of Phosphoenolpyruvate Carboxykinase Expression

Glucocorticoids modulate diverse metabolic activities in the liver, adipose tissue, and connective tissues, such as those of carbohydrate, lipid, and amino acid/protein.[1] Changes induced by pharmachologic or stressed-induced doses of glucocorticoids increase circulating concentrations of glucose, free fatty acids, amino acids, and glycerin by facilitating catabolism of fat, protein, and glycogen in peripheral tissues.[1] The liver takes up these compounds and uses them as substrates for gluconeogenesis and subsequent glycogen synthesis. Glucocorticoids influence glucose metabolism by acting at many steps of its metabolic pathways.[1,49] Glucocorticoids increase glycogen synthesis by activating glucokinase, glycogen synthase, and inactivation of glycogen phosphorylase, which helps catalyze glycogen.[49,50] Glucocorticoids also decrease the insulin-stimulated glucose uptake and utilization by

peripheral tissues.[51–53] Furthermore, glucocorticoids directly act on the β cells to reduce their insulin secretion.[54–56]

In addition to the above-described effects on glucose metabolism, glucocorticoids also increase hepatic gluconeogenesis, which converts amino acids, fatty acid, and glycerin supplied from peripheral tissues to glucose.[49] Glucocorticoids stimulate this metabolic activity by increasing the production of its rate-limiting enzyme, phosphoenol-pyruvate carboxykinase (PEPCK), by direct stimulation of its promoter activity via GR.[49,57,58]

GR is reported to be associated with the PEPCK promoter through its two GRE-like motifs, termed GR unit 1 and 2 (GRU1 and 2).[47,59–61] In addition, at least three accessory factor-binding elements, called AF-1, 2, and 3, are required for the activation of the PEPCK promoter by GR.[59,60] The factors that bind to these accessory elements include HNF-4/COUP-TFII, HNF-3, and COUP-TFII, respectively.[59–61] The GRU1 and GRU2 correspond to the classical GRE at only 7 and 6 of 12 nucleotides, respectively, and bind GR only with a very low affinity.[59,61] In addition, they are not able to confer glucocorticoid responsiveness if they are connected to the heterologous promoter-driven reported construct.[60] In contrast, mutations of any of the accessory elements significantly reduce the activation of the PEPCK promoter by glucocorticoids.[47,60] Thus, the accessory factors bound on the PEPCK promoter are essential for the GR-induced stimulation of this promoter activity.

Since we found that GR physically interacts with COUP-TFII, we examined the importance of their physical interaction on the PEPCK promoter activity.[24] We found that elimination of COUP-TFII with an antisense to this orphan receptor abolished the glucocorticoid-induced expression of PEPCK mRNA in HepG2 cells.[24] The same treatment also abolished glucocorticoid-induced enhancement of the PEPCK promoter activity. A COUP-TFII–binding defective GR mutant failed to enhance the activity of this promoter. These results indicate that COUP-TFII and its interaction with GR are necessary for the stimulation of the PEPCK promoter and subsequent PEPCK mRNA expression. Since COUP-TFII is one of the cofactors that bind the accessory elements of the PEPCK promoter, GR may be attracted to the PEPCK promoter through COUP-TFII via its protein–protein interaction. Thus, GRU1 and 2 might play a supportive role in attracting GR to the PEPCK promoter.

It has been recently shown that the peroxisome proliferator–activated receptor-γ coactivator-1 (PGC-1), which has a major role in the cellular respiration and adaptive thermogenesis in muscle and brown fat, stimulates the PEPCK expression in hepatocytes, through cooperation with HNF-4.[62,63] PGC-1 is also known to synergistically act with GR on this promoter.[62] Since HNF-4 is one of the molecules that occupy the first accessory elements (AF-1) of the PEPCK promoter and interact with COUP-TFII,[45,62] COUP-TFII and GR might also have functional/physical interaction with this coactivator to stimulate the PEPCK expression.

Cholesterol Metabolism and Apolipoprotein Synthesis

Glucocorticoids increase serum cholesterol levels and modulate serum concentration of the low-density (LDL), very-low-density (VLDL), and high-density (HDL) lipoproteins by affecting various steps of their synthesis/clearance/degradation pathways.[49,64] For example, glucocorticoids stimulate cholesterol synthesis by inducing the expression of its rate-limiting enzyme, HMG-CoA reductase, *in vitro.*[65]

Glucocorticoids also suppress the expression of the LDL receptor on the hepatocyte that may help increase serum LDL levels.[66] In addition, glucocorticoids affect the production of apolipoproteins, components of serum lipoproteins that regulate the metabolic process of lipoproteins in the liver and peripheral tissues. Administration of glucocorticoids increases the serum concentration of apolipoprotein A1, a major protein component of HDL,[67] both in normal subjects and cultured cells.[64,68] Glucocorticoids also increase serum concentrations of apolipoprotein E.[64]

COUP-TFII was first identified as a protein that binds to regulatory elements of the apolipoprotein A1 gene, and for this reason it was originally called ARP-1 (apolipoprotein A1 regulatory protein).[69] Through binding to its promoter and change in its transcriptional activity, COUP-TFII reduces the transcription of the apolipoprotein A1 gene.[69] Since both glucocorticoids and COUP-TFII regulate the expression of apolipoprotein A1, it is likely that glucocorticoids increase the expression of this protein through COUP-TFII via protein–protein interactions between GR and COUP-TFII. Since COUP-TFII also binds to the promoter region of the very-low-density apolipoprotein II, apolipoprotein CII, B, and AII genes, it regulates their transcriptional activity and modulates the lipoprotein lipase expression;[69–71] glucocorticoids may indirectly affect their expressions through COUP-TFII, and hence further regulate the metabolism of other lipoproteins.

In addition to the above-described apolipoprotein metabolism, glucocorticoids also stimulate the bile acid synthesis[72,73] by stimulating the expression of its rate-limiting enzyme cholesterol 7α-hydroxylase, a gene product of the CYP7A.[72] The COUP-TFII binds to the promoter region of the CYP7A and stimulates its transcriptional activity.[46] Since we showed that glucocorticoids enhanced this effect of COUP-TFII on the CYP7A promoter,[24] it is possible that glucocorticoids regulate the expression of this enzyme through protein–protein interaction between the GR and promoter-bound COUP-TFII.

Xenobiotic Metabolism: Regulation of the CYP3A Expression

Glucocorticoids have important effects on the xenobiotic metabolism in the liver, which catalyzes oxidative reactions of clinically used compounds and environmental chemicals.[74] The CYP3A, a microsomal cytochrome P450 monooxygenase enzyme, plays a major role in this reaction.[74] This molecule is the most abundant form in the enzymes that catalyze xenobiotic metabolism.[74] It is also known that several clinically important chemicals, such as pregnane compounds, amilorides, imigazole, PPARγ agonists, and phenobarbital, induce CYP3A expression.[74] Glucocorticoids also stimulate the transcription rate of this enzyme.[75]

Among the CYP3A family enzymes, the rat CYP3A23 gene has been most extensively examined in the regulation of its gene expression by glucocorticoids.[76–79] The promoter region of this enzyme has three regions—DEX-RE1, 2, and Site A—which support inducibility of CYP3A23 by glucocorticoids.[77] COUP-TFII and pregnane X receptor (PXR) bind the former two sites, while HNF-4 associates with the last site.[74,79–82] The mechanism of glucocorticoid-induced regulation of this promoter is complicated—at low concentrations, glucocorticoids stimulate the expression of the PXR protein and indirectly stimulate the activity of the CYP3A23 promoter, while at higher concentrations, glucocorticoids directly stimulate the promoter by acting as ligands for PXR.[83] Interestingly, COUP-TFII regulates this promoter activity by

binding to the same region of this promoter that PXR targets.[74,83] Thus, it is highly likely that glucocorticoids additionally regulate this promoter activity via associating with promoter-bound COUP-TFII, and their interaction might, in some part, explain the regulatory mechanisms of the CYP3A expression by glucocorticoids.

SUMMARY

We showed that GR and COUP-TFII physically interact and mutually influence each other's transcriptional activity. This interaction may be important for the expression of PEPCK, which is a rate-limiting enzyme of hepatic gluconeogenesis. GR and COUP-TFII may also act cooperatively on the induction of apolipoproteins and CYP7A and CYP3A, which are important enzymes that catalyze bile acid synthesis and xenobiotic metabolism, respectively. These observations indicate that COUP-TFII may play an important role in the glucocorticoid effects on the intermediary metabolism, such as glucose, cholesterol, and catabolism of chemical compounds. Since knockout animals of GR or COUP-TFII have become available, the biologic importance of their interaction should be tested *in vivo* in these animals. Modulation of their interaction may be also important for the pathologic states that impair glucose and cholesterol metabolism, such as diabetes mellitus and dyslipidemia. Further studies are required to determine the implications of COUP-TFII to these pathologic states.

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