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Nontranscriptional actions of the glucocorticoid receptor

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Abstract

Many cellular responses to corticosteroids involve the transcriptional modulation of target genes by a prototypical nuclear receptor, the glucocorticoid receptor (GR). In the classic model of steroid hormone action GR acts as ligand-dependent transcription factor by either activating or repressing gene expression through direct interactions with DNA or other transcription factors. Recent evidence suggests an important role for nontranscriptional effects of GR in the vascular system. The nontranscriptional actions of GR involve the rapid activation of protein kinases, such as phosphatidylinositol-3 kinase and Akt, leading to the activation of endothelial nitric oxide synthase. This novel pathway of steroid hormone action protects against ischemic injury by augmenting blood flow and decreasing vascular inflammation.

Keywords

Glucocorticoid receptor; Nontranscriptional; Nitric oxide; Ischemia; Akt

Introduction

The study of steroid hormone action has provided many important insights into the regulation of cellular functions by nuclear receptors and at the same time has revealed surprising levels of biological complexity. Corticosteroids are essential for normal development and the stress responses through the regulation of intermediary metabolism and the immune system [1,2]. Produced by the adrenal glands under the regulation of the hypothalamus-pituitary gland axis, corticosteroids were among the first hormones to be identified and later found to exert their effects through a specific DNA-binding protein, the glucocorticoid receptor (GR) [3]. It was subsequently shown that GR can act as a ligand-dependent transcription factor that positively regulates genes through interaction with DNA enhancer sequences, called glucocorticoid response elements (GRE) [4,5,6]. Molecular analysis of the anti-inflammatory actions of GR later revealed a second important mechanism of GR function in the nucleus. Activated GR can negatively regulate expression of inflammatory genes through direct protein-protein interaction with proinflammatory transcription factors without DNA binding (termed transrepression) [7,8]. Recent findings suggest that there is yet another level of GR action with particular importance for the

vascular system. A rapid, nontranscriptional effect of GR was found to mediate tissue protection in myocardial infarction and stroke through activation of endothelial nitric oxide synthase (eNOS), which is mediated by the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [9,10].

In this contribution we review and discuss existing data on the use of corticosteroids in vascular inflammation and acute ischemia, provide an overview of the molecular mechanisms of GR action with emphasis on physiological and pharmacological significance, and discuss the implications for potential future therapeutic approaches.

Beneficial and harmful effects of corticosteroids in acute ischemia

Corticosteroids have been used in the treatment of cardiovascular disease, such as acute myocardial infarction and stroke, with conflicting results [11]. Several features make steroids attractive candidates for the treatment of acute ischemia. The degree of ischemic tissue damage is related to the extent of the inflammatory response, which is coordinated through the interaction of leukocytes with activated vascular endothelium. Corticosteroids inhibit endothelial cell activation and leukocyte-endothelial interaction, thereby exerting prominent, and very rapid, anti-inflammatory effects on the vasculature [12]. Steroid treatment improves survival of patients with acute myocardial infarction and protects the myocardium from experimental ischemic injury [13,14,15]. In ischemic stroke steroids can improve clinical outcome in a subset of patients with severe stroke and administration of high doses of corticosteroids substantially reduces tissue damage in experimental focal cerebral ischemia [16,17,18,19]. In addition, our group has recently shown that high-dose steroid treatment decreases vascular inflammation and ischemic tissue damage after myocardial infarction and stroke through direct vascular effects involving the nontranscriptional activation of eNOS [9,10].

The prolonged use of steroids, however, is often limited by their adverse effects attributed to a delayed genomic response initiated by GR. This is exemplified by the clinical syndrome of hyperglycemia, dyslipidemia, hypertension, osteoporosis, and impaired wound healing in patients with chronic corticosteroid excess (i.e., Cushing syndrome) [20]. Furthermore, down-regulation of eNOS is thought to contribute to steroid-induced hypertension [21]. Indeed, continued low-dose steroid administration increases ischemic injury after global ischemia of the brain [22]. In myocardial infarction impaired wound healing and cardiac remodeling can lead to cardiac rupture within 2 weeks of myocardial infarction [23,24], and this has led to discontinuation of steroid treatment for acute ischemia. Therefore defining the exact molecular mechanisms involved in the beneficial and detrimental effects of GR could have important therapeutic implications.

Molecular structure of GR

GR is expressed ubiquitously and belongs to the nuclear receptor superfamily, which also includes receptors for the mineralocorticoids, estrogens (ER), progestins, and androgens, as well as receptors for peroxisome proliferators, vitamin D, and thyroid hormones. Phylogenetic and sequence analysis has shown that GR with the receptors for progestins, mineralocorticoids, and androgens form a subfamily of oxosteroid receptors that is distinct from the ER subfamily [25]. GR has the prototypical modular structure of nuclear receptors: an N-terminal transcriptional activation function 1 domain, a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) harboring a ligand-dependent transcriptional activation function-2 domain [6,26]. Alternative mRNA splicing results in a second GR isoform, GR β , that is defective in steroid binding and acts as a dominant negative inhibitor of GR α in vitro [3,27,28]. However, a clear functional role for GR β has yet to be established.

The activation of GR is regulated by the LBD. In the absence of ligand, GR is assembled into a multiprotein complex that includes heat shock proteins and immunophilins. This retains GR in the cytoplasm but enables high-affinity ligand binding [29,30]. Hormone binding to the LBD leads to a conformational change in the receptor followed by dissociation of the multiprotein complex and rapid nuclear entry. Nuclear translocation is mediated by two nuclear localization sequences located adjacent to the DBD, and in the LBD, respectively [31,32]. Interestingly, hormone-bound GR is not permanently localized to the nucleus. Rather, GR rapidly and continuously shuttles between the nucleus and the cytoplasm [33,34].

Transcriptional modulation by GR: DNA binding vs. protein-protein interaction

In the nucleus GR regulates transcription by two distinct mechanisms: DNA binding-dependent transcriptional modulation or DNA binding-independent modulation mediated by protein-protein interaction. In the first instance, activated GR binds to GRE present in the regulatory regions of target genes and activates transcription through recruitment of transcriptional coactivators, such as steroid receptor coactivator-1, and the basic transcription machinery [6,35]. Transcriptional activation by GR requires homodimerization through two different dimerization interfaces. A five amino acid sequence (D loop) in the second zinc-finger of the DBD mediates cooperative DNA-binding to the palindromic motive of the GRE, and mutations in the D loop abrogate transactivation by GR [36,37]. In addition, dimerization requires reciprocal interactions of two hydrophobic amino acids in the LBD. Consequently mutations in this region also interfere with transactivation [38]. By the same mechanism of direct interaction with DNA, GR has also been reported to repress transcription through negative GREs [39].

The second instance of GR nuclear action is independent of DNA binding and involves modulation of transcriptional activity through direct protein-protein interaction with inducible transcription factors [40]. An important example of DNA-independent actions of steroids is transrepression of the proinflammatory transcription factors activator protein (AP) 1 and nuclear transcription factor κ B (NF- κ B). GR weakly interacts with and inhibits AP-1-dependent transcription without altering its DNA binding [7,41,42]. Importantly, DNA binding inactive mutants of GR are fully capable of AP-1 transrepression [37]. Interaction between activated GR and NF- κ B is mediated by the second zinc-finger in the DBD of GR, and transrepression does not require DNA binding of GR. Instead, GR interferes with the transactivation potential of the p65/RelA subunit of NF- κ B (see [43] and references herein). In addition to transrepression, protein-protein interaction can also lead to synergistic induction of promoter activity, as shown for interaction of GR with Stat-5 on the β -casein promoter [44].

To link the pleiotropic molecular actions of GR to biologically significant functions, Reichardt and Schutz [45] have generated different genetically modified mice. Mice lacking GR (GR^{-/-}) die shortly after birth due to impaired maturation of several organs including the lungs [46]. In contrast, overexpression of GR renders mice resistant to stress and endotoxic shock, which seem to involve a decrease in the inflammatory response [47]. While the absence of GR is incompatible with life, DNA binding and transactivation of target genes by GR is not essential for development or survival. Mice with a targeted mutation in the D loop dimerization domain of GR (GR^{dim}), which impairs cooperative DNA binding and transactivation by GR, lack GRE-dependent gene expression but survive to adulthood [48]. GR^{dim} mice, however, do not respond to corticosteroids with induction of gluconeogenic enzymes, such as tyrosine aminotransferase, demonstrating that they are defective in mounting a positive GRE-mediated transcriptional response. Interestingly, the

anti-inflammatory actions of steroids are preserved in these mice. Steroids potently suppress the local and systemic inflammatory response in GR^{dim} mice, and repression of AP-1 and NF-κB-dependent genes, such as matrix metalloproteinase-3 and collagenase-3, is comparable to wild-type mice [48,49,50]. Indeed, analysis of GR^{dim} mice has recently provided insight into the mechanism of impaired wound repair [51]. After skin injury there is enhanced granulation tissue and increased numbers of fibroblasts in GR^{dim} mice as compared to control, suggesting that the inhibitory effect of steroids on wound healing is mediated by the DNA binding activity of GR (see Table 1).

Rapid nontranscriptional effects of GR

Some effects of corticosteroids are very rapid, making primarily transcriptional mechanisms of action unlikely and hence have been referred to as “nontranscriptional” effects [52]. However, it should be noted that this concept does not exclude the possibility of indirect downstream transcriptional modulation secondary to the initiated signaling pathways. Corticosteroids alter amphibian behavior and increase inositol trisphosphate in vascular smooth muscle cells within minutes of administration [53,54]. In mice the antianaphylactic effect of high-dose corticosteroids occurs acutely and is unaltered by the transcriptional inhibitor actinomycin D [55]. Furthermore, in patients with active rheumatoid arthritis steroids rapidly inhibit leukocyte recruitment into inflamed joints [56]. In myocardial infarction and ischemic stroke, high-dose steroids cause a transient decrease in blood pressure and systemic vascular resistance accompanied by an increase in coronary and cerebral blood flow within minutes of administration [10,14,57].

Although some effects of steroids do not seem to be mediated by GR, and are therefore designated nonspecific, there is growing evidence for rapid nontranscriptional actions of GR, especially with regard to inflammation [58,59]. The synthetic corticosteroid dexamethasone (Dex) rapidly inhibits cytosolic phospholipase A₂ activity and release of arachidonic acid, which are important mediators of inflammation. Inhibition was reversed by the GR antagonist RU486 (mifepristone), and by pharmacological inhibition of Src, suggesting that rapid inhibition of cytosolic phospholipase A₂ by GR is mediated by Src [60]. Interestingly, a nonclassical effect of GR on rapid kinase signaling is also involved in the inhibition of AP-1 by steroids. The transcriptional activity of the AP-1 subunit c-Jun is enhanced through phosphorylation on Ser63/73 by members of the Jun N-terminal kinase (JNK) subfamily in response to inflammatory cytokines [61]. Activated GR prevented phosphorylation of c-Jun, and transcriptional enhancement of AP-1, by blocking the JNK signaling cascade [62]. Acute Dex treatment interfered with JNK activation in the cytoplasm and nucleus without altering JNK subcellular distribution. These effects did not involve direct interaction between JNK and GR and were independent of DNA binding, since a dimerization defective GR mutant, which lacks transcriptional capacity, is still able to suppress JNK activation in response to Dex [63].

There is growing evidence that the nontranscriptional actions of several other steroid hormone receptors regulate physiologically important processes [64]. For example, osteocyte apoptosis and bone loss is prevented by the androgen and estrogen steroid receptors through nontranscriptional activation of protein kinase Src and mitogen-activated protein kinase [65,66]. Vascular nitric oxide production and vasodilation by estrogen depends on nontranscriptional activation of eNOS and are mediated by ERα-dependent activation of the PI3 K/Akt pathway [67,68,69]. Indeed, the vascular protective effects of estrogen are dependent on the nontranscriptional activation of eNOS via PI3 K/Akt [68].

Nontranscriptional activation of eNOS by corticosteroids

Many beneficial actions of corticosteroids in vivo involve the vascular endothelium. An important endogenous mediator of vascular integrity is endothelium-derived NO [70]. NO produced by eNOS possesses anti-inflammatory, antiatherogenic, and anti-ischemic properties [71,72]. Enhanced NO production by administration of the eNOS substrate L-arginine or upregulation of eNOS by statins confers stroke protection [73,74,75], and transgenic mice overexpressing eNOS show decreased leukocyte accumulation and reduced vascular lesion formation following vascular injury [76]. Conversely, mice with targeted disruption of eNOS (eNOS^{-/-}) exhibit increased vascular inflammation and larger cerebral infarctions following experimental ischemia [77,78], while inhibition of NOS activity decreases cerebral blood flow and increases infarct size after ischemia [79].

In two different mouse models of ischemic injury, transient myocardial ischemia and transient focal cerebral ischemia, the protective effects of steroids were mediated by the nontranscriptional activation of eNOS by GR [9,10]. High-dose corticosteroids significantly reduced ischemic tissue damage to the heart and the brain, while low doses were ineffective. Cotreatment with the GR antagonist RU486 completely reversed the effects of Dex, suggesting that GR mediated tissue protection. Furthermore, ischemia protection was mediated by NO, since the beneficial actions of steroids were absent in eNOS^{-/-} mice or blocked by cotreatment with the NOS inhibitor N^ω-Nitro-L-arginine methylester. Steroid treatment acutely increased eNOS activity in vitro and in vivo, which led to decreased vascular inflammation and increased vasorelaxation and regional cerebral blood flow in a NO-dependent manner.

Activation of PI3 K/Akt by corticosteroids

Corticosteroids have recently been reported to activate protein kinase Akt [9,10,68,80], which is an important regulator of cell cycle progression and mediator of cellular survival downstream of PI3 K [81]. Phosphorylation of eNOS by Akt leads to increase endothelial NO release [82,83]. In a ligand-dependent manner corticosteroids activate PI3 K and Akt, which is blocked by cotreatment with RU486 or the PI3 K inhibitor LY294002 but not by transcriptional inhibitors [9,10] (Fig. 1). Although induction of nontranscriptional actions requires high doses of steroids, these effects are nevertheless specifically mediated by GR. This was demonstrated in transfection studies in COS7 cells, which lack endogenous GR. Whereas Dex did not activate PI3 K or Akt in the absence of GR, it readily induced Akt kinase activation after transfection with GR, or the dimerization defective GR mutant (A458T), which is unable to transactivate target genes [10]. This suggests a DNA binding independent mechanism of activation. The fact that PI3 K activation can be suppressed by the GR antagonist RU486 further argues against the involvement of transrepression in this process, since the RU486 compound still induces transrepression of AP-1 dependent gene expression in reporter assays [37]. Akt

The mechanism of PI3 K activation by GR is currently unknown but seems to involve association of GR with the regulatory p85 α subunit of PI3 K. Also, activation does not require dimerization of the receptor since the dimerization defective mutant is equally active [10]. A model of the nontranscriptional actions of GR is depicted in Fig. 2. Analogies might be drawn to PI3 K activation by another steroid receptor, ER α , although there is only modest sequence homology between the two receptors. Activation of PI3 K by ER α involves activation of Src and formation of a ternary complex of p85 and Src with the activated receptor, which was mediated by the C-terminus of ER α including the LBD [84]. Since positive cross talk between Src and PI3 K has already been described it might be speculated that this mechanism is also involved in activation of PI3 K by GR [85].

Corticosteroids as anti-ischemic therapy

The study of corticosteroid action in acute ischemia has yielded conflicting results, which may reflect differences in dosage regimens, time to onset of treatment, or the specific clinical or experimental condition. Several new aspects concerning the mechanism of ischemia protection have emerged from recent studies, which might allow for optimized treatment strategies.

A consistent feature of the beneficial actions of corticosteroids is the requirement for high-dose steroids [9,10,14,19,57]. Ischemia protection usually requires acute treatment (e.g., less than 6 h after the onset of ischemia). These features are in agreement with a nontranscriptional mechanism of ischemia protection by GR mediated by NO. By enhancing endothelial NO production corticosteroids decrease postischemic vascular inflammation and increase ischemic blood flow [9,10]. The therapeutic use of corticosteroids is limited by side effects, which are typically associated with chronic treatment. Thus it appears that these detrimental effects are related to the genomic actions of corticosteroids. By exploring the rapid, nontranscriptional actions of GR the biological and pharmacological actions of corticosteroids could be considerably broadened. This could lead to the development of a novel class of drugs that selectively activates the nontranscriptional actions of GR. Indeed, synthetic compounds that separate specific GR functions, such as transactivation from transrepression by GR, have recently been identified [86]. It remains to be determined whether these compounds are capable of separating the beneficial from the detrimental effects of corticosteroids.

Biography



FLORIAN P. LIMBOURG received his M.D. degree from Freiburg University Medical School in Germany. He is currently a postdoctoral research fellow at the Brigham and Women's Hospital and Harvard Medical School, Boston, Mass., USA. His fellowship is supported by the Deutsche Forschungsgemeinschaft.



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Abbreviations

AP	Activator protein
DBD	DNA-binding domain
Dex	Dexamethasone
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
GR	Glucocorticoid receptor
GRE	Glucocorticoid response elements
JNK	Jun N-terminal kinase
LBD	Ligand-binding domain
NF-κB	Nuclear transcription factor κ B
PI3K	Phosphatidylinositol 3-kinase

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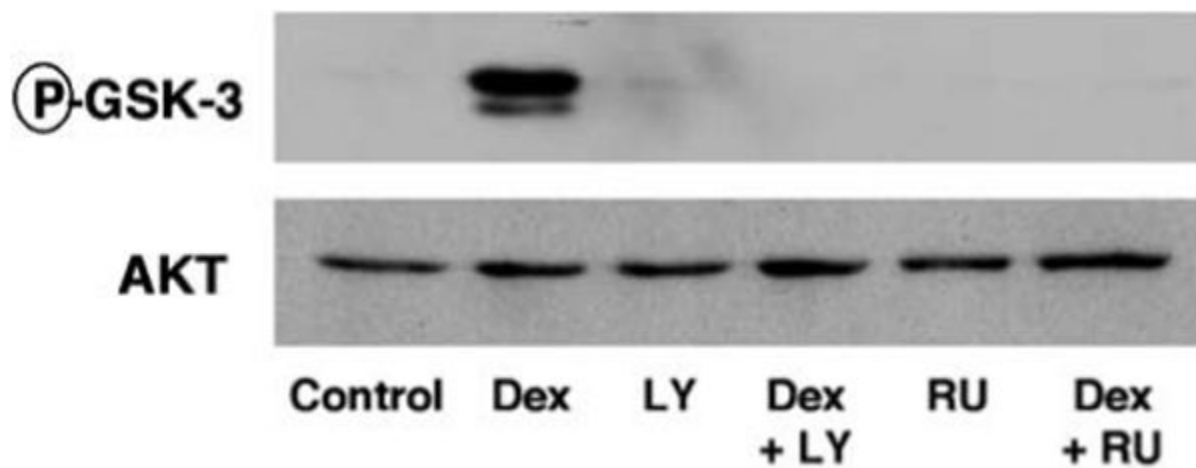


Fig. 1. GR activates Akt through PI3 K. *Above* Effect of Dex with and without RU486 (*RU*) and LY294002 (*LY*) on Akt kinase activity, measured by phosphorylation of the Akt downstream target GSK-3 by immunoblotting (*P-GSK-3*). *Below* Immunoblotting for total Akt levels in the kinase reaction. (Reprinted from [9])

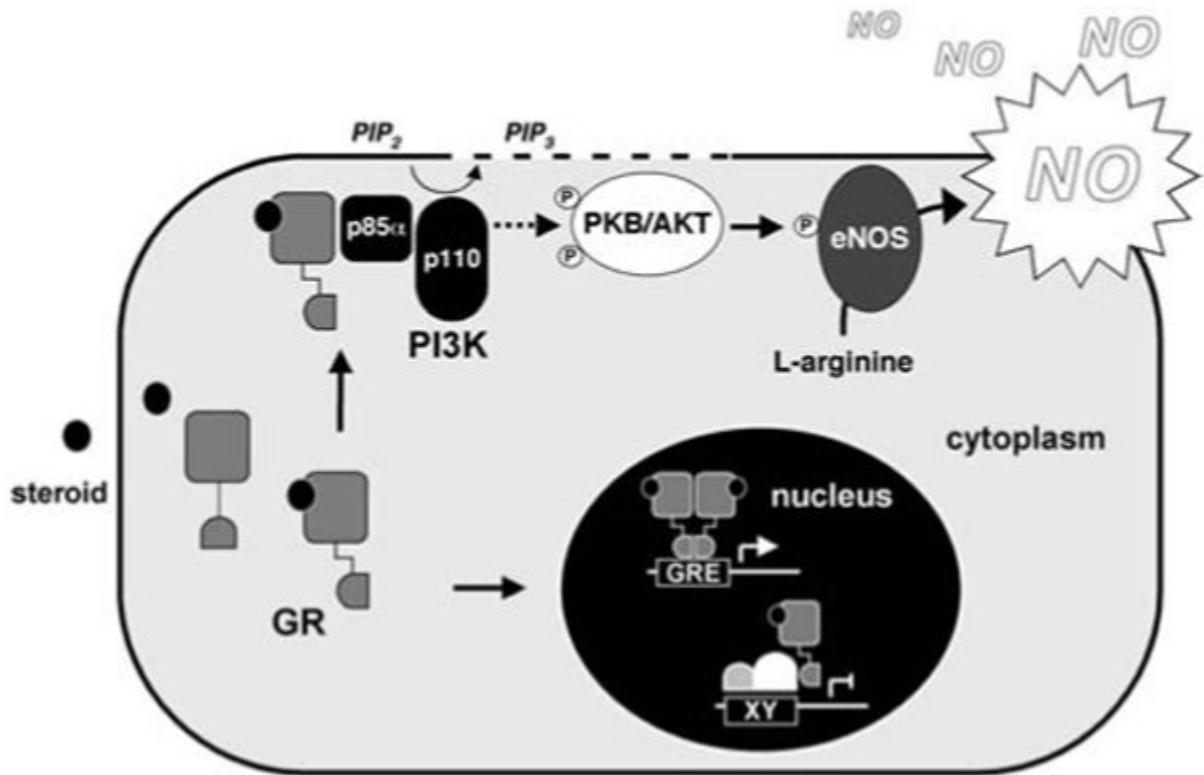


Fig. 2. Nuclear and nonnuclear actions of the glucocorticoid receptor. Association of GR with the regulatory p85 subunit of PI3 K stimulates formation of 3'-phosphorylated phosphatidylinositols (e.g., phosphatidylinositol 3,4,5-trisphosphate, *PIP*₃) from phosphatidylinositol phosphate precursors (e.g., phosphatidylinositol 4,5-trisphosphate, *PIP*₂). This leads to the subsequent recruitment and activation of protein kinase Akt, which enhances NO release through phosphorylation of eNOS. In the nucleus GR binds either directly to glucocorticoid response elements (*GRE*) or modulates the function of other transcription factors

Table 1

Different GR actions mediate the response to steroids. The mode of GR action mediating the response to steroids is indicated (*D* DNA binding-dependent action, *I* DNA binding-independent action, *NT* nontranscriptional action) (adapted from [1,87])

Organ/system	Effect	GR action
Brain	Anxiety reaction	?
	Hippocampal neuron response	D
Lung	Postnatal maturation	I
Cardiovascular system	Ischemia protection	NT
	Suppression of vascular inflammation	NT
Hematopoietic system	Erythroblast proliferation	D
Liver	Induction of gluconeogenic enzymes	D
Bone	Osteoporosis	?
Immune system	Thymocyte apoptosis	D
Inflammation	Suppression of local and systemic inflammatory response	I