Nuclear Integration of Glucocorticoid Receptor and Nuclear Factor-κB Signaling by CREB-binding Protein and Steroid Receptor Coactivator-1*

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The p65 (RelA) component of nuclear factor-κB (NF-κB) and the glucocorticoid receptor (GR) mutually repress each other’s ability to activate transcription. Both of these transcriptional activators depend upon the coactivators CREB-binding protein (CBP) and steroid receptor coactivator-1 (SRC-1) for maximal activity. Here we show that increased levels of CBP relieves the inhibition of glucocorticoid-mediated repression of NF-κB activity and the NF-κB-mediated repression of GR activity. SRC-1 can relieve the NF-κB-mediated repression of GR activity. We propose that cross-talk between the p65 component of NF-κB and glucocorticoid receptors is due, at least in part, to nuclear competition for limiting amounts of the coactivators CBP and SRC-1, thus providing a novel mechanism for decreasing expression of genes involved in the inflammatory response.

Nuclear factor-κB (NF-κB) is an inducible transcription factor that plays an essential role in the regulation of gene expression in response to inflammatory stimuli (1–3). It is composed of members of the Rel family (p50, p52, p65 (RelA), c-Rel, and RelB), which share a region of homology known as the Rel homology domain capable of directing DNA binding and mediating dimerization. In many cells, NF-κB is found in an inactive form in the cytoplasm bound to an inhibitory protein, IκB. In response to many activating signals, the inhibitor is degraded by the proteasome and NF-κB translocates to the nucleus where it interacts with transcription coactivator proteins to activate target genes. CREB-binding protein (CBP) and its structural homolog p300 are transcriptional coactivators of the p65 subunit (4, 5). The steroid receptor coactivator-1 (SRC-1) also potentiates NF-κB-mediated transactivation (6).

Glucocorticoid hormones modulate the expression of a variety of genes associated with inflammatory responses by binding to a widely expressed intracellular receptor (GR). The GR is a member of the nuclear receptor family of proteins, which have a conserved modular architecture consisting of three functional domains: a DNA-binding domain, transcriptional activating domain, and a ligand-binding domain (7, 8). Transcriptional activation by this class of receptors requires that part of the C-terminal ligand-binding domain (called AF2) undergo a conformational change upon binding of ligand. This enables the receptors to bind a series of coactivator proteins, such as SRC-1 and CBP (9). Induction of gene expression by GR may contribute to the effects of glucocorticoid hormones, but recent findings suggest that DNA binding by GR is not required for some of the physiologic functions of the receptor (10, 11).

Previous studies found that activation of NF-κB is inhibited by glucocorticoid-induced expression of the inhibitor, IκBα, which suggested an explanation for how these steroids suppress an inflammatory response (12, 13). However, in endothelial cells (14, 15), as well as some other cells, glucocorticoid-mediated repression of NF-κB activity does not involve induction of IκBα synthesis. These cells, the GR does not inhibit nuclear accumulation of NF-κB, or inhibit DNA binding, suggesting that GR blocks NF-κB-mediated transactivation. Analysis of cell lines stably expressing a fusion protein between the DNA-binding domain of the yeast GAL4 protein and the transactivating region of p65 revealed that expression of the GAL4-dependent reporter gene was strongly suppressed by the action of the dexamethasone (16). This suggests that reduction of p65-dependent gene expression by the activated GR involves interference with transactivation. Because transactivation by p65 involves both CBP (4, 5) and SRC-1 (6), we investigated the role of these coactivators in the antagonistic interaction between the nuclear receptor and the NF-κB signaling pathways. We find that GR-mediated repression of p65-dependent gene expression results, at least in part, from competition for a limiting amount of these versatile transcriptional coactivators.

EXPERIMENTAL PROCEDURES

Cells and Transfections—Bovine aortic endothelial cells were obtained as described previously (17). COS-7 cells were obtained from the American Type Culture Collection (ATCC). The COS cells were transfected by the modified CaCl2 method as described in Ref. 17 and in the figure legends. Whole cell extracts were prepared from the transfected cells, and CAT activity was determined, as described previously (17). In studies with the GRE-NF-κB reporter construct, bovine aortic endothelial cells were transfected using LipofectAMINE and OptiMem (Life Technologies, Inc.) for 5 h at 37 °C in 5% CO2, prior to a medium exchange. Cells were harvested the next day and lysed, and luciferase assays performed according to the manufacturer’s instructions (Promega).

Plasmids—The plasmid construct p-578 E-selectin CAT was prepared as described previously (4). CBP regions 1–100, 1–771, 706–1069, 1069–1892, and 1892–2441 were amplified by polymerase chain reac-
Overexpression of CBP increases GR-dependent (sion.

...the 1069–1892 region of

CBP—

To compete effectively with p65 for CBP, GR should interact with CBP. Previous studies indicated that binding sites for the thyroid hormone, estrogen, and retinoic acid receptors (19) and that CBP in-



Fig. 2. GR interacts with CBP. Regions of CBP were tested for interaction with GR from programmed COS cell lysates. Sepharose-

GST Binding Assay—Fragments of the CBP coding sequence were subcloned in-frame into pGEX vectors (Amersham Pharmacia Biotech). GST fusion proteins were expressed in Escherichia coli DH5a (4). Coo-

GST and p65, respectively (Fig. 1, A and B). Cotransfection with CBP stimulated expression of the reporters 5–10-fold above the levels seen with the transcriptional activators alone.

SRC-1 modulates ligand-dependent transcriptional activation of the estrogen, thyroid hormone, and retinoic acid receptors (19) and that CBP increases p65-dependent transcription of the E-selectin, vascular cell adhesion molecule (4), and interferon-β-promoter-reporter constructs (20). To determine whether CBP limits expression of GR and p65, we examined the effect of overexpression of CBP on the expression of reporters with isolated recognition elements in transient transfection experiments. GRE and kB promoter-reporter constructs were activated severalfold by GR and p65, respectively (Fig. 1, A and B). Cotransfection with CBP stimulated expression of the reporters 5–10-fold above the levels seen with the transcriptional activators alone.

SRC-1 overexpression studies were also performed to verify that SRC-1 limits expression of GR-dependent gene expression and that SRC-1 modulates p65-dependent expression of an authentic NF-κB-dependent promoter. GRE and kB promoter-reporter constructs were activated severalfold by GR and p65, respectively (Fig. 1, C and D). Cotransfection with SRC-1 stimulated expression of the reporters 5-fold above the levels seen with the transcriptional activators alone. Control studies demonstrate that overex-

expression constructs (data not shown). These findings demonstrate that overexpression of CBP or SRC-1 increases both p65 and GR-dependent gene expression and suggests that the coactivators are present in limiting amounts.

To investigate a direct interaction between GR and CBP, a series of fragments of the coactivator were bacterially ex-

Regions of CBP were tested for interaction with GR from programmed COS cell lysates. Sepharose-

nion and inserted into pGex vectors as described previously (4). Full-

length CBP expression vector was provided by R. Goodman (Oregon Health Science University, Portland, OR).

Three minimal promoter-reporter constructs were used in these studies. First, a GRE-CAT construct was provided by Dr. Robert Scheinman (University of Colorado, Denver, CO) and consists of two GRE sites in a minimal TATA box containing E1B promoter (18). The isolated NF-κB promoter-CAT reporter construct contains two NF-κB elements in a minimal TATA box containing promoter derived from the vascular cell adhesion molecule-1 promoter (17). To create the GRE-NF-κB reporter construct, the following oligonucleotides were synthesized: oligonucleotide 1, 5′-GATCCGTACAGGATGTCTAGATGATCTGGGAAATT

CCTC-3′ and oligonucleotide 2, 5′-GATCCGAGAATTTTCCATGATCATTAGAACATCCTTACAG-3′. The oligonucleotides were phospho-

rlylated, annealed, and ligated into the Bgl II site of an SV40 minimal promoter-luciferase reporter vector, pGL3 Promoter (Promega).
Coactivator Integration of GR and NF-κB Signaling

In A COS cells were transfected with 1 µg of an E-selectin-promoter reporter (-578-CAT), 1 µg of the p65 vector, and increasing amounts of the GR expression vector (0–0.5 µg); 10 µg of the CBP or an equivalent amount of an empty expression vector were cotransfected as indicated. In B COS cells were cotransfected with 3 µg of the GRE-CAT reporter construct, 100 ng of GR, 500 ng of p65, and either 5 µg (lanes 6 and 7) or 10 µg (lanes 8 and 9) of the CBP expression vector. Total plasmid DNA was kept constant at 12 µg by the addition of the respective empty expression vector. In C COS cells were transfected with 2 µg of GRE-CAT reporter construct, 100 ng of GR and 100 ng, 250 ng, 1 µg, 2.5 µg, and 5 µg of p65. For SRC-1 rescue, 5 µg of p65 was used with 5 and 10 µg SRC-1 expression vector. The total DNA concentration was adjusted to 20 µg/10-cm tissue culture dish with the pCR3 expression plasmid. Portions of the cellular extracts from A–C were analyzed for p65 and GR by Western blot analysis using rabbit anti-p65 (Rockland) or rabbit anti-GR (Santa Cruz). Dexamethasone (dex) was added to appropriate cultures (10 nM) immediately after transfection. 2–3 days after transfection, CAT activity in cell extracts was measured as described previously (17). Data presented are representative of at least three independent transfections.

the coactivator (Fig. 2). Binding between the transcriptional activators and CBP was specific because no interactions were observed either with GST alone or with several other regions of CBP. Further mapping of the N-terminal interaction site revealed that GR bound to the 100–446 region of CBP (Fig. 2 and data not shown) and not to the N-terminal 100 amino acids of CBP, similar to many of the other nuclear receptors (19).

CBP and SRC-1 Rescue GR Inhibited p65-dependent Transactivation—If either CBP or SRC-1 function as signal integrators for the NF-κB and GR pathways, we would expect mutual transcriptional interference between these two signal-dependent activators. Using transient transfection assays, p65-mediated transcriptional activation of either E-selectin-promoter reporter constructs (Fig. 3A) or an isolated NF-κB element-reporter (data not shown) are repressed by dexamethasone-induced activation of GR in a dose-dependent manner (Fig. 3A, lanes 1–6). Control studies demonstrate that overexpressed GR did not decrease production of p65 from the corresponding expression constructs (Fig. 3A, inset). Parallel transfection studies demonstrate that GR-dependent gene expression is repressed by p65 (Fig. 3, B and C, lanes 3–7). Thus p65-mediated transcriptional activation was repressed by GR, and GR-dependent gene expression was repressed by p65, consistent with previous reports (18, 22).

One possibility suggested by the preceding findings is that the formation of complexes between either p65 or GR and the coactivators would reduce the amount of coactivator available for transcriptional activation. If competition for limiting amounts of CBP or SRC-1 account for the inhibitory effect of GR, then increased levels of the coactivators should restore or rescue p65-dependent gene expression. Indeed, the inhibitory effect of GR on p65-dependent gene expression was completely abolished by cotransfection of vectors expressing CBP, but not by a comparable amount of empty expression vector (Fig. 3A, compare lanes 7 and 8). The inhibitory effect of p65 on GR-dependent gene expression was eliminated by expression of CBP, in a dose-dependent manner (Fig. 3B, lanes 6–9). In a similar manner, the suppression of p65 on GR-dependent gene expression was significantly decreased by SRC-1 (Fig. 3C, compare lane 7 with lanes 8 and 9). Control studies demonstrated that CBP or SRC-1 overexpression did not significantly alter levels of either p65 (Fig. 3, A, B, and C, insets) or GR (data not shown).

Additionally, overexpression of an irrelevant transcriptional activator or mutated forms of CBP did not result in rescue, and overexpression of CBP did not modify the amount or composition of nuclear NF-κB complexes (data not shown). Collectively, these functional studies demonstrate that CBP and SRC-1 are limiting for both p65- and GR-dependent transactivation and that the coactivators can rescue the repressive interaction between p65 and the GR.

Stimulatory Interactions between the Activated GR and p65—The previous functional studies suggest that competition between p65 and the GR for limiting amounts of coactivator...
Coactivator Integration of GR and NF-κB Signaling

Protein kinase A stimulates p65 transcriptional activation, in part, by promoting an interaction with CBP/p300 (29). Additionally, the p38 and MAP kinase pathways increase p65 transcriptional activity mediated by tumor necrosis factor (30). It is possible that several of these phosphorylation events facilitate CBP binding and are decreased by activated GR. Decreased phosphorylation of p65 would constitute an additional overlapping mechanism to blunt NF-κB-dependent action by reducing the amount of competent NF-κB complex capable of competing for and interacting with CBP.

Because many of the large class of genes encoding inflammatory mediators such as cytokines, chemokines, and adhesion molecules are NF-κB-dependent (1), coactivator-mediated signal integration could explain, at least in part, the profound effects of glucocorticoids on suppressing immune and inflammatory responses. The extent of the inhibition would depend upon the abundance of the transcriptional activators, the level of the coactivator, and the relative affinity of the transcription factor for the coactivator.

FIG. 4. Stimulatory interactions between p65 and the activated GR. Bovine aortic endothelial cells were co-transfected with 1 μg of the GRE-NfκB reporter construct, 1 μg of GR or 100 ng p65, in the absence or presence of either 4 μg of CBP, 4 μg of SRC-1, or a combination of the expression vectors. The total concentration of DNA was adjusted to 10 μg with empty pCR3 expression vector. Dexamethasone (dex) was added to appropriate cultures (10 nM) immediately after transfection. Data are from four independent transfections, and the standard deviations are shown.