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The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease

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Glucocorticoids are primary stress hormones necessary for life that regulate numerous physiologic processes in an effort to maintain homeostasis. Synthetic derivatives of these hormones have been mainstays in the clinic for treating inflammatory diseases, autoimmune disorders, and hematologic cancers. The physiologic and pharmacologic actions of glucocorticoids are mediated by the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Ligand-occupied GR induces or represses the transcription of thousands of genes through direct binding to DNA response elements, physically associating with other transcription factors, or both. The traditional view that glucocorticoids act through a single GR protein has changed dramatically with the discovery of a large cohort of receptor isoforms with unique expression, gene-regulatory, and functional profiles. These GR subtypes are derived from a single gene by means of alternative splicing and alternative translation initiation mechanisms. Posttranslational modification of these GR isoforms further expands the diversity of glucocorticoid responses. Here we discuss the origin and molecular properties of the GR isoforms and their contribution to the specificity and sensitivity of glucocorticoid signaling in healthy and diseased tissues. (J Allergy Clin Immunol 2013;132:1033-44.)

Key words: *Glucocorticoid receptor, glucocorticoid, isoforms, glucocorticoid signaling*

Abbreviations used

ACTH:	Adrenocorticotropic hormone
AF:	Activation function
AP1:	Activator protein 1
β2AR:	β_2-Adrenergic receptor
CBG:	Corticosteroid-binding globulin
DBD:	DNA-binding domain
GPCR:	G protein–coupled receptor
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid-responsive element
LBD:	Ligand-binding domain
MAPK:	Mitogen-activated protein kinase
NF-κB:	Nuclear factor κB
nGRE:	Negative glucocorticoid-responsive element
NTD:	N-terminal transactivation domain
SEGRA:	Selective glucocorticoid receptor agonist

Glucocorticoids are hormones essential for life that are synthesized and released by the ***adrenal cortex*** in a ***circadian*** manner and in response to stress. The secretion of these hormones is controlled by the hypothalamic-pituitary-adrenal axis (Fig 1). Internal and external signals trigger the hypothalamus to release corticotropin-releasing hormone, which acts on the anterior pituitary to stimulate the synthesis and secretion of adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex to stimulate the production and secretion of glucocorticoids. Acting on nearly every tissue and organ in the body, glucocorticoids function to maintain homeostasis both in response to normal diurnal changes in metabolism and in the face of stressful perturbations. Glucocorticoids regulate a plethora of physiologic processes, including intermediary metabolism, immune function, skeletal growth, cardiovascular function, reproduction, and cognition.[1,2] In a classic negative feedback loop, glucocorticoids also target the hypothalamus and anterior pituitary to inhibit the production and release of corticotropin-releasing hormone and ACTH and thereby limit both the magnitude and duration of the glucocorticoid increase (Fig 1).

Because of their powerful anti-inflammatory and immunosuppressive actions, glucocorticoids are one of the most widely prescribed drugs in the world today.[3,4] Synthetic glucocorticoids have been indispensable over the last half century for treating inflammatory and autoimmune diseases, such as asthma, allergy, sepsis, rheumatoid arthritis, ulcerative colitis, and multiple sclerosis. They are also commonly prescribed to prevent organ transplant rejection and to treat cancers of the lymphoid system, such as leukemias, lymphomas, and myelomas. Unfortunately, the therapeutic benefits of glucocorticoids are limited by severe side effects that develop in patients chronically treated with these steroids.[3,5] Adverse effects include osteoporosis, skin atrophy, diabetes, abdominal obesity, glaucoma, growth retardation in children, and hypertension. In addition, patients receiving long-term glucocorticoid therapy frequently have tissue-specific glucocorticoid resistance. Understanding the factors at a molecular

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Terms in boldface and italics are defined in the glossary on page 1034.

level that control the cellular response to glucocorticoids is a critical goal of current research because progress in this area will facilitate the development of novel glucocorticoids with improved benefit/risk ratios.

The physiologic and pharmacologic actions of glucocorticoids are mediated by the glucocorticoid receptor (GR; NR3C1), a member of the nuclear receptor superfamily of ligand-dependent transcription factors.[6] After glucocorticoid binding, the GR induces or represses the transcription of target genes, which can comprise up to 10% to 20% of the human genome.[7-9] Consistent with the pleiotropic actions of glucocorticoids, the GR is expressed in nearly every cell of the body and is necessary for life after birth.[10] The cellular response to glucocorticoids is remarkably diverse, exhibiting profound variability in specificity and sensitivity.[11,12] For example, glucocorticoids induce the killing of thymocytes and osteoblasts but promote the survival of hepatocytes and cardiomyocytes. In addition, glucocorticoid sensitivity varies among subjects, within tissues of the same subject, and even within the same cell during different stages of the cell cycle.[13,14] Although cell type–specific alterations in ligand bioavailability and cofactor expression can modulate the glucocorticoid response, recent studies have also identified a major role for an expanding array of GR isoforms.[15,16]

The GR is derived from a single gene; however, multiple GR proteins exist because of ***alternative splicing*** and alternative translation initiation mechanisms. This large cohort of functionally distinct receptor subtypes are subject to various posttranslational modifications that further regulate their signaling properties. Consequently, the cellular response to glucocorticoids is determined in large measure by the expressed complement and composite actions of the individual GR isoforms. In this review we discuss the molecular heterogeneity of the GR and its potential contribution to the regulation and dysregulation of glucocorticoid signaling.

GR SIGNALING

Classic GR signaling pathway

The GR is a modular protein composed of 3 major domains: an N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD; Fig 2).[17] The DBD and LBD are separated by a flexible region of the protein termed the hinge region. The DBD is the most conserved domain across the nuclear receptor family and contains 2 zinc finger motifs that recognize and bind target DNA sequences called glucocorticoid-responsive elements (GREs). The NTD houses a powerful transcriptional activation function (AF1) that interacts with coregulators and the basal transcription machinery and is the primary site for posttranslational modifications (Fig 2). The LBD, consisting of 12 α-helices and 4 β-sheets, forms a hydrophobic pocket for binding glucocorticoids and also contains an AF2 domain that interacts with coregulators in a ligand-dependent manner.[18] Two ***nuclear localization signals*** (NL1 and NL2) are located at the DBD/hinge region junction and within the LBD, respectively.

In the absence of hormone, the GR resides predominantly in the cytoplasm of cells as part of a large multiprotein complex that includes chaperone proteins (hsp90, hsp70, and p23) and immunophilins of the FK506 family (FKBP51 and FKBP52; Fig 3).[19,20] These proteins maintain the receptor in a conformation that is transcriptionally inactive but favors high-affinity ligand binding. Cortisol, the most abundant endogenous glucocorticoid in human subjects, is transported in the blood predominantly bound to corticosteroid-binding globulin (CBG). CBG not only facilitates cortisol distribution but also plays a role in its release to tissues. CBG-free cortisol passively diffuses across the plasma membrane; however, its bioavailability within the cell is controlled by 2 enzymes working in an opposing manner.[21] 11β-Hydroxysteroid dehydrogenase type 2 oxidizes cortisol into

GLOSSARY

ACETYLATION: The addition of an acetyl group (CH_3CO) to an organic compound.

ADRENAL CORTEX: An endocrine organ made up of specific zones that synthesizes 3 different classes of steroids: (1) glucocorticoids (cortisol), which are synthesized primarily in the fasciculata, the middle layer; (2) mineralocorticoids (aldosterone), which are generated in the glomerulosa, the outermost layer; and (3) sex steroids (estrogens and androgens), which are produced largely in the innermost layer, the reticularis.

ALTERNATIVE SPLICING: The process by which a given gene is spliced into more than 1 type of mRNA molecule.

ARACHIDONIC ACID: A polyunsaturated fatty acid derived from membrane phospholipids by the action of cytosolic phospholipase A_2.

β-ARRESTINS: A family of proteins that function to transduce and terminate (desensitize) G protein–coupled receptor signals.

CAVEOLAE: Latin for "little caves"; a membrane compartment at the surface of most cells capable of endocytosis and exocytosis, as well as compartmentalizing a variety of signaling activities. Caveolin-1 is the marker protein used to isolate caveolae by means of cell fractionation.

CIRCADIAN: Latin for "approximately a day"; biological processes under daily rhythmicity through an internal clock. Diurnal species demonstrate a gradual increase in plasma cortisol several hours before awakening. Plasma cortisol levels reach their nadir around midnight.

HISTONE: Proteins that are rich in the basic amino acids lysine and arginine and complexed with DNA in chromatin.

MIFEPRISTONE: A synthetic steroid that competitively binds to the glucocorticoid receptor and progesterone receptor, blocking their effects. Clinically, it can be used to terminate a pregnancy. At high doses, it blocks the effects of cortisol in patients with Cushing syndrome.

NUCLEAR LOCALIZATION SIGNALS: Factors necessary for nuclear import (eg, transcription factors, coactivators or corepressors, DNA repair enzymes, ribosomal proteins, and mRNA processing factors) that allow molecules to pass through nuclear pore complexes through shuttling receptors, such as importins.

POLYMORPHISM: One of 2 or more variants of a particular DNA sequence. The most common type of polymorphism involves variation at a single base pair (single nucleotide polymorphism). Polymorphisms can also involve long stretches of DNA.

SEX-SPECIFIC DIFFERENCE: The female/male ratio among patients with rheumatoid arthritis is 2:1 to 3:1, and that among patients with systemic lupus erythematosus is 9:1.

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION FAMILY: Transcription factors that are substrates for the Jak family of tyrosine kinases. STATs are activated by Jak-dependant tyrosine phosphorylation and form dimers that then translocate into the nucleus.

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