Overcoming Steroid Insensitivity in Respiratory Disease

Edited by

Ian M. Adcock and Kian Fan Chung

National Heart and Lung Institute,
Imperial College London,
London, UK

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Preface

The treatment of chronic inflammatory diseases was revolutionized by the discovery of the therapeutic utility of corticosteroids in the 1950s. Since this time they have been the mainstay of treatment for chronic inflammatory diseases. Their utility has been tempered, however, by the increasing risk of debilitating side effects with higher dose therapy. This is important because a reasonable proportion of patients with severe asthma do not respond well to high doses of inhaled or even oral corticosteroids. Thus 5% of asthmatics who do not respond to corticosteroid therapy account for >50% of the total healthcare costs for asthma. In addition, patients with chronic obstructive pulmonary disease also show little or no responsiveness to conventional corticosteroid therapy.

In the treatment of airways diseases side effects can be limited by targeted delivery to the airway and lung. Significant progress has been made through the use of increasingly selective molecules, and through a variety of lung-targeting strategies. Moreover, the recent developments in our understanding of the molecular and structural mechanisms of corticosteroid actions have suggested that it may be possible to develop a new corticosteroid, with intrinsically different pharmacology, that does not induce many of the pathways involved in the manifestation of side effects. A combination of these developments will enable the design of agents with an enhanced therapeutic index.

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1

Molecular Mechanisms of Glucocorticoid Receptor Action

Pankaj Bhavsar and Ian M. Adcock

1.1 Introduction

Glucocorticoids are the most effective therapy for the treatment of many chronic inflammatory diseases such as asthma and inflammatory bowel disease (Ito et al., 2006a). In contrast to the situation in asthma, chronic obstructive pulmonary disease (COPD), a common and debilitating chronic inflammatory disease of the lung, is glucocorticoid insensitive (Barnes, 2000a, b; Culpitt et al., 2003).

Glucocorticoids act by binding to cytosolic glucocorticoid receptors (GRs), which upon binding become activated and rapidly translocate to the nucleus. Within the nucleus, GR either induces transcription of genes such as secretary leukocyte proteinase inhibitor (SLPI)(Abbinante-Nissen et al., 1995) and mitogen-activated kinase phosphatase-1(Lasa et al., 2002) by binding to specific DNA elements (glucocorticoid response element, GRE) at the promoter/enhancer of responsive genes, or reduces inflammatory gene transcription induced by nuclear factor-kappa B (NF-κB) or other pro-inflammatory transcription factors (Ito et al., 2006a). Binding of GR to p65-NF-κB is crucial for transrepression by glucocorticoids, however, it is not clear how the GR dissociates its ability to control inflammation by suppressing NF-κB from its ability to directly transactivate genes via binding to GRE (Ito et al., 2006b).

In the resting cell, chromatin is tightly compacted to prevent transcription factor accessibility. During activation of the cell this compact inaccessible chromatin is made available to DNA-binding proteins, thus allowing the induction of gene transcription (Lee and Workman, 2007; Li et al., 2007). There is compelling evidence that increased inflammatory gene transcription is associated with an increase in histone acetylation induced by transcriptional coactivators containing...
histone acetyltransferase (HAT) activity, whereas hypoacetylation is correlated with reduced transcription or gene silencing (Lee and Workman, 2007; Li et al., 2007), which is controlled by histone deacetylases (HDACs). Investigation of the expression of key components of GR function in asthma and COPD may reveal the critical protein(s) required for glucocorticoid function in lung disease.

1.2 Glucocorticoid Receptor

Glucocorticoids exert their effects by freely diffusing across the plasma membrane of cells and binding to a ubiquitously expressed GR that is localized in the cytoplasm of target cells. Although unliganded GR is thought to remain in the cytoplasm, evidence using nuclear export inhibitors suggests that a rapid active cycling of GR between the nucleus and cytoplasm may occur (Hache et al., 1999; Kumar et al., 2004). Two GR isoforms (α and β) were originally described, with the nuclear GRβ having a dominant negative effect on GRα via the formation of GRα/GRβ heterodimers. Evidence is accumulating that this isoform may be important in certain disease states where GR nuclear translocation is deficient (Ito et al., 2006a; Zhou and Cidlowski, 2005). In addition, the use of different start sites has increased the number of potential isoforms present and differential expression may affect glucocorticoid function in an organ- or disease-specific manner (Lu and Cidlowski, 2005).

The crystal structure of the GR ligand binding domain (LBD) has been determined in a ternary complex with dexamethasone and a TIF2 coactivator peptide following point mutation of F602 (Bledsoe et al., 2002). The overall structure is similar to LBDs of other nuclear hormone receptors (NHRs) but contains a unique dimerization interface and a second charge clamp that may be important for cofactor selectivity. As with the results seen with the oestrogen antagonist raloxifene binding to oestrogen receptor, RU486 binding to the GR LBD results in a failure of helix 12 to correctly close over the binding cleft (Kauppi et al., 2003). This results in a conformation more able to recruit corepressors than the closed helix 12, which efficiently recruits coactivators (Garside et al., 2004; Kauppi et al., 2003) and has led to rational-based design of selective dissociated GR agonists (Barker et al., 2005). Modification of the dimerization interface by mutation of I628A resulted in reduced transactivation ability without affecting the ability to repress an NF-κB-driven reporter gene. The contrasting effects of this mutant suggest that the monomer and dimer forms of GR may regulate distinct signalling pathways confirming data obtained from the dim(−/−) mouse (Karin, 1998).

1.3 Gene Induction by GR

Glucocorticoid binding to the cytoplasmic GR enables dissociation of chaperone proteins, including heat shock protein (hsp) 90, thereby allowing nuclear localization of
the activated GR–glucocorticoid complex utilizing selective importins (Chook and Blobel, 2001; Picard and Yamamoto, 1987; Savory et al., 1999). GR nuclear export is also tightly regulated; however, the role of the exportin-1 (CRM-1) pathway is currently unclear (Kumar et al., 2004; Picard and Yamamoto, 1987). Importantly, the nuclear localization signal (NLS)–importin-α interaction is often influenced directly by the phosphorylation status of the imported proteins (Chook and Blobel, 2001).

Within the nucleus GR dimerizes with another GR and binds to consensus DNA sites termed glucocorticoid response elements (GREs, GGTACAAnnnTGGTCT) in the regulatory regions of glucocorticoid-responsive genes (Figure 1.1). This interaction allows GRs to recruit activated transcriptional coactivator proteins, including steroid receptor coactivator-1 (SRC-1) and cAMP response element binding protein (CBP), through LXXLL motifs (Smith and O’Malley, 2004). This produces a DNA-protein structure that allows enhanced gene transcription (Karin, 1998). Individual GR ligands can target GR to specific nuclear subdomains (Schaaf et al., 2005) and this, along with differences in the number of GREs, and the position of the GREs relative to the transcriptional start site (Wang et al., 2004), controls the magnitude and direction of the transcriptional response to glucocorticoids, dependent upon the particular ligand.

1.4 GR Transactivation and Histone Acetylation

Expression and repression of genes are associated with alterations in chromatin structure by enzymatic modification of core histones (Lee and Workman, 2007; Li et al., 2007).
Specific residues (lysines, arginines and serines) within the N-terminal tails of core histones are capable of being post-translationally modified by acetylation, methylation, ubiquitination or phosphorylation, all of which have been implicated in the regulation of gene expression (Lee and Workman, 2007; Li et al., 2007). The “histone code” refers to these modifications, which are set and maintained by histone-modifying enzymes and contribute to coactivator recruitment and subsequent increases in transcription (Jenuwein and Allis, 2001; Rice and Allis, 2001).

Transcriptional coactivators such as CBP, SRC-1, TIF2, GRIP-1 and p300/CBP associated factor (PCAF) have intrinsic HAT activity (Lee and Workman, 2007; Li et al., 2007), and increased GR-mediated gene transcription is associated with an increase in histone acetylation. In lung epithelial cells this occurs predominantly on histone H4 rather than H3, although it may be cell and/or gene specific (Ito et al., 2000; Li et al., 2003; Wu et al., 2005). In contrast, hypoacetylation induced by HDACs is correlated with reduced transcription or gene silencing (Lee and Workman, 2007; Li et al., 2007). Initially it was thought that acetylation neutralized positively charged lysine residues, thereby allowing reduced contacts with the negatively charged DNA. Subsequent data, however, have suggested that acetylation and other epigenetic tags on histones function as signalling marks enabling the recruitment of specific factors involved in gene transcription (Lee and Workman, 2007; Li et al., 2007). It is becoming clear that these two hypotheses are not mutually exclusive and interdependence can occur (Lee and Workman, 2007; Li et al., 2007). NHRs including GR do not necessarily recruit all these co-factors directly (Huang et al., 2003) as NHR-recruited cofactors can sequentially recruit other coactivators and chromatin remodelling complexes (Wu et al., 2005) that aid the formation of the transcription initiation complex and result in local chromatin remodelling (Lee and Workman, 2007; Li et al., 2007) (Figure 1.1).

A series of studies from Archer and others (Trotter and Archer, 2007) indicate that the ATP-dependent chromatin remodelling protein Brg1 is essential for GR-mediated transactivation at the MMTV promoter and a select number of endogenous promoters such as 11β-HSD and p21CIP/WAF. Brg1 and associated factors are targeted to acetylated histone tails by bromodomains but they can also interact directly or indirectly with GR following GR ligand binding (Nie et al., 2000). More recently, it has become clear that Brg1 is also involved in GR-mediated transrepression and that the ultimate fate depends upon GR recruitment of cofactors (repressors or activators) at specific genes (Hebbar and Archer, 2007). In a similar manner perhaps STAMP (SRC-1- and TIF2-associated modulatory protein) can interact with TIF2 and SRC-1 to enhance both the transactivation and transrepression activities of GR (He and Simons, 2007).

In addition to the more classical modes of action, it is now clear that GR can bind to DNA as heterodimers with other transcription factors, such as members of the signal transducer and activator of transcription (STAT) family (Biola et al., 2000; Stocklin et al., 1996) and the ETS transcription factors (Mullick et al., 2001), leading to the recruitment of distinct coactivator (e.g. GRIP-1) or corepressor (e.g.
1.5 Post-translational Modifications of GR

GR is a phosphoprotein containing numerous potential phosphorylation sites, including those for extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), protein kinase C and protein kinase A. Altered GR phosphorylation status can affect GR-ligand binding (Irusen et al., 2002), hsp90 interactions (Hu et al., 1994), subcellular localization (Somers and DeFranco, 1992; Zuo et al., 1999), nuclear–cytoplasmic shuttling (Galigniana et al., 1999a; Hsu et al., 1992) and transactivation potential (Zuo et al., 1999), possibly through association with coactivator molecules (Somers and DeFranco, 1992) (Figure 1.2). The specific roles of these phosphorylation events are unclear (Zhou and Cidlowski, 2005) but phosphorylation of Ser-211 is a good marker of GR activation (Zhou and Cidlowski, 2005). MAPK activation or overexpression can also target other serine/threonine residues in GR, decreasing GR-mediated transactivation (Irusen et al., 2002; Szatmary et al., 2004) possibly via an effect on Ser-226 phosphorylation and increasing GR nuclear export (Itoh et al., 2002).

More recent evidence suggests that GR phosphorylation is also involved in receptor turnover and that phosphorylation can target the receptor for hormone-mediated

Figure 1.2  Post-translational modification of the glucocorticoid receptor (GR), particularly by phosphorylation and nitration, alters GR function and contributes to the potential for diverse function in distinct tissues. Other modifications such as ubiquitination (Ub), sumoylation (Su) and acetylation (Ac) are also shown.
proteasomal degradation (Wallace and Cidlowski, 2001). GR sumoylation appears to have the opposite effect of ubiquitination and results in increased GR activity (Le et al., 2002) possibly through changes in cofactor recruitment (Lin et al., 2003) (Figure 1.2). Interestingly, nitrosylation of GR at an hsp90 interaction site induced by the NO donor S-nitroso-dl-penicillamine has also been shown to prevent GR dissociation from the hsp90 complex and to reduced ligand binding (Galigniana et al., 1999b).

It has become clear that histones are not the only targets for histone acetylases and recent evidence has suggested that acetylation of transcription factors can modify their activity. For example, the p65 component of NF-κB can also be acetylated, thus modifying its transcriptional activity (Chen et al., 2001). Recent evidence suggests that GR is also acetylated (Figure 1.2) upon ligand binding and that deacetylation is critical for interaction with p65 at least at low (µM) dexamethasone concentrations (Ito et al., 2006b). Acetylation occurs within the GR nuclear retention signal (NRS) and may therefore possibly modify GR nuclear retention and GR-mediated transactivation and/or transrepression (Carrigan et al., 2007).

1.6 Repression of NF-κB-induced Inflammatory Gene Expression by GR–NF-κB

GR dimerization-deficient mice (Reichardt et al., 1998; Reichardt et al., 2001) indicate that the major anti-inflammatory effects of glucocorticoids are due mainly to an interaction between GR and transcription factors such as NF-κB, which mediate the expression of inflammatory genes (De Bosscher et al., 2006; Karin, 1998) (Figure 1.3). NF-κB is activated by numerous extracellular stimuli, including cytokines such as tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β), viruses and immune challenges (Baldwin, 2001). NF-κB is able not only to control induction of inflammatory genes in its own right but can enhance the activity of other cell- and signal-specific transcription factors (Barnes and Karin, 1997). Activation of NF-κB involves stimulation of a phosphorylation cascade resulting in phosphorylation and ubiquitination of a cytoplasmic inhibitor (IκBα) and release of NF-κB (generally a p65/p50 heterodimer) and its nuclear translocation (Ghosh and Karin, 2002). Subtle changes in p65 phosphorylation are also important in its activation (Sarkar et al., 2007); for example, inactive p65 is non-phosphorylated and is associated predominantly with HDAC1, whereas p65 is phosphorylated following IKK-2 stimulation and is able to bind to coactivator molecules such as p300/CBP (Zhong et al., 2002). NF-κB, as with GR, can induce histone acetylation and other histone modifications in a temporal manner (Ito et al., 2000; Lee et al., 2006) leading to recruitment of distinct coactivator and remodelling complexes and the induction of inflammatory gene expression. Adding to the complexity of NF-κB activation, p65 is also acetylated and its acetylation status by selective HDACs controls its association with, and loss of activity by, IκBα (Chen et al., 2001).
NF-κB activated by distinct cellular stimuli can control the expression of different patterns of genes (Covert et al., 2005; Ogawa et al., 2005; Werner et al., 2005). Thus, lipopolysaccharide (LPS) and TNFα induced distinct gene profiles as a result of differences in the amplitude and duration of NF-κB activation, rate of IκBα decay and association with other factors such as IRF-3 (Covert et al., 2005; Nelson et al., 2004; Ogawa et al., 2005; Werner et al., 2005). Other signalling pathways such as the MAPKs may also affect the pattern and/or duration of NF-κB-mediated gene expression. Furthermore, it has also become clear that small changes in the consensus κB binding site and surrounding bases can have profound effects on the subsequent ability of activated NF-κB to activate gene expression (Luecke and Yamamoto, 2005).

1.7 GR–NF-κB Cross-talk

The precise mechanism for the ability of activated GR to repress NF-κB-induced gene transcription is still under debate and may alter depending upon GR expression levels (Simons, 2006) but it includes binding to, or recruiting, nuclear receptor
corepressors (Ito et al., 2000; Nie et al., 2005; Rosenfeld and Glass, 2001), direct repression of coactivator complexes (Ito et al., 2000; Pascual et al., 2005), actions on histone phosphorylation status (Hasegawa et al., 2005) or effects on RNA polymerase II phosphorylation (Luecke and Yamamoto, 2005; Nissen and Yamamoto, 2000). We have previously reported that dexamethasone-induced NF-κB-mediated gene expression in epithelial cells is associated with changes in histone acetylation (Ito et al., 2000). Similar data have also been reported in primary airway smooth-muscle cells where fluticasone was able to attenuate TNFα-induced p65 association with the native CCL11 promoter and block TNFα-induced histone H4 acetylation (Nie et al., 2005).

Furthermore, these effects are context/gene dependent and repression often depends upon factors complexed with NF-κB. Thus, activated GR represses a large set of functionally related inflammatory genes stimulated by p65/IRF-3 complexes (Ogawa et al., 2005), perhaps by targeting the IRF-3–GRIP-1 interaction (Reily et al., 2006). In contrast, peroxisome proliferator -activated receptor gamma (PPARγ) and liver X receptors (LXRs) repress overlapping transcriptional targets in a p65/IRF-3-independent manner and cooperate with GR to suppress distinct subsets of pattern recognition receptor (PRR)-responsive genes (Ogawa et al., 2005). Designing drugs with the capacity to activate GR and other nuclear hormone receptors may, therefore, enhance the anti-inflammatory profile of glucocorticoids. Moreover, as the expression of many cofactors and nuclear receptors is tissue specific, there is the attractive possibility of designing tissue-specific ligands, although this approach will require a clearer understanding of the key tissues that are targeted by glucocorticoids.

Furthermore, GR–NF-κB interactions do not always result in gene repression. The context in which GRE and κB sites are found within a promoter of specific genes may drastically affect the final effect on gene expression (Hermoso et al., 2004; Sakai et al., 2004). For example, dexamethasone can enhance cytokine-inducible expression of TLR2 via a GR–p65 association on the promoter where the GRE overlaps with the κB site. This does not absolutely require the GRE and κB-RE to be in a close sequence alignment as similar effects of p65 and GR can be seen during the induction of stem cell factor (SCF) in human fibroblasts, despite the GRE and κB-RE being separated by ~1700 bp (Da Silva et al., 2004).

1.8 Role of HDAC2 in Glucocorticoid Function

We and others have reported that non-selective HDAC inhibitors are able to prevent GR repression of inflammatory genes (Ito et al., 2000; Jee et al., 2005; Marwick et al., 2004). Using siRNA we were able to show that HDAC2 was the only class I HDAC involved in GR-mediated gene repression (Ito et al., 2006b) and that this did not affect the ability of activated GR to undergo nuclear translocation or transactivation (Ito et al., 2006b). GR is rapidly acetylated upon ligand binding at
aa492-495 (KTKK) within the GR hinge region and only the deacetylated form of GR is able to associate with p65. Thus, recruitment of HDAC2 by acetylated GR leads to deacetylation of GR, interaction with the p65-NF-κB activated complex and subsequent suppression of inflammatory gene expression. This mechanism provides a molecular explanation for the ability of GR to distinguish between recruitment of coactivator and corepressor proteins as previously demonstrated for GRIP (Rogatsky et al., 2002) and the subsequent ability to transactivate or repress gene transcription (Ito et al., 2006b).

1.9 Overexpression of HDAC2 Restores Glucocorticoid Sensitivity in Alveolar Macrophages

HDAC2 expression and activity are decreased in smokers (Ito et al., 2001) and patients with COPD (Ito et al., 2005), who are known to be insensitive to the anti-inflammatory effects of glucocorticoids (Barnes, 2000a). In addition, there is a negative correlation between the repressive effect of dexamethasone on cytokine production and total HDAC activity in alveolar macrophages from smokers and non-smokers (Ito et al., 2001). In order to confirm a role for HDAC2 in glucocorticoid responsiveness in primary cells from these subjects, we overexpressed HDAC2 and examined cytokine repression by dexamethasone (Ito et al., 2006b). GR acetylation following dexamethasone treatment was increased in alveolar macrophages obtained from patients with COPD compared to normal subjects and these cells did not respond well to dexamethasone (Ito et al., 2006b). Overexpression of HDAC2, but not HDAC1, in primary macrophages from COPD patients restored dexamethasone efficacy towards suppressing LPS-induced GM-CSF release to levels seen in cells from healthy control subjects (Ito et al., 2006b). Furthermore, knockdown of HDAC2 in sputum macrophages from healthy non-smokers by RNAi reduced the inhibitory effect of dexamethasone (Ito et al., 2006b).

In support of this hypothesis, Bilodeau and colleagues (Bilodeau et al., 2006) have reported that GR-mediated repression of proopiomelanocortin (POMC) requires the ATPase-dependent chromatin remodelling enzyme Brg1 and the recruitment of HDAC2 to enable deacetylation of the POMC promoter. Bilodeau further hypothesized that loss of Brg1 and/or HDAC2 should induce glucocorticoid insensitivity and reports that 50% of glucocorticoid-resistant human and dog corticotroph adenomas, which are the hallmark of Cushing’s disease, are deficient in the nuclear expression of either protein (Bilodeau et al., 2006).

Brg1-induced remodelling is essential for RNP2 initiation and re-initiation (Bilodeau et al., 2006), which involves changes in RNA polymerase II C-terminal domain (CTD) phosphorylation. Yamamoto and co-workers have shown previously that dexamethasone was able to reduce CTD serine 2 (Ser-2) phosphorylation induced by TNFα-induced NF-κB at the IL-8 promoter (Nissen and Yamamoto, 2000) via a reduction in the association of the Ser-2 CTD kinase, P-TEFb (Luecke
and Yamamoto, 2005). Importantly, Bilodeau was able to show that a lack of HDAC2 leads to loss of Brg1 function resulting in RNP2 stalling on the promoter of responsive genes and a failure to remove the phospho tag from Ser-2 in the CTD (Bilodeau et al., 2006).

Taken together, these results indicate that HDAC2 is a key protein involved in the suppression of p65-mediated inflammatory gene expression. HDAC2 acts by deacetylation of GR, thereby enabling p65 association and, in conjunction with Brg1, this results in subsequent attenuation of pro-inflammatory gene transcription. The importance of this mechanism in COPD and Cushing’s disease, both glucocorticoid-insensitive diseases, is emphasized by: i) overexpression of HDAC2 restores glucocorticoid sensitivity in primary cells from COPD patients (Ito et al., 2006b), and ii) a lack of HDAC2 is associated with a lack of glucocorticoid responsiveness in adenocarcinomas from Cushing’s patients (Bilodeau et al., 2006).

1.10 Acetylation of hsp90 and Regulation of GR Function

The actions of trichostatin A (TSA) on GR-mediated functions may not result from changes in GR acetylation. Chaperone proteins, including Hsp90 and p23 but not hsp70, also associate with GR at native GREs and modulate GR-mediated transactivation (Freeman and Yamamoto, 2002) and/or nuclear export (Kakar et al., 2006). The function of hsp90 is also regulated by its acetylation by chaperone acetyltransferase (CHAT) (Scroggins et al., 2007), a process that is specifically reversed by HDAC6 (Kovacs et al., 2005). Inactivation of HDAC6 by TSA or by selective knockdown using siRNA results in the accumulation of acetylated Hsp90, which is unable to interact stably with GR or with the critical co-chaperone p23 in A549 cells. This leads to defective GR ligand binding, nuclear translocation and transcriptional activation (Murphy et al., 2005).

1.11 Other Mechanisms of GR Action

Suppression of AP-1-induced gene transcription

The GR monomer can bind directly or indirectly with AP-1, which is also upregulated during inflammation (Demoly et al., 1995). Regulation of AP-1 activity has been thought to be similar to that of NF-κB. However, recent data has reported that a point mutation in the second zinc finger of the GR DNA binding domain (R488Q) results in a GR able to repress AP-1-mediated transcriptional responses but not those activated by NF-κB in three different cell lines, despite being able to physically associate with NF-κB (Bladh et al., 2005). In addition, this mutant had no effect on GRE-mediated events. The genes differentially regulated by this mutant were mainly involved in control of transcription and cell growth. The results
indicate that different GR interaction surfaces or mechanisms are involved in the repression of NF-κB and AP-1, respectively (Bladh et al., 2005).

Other mechanisms for GR suppression of AP-1 and NF-κB have been proposed. The GR dimer can induce the expression of the NF-κB inhibitor IκB-α in certain cell types (Auphan et al., 1995; Heck et al., 1997). Similarly, induction of GILZ (glucocorticoid inducible leucine zipper) can prevent AP-1 DNA binding and activity in some cells (Mittelstadt and Ashwell, 2001).

### Mutual suppression of MAPK activity

In addition, glucocorticoids may play a role in repressing the action of MAPKs, such as ERK and c-Jun N-terminal kinase (JNK) (Adcock, 2003). Caelles and colleagues have demonstrated that dexamethasone inhibits the phosphorylation and activation of JNK, resulting in a failure to phosphorylate c-Jun and Elk-1, reduced c-fos transcription and a marked reduction in AP-1 activity (Caelles et al., 1997). More recently, it has been shown that dexamethasone can rapidly induce the dual-specificity MAPK phosphatase-1 (MKP-1) and thereby attenuate p38-MAPK activation (Kassel et al., 2001; Lasa et al., 2001). Rogatsky and colleagues have, in turn, shown reciprocal inhibition of rat GR reporter gene activity by JNKs by direct phosphorylation of Ser-246, whereas ERK can inhibit GR action by an indirect effect, possibly through phosphorylation of a cofactor (Rogatsky et al., 1998). Furthermore, we and others have shown that p38-MAPK-mediated GR phosphorylation can attenuate GR function (Irusen et al., 2002; Szatmary et al., 2004).

In addition, p38-MAPK-mediated phosphorylation of Ser-10 of histone H3 is rapidly inhibited and redistributed away from sites of active gene transcription in a time- and concentration-dependent manner by dexamethasone in BEAS-2B cells (Hasegawa et al., 2005).

### Regulation of mRNA stability

Glucocorticoids also appear to exert anti-inflammatory actions that do not depend on the receptor’s ability to regulate transcription in the nucleus. Adenylate–uridylate-rich elements (AREs) are found within the 3'-UTR of many inflammatory genes and control the stability of mRNA (Fan et al., 2005; Meyer et al., 2004). These sequences are very heterogeneous and include both AUUUA pentamers and AT-rich stretches. Binding of mRNA to ARE-binding proteins results in the formation of messenger ribonucleoprotein (mRNP) complexes which control mRNA decay (Fan et al., 2005; Meyer et al., 2004). Several ARE-binding proteins have been reported and include tristetrapolin (TTP), which promotes mRNA decay, and HuR family members, which are associated with mRNA stability. Importantly, HuR binding to AREs is dependent upon p38 MAPK (Fan et al., 2005; Meyer et al., 2004). Dexamethasone has been
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reported to regulate the levels of HuR and TTP, thereby reducing the levels of inflammatory gene mRNAs such as COX-2 and CCL11 (Lu and Cidlowski, 2005; Shim and Karin, 2002) through a p38-MAPK-mediated pathway subsequent to induction of MKP-1 (Bergmann et al., 2004; Lasa et al., 2001). However, significant modulation of these genes often appears at 10 nM dexamethasone rather than the 1 nM concentrations associated with suppression of many inflammatory genes, although again these effects may be cell selective (Jalonen et al., 2005). Intriguingly, dexamethasone has recently been reported to decrease TTP expression in LPS-stimulated murine macrophages (Jalonen et al., 2005).

Non-genomic actions of glucocorticoids

Some very rapid effects of glucocorticoids, such as changes in bronchial blood flow, occur within minutes after inhaled corticosteroid dosing in asthmatics (Mendes et al., 2003) and this cannot be explained by the traditional genomic theory of steroid action. It has been proposed that these non-genomic actions are mediated by a distinct membrane receptor (Chen and Qiu, 1999; Norman and Mizwicki, 2004). Initially described in amphibians, these receptors have been described in mammalian cells and have distinctive hormone binding properties compared to the well-characterized cytoplasmic receptors and are probably linked to a number of intracellular signalling pathways acting through G-protein-coupled receptors and a number of kinase pathways (Evans et al., 2000; Norman and Mizwicki, 2004; Powell et al., 1999). In addition, the classical GR–hsp90 complex is associated with a number of kinases, phosphatases and acetylases (Adcock et al., 2002). These enzymes are released upon hormone binding and may also account for the rapid induction of tyrosine kinases seen in some cell types by glucocorticoids (Croxtall et al., 2000; Croxtall et al., 2002).

1.12 Conclusions

Overall, GR is able to selectively repress specific target genes by differing actions on promoter-specific components of NF-κB and AP-1 activation complexes and by effects on MAPKs. Explanations for the differences between the models may involve the concentration and timing of dexamethasone treatment of cells, GR expression levels and the precise inflammatory stimulus used (Simons, 2006). These pleiotropic effects of GR on suppressing inflammatory gene expression may underlie their effectiveness in most patients with airways disease but this also suggests that alteration of any of these pathways may result in a reduced ability of glucocorticoids to function effectively in patients with chronic inflammatory diseases of the lung. Understanding the molecular mechanisms of GR action may lead to the development of new anti-inflammatory drugs or enable clinicians to
reverse the relative steroid insensitivity that is characteristic of severe asthma and COPD for example.

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References


Barnes PJ. Mechanisms in COPD: differences from asthma. Chest 2000b; 117: 10S–14S.


Caelles C, Gonzalez-Sancho JM, Munoz A. Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. *Genes Dev* 1997; **11**: 3351–3364.


Croxtall JD, Choudhury Q, Flower RJ. Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *Br J Pharmacol* 2000; **130**: 289–298.


Galigniana MD, Housley PR, DeFranco DB, Pratt WB. Inhibition of glucocorticoid receptor nucleocytoplasmic shuttling by okadaic acid requires intact cytoskeleton. *J Biol Chem* 1999a; **274**: 16222–16227.

REFERENCES


Mittelstadt PR, Ashwell JD. Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ. *J Biol Chem* 2001; 276: 29603–29610.