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Review

Effects of glucocorticoids on gene transcription

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Abstract

Glucocorticoids bind to and activate a cytoplasmic glucocorticoid receptor. The activated glucocorticoid receptor translocates into the nucleus and binds to specific response elements in the promoter regions of anti-inflammatory genes such as lipocortin-1 and secretory leukocyte protease inhibitor (SLPI). However, the major anti-inflammatory effects of glucocorticoids appear to be due largely to interaction between the activated glucocorticoid receptor and transcription factors, notably nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), that mediate the expression of inflammatory genes. NF-κB switches on inflammatory genes via a process involving recruitment of transcriptional co-activator proteins and changes in chromatin modifications such as histone acetylation. This process must occur in the correct temporal manner to allow for effective inflammatory gene expression to occur. The interactions between NF-κB and the glucocorticoid receptor result in differing effects on histone modifications and chromatin remodelling. Drugs that enhance glucocorticoid receptor nuclear translocation (long acting β-agonists) and GR-associated histone deacetylases activity (theophylline) have been shown to be effective add-on therapies. In addition, dissociated glucocorticoids that target NF-κB preferentially have also been successful in the treatment of allergic disease.

Keywords: Asthma; Histone acetylation/deacetylation; Glucocorticoid resistance; Nuclear factor-κB

Contents

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1. The molecular basis of inflammation in bronchial asthma

Inflammation is a central feature of bronchial asthma and involves the recruitment and activation of inflammatory cells and changes in the structural cells of the lung. These structural changes include basement membrane thickening, epithelial cell loss, airway smooth muscle hypertrophy, hyperplasia and migration in asthma and matrix destruction in emphysema. Asthma is characterized by increased expression of many proteins involved in the complex inflammatory cascade, including cytokines, chemokines, receptors, and adhesion molecules (reviewed in Barnes et al., 1998).

Many of the genes are not expressed in normal cells under resting conditions but are induced in a cell-specific manner suggesting a role for de novo gene transcription. Changes in gene transcription are regulated by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) (Barnes and Karin, 1997). For example, NF-κB is markedly activated in epithelial cells of asthmatic patients (Hart et al., 1998), and it regulates many of the inflammatory genes that are abnormally expressed in asthma (Barnes and Karin, 1997). NF-κB may be activated by rhinovirus infection and allergen exposure, both of which exacerbate asthmatic inflammation (Zhu et al., 1996;Donovan et al., 1999;). AP-1 expression is also enhanced in asthmatic airways (Demoly et al., 1992) and it is reduced following glucocorticoid therapy (Demoly et al., 1995). This enhanced AP-1 expression is further elevated in severe steroid-insensitive asthma resulting from increased expression or an insensitivity to downregulation by glucocorticoids(Lane et al., 1998). In addition, its expression and activity is enhanced by factors associated with asthma and airway hyperresponsiveness and remodeling (Jibiki et al., 2003; Panettieri et al., 1990).

2. Chromatin remodeling

DNA is tightly compacted around a protein core. This chromatin structure is composed of nucleosomes, which are particles consisting of ~146-bp DNA associated with an octamer of two molecules each of core histone proteins (H2A, H2B, H3, and H4). Expression and repression of genes is associated with alterations in chromatin structure by enzymatic modification of core histones (Urnov and Wolffe, 2001). In the resting cell, DNA is tightly compacted around these basic core histones, excluding the binding of the enzyme RNA polymerase II, which activates the formation of messenger RNA. This conformation of the chromatin structure is described as closed and is associated with suppression of gene expression (Urnov and Wolffe, 2001) (Fig. 1).

The irregular 30-nm chromatin fibre is stabilized and further compacted by interactions between nucleosomes and linker histones such as H1. Histone H1 has long been regarded as a general repressor of transcription, but there are indications that it has a role in transcriptional regulation (Zlatanova et al., 2000). In this newer model, histone H1 acts as a "gate" to nucleosomal DNA, preventing transcription factor DNA binding. Histone H1 removal is needed for high mobility group (HMG)B proteins to bind to the same sites as histone H1 and induce a more fluid nucleosomal structure and thus making nucleosomal DNA accessible to further transcription-factor binding and stimulation of transcription. Thus, phosphorylation of histone H1 may play an essential role in activation of gene transcription (Zlatanova et al., 2000).

2.1. Histone acetylation and gene transcription

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 (Urnov

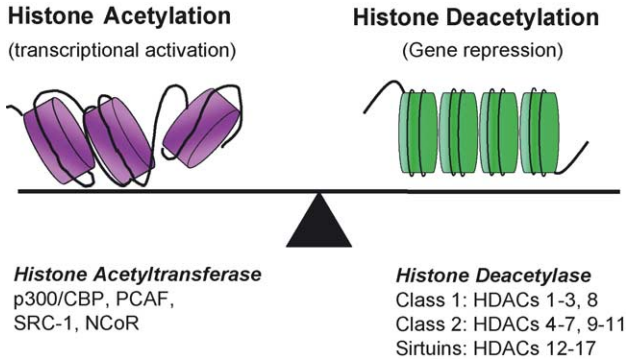

Fig. 1. Gene repression and activation are regulated by acetylation of core histones. In the resting state, DNA is tightly coiled around histones, forming a dense nucleosomal structure due to electrostatic attraction between negatively charged DNA and positively charged lysine residues. Acetylation of histones removes this charge, allowing loosening of the nucleosomal structure. Histone acetylation is mediated by transcriptional coactivators, which have intrinsic histone acetyltransferase (HAT) activity, whereas repression is induced by histone deacetylases (HDACs), which reverse this acetylation, allowing repackaging of the nucleosomes. Adapted from Urnov and Wolffe (2001).

and Wolffe, 2001). Acetylation of the σ-group on lysines reduces the charge of the histone residue and subsequently releases the tightly wound DNA, allowing the recruitment of further large protein complexes (Urnov and Wolffe, 2001). Changes in histone acetylation have been implicated in gene transcription since the seminal studies of Allfrey et al. (1964), however, its precise role was unknown. A breakthrough in the discovery of the role of histone acetylation was the demonstration 30 years later that transcriptional coactivators such as cAMP response element binding protein (CREB) binding protein (CBP) and p300/CBP-associated factor have intrinsic histone acetyltransferase (HAT) activity (Janknecht and Hunter, 1996). This activity is therefore recruited to the site of active gene transcription by the binding of transcription factors to DNA. This association between co-activator and transcription factor may, in addition, further enhance co-activator HAT activity (Urnov and Wolffe, 2001; Janknecht and Hunter, 1996).

Increased gene transcription is associated with an increase in histone acetylation, whereas hypoacetylation is correlated with reduced transcription or gene silencing (Urnov and Wolffe, 2001). Histone acetylation is an active process whereby small changes in the activity of HATs or histone deacetylases (HDACs) can markedly affect the overall histone acetylase activity associated with inflammatory genes (Urnov and Wolffe, 2001). Importantly, these changes in histone acetylation appear to be targeted toward regions of DNA associated with specific activator sites within the regulatory regions of induced inflammatory genes (Urnov and Wolffe, 2001), although a global loosening of histone structure has also been proposed (Waterborg, 2000).

2.2. Histone deacetylation

Repression of genes is associated with the reversal of histone acetylation, or histone deacetylation, a process controlled by HDACs (De Ruijter et al., 2003). The number of known HDACs is growing; so far, at least 11 mammalian forms have been identified along with at least seven sirtuins (De Ruijter et al., 2003). These HDACs are categorized into two classes according to homology with yeast HDACs. Class 1 HDACs (HDAC1, 2, 3 and 8) are most closely related to the yeast (*Saccharomyces cerevisiae*) transcriptional regulator RPD3. Class II HDACs (HDAC4, 5, 6, 7, 9 and 10) share domains with similarity to HDA1, another deacetylase found in yeast (De Ruijter et al., 2003). Currently, it is thought that HDACs of class I are expressed in most cell types, whereas the expression pattern of class II HDACs is more restricted, suggesting that they might be involved in cellular differentiation and developmental processes (De Ruijter et al., 2003). Class II HDACs often shuttle between the nucleus and the cytoplasm and in the case of HDAC4 and 5 are important in cardiac myocyte development (De Ruijter et al., 2003). The regulation of this shuttling process is linked to cellular kinase signaling networks changing HDAC phosphorylation and association with 14–3–3 docking proteins (De Ruijter et al., 2003; Fischle et al., 2001). Deacetylation of histones increases the winding of DNA around histone residues, resulting in a dense chromatin structure and reduced access of transcription factors to their binding sites, thereby leading to repressed transcription of inflammatory genes (Urnov and Wolffe, 2001).

However, the simple model described above does not tell the full story. Under resting conditions, less than half of the potential lysine residues available for acetylation are in fact acetylated, and these residues have a rapid turnover (Waterborg, 2000). This situation suggests that even small changes above or below the resting level are enough to lead to an activated chromatin state. Furthermore, this model predicts that changes in the "histone code" (Jenuwein and Allis, 2001) must be translated into downstream events extremely rapidly (Waterborg, 2000). The "histone code" refers to the diverse range of histone tail post-translational modifications such as acetylation, methylation, phosphorylation and ubiquitination which are set and maintained by histone-modifying enzymes and contribute to co-activator recruitment and subsequent increases in transcription.

3. Nuclear factor-κB

Although numerous different pathways are activated during the inflammatory response, NF-κB is thought to be of paramount importance in asthmatic inflammation because it is activated by all the stimuli considered important in the inflammatory response to allergen exposure (Baldwin, 2001). In addition, it is a major target for glucocorticoids (Barnes and Karin, 1997). NF-κB is ubiquitously expressed within cells, and it not only controls induction of inflammatory genes in its own right but also enhances the activity of other cell- and signal-specific transcription factors (Baldwin, 2001; Ohmori et al., 1997).

NF-κB is activated by numerous extracellular stimuli, including cytokines such as tumor necrosis factor (TNF)-α and interleukin-1β, viruses, and immune challenges (Baldwin, 2001). Activation of cell-surface receptors leads to phosphorylation of receptor-associated kinases (Baldwin, 2001), which in turn phosphorylate the inhibitors of NF-κB kinase (IKKs). Phosphorylation of IKKs results in phosphorylation of the NF-κB cytoplasmic inhibitor (IκBα), so that IκBα is targeted for proteosomal degradation. This degradation precipitates the release of NF-κB from its inactive state, enabling nuclear translocation and binding to specific DNA response elements within the regulatory regions of responsive genes (Baldwin, 2001).

NF-κB is predominantly composed of the p50/p65 heterodimer (Baldwin, 2001). Subtle changes in p65 phosphorylation are influential; for example, inactive p65

is nonphosphorylated 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). Interleukin-1β can also activate other pathways, distinct from NF-κB, which can impinge on NF-κB activation (Nasuhara et al., 1999). These additional pathways, such as protein kinase C and nonreceptor tyrosine kinases, may enhance NF-κB activity, either by phosphorylating p65 and thereby enhancing cofactor recruitment (Zhong et al., 2002) or by phosphorylating NF-κB-associated cofactors (Nasuhara et al., 1999).

3.1. NF-κB induces histone acetylation

Cytokines such as TNF-α and interleukin-1β, acting via NF-κB, can induce histone acetylation in both a time- and concentration-dependent manner (Ito et al., 2000). This NF-κB-induced acetylation occurs on histone H4 and H3 with a greater effect on histone H4 and it is directed primarily toward lysine residues 8 and 12 at NF-κB-responsive regulatory elements (Ito et al., 2000). It is not known whether modifications on H2A and H2B occur after NF-κB association/activation. The "histone code" would suggest that even small changes in histone tail modifications could have marked structural changes and allow recruitment of distinct co-activator complexes. Upon DNA binding, NF-κB recruits a large coactivator complex that contains the HAT proteins CBP and p300/CBP-associated factor. Several other HATs have been reported to be associated with NF-κB, including transcriptional intermediary factor-2 (TIF-2), also known as glucocorticoid receptor interacting protein-1 (GRIP)-1; p300; and members of the p160 family and steroid receptor coactivator-1 (SRC-1) (Jenkins et al., 2001).

The histone deacetylase inhibitor trichostatin A has been reported to enhance NF-κB-driven inflammatory gene transcription in a number of cell lines (Ito et al., 2000; Ashburner et al., 2001; Chen et al., 2001; Zhong et al., 2002). Two major mechanisms for this effect have been proposed. In the first case, it has been reported that NF-κB has an associated HDAC when bound to DNA that acts as a break on the ability of NF-κB to activate local HAT activity. Inhibition of this associated HDAC leads to increased local HAT activity and elevated inflammatory gene transcription (Ito et al., 2000; Ashburner et al., 2001; Zhong et al., 2002). Chen et al. (2001) have proposed an alternative mechanism. Here, HDAC3 can modify NF-κB nuclear-cytoplasmic shuttling and association with IκBα resulting in enhance nuclear retention of activated NF-κB that is insensitive to inactivation by IκBα. More recently using overexpression systems, it has been suggested that IκBα can sequester HDAC1 and HDAC3 in the cytoplasm enhancing NF-κB activity (Viatour et al., 2003).

3.2. Temporal association of NF-κB with DNA, cofactors, and gene induction

This model described above predicts that activation of NF-κB results in NF-κB binding to κB sites in the

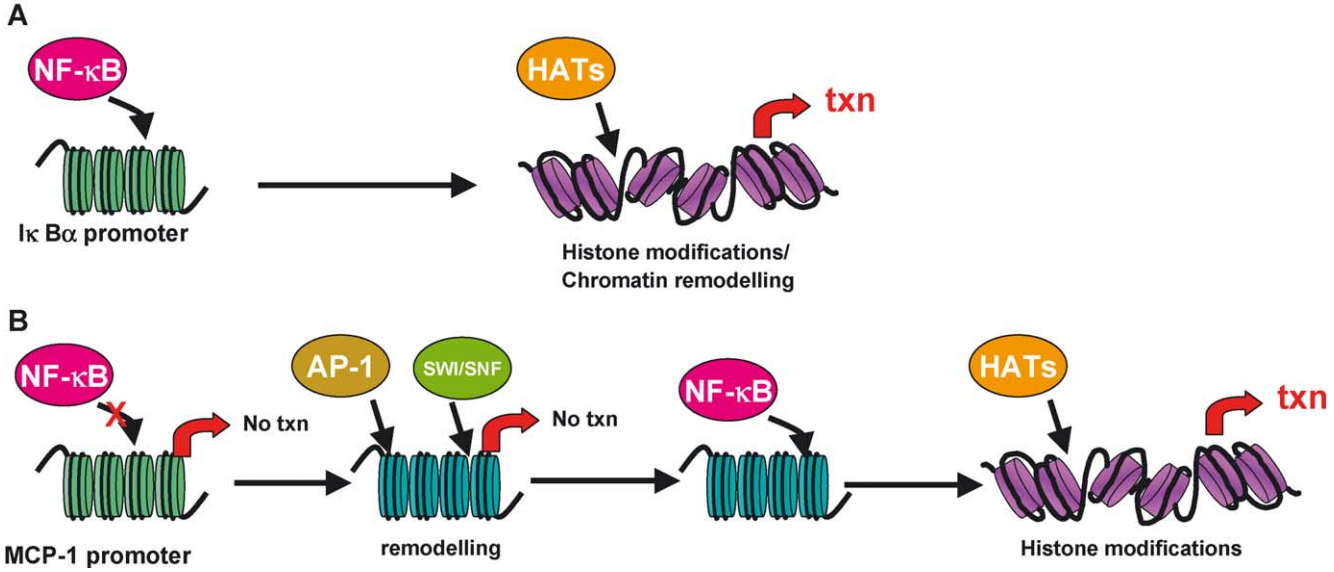

Fig. 2. NF-κB activation of inflammatory genes. (A) Simple model of NF-κB activation of IκBα gene expression. Activation of NF-κB by exogenous stimuli results in NF-κB binding to κB-sites in the regulatory regions of the IκBα promoter, subsequent recruitment of HATs, and chromatin modification, leading to chromatin remodeling and induction of gene transcription (txn). In the case of IκBα, NF-κB is rapidly ejected from DNA within 10 min. (B) A more complex model accounts for the fact that many genes, such as monocyte chemoattractant protein-1 (MCP-1), do not show NF-κB promoter binding for up to 2 h following LPS stimulation of NF-κB nuclear translocation, and no transcription of these genes occurs during this time. MCP-1 transcription requires AP-1 binding to its consensus site within the MCP-1 regulatory domain and subsequent ATP-dependent chromatin remodeling using the SWI/SNF complex to reveal the NF-κB DNA binding site. Once the regulatory regions are remodeled, NF-κB is able to bind to DNA, recruit HATs, and drive MCP-1 gene expression.

regulatory regions of inflammatory genes, subsequent recruitment of HATs and chromatin modification leading to chromatin remodeling and induction of gene transcription. However, in a series of studies using chromatin immunoprecipitation assays, Saccani et al. (2001) have shown that the simplistic model described here previously needs modification. Immediate early genes such as IκBα do indeed bind NF-κB to their promoters rapidly after lipopolysaccharide stimulation, but within 10 min NF-κB dissociates from the IκBα promoter site and never reassociates. In contrast, NF-κB binds to its promoter sites in DNA for up to 2 h before dissociation in distinct sets of genes (manganese superoxide dismutase and macrophage inflammatory protein-2), in spite of stimulation by lipopolysaccharide at the same time. However, other NF-κB-regulated genes, such as RANTES (the chemokine regulated on activation, normal T cell expressed and secreted), monocyte chemoattractant protein-1, and IL-6, do not show NF-κB binding to their promoters until 2 h after activation. NF-κB sites in the promoter regions of these genes are originally in a repressed chromatin environment that prevents NF-κB DNA binding and subsequent gene expression. These become accessible only after AP-1-mediated histone acetylation and subsequent alteration in the local nucleosomal structure (Saccani et al., 2001) (Fig. 2). Thus, there are subtle changes in NF-κB DNA binding that are promoter context-dependent, precede coactivator recruitment, and are not detectable using conventional band shift and reporter gene assays.

3.3. Acetylation of transcription factors can modify their activity

Many studies have used histone deacetylases inhibitors such as trichostatin A, butyrate and Suberoylanilide hydroxamic acid as a means of implicating changes in histone acetylation with changes in gene transcription. However, recently several reports have shown a paradoxical suppression of gene expression by these agents (Nair et al., 2001; Tong et al., 2004; Kim et al., 2001). It has become clear that histones are not the only targets for acetylases such as CBP/p300 and recent evidence has suggested that acetylation of transcription factors can modify their activity (Gu and Roeder, 1997; Barlev et al., 2001). For example, the tumour suppressor p53 can be acetylated by p300 resulting in enhanced DNA binding (Gu and Roeder, 1997) on the p21 promoter and further leads to increased histone H3 and H4 acetylation (Barlev et al., 2001). As described above, the p65 component of NF-κB can also be acetylated thus modifying its transcriptional activity (Chen et al., 2001). Other transcription factors reported to have altered DNA binding or transcriptional activity following acetylation include GATA-1 (Boyes et al., 1998), c-Jun (Vries et al., 2001), YY1 (Yao et al., 2001), MyoD (Polesskaya et al., 2001), E2F (Marzio et al., 2000) and Sp3 (Braun et al., 2001). It is clear therefore, that acetylation of transcription factors can alter interactions between these factors and DNA and among different transcription factors, and is an integral part of transcription and differentiation processes. These data emphasise the fact that the activity of transcription factors can be regulated by post-transcriptional modifications and the need to confirm that inhibitors are working as expected.

4. Glucocorticoid-induced gene transcription

Glucocorticoids exert their effects by binding to a cytoplasmic glucocorticoid receptor that has several functional domains, including a ligand binding domain, a DNA binding domain, and two domains that are involved in transactivation of genes once binding to DNA has occurred via association with other proteins (activation function-1 and -2) (Karin, 1998). An inactive glucocorticoid receptor is bound to a protein complex that includes two subunits of the heat shock protein hsp90, which thus act as molecular chaperones, preventing the nuclear localization of the unoccupied glucocorticoid receptor (Karin, 1998). Once the ligand binds to the glucocorticoid receptor, hsp90 dissociates, allowing the nuclear localization of the activated glucocorticoid receptor-steroid complex, its binding as a dimer to glucocorticoid response elements, and its interaction with coactivator complexes (Karin, 1998). The degree of glucocorticoid receptor nuclear localisation is a critical factor in determining the level of glucocorticoid receptor function.

Glucocorticoids produce their effect on responsive cells by activating the glucocorticoid receptor to directly or indirectly regulate the transcription of target genes (Karin,

Table 1
Glucocorticoid-sensitive genes

Decreased transcription	Increased transcription
•Chemokines IL-8, RANTES, macrophage Inflammatory protein-1α, monocyte chemoattractant protein (MCP)-1, MCP-3, MCP-4, eotaxin •Cytokines Interleukins-1, 2, 3, 4, 5, 6, 9, 11, 12, 13, 16, 17, 18, TNF-α, granulocyte macrophage colony-stimulating factor, stem cell factor •Inducible enzymes Inducible nitric oxide synthase, cyclooxygenase-2, cytoplasmic phospholipase A_2 •Endothelin-1 receptors Neurokinin NK_1-receptors, NK_2-receptors •Adhesion molecules Intercellular cell adhesion molecule-1, E-selectin	•Lipocortin-1/annexin-1 (phospholipase A_2 inhibitor) •Clara cell protein (CC10, phospholipase A_2 inhibitor) •β_2-adrenoceptor •Secretory leukocyte inhibitory protein (SLPI) •IL-1 receptor antagonist •IL-1R2 (decoy receptor) •IκBα (inhibitor of NF-κB) •CD163 (scavenger receptor) •MAP Kinase phosphatase 1 (MKP-1)

1998). The number of genes per cell directly regulated by glucocorticoids is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors and coactivators. It is unlikely that the widespread anti-inflammatory actions of glucocorticoids could be explained in full by increased transcription of the small numbers of anti-inflammatory genes, such as annexin-1, interleukin-10, and the inhibitor of NF-κB, IκBα (Table 1).

Glucocorticoid receptors, like other transcription factors, increase gene transcription through an action on chromatin remodeling and recruitment of RNA polymerase II to the site of local DNA unwinding, as described above for NF-κB (Fig. 3). The glucocorticoid receptor interacts with CBP and other coactivator proteins, including SRC-1, TIF-2, p300/CBP cointegrator protein, and GRIP-1, that enhance local HAT activity (Jenkins et al., 2001; Rosenfeld and Glass, 2001). Dexamethasone at concentrations ($\geq 10^{-8}$ M) in A549 cells enhances binding of activated glucocorticoid receptor to CBP and/or associated coactivators, resulting in histone acetylation on lysines 5 and 16 of histone H4 recruitment of the activated transcription complex, RNA polymerase II and subsequently increased gene transcription (Ito et al., 2000). Furthermore, recent data from Li et al. (2003) show that differential recruitment of coactivators by nuclear receptors determines the assembly of coactivator complexes on glucocorticoid receptor target promoters, such as the highly inducible mouse mammary tumour virus (MMTV) promoter, resulting in acetylation of distinct lysine residues at the promoter start site.

The question arises: how can the glucocorticoid receptor, or any other transcription factor, interact with its recognition site when DNA is compacted? The glucocorticoid receptor may bind to a glucocorticoid response element within the linker DNA between nucleosomes or, alternatively, when the glucocorticoid response element is wound around histones, as long as the core residues are facing outward (Belikov et al., 2001). Binding to the glucocorticoid response element may then modify the local chromatin structure, altering glucocorticoid receptor access. High-resolution mapping of glucocorticoid receptor interactions with the MMTV-long terminal repeat (MMTV-LTR) in Xenopus oocytes suggests that the glucocorticoid receptor not only reorganizes the chromatin immediately surrounding its binding site but also can have effects elsewhere, thereby enforcing a particular translational frame on the chromatin template and modifying the effects of other DNA binding proteins (Belikov et al., 2001).

One other important question that needs to be addressed is whether there is a specific order of recruitment of distinct factors to the activated glucocorticoid receptor complex to gene transcription. Recent work examining androgen and thyroid hormone receptor gene activation shows that nuclear hormone receptors do not in themselves recruit all the cofactors required at the target promoters (Huang et al., 2003). Steroid receptor coactivators, recruited by receptors,

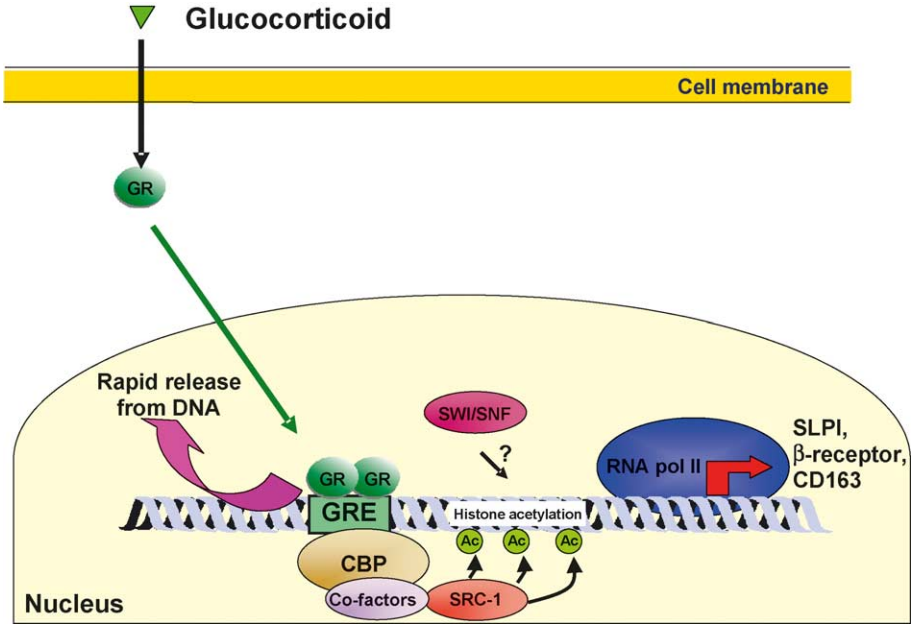

Fig. 3. How glucocorticoids switch on anti-inflammatory gene expression. Glucocorticoids bind to cytoplasmic glucocorticoid receptors (GR) that translocate to the nucleus, where they bind to glucocorticoid response elements (GREs) in the promoter region of glucocorticoid-sensitive genes. This leads to recruitment and activation of transcriptional coactivator molecules such as CBP, SRC-1, or other cofactors that have intrinsic HAT activity. This, in turn, results in acetylation of specific lysine residues on core histone proteins. Chromatin modification leads to local unwinding of the DNA structure, allowing recruitment of large protein complexes, including RNA polymerase II (RNA pol II). It is unclear whether chromatin remodeling by SWI/SNF is essential for this process. The process results in activation of genes encoding anti-inflammatory proteins, such as secretory leukoprotease inhibitor (SLPI), β_2-adrenoceptors, and CD163. As an added complication, it is now clear that DNA binding by the GR is transient, lasting only seconds, while the subsequent changes are prolonged, suggesting epigenetic memory of the GR–GRE binding.

can in turn recruit other coactivators, such as p300/CBP, and p300/CBP can subsequently recruit SWI/SNF (a large multisubunit protein complex) and mediator complexes. SWI/SNF enables chromatin remodeling to occur in an adenosine triphosphate (ATP)-dependent manner, but histone acetylation by p300/CBP facilitates the recruitment of SWI/SNF and mediator complexes. Thus, cofactor–cofactor interactions are essential for effective gene expression. The interactions do not have to occur sequentially, but histone acetylation can enhance the recruitment of large multiprotein complexes in a coordinated manner (Huang et al., 2003).

However, again the story must be more complex. The intriguing data of McNally et al. (2000) provide clear evidence in vitro that the glucocorticoid receptor has a "hit-and-run" mechanism of action rather than a stable association with glucocorticoid response element. This group used fluorescence recovery after photobleaching and fluorescence loss in photobleaching to examine green fluorescent protein/glucocorticoid receptor association with a multimer of 200 copies of stably integrated MMTV-LTR. The results showed that the glucocorticoid receptor resided on DNA for less than 10 s before being ejected and replaced by another glucocorticoid receptor. This ejection may allow binding of additional regulatory factors that enhance gene transcription, such as HAT-containing complexes, and may also play a role in feedback regulation. Interestingly, in the absence of ATP and chromatin remodelling factors, the glucocorticoid receptor stably interacts with the MMTV-LTR (Fletcher et al., 2000).

4.1. Switching off inflammatory genes

4.1.1. Cross-talk between the glucocorticoid receptor and other transcription factors

In spite of the ability of glucocorticoids to induce gene transcription, the major anti-inflammatory effects of glucocorticoids are through repression of inflammatory and immune genes. The inhibitory effect of glucocorticoids appears to be due largely to interaction between the activated glucocorticoid receptor and the transcription factors, such as NF-B and AP-1, that mediate the expression of these inflammatory genes (Barnes and Karin, 1997).

An important question is why glucocorticoids switch off only inflammatory genes, as they clearly do not suppress all activated genes and are well tolerated as long-term treatments. It is possible that the glucocorticoid receptor, acting as a monomer, binds only to specific coactivator complexes that are activated by proinflammatory transcription factors, such as NF-κB and AP-1, although we do not understand how this specific recognition occurs. It is possible that the required residency time of glucocorticoid receptor on glucocorticoid response element may be a factor in distinguishing transactivation from transrepression. In this model, low concentrations of glucocorticoids leads to fewer activated glucocorticoid receptors having enough residency on DNA to recruit co-activator complexes and so transcription doesn't occur. In contrast, this requirement for residency does not affect the association between p65 and glucocorticoid receptor, which can therefore occur at low concentrations.

The interaction between proinflammatory transcription factors and the glucocorticoid receptor may result in differing effects on histone acetylation/deacetylation, through one of several mechanisms that are probably not exclusive. The repressive action of glucocorticoids may be due to competition-activated glucocorticoid receptor binding to one of several transcription corepressor molecules, such as nuclear receptor interacting protein-1 and nuclear receptor corepressor-1, which associate with proteins that have differing histone deacetylase activity (Rosenfeld and Glass, 2001). In addition, low concentrations of dexamethasone ($>10^{-9}$ M) can repress this TNFα- and interleukin-1β-stimulated histone acetylation. This occurs by a combination of direct inhibition of CBP-associated HAT activity and by active recruitment of HDAC proteins to the promoter of actively transcribed inflammatory genes (Ito et al., 2000) (Fig. 4). In addition, high concentrations of glucocorticoids can induce HDAC expression in a time-dependent manner and overall, this process results in the deacetylation of histones and repression of inflammatory genes (Ito et al., 2000).

According to the "histone code" (Jenuwein and Allis, 2001), other histone modifications would be expected to play a role in glucocorticoid receptor/NF-κB cross-talk at the level of chromatin. Recent evidence has indicated that the off-switch for NF-κB-mediated inflammatory gene transcription correlates with histone H3 K9 methylation rather than decreased H4 acetylation (Saccani et al., 2002). We have recently shown that suppression of histone methylation blocks some glucocorticoid receptor functions, synergistically with inhibition of histone deacetylases (Kagoshima et al., 2001).

Alternatively, there may be competition between pro- and anti-inflammatory transcription factors for limited amounts of cofactors, such as CBP, resulting in a reduction in the expression of inflammatory genes. However, this does not explain the specificity of the cross-talk between the glucocorticoid receptor and NF-κB or between the glucocorticoid receptor and AP-1. De Bosscher et al. (2000) have cast further doubt on this hypothesis, showing that overexpression of CBP and other cofactors did not affect the glucocorticoid concentration–response curve.

Recent data have suggested a further mechanism for glucocorticoid receptor action (Nissen and Yamamoto, 2000). Serine 2 phosphorylation of the C-terminal repeat region of RNA polymerase II, induced by NF-κB activity at the IL-8 promoter, is reduced by the glucocorticoid receptor without affecting the assembly of the preinitiation complex. This again suggests an action for the glucocorticoid receptor downstream of NF-κB DNA binding; however, here it is acting downstream of coactivator

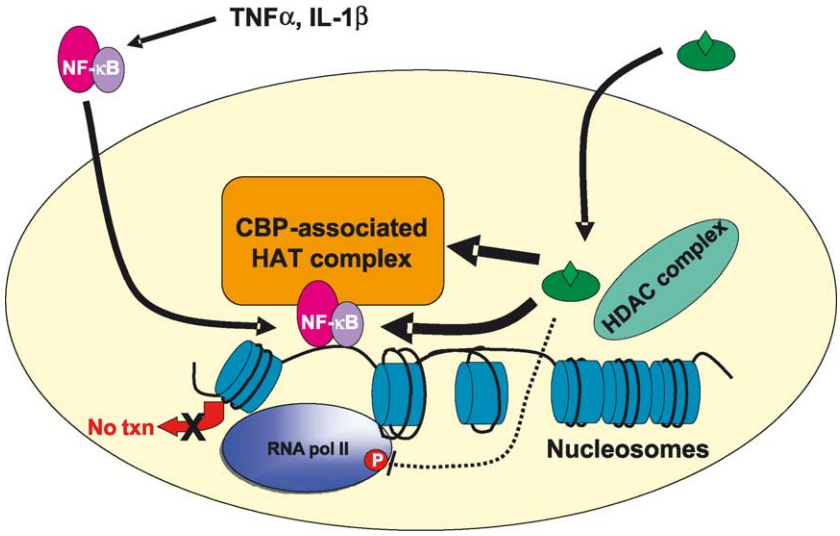

Fig. 4. How glucocorticoids switch off inflammatory gene expression. Many inflammatory genes are activated by stimuli, such as interleukin (IL)-1β or tumor necrosis factor (TNF)-α, and activate NF-κB, which translocates to the nucleus. NF-κB binds to specific κB recognition sites in the promoter regions of responsive genes and subsequently recruits transcriptional coactivators, such as CBP or p300/CBP-associated factor, that have intrinsic HAT activity. This results in acetylation of lysines in core histones, leading to recruitment of large protein complexes, including RNA polymerase II (RNA pol II), and in turn leading to increased transcription of inflammatory genes (txn). Glucocorticoid receptors (GR) translocate to the nucleus after activation by glucocorticoids and act as monomers, reducing histone acetylation. The activated GR monomer interacts with and inhibits the HAT activity of coactivator complexes. In addition, the GR is able to recruit HDAC to the NF-κB complex, leading to suppression of inflammatory genes (no txn). Furthermore, the GR may also be able to reduce phosphorylation of serine 2 residues within the C-terminal repeat region of RNA polymerase II, reducing its capacity to cause mRNA elongation and re-initiation.

function and the kinase/phosphatase target of the glucocorticoid receptor in unknown.

Full inflammatory gene expression probably requires that a number of transcription factors act together in a coordinated manner, and repression of a single transcription factor may only partially modify the full response. Glucocorticoids may be able to reduce inflammatory gene expression by repressing downstream targets of transcription factor activation, irrespective of the precise activated transcription factors involved.

The importance of cross-talk in glucocorticoid receptor actions is indicated by the construction of a glucocorticoid receptor dimerization-deficient mutant mouse in which the glucocorticoid receptor is unable to dimerize and therefore bind to DNA, so that the transactivation and transrepression activities of glucocorticoids are separated (Reichardt et al., 2001). These animals survive to adulthood, in contrast to glucocorticoid receptor-knockout animals. In these animals, dexamethasone was able to inhibit AP-1-driven and NF-κB-driven gene transcription, but the ability to facilitate glucocorticoid response element-mediated effects such as cortisol suppression and T-cell apoptosis was markedly attenuated. This suggests that it will be possible to develop glucocorticoids with a greater therapeutic window.

In addition, corticosteroids may also play a role in repressing the action of mitogen-activated protein kinases (MAPK)s such as the extracellular regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) (Swantek et al., 1997; Caelles et al., 1997; Rider et al., 1996; Hirasawa et al., 1998). Thus, Caelles et al. have demonstrated that corticosteroids inhibit 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. More recently, it has been shown that dexamethasone can rapidly induce the dual specificity MAPK phosphatase MKP-1 and thereby attenuate p38 MAPK activation (Lasa et al., 2001; Lasa et al., 2002; Kassel et al., 2001). Rogatsky et al. (1998) have in turn shown reciprocal inhibition of rat glucocorticoid receptor reporter gene activity by JNKs by a direct phosphorylation of serine 246 whereas ERK can inhibit glucocorticoid receptor action by an indirect effect possibly through phosphorylation of a co-factor.

4.2. Non-genomic actions of glucocorticoids

The traditional genomic theory of glucocorticoid action, whether directly interacting with DNA or involving cross-talk with other transcription factors, does not fully explain some very rapid effects of glucocorticoids, and it is thought that some nongenomic actions are mediated by a distinct membrane receptor (Norman et al., 2004; Chen and Qiu, 1999). Initially described in amphibians, these receptors have subsequently been described in mammalian cells and has distinctive hormone binding properties compared to the well-characterized cytoplasmic receptor and is probably linked to a number of intracellular signaling pathways

acting through G-protein coupled receptors and a number of kinase pathways (Chen and Qiu, 1999; Norman et al., 2004; Powell et al., 1999; Evans et al., 2000). There are a number of reviews providing a summary of the evidence for these rapid effects seen and, of these, one important in respiratory disease may include changes in bronchial blood flow induced by inhaled glucocorticoids (Mendes et al., 2003). In addition, the classical receptor is associated with a number of kinases and phosphatases within the inactive glucocorticoid receptor/hsp90 complex (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., 2002; Croxtall et al., 2000).

4.3. Effects of long acting β-receptor agonists on glucocorticoid receptor function

Long-acting β_2-adrenoceptor agonists (LABAs) and glucocorticoids are the two most effective treatments for asthma and are more potent in combination than either drug alone (Barnes, 2002; Nelson et al., 2003). Whereas glucocorticoids are used to treat airway inflammation, LABAs are used as bronchodilatory agents to bring rapid relief of airway bronchoconstriction (Barnes, 2002). LABAs do not have major anti-inflammatory actions in themselves in vivo as opposed to their potent effects in vitro (Johnson, 2002), suggesting that the added benefit of combination therapy probably relates to cross-talk between the two drugs.

In an important in vitro study, Eickelberg et al. (1999a,b) found that in primary human lung fibroblasts and vascular smooth muscle cells both salbutamol and salmeterol could induce glucocorticoid receptor nuclear translocation and enhance glucocorticoid receptor-glucocorticoid response element binding in the absence of ligand. Translocation of glucocorticoid receptor by β_2-agonists was less effective than that seen with dexamethasone and was protein kinase A (PKA) dependent. This study has since been confirmed in preliminary reports in vivo (Adcock et al., 2002; Usmani et al., 2001, 2002), which have indicated that salmeterol can induce glucocorticoid receptor nuclear translocation and that this may prime the receptor to be more responsive to a concomitant or subsequent challenge with glucocorticoid. Salmeterol also enhanced fluticasone repression of NF-κB reporter genes (Adcock et al., 2002). We have proposed that phosphorylation of glucocorticoid receptor and glucocorticoid receptor-associated factors may alter glucocorticoid receptor nuclear translocation of the unliganded receptor, but more importantly it may alter cofactor recruitment (Adcock et al., 2002). In addition, the activity of these cofactors may be enhanced by PKA and MAPK pathways (Adcock et al., 2002). Functionally, this enhances glucocorticoid receptor responsiveness to both transactivation and transrepression actions and leads to modification in proinflammatory and anti-inflammatory mediator release. In vivo studies have also suggested another mechanism for glucocorticoid receptor activity in that salmeterol and fluticasone can act together to enhance nuclear export of the Th2-specific transcription factor GATA3 (Maneechotesuwan et al., 2002).

5. Therapeutic implications

Inhaled glucocorticoids are now used as first-line therapy for the treatment of persistent asthma in adults and children in many countries, as they are the most effective treatments for asthma currently available (Barnes and Adcock, 1998). However, at high doses systemic absorption of inhaled glucocorticoids may have deleterious effects, so there has been a search for safer steroids for inhalation and even for oral administration.

5.1. Dissociated glucocorticoids

Whilst the major anti-inflammatory effects of corticosteroids are almost certainly due to transrepression, the underlying molecular mechanisms for the side-effects of glucocorticoids are complex and not fully understood (Schacke et al., 2002a,b). Certain side effects such as diabetes and glaucoma are due to transactivation events whilst others are due transrepression (Hypothalamic–Preoptic Area–Adrenal [HPA] suppression). In addition, the precise molecular events underlying glucocorticoid induction of osteoporosis is unclear but probably requires both gene induction and gene repression (Schacke et al., 2002a,b). In mice with glucocorticoid receptor that do not dimerize, there is no transactivation, but transrepression appears to be normal (Reichardt et al., 1998, 2001).

Despite this uncertainty, there has been a search for "dissociated" glucocorticoids that selectively transrepress without significant transactivation, thus potentially reducing the risk of systemic side effects. Several non-steroidal "selective glucocorticoid receptoragonists" (SEGRA) have recently been reported that show dissociated properties in human cells and are now in clinical development where they show good separation between transrepression and transactivation actions in the skin (Schacke et al., 2002a,b, 2004). This suggests that the development of glucocorticoids and SEGRA with a greater margin of safety is possible and may even lead to the development of oral compounds that have reduced adverse effects. Furthermore, the newer topical glucocorticoids used today, such as fluticasone, mometasone and budesonide, appear to have more potent transrepression than transactivation effects, which may account, at least in part, for their selection as potent anti-inflammatory agents (Adcock et al., 1999). This suggests that the development of glucocorticoids with a greater margin of safety is possible and may even lead to the development of oral glucocorticoids that do not have significant adverse effects. The recent resolution

of the crystal structure of the glucocorticoid receptor may help in better design of dissociated glucocorticoids (Bledsoe et al., 2002).

5.2. Other approaches to anti-inflammatory therapy

The elucidation of the molecular mechanisms of glucocorticoids raises the possibility that novel non-steroidal anti-inflammatory treatments might be developed that mimic the actions of glucocorticoids on inflammatory gene regulation. Inhibition of specific HATs activated by NF-κB may prove to be useful targets, especially if they also repress the action of other proinflammatory transcription factors (Turlais et al., 2001). Alternatively, activation of HDACs may have therapeutic potential, and theophylline has been shown to have this property, resulting in marked potentiation of the anti-inflammatory effects of glucocorticoids both in vitro and in vivo (Ito et al., 2002). This action of theophylline is not mediated via phosphodiesterase inhibition or adenosine receptor antagonism and, therefore, appears to be a novel action of theophylline (Ito et al., 2002). It may be possible to discover similar drugs that could form the basis of a new class of anti-inflammatory drugs without the side effects that limit the use of theophylline (Ito et al., 2002).

Many of the anti-inflammatory effects of glucocorticoids appear to be mediated via inhibition of the transcriptional effects of NF-κB, and small-molecule inhibitors of IKK-2, which activate NF-κB, are in development. However, glucocorticoids have additional effects, so it is uncertain whether IKK-2 inhibitors will parallel the clinical effectiveness of glucocorticoids. They may have side effects, such as increased susceptibility to infections, however as a corollary to this if glucocorticoids were discovered today, they would be unlikely to be used in humans because of the low therapeutic ratio and their side effect profile.

6. Conclusion

Advances in delineating the fundamental mechanisms of gene transcription, especially recruitment of histone-modifying cofactors, have resulted in better understanding of the molecular mechanisms whereby glucocorticoids suppress inflammation. The challenge is to see if these mechanisms hold true in primary cells in vivo. This will undoubtedly lead to the development of drugs that target novel aspects of GR function and potentially restore glucocorticoid sensitivity to diseases that are unresponsive to current therapeutic strategies.

Acknowledgments

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