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Review article
Pregledni članak

GLUCOCORTICOID RECEPTOR IN HEALTH AND DISEASE

GLUKOKORTIKOIDNI RECEPTOR U ZDRAVLJU I BOLESTI

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Summary: Glucocorticoid hormones are essential for life, have a vital place in the treatment of inflammatory and autoimmune diseases and are increasingly implicated in the pathogenesis of a number of common disorders. Their action is mediated by an intracellular receptor protein, the glucocorticoid receptor (GR), functioning as a ligand-inducible transcription factor. Multiple synthetic glucocorticoids are used as potent antiinflammatory and immunosuppressive agents, but their therapeutic usefulness is limited by a wide range and severity of side-effects. One of the most important pharmaceutical goals has been to design steroidal and non-steroidal GR ligands with profound therapeutic efficacy and reduced unwanted effects. The therapeutic benefit of glucocorticoid agonists is frequently compromised by resistance to glucocorticoids, which may depend on: access of the hormones to target cells, steroid metabolism, expression level and isoform composition of the GR protein, mutations and polymorphisms in the GR gene and association of the receptor with chaperone proteins. The major breakthrough into the critical role of glucocorticoid signaling in the maintenance of homeostasis and pathogenesis of diseases, as well as into the molecular mechanisms underlying the therapeutic usefulness of antiinflammatory drugs acting through the GR is expected to result from the current progress in large-scale gene expression profiling technologies and computational biology.

Keywords: glucocorticoid hormones, glucocorticoid receptor, mechanism of action, pathogenesis of disease, antiinflammatory drugs

Kratak sadržaj: Glukokortikoidni hormoni su neophodni za održavanje homeostaze, imaju ključnu ulogu u terapiji inflamatornih i autoimunih poremećaja i učestvuju u patogenezi mnogih bolesti. Ovi hormoni deluju posredstvom unutarćelijskog receptornog proteina, glukokortikoidnog receptora, koji funkcioniše kao inducibilan transkripcioni faktor aktiviran ligandom. Mnogi sintetski glukokortikoidi koriste se kao efikasni antiinflamatorni i imunosupresivni agensi, ali je njihov terapeutski učinak ograničen širokim spektrom i intenzitetom sporednih efekata. Jedan od najvažnijih ciljeva farmaceutske industrije jeste sinteza steroidnih i nesteroidnih liganada GR-a sa izraženom terapeutskom efikasnošću i redukovanim neželjenim efektima. Terapeutski učinak glukokortikoidnih agonista često je umanjen zbog rezistencije na glukokortikoide koja zavisi od: dostupnosti ciljnih ćelija hormonima, metabolizma steroida, nivoa ekspresije i izoformskog sastava GR, mutacija i polimorfizama u genu za receptor i interakcije receptora sa šaperonima. Očekuje se da veliki prodor u upoznavanju ključne uloge glukokortikoidnih hormona u održavanju homeostaze i patogenezi bolesti, kao i u rasvetljavanju molekularnih mehanizama terapeutskih efekata antiinflamatornih lekova koji deluju posredstvom GR rezultira iz napretka savremenih tehnologija za globalno izučavanje ekspresije gena i bioinformatike.

Ključne reči: glukokortikoidni hormoni, glukokortikoidni receptor, mehanizam delovanja, patogeneza bolesti, antiinflamatorni lekovi

Introduction

Glucocorticoids are adrenocortical hormones contributing fundamentally to the maintenance of basal and stress-related homeostasis in all higher organisms. They influence the activity of almost every cell in the body, modulate the expression of approximately 10% of human genes and are essential for growth, development and differentiation. Consequently, these hormones are implicated in the pathogenesis of a number of diseases and, because of their

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antiinflammatory and immunosuppressive actions, are widely used in the therapy. However, when given therapeutically they produce many adverse side-effects. For decades, many researchers focused their efforts on these hormones, attempting to design synthetic analogues with maximal therapeutic efficacy and reduced undesired effects.

Glucocorticoid actions are mediated by an intracellular receptor protein, the glucocorticoid receptor (GR). GR is a member of the superfamily of nuclear receptors, which function as ligand-inducible transcription factors. Unlike other receptors of this superfamily, unliganded GR is predominantly localized within the cytoplasm of target cells, but after hormone binding it rapidly translocates to the nucleus. The GR is a multifunctional protein consisting of several functional domains. It interacts with many other signaling proteins, which in addition to the receptor itself, represent potential drug targets for the manipulation of cellular responses to glucocorticoids.

The numerous actions of glucocorticoids are mediated by a number of GR isoforms capable of forming homo- and hetero-dimers. Multiple GR monomers and dimers expressed at different levels in a cell-specific manner exert quantitatively and qualitatively different transcriptional activities, making the glucocorticoid signaling system highly versatile.

It is of utmost clinical importance to elucidate the molecular mechanisms by which glucocorticoids and their synthetic analogues produce their complex and varied actions, because they are used in the treatment of inflammatory and autoimmune disorders, such as asthma, rheumatoid arthritis, inflammatory bowel disease, and also as immunosuppresive therapy after organ transplantation. The additional reason for studying the molecular mechanisms of glucocorticoid action lies in the fact that deregulation of the secretion and/or activity of endogenous glucocorticoids is implicated in the pathogenesis of a number of common disorders that pose a growing clinical burden, including obesity, type 2 diabetes, the metabolic syndrome, essential hypertension, atherosclerosis with its cardiovascular sequelae, osteoporosis and stress-related psychiatric disorders, such as anxiety, depression, posttraumatic stress disorder, insomnia, and chronic pain and fatigue syndromes.

Glucocorticoid hormones

Physiological effects

The principal endogenous glucocorticoids are cortisol and corticosterone, cortisol being the predominant glucocorticoid in man. Glucocorticoids exert widespread actions essential for the maintenance of homeostasis and enable the organism to prepare for, respond to and cope with physical and emotional stress (1). Their influence on metabolism encompasses stimulation of carbohydrate and protein breakdown, as well as complex effects on lipid deposition and breakdown. They are also important regulators of immune and inflammatory processes and are required for host defense. Stress-protective actions of these hormones are now considered to be based on their antiinflammatory and immunosuppressive activity, since they quench the pathophysiological responses to tissue injury and inflammation preventing them from overshooting and threatening the homeostasis themselves (2). A wide spectrum of physiological actions of the endogenous glucocorticoids also includes their effects on blood pressure, bone, cell growth and apoptosis. Within the central nervous system (CNS), glucocorticoids target both neurons and glial cells, and are equally important during development, when they play an organizational role in the brain, and in the adulthood, when they contribute to neuronal plasticity. Other central effects of glucocorticoids include complex changes in mood, arousal, cognition, sleep, behavior, modulation of food intake, body temperature, pain perception and neuroendocrine function (3).

Persistent and pronounced elevations in circulating glucocorticoids, due to hypersecretion of endogenous hormones, like in Cushing's syndrome/disease, or to prolonged administration of exogenous steroids, frequently cause amplification of their metabolic and physiological effects and lead to a variety of pathological states. Likewise, insufficient glucocorticoid secretion, which may arise from Addison's disease, the adrenogenital syndrome or pituitary disease, is also accompanied by pathologies, but with the opposite characteristics (3, 4).

Secretion

The release of glucocorticoids from the zona fasciculata of the adrenal cortex into the systemic circulation is pulsatile, with the pulse amplitude following a circadian rhythm. The circadian and stress-induced secretion of the glucocorticoids is governed by the hypothalamic-pituitary-adrenocortical (HPA) axis. The hypothalamus, as a sensor of changes in the external and internal environment, receives and integrates neural and humoral information from many sources. Two hypothalamic neurohormones, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), reach the anterior pituitary gland via the hypophyseal–portal blood vessels, and act through specific receptors to trigger the release of the adrenocorticotrophic hormone (ACTH) into the systemic circulation. ACTH, in turn, acts on the adrenal cortex via type 2 melanocortin receptors to initiate the synthesis and release of cortisol. The sensitivity of the HPA axis to incoming stimuli is modulated by a negative feedback mechanism, in which the sequential release of CRH/AVP and ACTH is suppressed by endogenous glucocorticoids (5).

Antiinflammatory and immunosuppressive actions

Corticosteroids are the most effective anti-inflammatory therapy for many chronic inflammatory diseases, such as asthma, rheumatoid arthritis and inflammatory bowel disease, but are relatively ineffective in other diseases, such as chronic obstructive pulmonary disease (COPD). Chronic inflammation involves the infiltration and activation of many inflammatory and immune cells, which release inflammatory mediators that interact and activate structural cells at the site of inflammation. Different inflammatory diseases involve different cells and mediators (6), but common to all of them is increased expression of multiple inflammatory proteins, which are regulated at the level of gene transcription by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB) and activating protein-1 (AP-1). It is now believed that chromatin remodeling plays a critical role in the transcriptional control of inflammatory genes. Stimuli that switch these genes on act by changing the chromatin structure of the genes, whereas corticosteroids reverse this process mainly by the binding of liganded GR to coactivators and recruitment of histone deacetylase-2 (HDAC2) to the activated transcription complex (7).

Glucocorticoid receptor: structure and function

Functional domains

Similar to other steroid hormone receptors, GR is a modular protein organized into three major structural/functional domains: a ligand binding domain (LBD) at the C-terminus, a DNA-binding domain (DBD) in the central part of the protein and an N-terminal regulatory domain harboring a strong transcriptional activation function AF-1 (8). Recently, the crystal structure of the LBD of the human GRα was resolved (9, 10). The LBD is composed of α-helices and β-strands folded into a three-layer helical sandwich and forming a hydrophobic steroid binding pocket. In addition to the steroid binding pocket the LBD also harbors a secondary, ligand-dependent trans-activation function AF-2 (11). Upon ligand binding, the LBD undergoes a conformational change that results in the »closing« of the pocket by helix 12. The interaction between co-activators and GR is very sensitive to the structure of the ligand that is bound in the pocket. Thus, agonist binding to the LBD induces the reorientation of a critical Q-helix and the formation of a binding pocket for a family of co-activator proteins that play essential roles in transactivation (12, 13).

The DBD contains two Zn-fingers one of which is required for the specific recognition of the response element in the major groove of the DNA helix (14), while the other is involved in the dimerization of the receptor occurring upon its interaction with the DNA response element.

The N-terminal domain of the GR contains the glucocorticoid-independent AF1 transactivation subdomain. This region has been shown to interact with the TFIID complex and TBP of the general transcription machinery (15).

Signaling cascade of the glucocorticoid receptor

Nuclear translocation

GR is expressed in many cell types at a density of 2000 to 30,000 molecules per cell (16). In the unliganded state it is located in the cytoplasm within a multiprotein chaperone complex, the key component of which is a heat shock protein Hsp90, which maintains the receptor in an inactive state poised to bind its ligand. Apart from Hsp90 this complex contains the heat shock proteins Hsp40 and Hsp70, and other proteins, such as p23 and p60 (17). It is postulated that Hsp70 initiates the opening of the hydrophobic steroid-binding pocket in an ATP-dependent manner, enabling association of Hsp90 with the LBD of the receptor, which is a prerequisite for keeping the receptor in a ligand-receptive state (18).

The receptor is a phosphoprotein that becomes hyper-phosphorylated upon ligand binding. In the absence of the hormone, GR is predominantly phosphorylated at Ser203, and addition of the hormone increases phosphorylation at both Ser203 and Ser211 (19). Phosphorylation at Ser211 coincides with increased trans-activation properties of GR, possibly reflecting a conformational change that modulates the interaction with co-activators (19, 20). Phosphorylation status of the GR influences its intracellular localization: when phosphorylated at Ser203, the receptor localizes predominantly to the cytoplasm, whereas phosphorylation at Ser211 causes partition between the cytoplasmic and the nuclear compartment. When leaving the nuclear compartment, GR becomes dephosphorylated at Ser211 and can either be recycled by recruitment in a chaperone complex, which facilitates Ser203 phosphorylation, or is degraded (21).

Interaction with DNA regulatory elements

In the nucleus, activated GR can interact with the regulatory regions of responsive genes to alter the level of gene expression. GR can activate gene transcription by direct binding to the so-called simple glucocorticoid-responsive elements (GREs). A consensus GRE is the pentadecameric imperfect palindrome GGTACAnnnTGTTCT (22) with the 3′ half conserved, and the 5′ half highly variable. A GR

monomer first binds to the 3′ half-site, after which the 5′ half-site is occupied by a second monomer to form a DNA-bound dimer (23). The 3-basepair spacer between the half-sites is a strict requirement for cooperative binding of the two GR monomers to the palindromic GRE (24).

The response of many genes to glucocorticoids depends not only on GR binding to the GRE, but additionally requires the binding of other transcription factors to adjacent binding sites. Since these response elements are both spatially and functionally clustered, they are referred to as glucocorticoid responsive units (GRUs) (25). Therefore, GRUs act by integrating multiple signal inputs into one response. Different GRUs diverge widely in the identity of the bound transcription factors, and in the order and number of response elements in each unit. Thus, a tissue-specific response to a glucocorticoid is likely to result from the composition of its regulatory regions allowing for specific combinations of the GR with tissue specific accessory factors. GREs that negatively influence the transcription of responsive genes are referred to as negative GREs (nGREs). In this mode of regulation, direct binding of GR to the nGRE is required. A nGRE has a similar recognition sequence as a GRE, although the consensus sequence of a nGRE is more variable (ATYACnnTnTGATCn) than that of a GRE (26).

In certain genes, GR does not bind directly to the DNA to exert its effect, but is recruited to DNA-bound transcription factors in a regulatory complex, a mechanism known as tethering. In these cases the receptor seems to behave as a ligand-inducible co-regulator that employs protein-protein interactions to exert its effect, which, interestingly, can be both positive and negative (8).

Activation of transcription

The N-terminal domain of GR can interact with the general transcription factor TFIID (15), providing a mechanism by which the receptor can directly influence the rate of transcription. In addition, several transcription co-factors, which can associate with the DNA-bound receptor, can act as protein bridges with the transcription machinery to regulate gene expression (13). Thus, co-activators of the p160 family of transcription factors (SRC1, TIF-2/GRIP1, pCIP) interact with ligand-bound receptors (27) and recruit secondary co-activators essential for activation, including CBP/p300 (28, 29). Some of these possess histone acethyltransferase (HAT) activity required for chromatin remodeling and subsequent access of the transcriptional machinery to promoters (29, 30). However, some of these co-activators can also interact with the basal transcription machinery and activate the transcription of target genes (31).

Repression of transcription

Repression of transcription by GR is mostly achieved by antagonizing the trans-activating properties of other transcription factors via different regulatory mechanisms. First, the receptor can intrinsically inhibit gene expression by the direct binding to nGRE sequences. More complex mechanisms involve the receptor interaction with other transcription factors. Antiinflammatory actions of glucocorticoids are typically mediated by proinflammatory transcription factors, such as NF-κB and AP-1 (32). Several models exist to explain the glucocorticoid-induced repression of AP-1 and NF-κB signaling. In the direct-interaction model, GR and AP-1 interact and prevent the binding of each other to their response elements (33). In the competition model, GR and AP-1 compete with one another for binding to their overlapping response elements, while in the co-activator competition model, GR competes with NF-κB and/or AP-1 for binding to CBP, which is present in the cell in limiting amounts (28, 34). Furthermore, the GR-mediated repression of trans-activation by the p65 subunit of NF-κB is increased in the presence of CBP, suggesting that CBP functions as an integrator of the NF-κB/GR cross-talk (35). In the HAT inhibition model, GR binding to CBP inhibits recruitment of CBP by DNA-bound p65, reducing the HAT activity of the complex (32). Yet another possibility is an up-regulation of the inhibitor of NF-κB action (I-κB) in response to glucocorticoids (36). However, recent results showing that dimerization-impaired GR DNA-binding domain is capable of DNA-binding (37) and up-regulation of GRE-dependent genes (38) have shed some doubts on whether the antiinflammatory actions of glucocorticoids are indeed independent of DNA-binding.

Glucocorticoid receptor as a pharmacological target

The structure of the GR LBD has recently been solved (9) and it has been shown that the ligands with only slightly different structure can considerably alter the final conformation of the ligand–receptor complex, and thus influence the recruitment of various transcription co-factors and the biological function of the receptor. The molecular insights into the structure of the GR LBD provided important clues for a better understanding of the glucocorticoid signaling and for designing safer and more efficient medications. Namely, they led to concerted efforts by the pharmaceutical companies to develop selective GR ligands with preserved beneficial antiinflammatory activity, but reduced pleiotropic side-effect profile, which includes adversities such as fat redistribution, hyperglycemia, obesity, skin atrophy and osteoporosis (39). The tested compounds derive from different sources, such as from high throughput screening, where hundreds of thousands of compounds are randomly screened, or from rational drug design,

beginning with targeted modifications of the known GR-ligands. A screen for the ligands with a therapeutically beneficial profile usually consists of receptor binding assays and cellular in vitro tests for GR-mediated transactivation and transrepression, followed by in vivo analyses of their effects in various animal models of inflammation (40–42). At present various synthetic GR ligands with specific pharmacokinetic and pharmacodynamic properties are available for therapeutic use. Some of these novel compounds are highlighted in this section.

Long circulating liposomal glucocorticoids

One of the approaches to develop novel glucocorticoids or glucocorticoid-substituting compounds with an improved effect/side-effect ratio was the optimization of formulation for a systemic treatment with targeted release of the drug. The efforts toward that end led to the use of liposome-encapsulated steroids for the treatment of rheumatoid arthritis. One example is the encapsulation of prednisolone or dexamethasone in liposomes, an approach that enabled prolonged and more targeted availability of the drug in comparison with the drugs applied without liposomes (43). This administration route proved to be especially useful in rheumatoid arthritis and atherosclerosis, since liposomes display high affinity for macrophages in inflamed tissues, and these cells do not serve only as targets, but also play a crucial role in the release of glucocorticoids from liposomes and the generation of their relatively high and prolonged concentration in the synovium.

Nitro-steroids

A second approach to improve the ratio of desired and undesired effects was to create nitro-steroids by combining known steroidal GR-ligands with nitric oxide (NO). Nitroxy derivatives of the steroid scaffold slowly release NO, displaying enhanced antiinflammatory activity without increasing the dose of the steroid (44). Two interesting substances are NO-prednisolone (NCX-1015), which exerts 10-fold stronger antiinflammatory effects than conventional prednisolone, and NO-hydrocortisone (NCX-1004). At the same time, these drugs induce fewer side-effects, especially on the bones (44, 45). A possible explanation for the strong antiinflammatory activity of nitro-steroids may lie in the post-translational modification of GR through tyrosine nitration (44).

Selective glucocorticoid receptor agonists

Major efforts have been made and promising preclinical results achieved in the search for GR ligands that trigger molecular mechanisms of the GR action very selectively, so as to completely dissociate transactivation from transrepression by the receptor, with the goal of reducing the risk for side-effects, which are mainly based on transactivation, while maintaining the antiinflammatory efficacy, which relies on transrepression. These drugs, the so-called dissociating ligands or selective GR agonists (SEGRA), induce a conformational change of the receptor favoring GR/protein interactions, while disfavoring the receptor binding to DNA (39, 46).

In the last years, an increasing number of SEGRAs has been described. Very interesting substances are A276575 and its four enantiomeres (47). They all show equal affinity for the GR as dexamethasone, but posses less than 5% of its transactivation ability. Another potentially useful SEGRA is the substance ZK216348 with potent anti-inflammatory activity, but with a 60-fold weaker transactivation capacity compared to prednisolone, and even 300-fold weaker compared to dexamethasone (48). Haegeman's group described the first example of a dissociating compound, compound A, isolated from natural sources. It binds to the GR with an affinity similar to that of dexamethasone and selectively triggers transrepression. It seems to be as efficient an antiinflammatory agent as dexamethasone, and moreover, induces no increase in the blood glucose level (49).

The dissociating substances can be expected to be introduced into clinical medicine in the nearby future, but more in vivo studies are obviously needed to define their benefit/risk ratio in humans, since their performance in animals does not necessarily ensure efficacy in the complex situation of human disease. Secondly, the effects of SEGRAs observed at the cellular level in vitro may differ from those that occur on various cell types in vivo. Finally, the transactivation/transrepression model for the screening of anti-inflammatory glucocorticoids with reduced side-effects, although attractive, has some limitations. These mostly stem from the fact that there are genes that negatively regulate the processes in the immune system, but are transcriptionally upregulated by the GR (50, 51). Thus, SEGRAs acting exclusively via transrepression may not reach the overall immunosuppressive potential of conventional glucocorticoids. Likewise, the mechanisms underlying the occurrence of undesired effects are complex and subtle. For many of the metabolic effects transactivation seems to be the most prominent mechanism, as shown for genes encoding the enzymes of gluconeogenesis and protein catabolism (52). However, the mechanisms underpinning the induction of osteoporosis and skin atrophy are not very well understood and the dissociation between transactivation and transrepression does not promise to be a good enough indicator of the compound's therapeutic usefulness (46).

Physiological and pathological variations in tissue responsiveness to glucocorticoids

Glucocorticoids are necessary for life and are essential in all aspects of health and disease as they regulate vital processes from mitosis to apoptosis, from metabolism to growth and development. However, responses to glucocorticoids vary among individuals, cells and tissues. These hormones are used to treat a wide variety of allergic and inflammatory diseases, but owing to their ability to regulate the expression of genes involved in cell cycle progression and apoptosis, they are also effective in the treatment of lymphoproliferative diseases, such as leukemia and Hodgkin's disease, with particularly beneficial results being obtained in childhood acute lymphoblastic leukemia (ALL). Besides, they are applied as an indispensable part of the immunosuppressive regimes to prevent organ transplant rejection (39).

Unfortunately, in about 30% of the patients the response to glucocorticoids is poor, but since the glucocorticoid resistance in most cases is tissue specific, the unresponsive patients still suffer from numerous side-effects. As insensitivity to glucocorticoids limits the therapeutic benefit of glucocorticoid agonists and is usually positively correlated with poor prognosis in proliferative disorders (53, 54), it is of utmost clinical importance to elucidate the underlying molecular mechanisms. Within this context the following factors determining tissue responsiveness to glucocorticoid hormones are considered herein: mechanisms regulating the access of glucocorticoids to target cells, metabolism of the glucocorticoid hormones, expression level and isoform composition of the GR protein, mutations and polymorphisms in the GR gene, and association of the receptor with chaperone proteins.

Access of glucocorticoids to target cells

Approximately 95% of cortisol in the circulation is bound to a carrier protein, corticosteroid-binding globulin (CBG), while only the free steroid has ready access to target cells. The ability of glucocorticoids in the systemic circulation to reach target cells can be compromised by transporter proteins, called multidrug-resistant P-glycoproteins, which are expressed in a tissue-specific manner and act by actively pumping steroids out of the cells. They, thus, provide a mechanism for the tissue- and steroid-specific delivery of glucocorticoids to target cells, contributing to subtle differences in the pharmacological profile of various compounds and their deregulation may lead to the development of glucocorticoid resistance. Particular interest has been focused on the expression of these proteins in the blood–brain barrier, where they limit the access of steroids such as dexamethasone and, to a lesser extent, cortisol and corticosterone, to the brain (55).

Pre-receptor metabolism of glucocorticoids

Probably the most important factor regulating the access of endogenous glucocorticoids to their receptors is the metabolism of steroids within the target cells by 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, a phenomenon known as pre-receptor metabolism. In human tissues, these enzymes catalyze the interconversion of cortisol and its inactive metabolite, cortisone. Two distinct isoforms of 11β-HSD have been cloned and characterized: type 1 (11β-HSD1) and type 2 (11β-HSD2) (56). 11β-HSD2 is an NAD^+-dependent, constitutive enzyme that acts exclusively as a dehydrogenase and is colocalized with the mineralocorticoid receptor (MR) in tissues such as the kidney, parotid gland, sweat glands, colon and vascular smooth muscle cells. Patients with specific mutations in the 11β-HSD2 gene develop severe corticosterone-dependent hypertension and other features of apparent mineralocorticoid excess (56). The other isoform, 11β-HSD1, is expressed mainly in the liver, adipose tissue and brain, but is also found in other tissues, and is subject to regulation by a variety of factors including glucocorticoids, stress, sex steroids and cytokines. In vivo it functions solely as a reductase regenerating biologically active cortisol from inert steroids, cortisone or 11-dehydrocorticosterone (57). As 11β-HSD1 is found mainly in tissues in which the high affinity MR is sparse, whereas the low-affinity GR is abundant, it is thought that its principal role is to amplify the local concentration of active glucocorticoids in the tissues, such as the liver, in which the steroids have a key regulatory role. Several lines of evidence, including observations that 11β-HSD1 blockade promotes insulin secretion, support the view that 11β-HSD1 is an important factor in the development of insulin resistance, obesity and other metabolic disturbances. Consequently, drugs that selectively block 11β-HSD1 are now considered a promising pharmacological target.

Glucocorticoid receptor expression

In many cases, the level of GR expression is closely correlated with the magnitude of the glucocorticoid response (58). The cellular level of GR is dynamic and regulated in a cell-type specific manner by the surrounding concentration of the ligand (59). Administration of GR agonists results in down-regulation of the GR (60, 61) by the mechanisms that can be attributed to reduced transcription of the GR gene, as well as to decreased stability of the GR mRNA and protein (62). In contrast, hormone-induced upregulation of GR (autoinduction) is associated with glucocorticoid sensitivity. For example, T-cells that exhibit GR autoinduction at the protein and/or mRNA level are sensitive to glucocorticoid induced apoptosis (63), whereas those that fail to autoinduce GR are resistant (64). Taken together,

these data suggest that the GR expression level may be an important determinant of the response of malignant cells to glucocorticoids and that glucocorticoid sensitivity may critically depend on the effect of GR agonists on the number of intracellular GR molecules (65).

Glucocorticoid receptor isoforms

A host of GR isoforms, all deriving from a single GR gene, exhibit tissue-specific patterns of expression, as well as differences in subcellular localization and transcriptional activity (66–68). It is deemed that the repertoire of GR subtypes expressed by a particular cell may contribute to the ability of the cell to respond to glucocorticoids. Transcriptional, post-transcriptional, and translational mechanisms are all involved in controlling both the level of GR gene expression and the generation of a number of specific GR isoforms.

The GR gene has been mapped to chromosome 5 (5q31–32). The human GR cDNA was first cloned in 1985 (69) and subsequently it was found that the gene covers an 80 kb region and contains 9 exons (70). Recent studies have identified 9 alternative variants of exon 1 generated as a result of cell type-specific alternative promoter usage (71), as well as additional variability in exon 1 resulting from alternative splicing (72). The 5′-untranslated region occupies the whole exon 1 and a part of exon 2, so that the heterogeneity in exon 1 does not affect the sequence of the GR protein itself. However, the existing cell type-specific promoter usage may serve to regulate GR protein levels (71), alternative splicing at other sites in the primary GR transcript (73), and/or alternative translational initiation of mature GR mRNA (74), all of which are important factors in determining glucocorticoid sensitivity.

Alternative splicing of the GR precursor mRNA gives rise to five GR protein subtypes that have been termed GRα, GRβ, GRγ, GR-A, and GR-P (75). Upregulation of some of these isoforms may represent one of many mechanisms for modulating cellular responsiveness to glucocorticoids. The ubiquitously expressed GRα (777 amino acids) is the classic, functionally active receptor and is generated through splicing of exon 8 to the proximal end of exon 9 (9α), whereas GRβ (742 amino acids) is produced through splicing of exon 8 to the distal end of exon 9 (9β). Thus, GRα and GRβ proteins share identical N-termini encoded by exons 2–8 and are distinguished only by their C-termini (69). GRα is repressed by its ligand-binding domain (LBD) occupying the C-terminal part of the molecule, so that its nuclear import is allowed only upon ligand binding. In the GRβ the stretch of 50 C-terminal amino acids is replaced by a unique 15-amino acid tail that keeps the protein constitutively localized to the nucleus. GRβ is detected in most tissues and cell lines, but is generally expressed at lower levels than GRα (70, 76). It has been reported not to bind glucocorticoid agonists and was originally thought to control transcription only through a dominant-negative effect on GRα-induced gene expression (77). In support of a dominant-negative function for GRβ, resistance to glucocorticoid therapy in leukemia has been associated with high cellular levels of GRβ (78). Recent studies, however, suggest that GRβ may have a previously unappreciated role in cell signaling and the contribution of relative GRβ levels in determining cellular sensitivity to glucocorticoids in individuals undergoing glucocorticoid therapy for malignancies remains unclear (79). The GRγ isoform contains a three base insertion in the DBD between exons 3 and 4 that results in addition of arginine between the two zinc fingers of the DBD (80). Since it exhibits decreased transcriptional activity when compared to GRα, its elevated levels are associated with glucocorticoid resistance in childhood leukemia (81). The GR-A and GR-P splice variants lack portions of the LBD and studies from a number of laboratories have revealed a correlation between elevated levels of these isoforms and glucocorticoid resistance in myeloma and leukemia (82). However, more recent data suggest that GR-P may act in a cell type-specific manner to enhance glucocorticoid responsiveness (83).

Yet another mechanism for generating diversity in GR protein expression is alternative translation initiation. Yudt and Cidlowski (68) have shown that this mechanism gives rise to tissue-specific GRα and GRβ isoforms expression patterns. All translational isoforms exhibit a similar affinity for glucocorticoids and most of them undergo hormone-induced nuclear localization, followed by GRE binding and transcriptional regulation (66). However, they differ by their transcriptional activity in reporter assays, so that the specific intracellular pool of GRα subtypes may determine cellular sensitivity to glucocorticoids. Besides, microarray analysis revealed that GRα translational isoforms regulate unique, subtype-specific genes (66).

Mutations in the glucocorticoid receptor gene

Mutations in the GR gene are considered the primary cause of an inherited form of generalized glucocorticoid resistance and also of in vitro acquisition of glucocorticoid resistance in various malignant cell lines. Hillmann et al. were the first to discover a transactivation-deficient GR mutant (L753F) in cells from an individual with glucocorticoid-resistant ALL (84). Subsequently, an LBD-deficient GR mutant (△702) in cells from another individual with glucocorticoid-resistant ALL at relapse was identified (85). The two cases described above are the only known examples of resistance to glucocorticoid

therapy in malignancy attributable to acquired GR mutations in vivo. However, in light of the low sensitivity of conventional assays for identification of GR gene mutations in heterogeneous cell populations, it is likely that the acquisition of GR mutations in vivo is underestimated.

Glucocorticoid receptor gene polymorphisms

Several polymorphisms in the GR gene have been associated with variations in GR function. Thus, the ER22/23EK GR polymorphism within the N-terminal domain of the receptor has been associated with decreased GR transcriptional activity in reporter assays and with decreased expression of target genes when compared to wild type GR (86, 87). Upon further analysis, it was discovered that this polymorphism facilitated the expression of GRα-A, but had no effect on the expression of the GRβ-B translational isoforms (88). Since GRα-A is transcriptionally less active than GRβ-B (68), it can be proposed that ER22/23EK may correlate with glucocorticoid insensitivity (88). Similarly, the GRβ polymorphism A3669G located in the 3'untranslated region of the gene results in enhanced expression of the dominant-negative GRβ protein and is associated with favorable metabolic parameters (89). These data imply that both ER22/23EK and A3669G carriers have more a favorable metabolic profile due to relative insensitivity to endogenous glucocorticoids. In contrast to the polymorphisms described above, other two polymorphisms in the GR gene, Bcl I – located in intron 2 and N363S – located in the N-terminal domain, have been associated with generalized increase in glucocorticoid sensitivity (87, 90) and metabolic disorders (91). Interestingly, microarray analysis revealed a unique, 23 polymorphism-specific pattern of gene regulation for N363S when compared to GRα wild type (92). Moreover, some reports suggest that N363S is not only associated with glucocorticoid hypersensitivity, but also with decreased bone mineral density (93). Further analyses have led to the proposal that polymorphisms in the GR gene may be utilized as an indicator of disease, a predictor of adverse reactions and a prognostic factor in glucocorticoid-managed proliferative disorders (65).

Glucocorticoid receptor heterocomplex

Since the integrity of the mature GR heterocomplex is required for optimal ligand-binding and subsequent activation of the transcriptional response, abnormalities in the chaperones and co-chaperones that make up the GR heterocomplex may contribute to decreased glucocorticoid responsiveness. The alterations in Hsp90 and Hsp70 were shown to be associated with decreased cellular sensitivity to glucocorticoids. For example, an aberrant form of Hsp90 and low Hsp70 levels were identified in two out of nine glucocorticoid-resistant human leukemic cell lines (94). In addition, altered levels of Hsp90 were found in peripheral lymphocytes from individuals with steroid-resistant forms of asthma (95), multiple sclerosis (96) and idiopathic nephritic syndrome (97).

Furthermore, the relative levels of FKBP51 and FKBP52 immunophilins have also been regarded as important determinants of cellular sensitivity to glucocorticoids in various systems. For example, high levels of FKBP51 and low levels of FKBP52 were associated with glucocorticoid resistance in cell lines and tissues from several genera of New World primates (98), while FKBP51 overexpression inhibited hormone induced GR transactivation in mammalian cells (99). As mutated versions of chaperones and co-chaperones could alter signaling through the mature GR heterocomplex, potentially leading to decreased cellular sensitivity to glucocorticoid-induced cell death, the role of these proteins in glucocorticoid-resistant malignancy has been a matter of consideration (100).

Modulation of glucocorticoid receptor expression and function in disease

A part of the research conducted in our laboratory is concerned with the modulation of human GR structure, function and expression in various diseases, the etiology and pathophysiology of which are closely associated with the glucocorticoid hormones action. These studies are performed on the GR from isolated peripheral blood mononuclear cells (PBMCs), as readily accessible human cells that express high levels of GR and, being a part of the immune system, represent an excellent model for investigations of the molecular basis of antiinflammatory and immunosuppressive actions of the glucocorticoid hormones.

Moreover, there is an increasing body of evidence pointing to similarities between the receptor expression and mechanisms of action in the cells of the nervous system (e.g. neurons and glia) and lymphocytes, as well as to a central role of lymphocytes in integrating the CNS and immunological functions.

Considering the evidence along these lines Gladkevich et al. (101) provided strong arguments to support the exploiting of blood lymphocytes as a convenient neural probe and a possible genetic probe in studies of psychiatric disorders.

In this section we present our experimental data on functional modulation and alterations in the expression of GR in PBMCs from patients with asthma as a typical inflammatory disease, polycystic ovary syndrome (PCOS) as an endocrinopathy bearing

characteristics of a metabolic disorder and posttraumatic stress disorder (PTSD) as a psychiatric disorder associated with disturbances in the HPA axis regulation.

Asthma

Asthma is the most common chronic disease among children and adolescents. The pathogenesis and pathophysiology of this disease are known to be associated with alterations of GR function and also with persistent pulmonary inflammation, the important mediators of which are reactive oxygen and nitrogen species. To improve symptoms and bronchial hyperresponsiveness, and to limit the progressive decline in lung function, glucocorticoids are used as primary therapeutic agents, although the role of glucocorticoid hormones and their receptor in the pathophysiology and development of the disease is poorly understood. In our laboratory the GR hormone-binding parameters and the level of the receptor expression in PBMCs from stable asthmatic adolescents with different degrees of disease severity have been investigated. It was found that adolescent patients suffering from moderate asthma display altered GR functional characteristics in comparison to healthy subjects and mild asthmatics. Specifically, they present a higher number of GR per cell (B_{max}) and lower affinity of the receptor for the hormone ($1/K_D$). However, the GR protein level was found to be similar in PBMCs from the three groups of subjects (102). Subsequently we tested a hypothesis that GR functional alterations in asthma result from the action of oxidants. To that end we conducted a series of ex vivo treatments of PBMCs from healthy donors with various oxidizing agents and compared the resulting GR modifications with those previously noticed in asthmatic patients. The results showed that treatment of PBMCs by hydrogen peroxide (H_2O_2) provoked an increase in the level of GR protein, accompanied by a rise in the B_{max} and a decline of $1/K_D$. The H_2O_2-induced changes, including characteristic GR isoproteins expression pattern, were found to be very similar to the GR changes previously observed in PBMCs of moderate asthmatic patients, but not in mild asthmatics and healthy subjects. Treatment with other oxidants applied herein produced distinct effects, or exerted no influence on GR. Therefore, this study provided preliminary data suggesting that functional alterations of the GR associated with moderate asthma may be mediated by redox mechanisms that are based on oxidative and regulatory actions of H_2O_2 (103).

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a common endocrinopathy of the reproductive-aged women that is characterized by hyperandrogenic features, with an estimated prevalence of 5–10% (104). Apart from ovaries as the main source of androgens, excess adrenal androgens, as well as adrenocortical dysfunction were observed in women with PCOS (105). At present, PCOS is considered a metabolic disorder with a number of obesity-related risk factors for cardiovascular disease, specifically: insulin resistance, type 2 diabetes, and proatherogenic lipid profile (106). Metabolic derangements presented by central obesity, hyperlipidemia and insulin resistance are principally linked to the glucocorticoid excess (107), which might be explained by obesity-related increased tissue sensitivity to glucocorticoids (108), or decreased sensitivity to hypercholesterolemia (109). Therefore, we focused our study to analyze the possible causative relationship between the functional properties of the GR in PBMCs from women with PCOS and their clinical characteristics including biochemical blood parameters. The examined GR functional characteristics, B_{max} and $1/K_D$, as well as the B_{max}/K_D ratio called GR potency, were related to the anthropometric data, basal biochemical characteristics, insulin sensitivity indices and hormonal profile of the subjects. We observed a strong association between B_{max} and K_D, and also between GR potency and age of the PCOS women. The multiple regression analyses performed on the PCOS group showed that independent predictors of K_D were body mass index, and plasma levels of total cholesterol and dehydroepiandrosterone-sulphate (DHEA-S), while the independent predictors for the GR potency were age, body mass index, DHEA-S and basal cortisol concentration (Macut et al., 2009, submitted for publication). These findings should be elucidated in further studies analyzing the possible role of the GR in the pathogenesis of PCOS and metabolic consequences of the syndrome.

Posttraumatic stress disorder

Posttraumatic stress disorder (PTSD) is a chronic psychiatric disorder that can occur in subjects who have been exposed to or have witnessed a traumatic experience of an extreme nature. PTSD is typically accompanied by acute and chronic alterations in the stress response, i.e. with a deregulation of the HPA axis. In addition, previous studies have suggested altered GR expression and function in PBMC patients, but have yielded conflicting results, presumably because of not differentiating between the effects of PTSD pathology and war-related trauma. The aim of our study was to examine the GR expression and functional properties in PBMCs of Balkan war veterans with and without PTSD, in order to discriminate between PTSD- and war trauma-related alterations. GR hormone-binding parameters, B_{max} and equilibrium dissociation constant (K_D), were determined by saturation analysis in the PBMCs of war veterans with current or lifetime PTSD and

without PTSD, and of healthy male volunteers. Functional status of the receptor was assessed by measuring dexamethasone-induced inhibition of lysozyme synthesis. The levels of GR, mineralocorticoid receptor (MR) and heat shock proteins (Hsp90 and Hsp70) were evaluated by quantitative immunoblotting. An increase of B_{max} in the PBMCs of war veterans without PTSD vs. healthy controls and a rise of GR potency (B_{max}/K_D ratio) in patients with lifetime PTSD vs. those with the current disorder were noticed. Current PTSD coincided with disturbance of the correlation between B_{max} and K_D that normally exists in PBMCs of healthy subjects (110). Between-group differences in sensitivity of lymphocytes to dexamethasone were marginally significant, while those in the levels of GR, MR, Hsp90 and Hsp70 were not found. The results suggest that current PTSD may be associated with impairment of the compensation between GR number and its affinity for the hormone, resilience to PTSD with efficient regulation of the receptor's hormone-binding capacity and remission of the disorder with its elevated binding potency (Matić et al., in preparation). New techniques such as cDNA microarray and proteomics may give clues to define molecular abnormalities in psychiatric disorders and could eventually reveal information for diagnostic and treatment purposes.

Perspectives

Although many of the physiological effects of glucocorticoid hormones are well recognized, the underlying genomic mechanisms are only starting to be elucidated. During the last decade the field of genomics achieved a revolutionary advancement through development of a powerful large-scale gene expression profiling technology, including DNA microarrays, serial analysis of gene expression and proteomics, which allow rapid quantitative analysis of entire transcriptomes and proteomes. Understanding the molecular mechanisms by which glucocorticoid hormones exert their diverse metabolic and physiological effects requires identifying the direct target genes whose expression levels are modulated by the glucocorticoid signaling pathway. Moreover, a complete understanding of the glucocorticoid action also requires the transcription factors that may interact with the GR, and the loci where these interactions occur. The genome-wide in silico search for GRE sequences in combination with genome-wide location analysis, which may yield the set of promoters bound by the GR, and profiling of the expression levels of thousands of genes, which may identify genes that are functionally dependent upon the GR, enables the researchers to identify the set of glucocorticoid-regulated genes and also to propose interactions between the GR and other transcription factors at specific target genes. Such novel approaches may generate gene regulatory networks and promise to provide explanations of transcriptional regulation of candidate genes that underlie the glucocorticoid-mediated effects in different cell types, under diverse conditions and throughout time (111). Although linking physiology and genomics appears to be a complicated task, owing to a rapid progress of the large-scale gene expression profiling technologies and computational biology, our understanding of the complexity of glucocorticoid signaling has rapidly increased during the last decade. This progress has provided significant insight into the key role of these hormones not only in the maintenance of homeostasis, but also in the pathogenesis of disease. It has also made a major contribution to our understanding of the molecular mechanisms underlying both beneficial and adverse effects of the steroids used as drugs, and provided the basis for future development of more targetted glucocorticoid therapies.

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