**REVIEW ARTICLES**

**Molecular Mechanisms of Glucocorticoids**

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**Introduction**

Inflammatory processes are central to many respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and many types of diffuse interstitial lung disease. The specific characteristics of the inflammatory response and inflammation site vary according to the disease, but recruitment and activation of different inflammatory cells and changes in structural cells of the lung are implicated in all of them. Glucocorticoids are currently the most potent antiinflammatory drugs available. In fact, glucocorticoids, whether administered systemically or by inhalation, are the first-line drugs in the treatment of many of these inflammatory processes.

Many pharmacological applications have been found since the first molecule with glucocorticoid activity was identified in 1937, and the chemical properties of these molecules have been constantly improved. Their antiinflammatory properties in the treatment of rheumatoid arthritis were first discovered in the 1950s. We have only recently, however, made progress in understanding the mechanism of action of these drugs thanks to the development of new molecular biology techniques.

The aforementioned diseases are characterized by increased expression of a variety of proteins such as cytokines, chemokines, inflammatory enzymes, receptors, and adhesion molecules implicated in complex inflammatory cascades. Upregulation of these inflammatory proteins is caused by greater transcription of inflammatory genes which are not normally expressed by the affected cells. The expression of these inflammatory genes during inflammatory processes is specific to each cell. A better understanding of the mechanism of action of these drugs would help rationalize their use and favor the development of new compounds with specific effects.

**Glucocorticoids: Structure and Function**

Most of the glucocorticoid activity is associated with cortisol (or hydrocortisone). The use of cortisone in the treatment of rheumatoid arthritis earned Hensch the Nobel Prize for Medicine in 1950. This same year, cortisone started to be used in the treatment of asthma. At present, clinicians have at their disposal many types of glucocorticoid molecule, most of which are synthetic. The chemical structures of these molecules are based on natural corticosteroids, but with changes to optimize local antiinflammatory potency. For example, the molecule may be tailored to increase liposolubility to favor uptake by tissues to lower systemic bioavailability which, in turn, minimizes adverse reactions. Some of these molecules, such as budesonide, beclomethasone dipropionate, triamcinolone acetate, fluticasone propionate, flunisolide, or mometasone furoate have been widely used to treat inflammatory diseases of the airways such as asthma or rhinitis. They are essentially derived from modifications to the D ring of the cortisol molecule. Furthermore, better understanding of the mechanism of action of glucocorticoids, particularly regarding regulation of gene transcription and the structure of the glucocorticoid receptor (GR) and binding sites, has allowed new glucocorticoid molecular structures to be designed with dissociated properties. Such molecules might be of great clinical importance in the future and we will deal with them in more detail in this review.

Glucocorticoids are the most potent and effective drugs for preventing and suppressing inflammation caused by mechanical, chemical, infectious, and immunological stimuli. When inhaled glucocorticoids were introduced, they revolutionized the treatment of asthma because of their efficacy and safety. Some patients, however, respond poorly or not at all to antiinflammatory treatment. This small minority of asthma patients (<5%-7%) who are “corticosteroid resistant” or “corticosteroid dependent” present the biggest challenge because of the resulting health care costs and clinical complications and the limited number of effective therapeutic alternatives available. In COPD, on the other hand, use of inhaled glucocorticoids provides little clinical benefit and does not prevent
There are also phosphorylation sites and regions of hormone-independent activation function (AF2) related to transcription. The glucocorticoid receptor protein has 3 domains: the amino terminus, the DNA binding domain (DBD), and the carboxyl terminus for hormone binding. Two zinc fingers are located in the central region. There are also phosphorylation sites and regions of hormone-independent activation function (AF2) related to transcription.

Figure 1. Functional structure of the glucocorticoid receptor. The glucocorticoid receptor protein has 3 domains: the amino terminus, the DNA binding domain (DBD), and the carboxyl terminus for hormone binding. Two zinc fingers are located in the central region. There are also phosphorylation sites and regions of hormone-independent activation function (AF2) related to transcription.

**Glucocorticoid Receptor**

Glucocorticoids act by binding to a specific intracytoplasmic GR. The genetic code for the GR is located on the long arm of chromosome 5 (region 5q31-32). The genomic structure comprises 9 exons and there is evidence of 3 different gene promoters. Although the reason why different promoters are used in different cell types is not clear, it might be related to different forms of specific GR regulation according to cell type.

The GR belongs to a superfamily of receptors that also includes the mineralocorticoid, thyroid hormone, sex hormone, retinoic acid, and vitamin D receptors. All these receptors have a common DNA binding site—a short central region flanked by a variable N-terminal (or amino terminal) domain and a relatively variable C-terminal (or carboxyl terminal) domain (Figure 1). The N-terminus contains the hormone-independent activation function (AF1) domain, which has been associated with transcriptional activity and binding to coactivator proteins and transcriptional factors. The C-terminus, on the other hand, contains the AF2 domain, which is responsible for hormone binding, though growing evidence suggests that this region can also interact with other factors and coactivators implicated in gene transcription.

In addition to the humoral immune response, glucocorticoids have important effects on cell response. Glucocorticoids can shorten survival of eosinophils and reduce the number of (antigen-presenting) dendritic cells—effects which are partly responsible for the antiinflammatory effect observed in allergies. Glucocorticoids inhibit plasma exudation and glandular mucus secretion, and also suppress other cells such as lymphocytes or basophils, especially when used for long periods or at high doses. Other cell types, such as macrophages or neutrophils, do not seem to be influenced in vivo by topical treatments of rhinitis and asthma; thus the antibacterial response appears unaffected. Glucocorticoids also act on other cell groups such as endothelial cells (regulating permeability) and epithelial or glandular cells (inhibiting mucus secretion).5,11

The GR has two different molecular isoforms, GRα formed from 777 amino acid units and GRβ formed from 742 amino acid units.12 Both isoforms have been found together in almost all human tissue. GRα is the predominant isoform, the only one capable of binding the hormone, and the only one able to induce or suppress gene expression. GRβ is formed by alternative splicing of the GR pre-RNA transcript and only differs from the α isoform in the last amino acids of the C-terminus (Figure 2). This difference might be sufficient to prevent GRβ from binding to the hormone. The possibility that upregulation of the β isoform might strongly inhibit the active isoform by a competitive
mechanism and so reduce the efficacy of glucocorticoid has been the subject of a scientific debate about the true importance of the $\beta$ isoform in clinical response to glucocorticoids. However, the contradictory data obtained, the different methods used, and the great predominance of $\alpha$ isoform compared to the $\beta$ isoform have brought into question the functional importance of the $\beta$ isoform.

Inactive GR in the cytoplasm is bound by the C-terminus to an oligomeric complex of proteins that include 2 subunits of 90 kDa heat shock protein (hsp90), immunophilin p59, and the small phosphoprotein, p23. Interaction between the GR and hsp90 is important to mask the nuclear localization signal, necessary for subsequent migration of activated GR to the nucleus, and to conserve the configuration of the C-terminal hormone-binding domain. Moreover, hsp90s and other proteins associated with the receptor are probably necessary to ensure correct maturation of recently synthesized GRs.

A GR is released from its interactions with hsp90s on hormone binding. The receptor undergoes a conformational change and so becomes activated. The receptor then translocates to the cell nucleus where it binds as a dimer to DNA at its central domain. The central domain of the GR responsible for DNA binding consists of 2 zinc fingers. Nevertheless, new evidence suggests that cytoplasm-to-nucleus translocation of the GR also occurs by the nuclear localization signal without hormone binding.

The DNA binding domains are palindromic sequences of 15 base pairs denoted glucocorticoid response elements (GRE; GGTACAnnnTGTTCT). These are located in the 5' promoter region of the target genes. The interaction of GR-glucocorticoid dimers with the DNA double helix in these GRE regions and with certain coactivators will lead to induction or repression of gene transcription (transactivation). The interaction of a single activated GR homodimer with GRE can normally enhance transcription and so more protein is synthesized. In any case, it is still not entirely understood how part of this process can vary according to glucocorticoid dose, type of glucocorticoid, or the type of cell in which it is acting. The binding of glucocorticoid-GR complexes to DNA seems at least partly responsible for the endocrine actions of glucocorticoid, which include side effects such as osteoporosis, growth retardation in children, and metabolic disorders. However, the molecular mechanisms responsible for these effects are still not well understood.

Between 10 and 100 genes are thought to be directly regulated by glucocorticoids. There is evidence that the glucocorticoid-GR complex can also act by indirect gene regulation through induction of the synthesis of antiinflammatory proteins or, more importantly, through transrepression mechanisms. These mechanisms include direct inhibition of proinflammatory transcriptional factors such as nuclear factor-$\kappa$B (NF-$\kappa$B) or activator protein 1 (AP1), destabilization of enzymes, such as mitogen-activated protein kinases (MAPKs), involved in gene expression, and cell proliferation during inflammatory processes. Alternatively, as discussed later, the glucocorticoid-GR complex can participate in the recruitment and activation of histone acetyltransferase (HAT) and histone deacetylase (HDAC), both of which are responsible for the configuration of chromatin.

### Molecular Mechanisms of Action

#### Repression of Inflammatory Gene Transcription

We have yet to determine the most critical aspects of the antiinflammatory properties of the glucocorticoids, but it is clear that inhibition of cytokine and chemokine synthesis is particularly important. Glucocorticoids suppress synthesis of several cytokines and
activity the cyclic AMP response element-binding (CREB)
κB and AP1 bind to large coactivator molecules such as
and HDAC.
transcription and is regulated by enzymes such as HAT
of the K residues correlates with activation of
lysines (K) where acetylation takes place. Acetylation
termini of the histones have alternately conserved
are formed from an octomer of 4 histones. The N-
Chromatin is made up of nucleosomes, which in turn
inhibit the production of osteocalcin by a GRE that
promoter sequences, suggesting that we should look for
less direct inhibitory mechanisms. The osteocalcin gene
is an example of a negative GRE. Glucocorticoids
blocks binding of the transcription complex to the
DNA; therefore no messenger RNA is produced.19
The inhibitory effect of glucocorticoids seems to be
largely secondary to protein-protein interactions
between activated GR and nuclear transcription factors
such as AP1,20 NF-κB,21 or some signal transducer and
activator of transcription (STAT) proteins such as
STAT3,22 STAT5,23 and STAT6.24 Recent studies suggest
that glucocorticoids may affect the chromatin structure.
Chromatin structure is dynamic. Thus, the DNA is
tightly coiled around the histone residues of the nucleus
in a resting cell but this compact and inaccessible DNA
becomes more accessible to transcription factors during
cell activation, and gene transcription is initiated.25
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transcription and is regulated by enzymes such as HAT
and HDAC.26 Several transcription factors such as NF-
kB and AP1 bind to large coactivator molecules such as
the cyclic AMP response element-binding (CREB)
binding protein (CBP), which has intrinsic HAT
activity27 and provides a link with the basal

chemokines, such as GM-CSF, interleukin 1β, 4, 5, and
8, and eotaxin, which are of particular importance in
inflammatory lung diseases (Table).18
This inhibition was initially attributed to binding of
the GR to the GREs in DNA thereby repressing
transcription. Few of the genes that are deactivated by
glucocorticoid, however, have negative GRE in their
promoter sequences, suggesting that we should look for
less direct inhibitory mechanisms. The osteocalcin gene
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binding protein (CBP), which has intrinsic HAT
activity27 and provides a link with the basal
transcription machinery and RNA polymerase II to
initiate transcription.28 Binding of transcription factors
to the CBP leads to increased acetylation of histone and
gene transcription (Figure 3). The GR can compete with
the binding sites of other CBP transcription factors or,
alternatively, activate transcriptional corepressors with
HDAC activity. Ito et al29,30 have shown that the
glucocorticoids inhibit p65-associated HAT activity of
NF-κB and that the GR recruits HDAC2 to inhibit
interleukin 1β-induced acetylation of histone H4 at
lysines 8 and 12. This results in deacetylation of the
nuclear histones, and so the configuration of the
chromatin is once again modified, making it coil tightly
around the histones. Access of transcription factors such
as NF-κB and AP1 to the DNA binding sites is hindered
and inflammatory transcription is repressed as a result
(also known as transrepression). In other words, the
mechanism of the antiinflammatory action of
glucocorticoids is 2-fold: inhibition of histone
acetylation mediated by transcriptional factors with
intrinsic HAT activity and recruitment of HDAC to the
transcription sites.
Other nongenomic mechanisms have been proposed,
including antagonism of transcription factors dependent

| TABLE |
| Increased Gene Transcription |
| Lipocortin 1 |
| β2 receptors |
| Serum leukoprotease inhibitor (SLPI) |
| Clara cell protein (CC10, phospholipase A2 inhibitor) |
| IL1 receptor antagonist |
| Nuclear factor-κB inhibitor (IkB-α) |
| IL10 |
| Decreased Gene Transcription |
| Cytokines (IL1, IL2, IL3, IL4, IL5, IL6, IL8, IL11, IL13, tumor necrosis factor α, granulocyte and macrophage colony stimulating factor) |
| Chemokines (RANTES, eotaxin, macrophage inflammatory protein 1α, monocyte chemotactic proteins 1 and 3) |
| Enzymes (inducible nitric oxide synthetase, cyclooxygenase 2, cytoplasmic phospholipase A2, [cPLA2]) |
| Adhesion molecules (intracellular adhesion molecule 1, vascular cell adhesion molecule 1) |
| Receptors (IL2 receptor, tachykinin 1 receptor [NK1]) |

*IL indicates interleukin; RANTES, regulated on activation, normal T-cell–expressed and secreted chemokine.

Figure 3. Effect of glucocorticoids on chromatin structure. Transcription factors such as signal transducer and activator of transcription (STAT), activator protein 1 (AP1), and nuclear-κB (NF-κB) bind to coactivator molecules such as cyclic AMP response element-binding CREB-binding protein (CBP) or p300—both with intrinsic histone acetyltransferase (HAT) activity—leading to acetylation of histone residues. Chromatin therefore uncoils, allowing transcription factors to bind to the chromatin and transcription of inflammatory genes occurs. After activation by glucocorticoids, the glucocorticoid receptor (GR) also binds to CBP, inhibiting the HAT activity of this molecule and histone deacetylase is recruited. Thus the DNA coils once again around the histones, making it inaccessible to transcription factors and transcription is thereby inhibited.

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on the signaling pathway of C-Jun N-terminal kinase (JNK). Support for this mechanism comes from blockade of the activation cascade of JNK signals by inhibition of serine 63 and 73 phosphorylation by the GR.\textsuperscript{31} The GR also inhibits protein synthesis because the half-life of messenger RNA is shortened through enhanced transcription of specific ribonucleases that target adenosine uridine-rich regions in some genes, such as those that regulate GM-CSF\textsuperscript{32} or cyclooxygenase 2.\textsuperscript{33}

**Induction of Antiinflammatory Gene Transcription**

The antiinflammatory action of glucocorticoids also occurs through upregulation of antiinflammatory proteins, such as lipocortin 1, serum leukoprotease inhibitor (SLPI), interleukin 10, and the interleukin 1 receptor antagonist. This effect is mediated by GREs in promoter regions of these genes.\textsuperscript{1} The GR can also increase transcription by binding to coactivator factors such as CBP, which can induce activation of RNA polymerase II, so giving rise to the formation of messenger RNA. Binding between activated GR and CBP also increases acetylation of nuclear histones, essential for activation of RNA polymerase II. For example, elevated concentrations of glucocorticoid increase secretion of SLPI in epithelial cells. This is associated with selective acetylation of lysines 5 and 16 of histone H4.\textsuperscript{29} Glucocorticoids have also been implicated in the increase of NF-κB inhibitor (IκBα), which may induce inhibition of NF-κB in lymphocytes,\textsuperscript{34} though this has not been shown in other cell lines.\textsuperscript{35}

Lipocortin 1 is a 37 kDa protein that inhibits PLA\textsubscript{2} and therefore suppresses the production of lipid mediators such as leukotrienes, prostaglandins, and platelet activating factor in epithelial cells and leukocytes.\textsuperscript{36} The main effect of glucocorticoids was thought to be inhibition of lipocortin, but we now know that the effects of this protein are not very specific\textsuperscript{27} and that the inductive capacity of glucocorticoids varies according to cell type. Other proteins upregulated by glucocorticoids include interleukin 10, the levels of which are reduced in alveolar macrophages of asthma patients but increase with steroid treatment.\textsuperscript{38} and the interleukin 1 receptor antagonist, which inhibits the interleukin 1 inflammatory protein at its cell receptor.

**Other Target Genes**

**Inflammatory enzymes.** Glucocorticoids inhibit the synthesis of nitric oxide synthetase—produced by proinflammatory cytokines—and other enzymes implicated in asthmatic inflammation such as cyclooxygenase 2 and cytosolic PLA\textsubscript{2}.

**Adhesion molecules.** Adhesion molecules play a crucial role in the migration of cells towards the site of inflammation. Many inflammatory cytokines induce expression of adhesion molecules on the surface of endothelial cells, and glucocorticoids may influence this expression either indirectly by downregulating these inflammatory cytokines, or directly through transcription of genes that code for these molecules, such as the intracellular adhesion molecule 1, E-selectin, or vascular cell adhesion molecule 1.\textsuperscript{39}

**Inflammatory receptors.** Glucocorticoids suppress expression of genes that code for some receptors of inflammatory mediators. Thus, glucocorticoids have been shown to inhibit important mediators of inflammation and bronchoconstriction in asthma such as tachykinin receptors (NK1 and NK2 receptors) and bradykinin receptors (B1 and B2).\textsuperscript{40}

**Apoptosis.** Glucocorticoids shorten the half-life of some inflammatory cells such as eosinophils. The survival of these cells depends on the presence of cytokines such as interleukin 5 and GM-CSF.\textsuperscript{41} Glucocorticoids block the synthesis of these cytokines and so trigger their programmed death (apoptosis), but the molecular mechanism of this effect has yet to be elucidated. Glucocorticoids also prolong survival of neutrophils and inhibit apoptosis of these cells.\textsuperscript{42}

**β Adrenoreceptors.** Glucocorticoids enhance expression of β adrenoreceptors, as much as doubling the transcription rate. The effect has been shown in vitro and in vivo,\textsuperscript{43} and may be particularly relevant to asthma as it can prevent tachyphylaxis in patients treated with beta agonists.

**Clinical Implications**

**Dissociated Glucocorticoids**

Many of the antiinflammatory effects of glucocorticoids are due to inhibition of transcription factors (transrepression), whereas the endocrine and metabolic effects are mediated by binding to GREs in DNA (transactivation). This has prompted investigators to search for glucocorticoid molecules with selective transrepressive actions to prevent side effects due to transactivation. The GR must be present in the activated homodimeric form to bind to the GRE in DNA, whereas interaction with transcription factors such as AP1 and NF-κB occurs with monomeric GR. The dissociation of these effects has been demonstrated by creating mutations in the GR in cells that are then infected with a viral vector. Several glucocorticoid molecules such as RU24858, RU486, and ZK98299 showed greater transrepression than transactivation in such cells.\textsuperscript{44} In fact, the antiinflammatory potency of inhaled glucocorticoids currently used for asthma treatment such as fluticasone propionate and budesonide can be explained by their greater transrepressive effect. A new class of glucocorticoids has recently been described. These glucocorticoids have potent transrepressive activity but transactivation is minimal.
Steroids with dissociated effects, such as RU2458, RU40066, or ZK216348, a selective nonsteroidal GR agonist, have potent antiinflammatory activity that is even comparable to that of prednisone. These new steroids also have a much more acceptable profile of adverse effects than the usual glucocorticoids. Thus, the development of new glucocorticoid molecules with a better safety margin should be possible and this might lead to the discovery of oral corticosteroids with no significant adverse effects.

**Glucocorticoid Resistance**

Although glucocorticoids are very effective at controlling inflammation in asthma and other inflammatory and immunological diseases, a small percentage of asthmatic patients do not respond even when high doses of a glucocorticoid are used. Therapeutic doses of glucocorticoids are also not always effective in patients with other diseases such as rheumatoid arthritis or inflammatory bowel disease. Although lack of effectiveness is uncommon, it constitutes an important problem for the clinical management of these patients.

In corticosteroid-resistant asthma, the forced expiratory volume in 1 second and the peak expiratory flow of the patient increase by less than 15% after 2 weeks of treatment with oral prednisolone at doses of 30-40 mg/day. These patients do not present symptoms of Addison disease or abnormal levels of sex hormones, and their plasma cortisol response and adrenal suppression in response to exogenous cortisol are both normal so they suffer from the side effects of glucocorticoids. Total resistance to glucocorticoids is rare—the prevalence is 1 case per 1000 asthma patients. Nevertheless, partial resistance, with an attenuated response to glucocorticoid that requires an increase in dose to control the disease—that is, glucocorticoid-dependent asthma—is much more common.

Several mechanisms have been proposed to explain resistance to glucocorticoids. Monocytes and T lymphocytes isolated from these patients have reduced in vitro response to glucocorticoids. Some of these cells show a decreased affinity of the GR for glucocorticoid, as shown by incubating T cells with interleukin 2 and interleukin 4, leading to functional inhibition of the GR. 

**Conclusions**

Our growing understanding of the molecular mechanisms of action of glucocorticoids has shed light on the pathophysiology of inflammatory processes and also pointed to new lines of investigation for the development of new the antiinflammatory treatments. New drugs, such as MAPK inhibitors, NF-κB inhibitors, or inhibitors of histone acetylation, are being developed that target the interactions of the GR with the proteins implicated in transcription or activation of a variety of transcription pathways. One of the most important consequences of this research into the actions of glucocorticoids is that it can help us understand and modify the complex and potentially synergistic interactions between transcription factors and different activation pathways. This would enable more selective blockade of these interactions at different levels.

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