



The Pro-inflammatory Effects of Glucocorticoids in the Brain

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Glucocorticoids are a class of steroid hormones derived from cholesterol. Their actions are mediated by the glucocorticoid and mineralocorticoid receptors, members of the superfamily of nuclear receptors, which, once bound to their ligands, act as transcription factors that can directly modulate gene expression. Through protein–protein interactions with other transcription factors, they can also regulate the activity of many genes in a composite or tethering way. Rapid non-genomic signaling was also demonstrated since glucocorticoids can act through membrane receptors and activate signal transduction pathways, such as protein kinases cascades, to modulate other transcriptions factors and activate or repress various target genes. By all these different mechanisms, glucocorticoids regulate numerous important functions in a large variety of cells, not only in the peripheral organs but also in the central nervous system during development and adulthood. In general, glucocorticoids are considered anti-inflammatory and protective agents due to their ability to inhibit gene expression of pro-inflammatory mediators and other possible damaging molecules. Nonetheless, recent studies have uncovered situations in which these hormones can act as pro-inflammatory agents depending on the dose, chronicity of exposure, and the structure/organ analyzed. In this review, we will provide an overview of the conditions under which these phenomena occur, a discussion that will serve as a basis for exploring the mechanistic foundation of glucocorticoids pro-inflammatory gene regulation in the brain.

Keywords: glucocorticoids, glucocorticoids receptors, brain, steroid hormones, inflammation, cytokines, pro-inflammatory

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INTRODUCTION

Inflammation and Glucocorticoids

Inflammation is described as a response to infection or injury. The cells of innate immunity, such as neutrophils and macrophages in the periphery (1) and microglia (2) in the CNS, express receptors called pattern recognition receptors (PRRs) (3, 4) that are able to recognize parts of conserved evolutionarily microbial molecules called pathogen-associated molecular patterns (PAMPs) (5). They also can recognize endogenous molecules called alarmins or danger-associated molecular patterns (DAMPs), which are host-derived proteins released from cells following tissue injury (1, 6). It has been greatly demonstrated that chronic inflammation is associated with several pathologies, such as obesity (7), cardiovascular diseases (8), rheumatoid arthritis (9), inflammatory bowel disease (10, 11), asthma (12, 13), diabetes (14, 15), neurodegenerative diseases (16), and cancer (17). These diseases are mostly characterized by a dysregulated inflammatory response, when the normally

protective role of inflammation becomes detrimental as the response becomes excessive in magnitude or duration.

Glucocorticoids (GCs, cortisol in primates and corticosterone – CORT – in rodents) are steroid hormones that essentially regulate a variety of physiological processes, including embryonic development, metabolism of carbohydrates, lipids, and proteins, the stress response, and the resolution of inflammation (6). GCs have potent anti-inflammatory and immunosuppressive action, reflecting the fact that they are the most widely prescribed drug for the treatment of a variety of inflammatory and autoimmune diseases (18). They are mainly synthesized in the suprarenal cortex as result of the activation of the hypothalamic–pituitary–adrenal axis (HPA axis).

In general, GCs are considered anti-inflammatory and protective agents, since they inhibit gene expression of pro-inflammatory mediators and other possible damaging molecules, e.g., reactive oxygen species (ROS), in peripheral organs and in the CNS. Despite the primary anti-inflammatory actions of GCs to resolve inflammatory process and restore homeostasis, it is becoming increasingly common studies, suggesting that GCs are not uniformly anti-inflammatory and in some cases may even increase certain parameters of the inflammatory response, mainly in the CNS (19), depending on the dose, chronicity of exposure, and structure/organ analyzed.

In this review, we will provide an overview of the conditions under which these phenomena occur, a discussion that will serve as a basis for exploring the mechanistic foundation of glucocorticoid potentiation of pro-inflammatory gene regulation.

Glucocorticoid Receptor

As other steroids, GCs are lipophilic molecules that easily reach the cytoplasm of target cells *via* peripheral circulation, where they are bound to carrier proteins and then diffuse promptly through cell membranes to modulate gene transcription through their two different nuclear receptors: the mineralocorticoid receptor (MR) or the glucocorticoid receptor (GR) (20, 21). The affinity of CORT for MR is 10-fold higher than for GR, consequently, MR is heavily occupied by basal CORT levels, being mainly responsible for the physiological effects of GCs, and GR is only heavily occupied during pharmacological levels of CORT or during peak secretion of this hormone, commonly observed in stressful situations (22).

The GR molecular structure consists of three functional domains: (1) the N-terminal amino domain (NTD) that contains one of the main transactivation domain, called the activation function 1 (AF1). This domain is functionally important, since it is required for transcriptional activity of steroid hormones; (2) the DNA-binding domain (DBD), which binds to the glucocorticoid responsive elements (GRE) sites in the promoter region of target genes; and (3) ligand-binding domain (LBD) that contains the binding domain to glucocorticoids as well as important sequences for interaction with coregulators, whether coactivators and/or corepressors (23).

Two human isoforms of GR have been identified, GR α and GR β , which are originated by alternative splicing of the primary transcript GR. The GR α is the predominant isoform of the receptor, and it is the one that transduces GCs signal (23). The GR β differs from GR α in the carboxy terminal sequence, where the last

50 amino acids are replaced by a sequence of 15 non-homologous amino acids, making GR β non-responsive to GCs (23, 24), with no transcription of target genes when GCs binds to GR β through a mechanism involving the formation of heterodimers GR α –GR β (23). The GR β has been described in some cells, especially inflammatory cells, such as lymphocytes and macrophages, and related with GCs resistance in diseases, such as asthma (12) and chronic lymphocytic leukemia (25). Recently, GR β was reported in liver of mice (26) and rats (27) with a role in metabolism control by insulin.

Glucocorticoid receptor isoforms are subject to various posttranslational alterations that further modulate receptor activity. After binding to GC, the conformational changes make them susceptible to modifications, i.e., phosphorylation, acetylation, ubiquitination, and sumoylation (23, 28–30), which influence DNA-binding complex force, affecting transcription and selection of genes regulated by this complex. The multiple phosphorylation events affecting GR NTD lead to changes in the spectrum of GR target genes and is thought to influence both transcriptional activity and nuclear trafficking (23, 30–32).

In conclusion, gene regulation by GCs is complex. The sequence of the DNA-binding site influences the conformation of the GC–GR, leading to different patterns of gene expression, including the pro-inflammatory ones. In addition, the recruitment of the GC–GR and their regulatory elements, as coactivators and RNA polymerase II to DNA binding, is also dependent on the existence of accessible chromatin, which appears to be cell type specific (29).

Glucocorticoid Signal Transduction

Upon reaching the target tissue, GCs bind to nuclear receptors, classical GRs, in the cytoplasm (33). GR binding promotes their release from a multiprotein complex composed by chaperones, e.g., Hsp70, Hsp90, p23, and immunophilins (FKBP51, FKBP52) (18, 30), allowing the GC–GR complex to interact with other cytosolic proteins, or in most cases, to translocate to the nucleus as a result of the exposure of a nuclear localization domain (18). Upon ligation and translocation, GR dimerizes and binds to DNA at palindromic GRE sites, activating the promoter as a transcription factor (transactivation), altering gene expression of various proteins. The GRE structure is not exclusive for GR binding, as it is also common for other nuclear receptors, such as MR, progesterone, and androgen receptors. For that reason, it is referred from some authors as hormonal response elements (HREs) rather than GRE (34).

The GC–GR complex also acts without dimerization, as a monomeric complex, and binds to DNA together with other transcription factor, in this way cooperatively enhancing gene expression (composite transactivation). In addition, the GC–GR complex can also physically interact with transcription factors without interacting with DNA itself, thereby inhibiting the transcription factor and repressing transcription. This is the predominant transrepression mechanism and is called tethering; it is the mechanism responsible to inhibit the pro-inflammatory transcription factors, NF κ B (35, 36) and activator protein 1 (AP-1) (37), and also other transcription factors, such as CREB (38),

accounting for the anti-inflammatory and immunosuppressive GCs effects.

In addition to the GR-mediated genomic mechanisms, several rapid non-genomic pathways have also been reported. According to them, GCs can have direct effects on cell membrane, interacting with membrane GR (mGR), as reported in human lymphocytes and monocytes (39), and in skeletal muscle fibers in mice (40). GCs can also interact with classical GRs targeting signaling proteins, such as kinases, and/or with classic GRs translocating into the mitochondria, one of the probable mechanisms of apoptosis induced by GC-GR complex on T cells (41–43). These pathways can result in the induction of downstream signaling cascades, changes in cytoplasmic calcium, sodium, or potassium concentrations, and increase in mitochondrial production of ROS (21, 34, 44).

The Pro-inflammatory Glucocorticoid Effects

The evidence supporting the pro-inflammatory GC effects is very robust and suggests that they occur in specific and very intricate situations. Timing, concentration, and duration of GC exposure are all critical in determining whether these hormones will be immunoactivator or immunosuppressor, but the specific situations leading to each outcome are not well documented.

Munhoz et al. (45, 46) have shown that chronic unpredictable stress (CUS), which chronically augments plasma CORT, and mid-to-high levels of CORT enhance the LPS-induced NF κ B activation as well as the expression of pro-inflammatory genes (iNOS, IL-1 β , TNF- α), while decrease the expression of classical anti-inflammatory ones (IL-1ra, IL-10, and MKP-1) in the frontal cortex and in a lesser extent in the hippocampus of rats. These pro-inflammatory GCs effects were region-dependent and GR-mediated, since they were not evident in the hypothalamus or heart of rats, and the pretreatment with the RU486 reversed them (45, 46).

Microglia are an important player in the GC endangerment to neurons. Sorrells et al. (47) have shown that the concurrent treatment with minocycline, a microglia inhibitor, but not indomethacin, a non-steroid anti-inflammatory drug, prevented GC endangerment in a model of neuronal death using kainic acid. High GC levels did not substantially affect the levels of the chemokines, CCL2, CINC-1, or baseline NF κ B activity, but they did suppress mRNA levels of CX3CL1, CX3CR1, and CD22 in the hippocampus – factors that restrain inflammatory responses – while increased by twofold the IL-1 β mRNA expression (47). In another study, using transgenic mice lacking GR expression in myeloid cells (microglia included), the same group showed that GC effects on myeloid cells have detrimental consequences for neuron survival in a kainic acid and transitory MCAO occlusion model (48). Indeed, GCs can activate microglia, putting them in a “primed” state (49–51). The primed microglia can undergo changes such as cell surface upregulation of receptors (e.g., TLRs and NLRs) (52, 53) and myeloid markers (e.g., MHC II) (54, 55), promoting a “sensitized” state without production of inflammatory or anti-inflammatory mediators, but, with further stimulus, can produce exaggerated levels of inflammatory

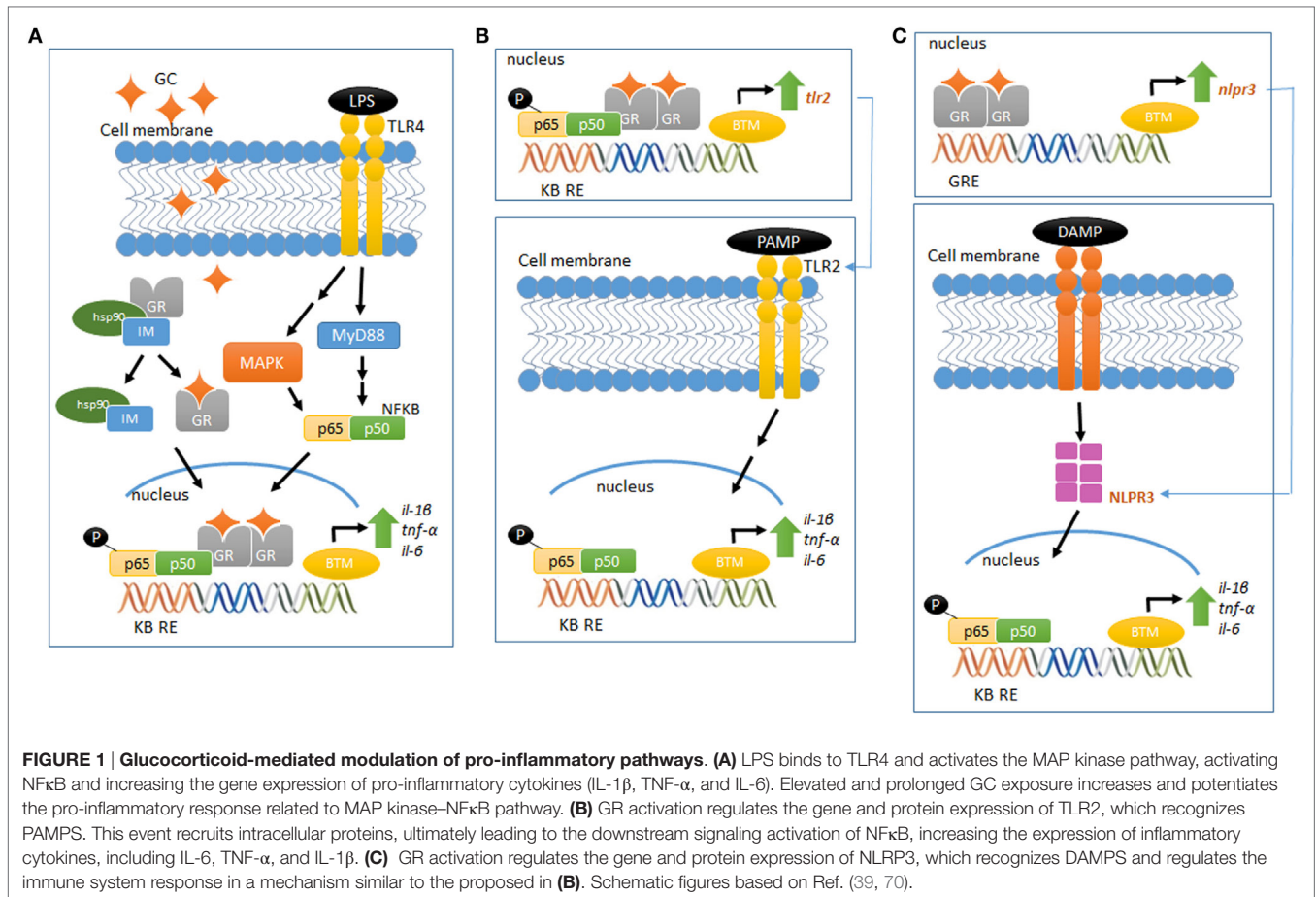
mediators. It has been showed increased plasma levels of TNF α and IL-1 β , and IL-1 β content in various CNS regions (pituitary, hypothalamus, hippocampus, and cerebellum) shortly after the LPS challenge (56).

Timing is an important variable to determine the outcome of GCs actions. Frank et al. (50, 57) reported that CORT administered acutely before an LPS challenge facilitated the inflammatory response to the challenge, both in the periphery and in the brain. In contrast, CORT administered after the same immune challenge resulted in suppression of the inflammatory response (50). They further demonstrated that prior *in vivo* administration of CORT potentiated the pro-inflammatory response of isolated hippocampal microglia exposed, *ex vivo*, to LPS (51), establishing a direct relationship between CORT elevation and sensitizing hippocampal microglia.

Corticosterone is also important in the sensitization of microglia in aging. Aged rats, submitted to an immune challenge, show potentiated and longer inflammatory response. Such potentiation has been associated with impairments in hippocampal synaptic plasticity and in contextual and spatial forms of memory (57–59). Interestingly, these aged rats exhibited higher corticosterone levels in the hippocampus, but not in the plasma, throughout the diurnal inactive phase only. Furthermore, the elevated diurnal hippocampal CORT was associated with an increased expression of hippocampal 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), the enzyme that catalyzes GCs inactive form, 1-dehydrocorticosterone, to the active one, CORT, and to a greater hippocampal GR activation, also only in the inactive phase of the day. Moreover, nuclear-specific expression of GR during the inactive phase was significantly higher in aged rats (59).

Although the molecular mechanism of GC pro-inflammatory actions is largely unknown, new players have been set in action. GCs upregulate the expression of the TLR2 and TLR4, both involved in the recognition of PAMPS and DAMPS and in the activation of signaling cascades that lead to the synthesis and release of inflammatory mediators (60). *In vitro* and *in vivo* studies have demonstrated that GCs can upregulate TLR2 expression by the activation of MAPK phosphatase-1 (MKP-1), which, in turn, inhibits p38 MAPK activity, a negative regulator for TLR2. The increased expression of TLR2 leads to enhanced cytokine expression, such as TNF- α , IL-1 β , and IL-8, upon challenge with an inflammatory stimulus. In addition, TNF- α and GCs cooperate to stimulate the promoter for TLR2 and potentially TLR4, increasing receptor expression (57, 61, 62) (Figures 1A,B).

The scientific literature provides growing information about the recently described new class of intracellular PRRs, the nucleotide-binding domain, leucine-rich repeat-containing (NBD-LRR, referred as NLRs) (63). Of the several NLRs recognized, NLRP1, NLRP3, AIM2, and NLRC4 are able to assemble and form a multiprotein complex known as the inflammasome. This complex regulates the activation of caspase-1 and the subsequent maturation and release of pro-inflammatory cytokines, such as IL-1 β and IL-18, in response to a wide variety of intracellular PRRs [for subject review, see Ref. (64)]. NLRP3 could be involved in GCs pro-inflammatory actions, as GCs potentiates the LPS-induced pro-inflammatory cytokine IL-1 β (45, 46). Indeed, Busillo et al. (65) demonstrated that NLRP3 is a GC-responsive gene in



macrophages, an effect GR-dependent. Glucocorticoid induction of NLRP3 sensitizes macrophages to extracellular ATP, which results in the secretion of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6 (65). Furthermore, GCs have been suggested to induce the expression of the purinergic receptor P2Y2R, which once activated, enhances IL-6 secretion by endothelial cells in response to ATP (66). Therefore, GC-mediated activation of TLR2, NLRP3, P2Y2R, and potentiation of TNF-α- and pro-inflammatory genes provide a potential mechanism by which these hormones exert pro-inflammatory actions (Figure 1C).

In addition, Weber and colleagues reported a new stress-induced pro-inflammatory mediator, the alarmin high-mobility group box-1 protein (HMGB-1) (67). HMGB-1 has been reported to be present in the CNS and to play a role in mediating neuro-inflammatory responses to ischemia and other injuries, such as immune disorders and neurodegenerative diseases [extensively reviewed in Ref. (68)]. Severe acute stress increases HMGB-1 protein levels in the hippocampus, specifically in the cytosol of hippocampal microglia isolated from severely acute stressed rats. NLRP3, IKBα, and NFκB proteins levels are also increased immediately after severe acute stress. Interestingly, Box A (a HMGB-1 antagonist) pretreatment blocked the stress-induced increase in microglial IL-1β, IKBα, and NLRP3 mRNA expression in response to a bacterial challenge (67). These results suggest that HMGB-1 participates in stress-inducing NLRP3 priming, adding

another mechanism by which GCs could have pro-inflammatory effects in the brain (67, 69).

MINERALOCORTICOIDS

Aldosterone is the steroidal hormone produced in the cortex of suprarenal gland that specifically binds to the MR. Although aldosterone is the related ligand of the MR, GCs can also bind to this receptor with equivalent affinity. The production and secretion of this hormone are majorly triggered in response to alterations in blood perfusion, perceived by cells in the juxtaglomerular complex. The main goal of this action is to maintain the blood pressure in a normal range in terms of control of water and electrolyte homeostasis (70). Mineralocorticoids can also modulate inflammation. MR expression is observed in cells of the immune system and aldosterone has been associated with pro-inflammatory immune effects, such as the release of pro-inflammatory cytokines, generating oxidative stress and inducing fibrosis (71).

Clinical studies have demonstrated that MR antagonism in cardiovascular diseases can blunt inflammatory damage, an important step in the induction of cardiac fibrosis (72). *In vitro* studies, using myeloid murine cells, have suggested an important role of mineralocorticoid in inducing changes in macrophages phenotype and, therefore, function.

In response to different inflammatory stimuli, macrophages can undergo classical M1 activation (stimulated by TLR ligands and IFN- γ) or alternative M2 activation (stimulated by IL-4/IL-13 cytokines); these states would correspond to the Th1–Th2 polarization of T cells [for review, see Ref. (73)]. In this sense, in dendritic cells from mice, it was shown that aldosterone induces the secretion of IL-6 and TGF- β , two pro-inflammatory cytokines necessary for the polarization of the adaptive immune response toward a Th17 phenotype, proning the system to autoimmunity (74). In peritoneal macrophages, aldosterone treatment promoted the activation of M1 macrophages, inducing TNF α , MCP-1, and IL12 gene expression, all those prevented by MR antagonism (75).

Regarding molecular mechanisms, it has been suggested that aldosterone, through MR, could regulate the expression of several genes. Indeed, cytokine promoters contain HREs, which can be modulated by GCs and potentially by mineralocorticoids (20). Despite it was suggested that ligand–receptor binding to simple HRE is enough to induce gene expression, many other proteins are able to modulate these interactions, adding specificity to gene transcription induced by MR. Also, this interaction could be more complex once the HRE also contains binding sites for non-receptor factors, the composite HREs (76).

In the brain, MR activation is important to maintain many functions of this tissue [for review, see Ref. (77)]. Under pathological conditions, such as cerebral stroke, MR antagonism or ablation has been associated with reduction of ischemic area, lower markers of remodeling process in cerebral vessels, and reduction of microglial/macrophages infiltration and activation, with decreased expression of cytokines and chemokines, such as IL-1 β , IL-6, TNF- α , MCP-1, and MIP-1. It was also reported that the MR activation in myeloid cells exacerbates inflammation and alters the M1/M2 inflammatory response to stroke (78). Finally, a study using microglial cells BV-2 reported that the stimulation with aldosterone resulted in NF κ B activation and the nuclear translocation of its p65 subunit, further upregulating the expression of IL-6 and TNF α , reinforcing the evidence of pro-inflammatory actions of mineralocorticoids in the brain (79).

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CONCLUDING REMARKS

Together, these findings suggest that exposure to steroid hormones in special conditions can shift the (neuro)immune microenvironment toward a pro-inflammatory state, predisposing certain regions of the CNS to a stronger pro-inflammatory response, if the organism is exposed to a subsequent challenge. Given the clinical implications of the pro-inflammatory steroid hormones actions, it is important to emphasize the need for continued basic investigations to understand the unexpected capacity of these hormones to increase CNS inflammation. Future research must be directed at determining how steroid hormones act on different cell types and regions to produce such markedly different immunomodulatory outcomes.

AUTHOR CONTRIBUTIONS

ED made substantial intellectual contributions to the conception, design, interpretation, drafting, and revising of the work, critically. CM made substantial contributions to conception, design, interpretation; revision critically for important intellectual content; and final approval of the version to be published.

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