

Mechanisms of Action of Glucocorticoids in Bronchial Asthma

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ABSTRACT

Asthma is a major health problem and their incidence is increasing and represent a recognized cause of morbidity and mortality. Glucocorticoids are the most effective drugs for asthma. Glucocorticoids suppress inflammation by several mechanisms. Its principal action at therapeutic doses is due to trans-repression of activated inflammatory genes, by the recruitment of the enzyme histone deacetylase-2 and the subsequent remodeling of chromatin. At higher concentration, glucocorticoids act as trans-activators, acetylating histones and stimulating the transcription of anti-inflammatory genes. Eventually, this mechanism could be involved in the activation of genes related to side effects. Post-transcriptional effects that modulate the stability of mRNA have been proved. This mechanism of action is known as genomic. In the last decades, a mechanism of local action with the use of glucocorticoids involving an interaction between glucocorticoids and noradrenaline has been described, through a membrane receptor in the smooth muscle of the blood vessels that reduces hyperperfusion and mucosal edema. This mechanism is known as non-genomic. This chapter discusses and compares both mechanisms by establishing similarities and differences between the two.

INTRODUCTION

The most commonly use of glucocorticoids (**GCs**) in respiratory diseases is in the treatment of bronchial asthma [1]. Inhaled GCs (**IGCs**) have been established as the first-line treatment in adults and children with persistent asthma: the most common chronic inflammatory disease [2,3]. This chapter describes the molecular mechanisms of GCs in bronchial asthma. For purposes of discussion such mechanisms are divided into genomic and non-genomic.

GENOMIC MECHANISM

This mechanism contemplates the action of GCs on the expression, activity and functioning of the genes encoding the synthesis of inflammatory and anti-inflammatory proteins in bronchial asthma.

Inflammation in Asthma: Molecular Basis

Patients with asthma have a specific pattern of airway inflammation, characterized by the presence of degranulated mastocytes, hypodense eosinophils and Th2 lymphocytes. These alterations are responsible for the clinical manifestations of the entity, including intermittent wheezing, dyspnea, cough and chest tightness [4]. GCs control and prevent these symptoms in many patients.

In asthma, approximately 100 inflammatory mediators can be released, including lipid mediators, inflammatory peptides, cytokines, chemokines, inflammatory enzymes, adhesion molecules, and growth factors [5]. Evidence suggests that airway structural cells (eg, epithelial cells, smooth muscle, endothelial cells and fibroblasts) are the major source of these mediators. The expression of inflammatory mediators is regulated by genetic transcription, which in turn is controlled by proinflammatory transcription factors such as NF- κ B (kappa-beta nuclear factor), AP-1 (activating protein-1), NF-AT (activated T-cell nuclear factor) and STATs (transducers of signals and transcription activators) [6,7]. For example, NF- κ B is markedly active in the epithelial cells of asthmatic patients [4,8]. Some of these factors may be activated by infection with rhinovirus and by allergens, potential intensifiers of asthmatic inflammation. These proinflammatory factors should be conceptualized as nuclear messenger proteins [9].

When proinflammatory transcription factors are activated, they bind to specific DNA recognition sequences. In this interaction, coactivating molecules such as p300 / CREB (cyclic AMP response element binding protein), CBP (CREB binding protein), pCAF (P300 / CBP activating factor) and SWI / SNF (SWI / SNF / non-fermentable sucrose) are involved. These molecular complexes have intrinsic histone acetyltransferase (**HAT**) activity, which leads to the expression of the corresponding inflammatory genes [10,11].

Chromatin remodeling

The chromatin structure is critical for the expression and repression of genes. Structurally, chromatin is constructed by units called nucleosomes. In turn, these are constituted by DNA associated with an octamer of histones (2H2A, 2H2B, 2H3, 2H4) and other proteins [12] [Figure 1].

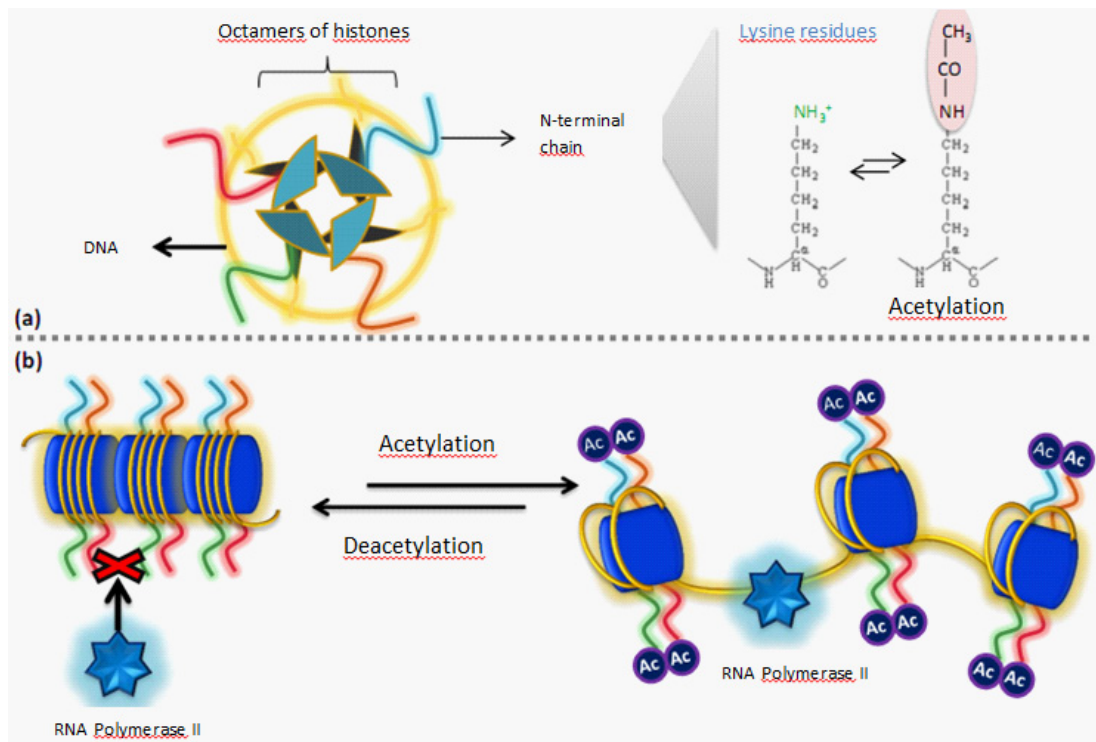


Figure 1: Structure of chromatin and histone modification by acetylation-deacetylation.

a) Note the acetylation of lysine residues in the N-terminal chain of histones (b) Acetylation of histones results in DNA cleavage, allowing the action of RNA polymerase II and gene transcription. Deacetylation produces the opposite: repression (genetic silence).

In the resting cell, the DNA is looped around the basic nucleus of histones. This conformation hinders the binding sites of RNA polymerase II, the enzyme responsible for the formation of messenger RNA (**mRNA**) (eg, gene transcription). Such entanglement is dictated by the attraction of electric charges. DNA typically has a negative charge, while the nucleus of histones has a positive charge. It is thus that chromatin adopts a “closed” distribution, which is associated with suppression of gene expression [9].

Each histone nucleus has an N-terminal chain rich in lysine residues. These residues can be acetylated, acquiring a more negative electric charge. It is thus that chromatin can change to the “open” conformation, losing the DNA-nucleus bond of histones, since there is now a repulsion of

charges [13]. DNA helices are unveiled and binding of RNA polymerase II to the corresponding sites in the DNA is possible, forming mRNA that will travel to the cytoplasm and will initiate the synthesis of inflammatory or anti-inflammatory mediators, depending on the gene involved [14]. This acetylation can be reversed by the activity of histone deacetylase enzymes (**HDACs**). It is thus that the role of HATs as co-activator and that of HDACs as co-repressors [15] [Figure 1]. In biopsies of patients with asthma HAT activity is increased and HDAC activity is reduced, favoring the expression of inflammatory genes [16,17].

The nucleus of histones can also be modified by methylation (and demethylation), phosphorylation, nitration (nitrating stress), SUMOylation and ubiquitination [14,18]. The concept of “histone code” refers to these modifications, which by altering the structure of chromatin, are associated with expression or repression of genes [19,21].

Biochemical Effects of GCs

To exert its effect, the GC must diffuse through the plasma membrane and bind to its receptor (**GR**) at the cytoplasmic level. Once activated the complex (the GC bound to the receptor), different mediators facilitate its translocation to the nucleus, where direct and indirect effects are generated on the control of inflammation [22]. This process is detailed below.

A) Glucocorticoid receptor (GR)

The GR is encoded by a single gene (the hGR gene consists of 9 exons and is located on chromosome 5), but several isoforms created through the alternative splicing in exon 9, are recognized. In the cytoplasm is the GR α , the only isoform of the receptor that binds to the GCs. The β isoform is bound to DNA, its functional significance is currently unknown [1,23].

While not stimulated by its ligand (**GCs**), the GR α remains attached to chaperone molecules (such as heat shock proteins 46, 50, 70 and 90) [22]. The binding of the ligand activates the GR α , which undergoes a structural change that liberates the chaperones [23]. With this, receptor sites that signal their nuclear translocation are exposed. Nuclear import proteins (eg, α -importin and importin-13) allow rapid mobilization of the complex [1].

For teaching purposes, the anti-inflammatory mechanisms of GCs can be studied from three perspectives: Activation of anti-inflammatory genes, deactivation of activated inflammatory genes and post-transcriptional effects. The first two models depend mainly on the dose of GCs and are closely related to the remodeling of the chromatin.

B) Genetic activation: high dose GCs

The activated GR-steroid dimerizes and translocates to the nucleus, and acquires the ability to bind to specific DNA sequences. Genes sensitive to GCs have in their promoter specific nucleotide sequences called “glucocorticoid response elements” (**GRE**) [24]. The binding of the homodimer, via zinc fingers, to the GRE causes unfolding of DNA. This allows CBP access which forms an anchor or scaffold for transcription factors (NF- κ B, SRC, AP-1; CREB, pCAF), which have HTA activity [23].

Genes that are activated by GCs include those encoding the $\beta 2$ adrenergic receptor, SLIP (secretory leukoprotease inhibitor), MPK-1 (MAPK inhibitor phosphatase-1), GILZ (leucine zipper protein induced by steroids), SRC-2 (steroid receptor coactivator), etc., [22]. In this way anti-inflammatory proteins are produced, which inactivates the inflammatory proteins generated in asthma. As mentioned, inhibition of gene expression is less frequent by this mechanism, and is more related to the adverse metabolic and endocrine effects of GCs.

C) Deactivation of activated inflammatory genes: GCs at low doses

In inflammation control, the main action of GCs is the inhibition of the synthesis of inflammatory mediators [22-24]. When low doses of GCs are used, the GC-GR complex does not dimerize and behaves like a monomer generating two mechanisms of action that repress gene expression. The first is repression, counteracting the co-activating response of pro-inflammatory factors. Histone acetylation, chromatin remodeling, and RNA polymerase II binding to genes encoding inflammatory protein synthesis are thus limited [23]. The second is the most important mechanism. HDAC-2 is recruited and activated causing the suppression of already activated inflammatory genes (gene silence) [23,24].

D) Post-transcriptional effects

In the last 15 years, the effects of GCs have been revealed in a series of genes that regulate post-transcriptional effects such as transport, replacement and transfer of mRNA [25]. For example, they may increase the degradation of mRNA by blocking the production of several pro-inflammatory cytokines [26]. GCs reduce mRNA stability for some inflammatory genes such as cyclooxygenase-2, through an inhibitory action on p38 mitogen-activated protein kinase (p38 MAP kinase) by inducing MAP kinase inhibitory phosphatase [27,28].

NON-GENOMIC MECHANISM

In bronchial asthma there is a biphasic response to treatment with a rapid component and a slow component. The initial phase of recovery with bronchodilators is due to a resolution of smooth muscle contraction (3-30 minutes for onset of action and a peak effect in 1-2 hours). The slower resolution phase probably represents the anti-inflammatory effect of systemic GCs (90-120 minutes for onset of action and 6-24 hours for improving lung function). When GCs enter cells it can take hours and even days to produce sufficient amounts of new anti-inflammatory proteins and block the already activated proinflammatory genes, which explains the slow period of beneficial activity of systemic GCs [29].

However, it has recently been shown that GCs have beneficial biological effects that are independent of the genetic transcription process [30,31]. This effect occurs by the local use of GCs in the airway. Much of the perfusion of the conducting airways occurs through the bronchial arteries, and most of the blood flow is distributed to the subepithelial tissue, the main site of airway inflammation in asthma [32,33]. In asthmatic patients there is an increased blood flow

in the mucosa and airway GCs reduce this blood flow by producing vasoconstriction due to a GC-catecholamine interaction [33]. The receptor mediating this mechanism is located on the smooth muscle cell membrane of the blood vessels of the airways [34]. This non-genomic mechanism is also known as “membrane-initiated GCs signaling”. This reduction is of rapid onset (30 minutes), transient (approximately 60 minutes) and returns to baseline at 90 minutes. It is conditioned by the dose administered and by the basal flow and is not a specific molecule, ie it occurs with any local GC but is higher with budesonide and fluticasone than with beclomethasone [35].

How does vasoconstriction occur? Figure 2 shows how the nerve endings form a synapse with the smooth muscle cells of the mucosal vessels releasing noradrenaline into the synaptic space, where it binds to alpha-adrenergic receptors producing contraction of the smooth muscle (via cyclic AMP) of the vessels, decreasing the flow and mucosal edema [31]. Some noradrenaline molecules in the synaptic space can be recaptured towards the presynaptic nerve end and can be reused later. But other noradrenaline molecules can also be captured by postsynaptic muscle termination and metabolized intracellularly by monoamine oxidase and catechol-ortho-methyl transferase (**MAO** and **COMT** respectively) [36]. The receptor that allows this capture is blocked by the local GCs, allowing the neurotransmitter to accumulate in the synaptic space and stimulating for longer the α -adrenergic receptor, perpetuating vasoconstriction. That is, the non-genomic effect [37].

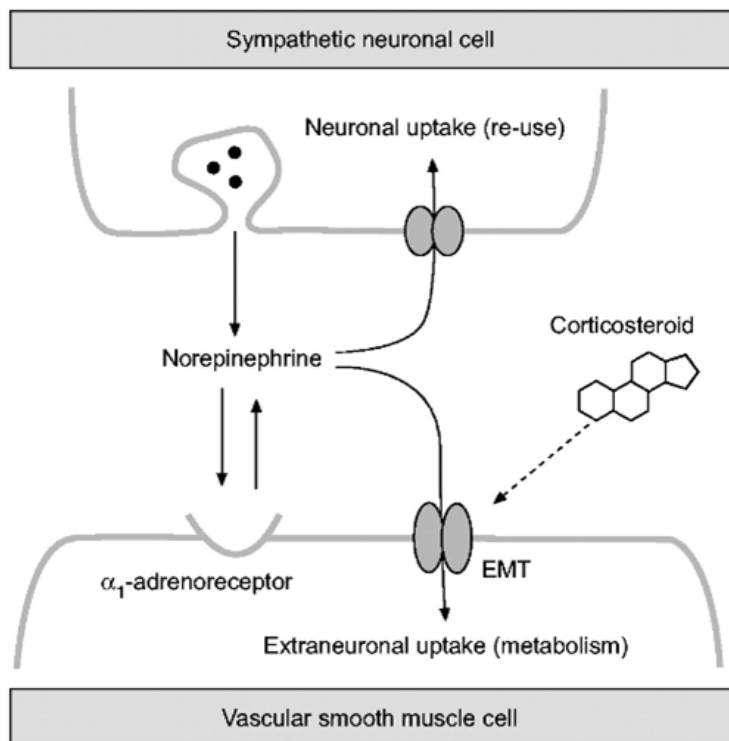


Figure 2: Non-genomic effect.

The effects of GCs produced on the smooth muscle cell of the blood vessels of the airway mucosa are shown. Note how GCs block neurotransmitter uptake allowing it to accumulate in neuromuscular synapses. EMT (Extraneuronal Monoamine Transporter) [31].

The nature of the receptor has been elusive although a transmembrane progesterin receptor has been characterized and cloned [38]. The topical GCs apparently interfere with the expression of an extra-neuronal cationic protein, which is the noradrenaline carrier. By blocking its transport, it allows its accumulation [39]. This non-genomic effect does not have systemic GCs. Local GCs have both mechanism of action, reducing inflammation and hyperperfusion. The introduction of inhaled GCs over 40 years ago has proven to be an effective and safe therapy, replacing oral therapy for the maintenance treatment of bronchial asthma except in very severe cases. International guidelines have been positioning inhaled GCs in bronchial asthma [40].

Also in the management of asthmatic crisis, the use of nebulized steroids has emerged as an alternative to systemic [41]. The efficacy may be comparable but safety is clearly superior with the nebulized route. More pharmacoeconomics studies are needed but the cost-benefit ratio may be in favor of nebulized suspensions, as demonstrated by the use of IGCs in the chronic treatment of the entity [42].

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