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Cytokine-Effects on Glucocorticoid Receptor Function: Relevance to Glucocorticoid Resistance and the Pathophysiology and Treatment of Major Depression

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Abstract

Glucocorticoids play an essential role in the response to environmental stressors, serving initially to mobilize bodily responses to challenge and ultimately serving to restrain neuroendocrine and immune reactions. A number of diseases including autoimmune, infectious and inflammatory disorders as well as certain neuropsychiatric disorders such as major depression have been associated with decreased responsiveness to glucocorticoids (glucocorticoid resistance), which is believed to be related in part to impaired functioning of the glucocorticoid receptor (GR). Glucocorticoid resistance, in turn, may contribute to excessive inflammation as well as hyperactivity of corticotropin releasing hormone and sympathetic nervous system pathways, which are known to contribute to a variety of diseases as well as behavioral alterations. Recent data indicate that glucocorticoid resistance may be a result of impaired GR function secondary to chronic exposure to inflammatory cytokines as may occur during chronic medical illness or chronic stress. Indeed, inflammatory cytokines and their signaling pathways including mitogen-activated protein kinases, nuclear factor-kB, signal transducers and activators of transcription, and cyclooxygenase have been found to inhibit GR function. Mechanisms include disruption of GR translocation and/or GR-DNA binding through protein-protein interactions of inflammatory mediators with the GR itself or relevant steroid receptor cofactors as well as alterations in GR phosphorylation status. Interestingly, cAMP signal transduction pathways can enhance GR function and inhibit cytokine signaling. Certain antidepressants have similar effects. Thus, further understanding the effects of cytokines on GR signaling and the mechanisms involved may reveal novel therapeutic targets for reversal of glucocorticoid resistance and restoration of glucocorticoid-mediated inhibition of relevant bodily/immune responses during stress and immune challenge.

Introduction

Glucocorticoids play a fundamental role in restraining inflammatory and neuroendocrine responses to a variety of challenges including pathogen exposure and stress (Raison and Miller, 2003). Indeed, glucocorticoids suppress critical inflammatory signaling pathways including nuclear factor-kB (NF-kB) and inhibit stress-related outflow pathways including corticotropin releasing hormone (CRH), the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Glucocorticoids therefore play a critical role in balancing bodily responses to challenge, serving to both restore and maintain homeostasis.

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Failure of glucocorticoids to inhibit inflammatory and neuroendocrine responses to challenge may contribute to disease development (Raison and Miller, 2003). For example, recent data indicate that excessive inflammation may play a significant role in a number of medical illnesses including cardiovascular disease, diabetes, and cancer (Raison et al., 2006). Moreover, excessive HPA axis responses, including increased production and release of CRH, and SNS hyperactivity are hallmarks of depression. Given the central role of glucocorticoids and their signaling pathways in the maintenance of health and the prevention of disease, it is not surprising that a number of disorders characterized by excessive inflammatory responses including rheumatoid arthritis, asthma, and inflammatory bowel disease as well as depression have been associated with resistance to the inhibitory effects of glucocorticoids (Pariante and Miller, 2001;Raison and Miller, 2003). In the case of major depression, a clinical disorder characterized by significant alterations in mood, neurovegetative function and cognition, glucocorticoid resistance has been one of the most reproducible biological findings in the disease, occurring in up to 80% of patients (see below) (Holsboer, 2000;Pariante and Miller, 2001;Heuser et al., 1994).

The etiology of glucocorticoid resistance in both inflammatory and neuropsychiatric disorders is unknown and likely involves multiple factors including genetic influences. Nevertheless, mounting data suggest that inflammation itself may contribute to reduced glucocorticoid sensitivity. For example, data have established that cytokine signaling pathways can interact with glucocorticoid receptor (GR) signaling pathways and thereby disrupt glucocorticoid action (Miller et al., 1999). Such effects of cytokines and their signaling pathways on hormone receptors have been demonstrated in a number of other conditions with pathophysiologic relevance including the effects of TNF-alpha and NF-kB on Vitamin D receptor signaling and its relevance to osteoporosis (Nanes, 2003); the effects of TNF-alpha on insulin receptor signaling and its relevance to diabetes (Hotamisligil, 1999); and the effects of IL-1 and TNF-alpha on insulin-like growth factor receptor signaling and its relevance to muscle wasting in disorders such as Acquired Immune Deficiency Syndrome(Broussard et al., 2004;Kelley, 2004). Thus, the effects of cytokines and their signaling pathways on hormone signaling in general, and GR signaling in particular, is an important area of investigation regarding both the pathophysiology and treatment of inflammatory and neuropsychiatric diseases. In this review, we will focus on the potential contribution of inflammation and activation of cytokine signaling pathways to glucocorticoid resistance and its relevance to major depression.

Glucocorticoid Resistance and the Pathophysiology of Depression

One of the most reliably reported neurobiological alterations in major depression is both HPA axis hyperactivity and impaired HPA axis glucocorticoid feedback sensitivity. Depressed patients have been shown to exhibit increased concentrations of the HPA axis hormone, cortisol, in plasma, urine, and cerebrospinal fluid (CSF) (Pariante and Miller, 2001). In addition, depressed patients have been found to exhibit an exaggerated cortisol response to adrenocorticotropin hormone (ACTH) (Holsboer, 2000;Nemeroff, 1996;Pariante and Miller, 2001). Of note, increases in HPA axis activity are especially apparent in individuals who are older and/or who are more severely depressed (Pariante, 2004).

HPA axis hyperactivity observed in patients with major depression is largely thought to result from hypersecretion of corticotropin-releasing hormone (CRH). Indeed, depressed patients exhibit increased concentrations of CRH in CSF, increased CRH mRNA and protein in the paraventricular nucleus of the hypothalamus (postmortem samples), and a blunted ACTH response to CRH challenge (likely reflecting downregulation of pituitary CRH receptors) (Nemeroff, 1996;Pariante and Miller, 2001). Moreover, downregulation of CRH receptors in frontal cortex of victims of suicide (many of whom were presumably depressed) has been

described (Nemeroff, 1996). Hypersecretion of CRH may contribute to the behavioral features of major depression, in that administration of CRH to laboratory animals has been shown to lead to a host of behavioral changes that are comparable to those seen in depression including alterations in mood, appetite, sleep, locomotor activity and cognition (Nemeroff, 1996).

CRH hyperactivity in major depression is believed to be related, in part, to the failure of cortisol to suppress CRH production through negative feedback regulation (Holsboer, 2000;Pariante and Miller, 2001) This phenomenon is referred to as glucocorticoid resistance. The presence of glucocorticoid resistance in mood disorders is supported by evidence of cortisol nonsuppression to dexamethasone in the dexamethasone suppression test (DST) and the more recently developed dexamethasone-CRH (DEX-CRH) test (Holsboer, 2000). Of note, the DEX-CRH test has been shown to be significantly more sensitive than the DST with a sensitivity of up to 80% in patients with major depression, compared to 25% for the DST (Heuser et al., 1994). Failure of dexamethasone to suppress HPA axis responses has also been shown to predict clinical outcome during antidepressant treatment and has been found in first degree relatives of depressed patients (Ising et al., 2005). Aside from abnormal in vivo responses to dexamethasone administration, glucocorticoid resistance in depressed patients has also been demonstrated in vitro. For example, following in vitro glucocorticoid exposure, peripheral blood immune cells from depressed patients have exhibited reduced dexamethasone-induced inhibition of immune cell responses, notably mitogen-induced lymphocyte proliferation and NK cell activity, compared to healthy controls (Pariante, 2004;Pariante and Miller, 2001). Taken together with observations from in vivo glucocorticoid sensitivity measures, these data indicate that glucocorticoid resistance (impaired glucocorticoid sensitivity) is widespread throughout the body in both neuroendocrine and immune tissues and is not solely a function of dexamethasone bioavailability in vivo. Moreover, indications of glucocorticoid resistance from both in vivo and in vitro measures in depressed individuals suggest that in vitro tests of glucocorticoid sensitivity may be useful biomarkers of central glucocorticoid resistance (Pariante, 2004).

Although the mechanism of glucocorticoid resistance is poorly understood, the glucocorticoid receptor (GR) is believed to be involved. Many nuclear factors and signal transduction pathways are known to modulate GR function; however, the relative contribution of these pathways to GR dysfunction in depression has not been determined. One possible set of factors that may play a role in altered GR function in major depression is proinflammatory cytokines. In addition to acutely stimulating CRH and activating the HPA axis, it is becoming increasingly recognized that a number of cytokines, including interleukin-1, IL-2, IL-4, tumor necrosis factor (TNF) alpha, and interferon (IFN) alpha, and their signaling pathways, may influence neuroendocrine function through the impairment of GR function (Miller et al., 1999;Raison et al., 2006).

Cytokines and Depression

Data indicate that major depression is associated with immune activation as reflected by increased plasma and CSF concentrations of a variety of cytokines and their receptors, including IL-1, IL-2, IL-6 and TNF-alpha, as well as increases in plasma concentrations of acute phase reactants (which reflect the effects of proinflammatory cytokines on the liver), chemokines, and cellular adhesion molecules (Raison et al., 2006). Probably the most reproducible finding of immune activation in patients with depression is increased plasma levels of IL-6 and its downstream product from the liver, C-reactive protein (CRP). Of note, immune activation has been associated with treatment responses in patients with major depression, and there is preliminary evidence that polymorphisms in relevant cytokine genes may predict antidepressant treatment responsiveness (Raison et al., 2006). In addition to evidence of increased immune activation under baseline conditions, recent data indicate that

stress-induced activation of inflammatory responses is exaggerated in depressed patients (Pace, In Press). For example, depressed patients challenged with a public speaking and metal arithmetic stressor exhibited significantly higher levels of stress-induced IL-6 and activation of the inflammatory signaling molecule, NF-kB, compared to healthy controls. NF-kB is a lynchpin in the inflammatory response to challenge, and when chronically activated, is believed to be a major contributor to the association between inflammation and a number of medical disorders including cardiovascular disease, diabetes, cancer and osteoporosis (Raison et al., 2006). Indeed, the high rate of comorbidity between depression and these medical conditions may in part be a consequence of the association of depression with immune activation (Evans et al., 1999). Of further relevance to the connection between depression and immune activation is the finding that a number of cytokines, especially innate immune cytokines including IFN-alpha, and the proinflammatory cytokines, IL-1, IL-6, and TNF-alpha, have been shown to lead to a syndrome referred to as “sickness behavior” in laboratory animals and humans. This syndrome shares many features with major depression including alterations in mood, neurovegetative function, and cognition (Dantzer, 2004). Indeed, patients treated with IFN-alpha to combat cancers and certain infectious diseases such as hepatitis C often develop many of the key diagnostic criteria of major depression, including depressed mood, irritability/anxiety, anhedonia, impaired sleep, decreased appetite, psychomotor retardation, fatigue and cognitive dysfunction (Raison et al., 2006). Individual symptoms of depression, especially fatigue and cognitive dysfunction have also been correlated with plasma concentrations of proinflammatory cytokines and/or their receptors in cancer patients (Bower et al., 2002;Meyers et al., 2005). Interestingly, cytokine effects on information processing have also been described and may contribute to depression. For example, our group has recently reported that HCV patients treated with IFN-alpha exhibit increased activity of the dorsal anterior cingluate cortex (dACC) as measured by functional magnetic resonance imaging during a task of visuospatial attention. The dACC plays an important role in performance monitoring and error processing. When performance decreases (errors increase), the dACC becomes activated, increases arousal through activation of the autonomic nervous system, and reallocates cognitive resources to improve performance (Critchley et al., 2005;Holroyd et al., 2004). Of note, increased activity in the dACC has been observed in individuals who are vulnerable to mood and anxiety disorders including patients with high-trait anxiety, neuroticism and obsessive compulsive disorder (Chang et al., 2004). Thus, cytokine-induced increases in dACC activity may reflect an increased sensitivity to error (and/or negatively perceived internal or external events) and thus potentially represent a cognitive pathway to psychopathology during cytokine exposure.

The mechanism of cytokine effects on behavior are believed to be related in part to their effects on neurotransmitter and neuropeptide function, synaptic plasticity, and neuroendocrine function (Raison et al., 2006). The effects of cytokines on neuroendocrine function in depression may be related in part to their effects on the GR and its signaling pathways leading to glucocorticoid resistance. Indeed, in patients with major depression, increased mitogen-induced IL-1 responses of peripheral blood mononuclear cells (PBMCs) were found to positively correlate with post-DST plasma levels of cortisol, suggesting that DST non-suppression may be related to the effects of pro-inflammatory cytokines on GR signaling (Maes et al., 1993). Interestingly, stressor exposure, a well-known precipitant of depression, has been shown to activate both proinflammatory cytokines and inflammatory signaling pathways (e.g. NF-kB) in humans and laboratory animals (Bierhaus et al., 2003). In addition, stress exposure has also been shown to induce glucocorticoid resistance in neuroendocrine and immune tissues in mice. For example, Avitsur and colleagues utilizing a social disruption paradigm observed that defeated, but not victorious, mice demonstrated decreased immune system sensitivity to glucocorticoid-mediated inhibition (Avitsur et al., 2002). Closer examination revealed that glucocorticoid resistance correlated with assumption of a subordinate behavioral profile following defeat and with number of wounds received while fighting aggressive intruder mice. The investigators proposed that because submissive behavior

is associated with increased wounding, the development of glucocorticoid resistance may be an adaptive mechanism, allowing inflammatory healing to occur despite stress-related increases in glucocorticoids. Taken together, these data indicate that glucocorticoid resistance is associated with proinflammatory cytokines not only in the context of depression and stress, but also in subgroups of patients with chronic inflammatory diseases, who also exhibit high rates of comorbid mood disorders (Evans et al., 1999;Lamberts, 1996).

Cytokines and Glucocorticoid Resistance

Given the multiple steps in GR signaling, there are many points at which cytokines and their signaling pathways may influence GR function (Holsboer, 2000;Pariante and Miller, 2001). Within the cell, GR reside primarily in the cytoplasm, stabilized in the non-activated state by association with a chaperone protein complex containing heat shock proteins. Upon ligand binding and activation, the GR dissociates from the chaperone protein complex, becomes hyperphosphorylated, undergoes a conformational change and translocates to the nucleus. Once in the nucleus dimerization occurs, and GR homodimers influence cellular function through either interaction with other nuclear transcription factors or binding to the glucocorticoid responsive promoter region (glucocorticoid responsive element; GRE) of relevant genes. GR-DNA binding requires a number of steroid receptor co-factors for initiation of promoter activity. Protein-protein interactions of the GR with NF-kB and GR-GRE binding leading to the induction of IkB (which stabilizes non-activated NF-kB in the cytosol) are major mechanisms by which the GR regulates inflammatory responses (McKay and Cidlowski, 1999). Interestingly, however, one recent report shows that glucocorticoid repression of NF-kB activity occurs in the absence of GR-DNA binding (in transgenic mice carrying a DNA binding-defective GR (Reichardt et al., 2001), suggesting that protein-protein interactions (GR-NF-kB) may be a more primary contributor to glucocorticoid inhibition of NF-kB than induction of IkB. In addition, overexpression of GR results in a marked inhibition of NF-kB activity even in the absence of steroid,(Raddatz et al., 2001) further supporting the notion that NF-kB-GR interactions play an important role in balancing responses to stress and inflammation.

Cytokines and GR

Effects of Cytokines on GR Expression

A number of studies have examined the effects of cytokines on the expression of whole cell and cytosolic GR protein in a variety of cell types using radioligand binding techniques. Results have been mixed, with evidence of both cytokine-induced increases and decreases of GR, in part related to experimental conditions and assay strategies (Miller et al., 1999;Miller et al., 2001). In general, studies using whole cell radioligand binding techniques have found increased GR numbers following primarily in vitro treatment with a host of cytokines including IL-1, IL-2, IL-4, IL-6, TNF-alpha and IFN-alpha, whereas the majority of studies measuring the GR using a cytosolic radioligand binding assay find GR to be decreased following treatment with the same group of cytokines (Miller et al., 1999;Miller et al., 2001).

Studies have also focused on the impact of inflammatory cytokines on the expression of GR isoforms. Two isoforms of the GR are known to exist in humans: human (h)GR alpha and hGR beta (Lewis-Tuffin and Cidlowski, 2006). While hGR alpha contains the full set of 12 alpha-helices required for ligand binding, hGR beta lacks the 12th helix and contains a modified 11th, making it both unable to bind glucocorticoids and unable to activate glucocorticoid-responsive genes. In addition, hGR beta has been found to limit hGR alpha-dependent activation of glucocorticoid-sensitive genes (Lewis-Tuffin and Cidlowski, 2006). Interestingly, a recent study demonstrated that treatment of HeLaS3 and CEMC7 cells with either TNF-alpha or IL-1 increased hGR beta expression 2-fold, while only increasing hGR alpha by 1.5 fold. The effect

of these cytokines was dependent on an NF-kB DNA binding site upstream of the GR promoter (Webster et al., 2001). Thus, the relative expression of GR alpha and GR beta is believed to contribute to conditions of glucocorticoid resistance in disorders such as glucocorticoid-resistant asthma, leukemia, and ulcerative colitis (Lewis-Tuffin and Cidlowski, 2006). Nevertheless, a role for hGR beta in major depression has not been established. Indeed, a recent report by Matsubara and coworkers suggests that expression of hGR beta is unchanged in patients with major depression, while expression of hGR alpha is decreased (Matsubara et al., 2006). Of note, an equivalent of hGR beta is has yet to be described in other species, including mice and rats (de Kloet et al., 1998;Otto et al., 1997).

Effects of Cytokines on GR Function

Studies on the effects of cytokines on GR function have consistently demonstrated that a variety of cytokines can inhibit GR signaling as reflected by decreased GR translocation and decreased activation of relevant GR-inducible enzymes or reporter gene constructs (Miller et al., 1999;Miller et al., 2001;Wang et al., 2004). Multiple inflammatory and immunoregulatory signaling pathways may play a role in disrupting GR translocation and function and thereby contribute to glucocorticoid resistance. Pathways that have received the most attention include those involving mitogen activated protein kinase (MAPK), NF-kB, and Januskinase (Jak) - signal transducers and activators of transcription (STAT) (Figure 1). While it is somewhat daunting to imagine that there may be multiple pathways involved in GR regulation, each of these pathways represents a potential therapeutic target for the reversal of GR resistance and will therefore be considered below.

Mitogen-Activated Protein Kinase (MAPK)—Some of the major pathways that may contribute to the development of glucocorticoid resistance following cytokine exposure are those associated with the activation of MAPK (see Figure 1). MAPK cascades are generally divided into four groups: extracellular signal-related kinases (ERK)-1/2, jun amino-terminal kinases (JNK1/2/3), p38 proteins (p38 alpha/beta/gamma/delta) and ERK5. Each group of MAPK is phosphorylated by a different MAPK kinase (MAPKK): MEK1/2 activates ERK1/2, MKK4/7 (JNKK1/2) activates JNKs, MKK3/6 activates p38 and MEK5 activates ERK5 (Chang and Karin, 2001). In response to stress, infection or other environmental stimuli, MAPKs such as ERK, JNK and p38 activate nuclear transcription factors and promote proliferative and inflammatory responses. For example, the ERK pathway is stimulated in response to growth factors through Ras activation (Johnson et al., 2005). JNK is activated by UV irradiation, TNF-alpha and other proinflammatory cytokines, and lipopolysaccharide (LPS) (Chang and Karin, 2001;Johnson et al., 2005). JNK phosphorylates the N-terminus of the transcription factor c-Jun, which increases its ability to activate transcription (Chang and Karin, 2001;Johnson et al., 2005;Su and Karin, 1996). p38 is stimulated by stress (e.g. osmotic shock), as well as by LPS, and inflammatory cytokines (Su and Karin, 1996). The substrates of p38 include the transcription factors ATF-2 and CHOP (Kyriakis and Avruch, 2001). Thus, the MAPKs are activated by a wide variety of extracellular stimuli and act upon a wide range of targets (Chang and Karin, 2001;Johnson et al., 2005;Kyriakis and Avruch, 2001;Su and Karin, 1996).

Of relevance to GR function, multiple MAPK pathways have been implicated in GR dysregulation. Activation of ERK or JNK has been shown to inhibit GR function by directly phosphorylating GR at Ser-246 (JNK) and indirectly by ERK on GR cofactor (Rogatsky et al., 1998). In addition, ERK has been shown to mediate super-antigen-induced corticosteroid resistance in human T cells (Li et al., 2004). Our group and others have shown that p38 plays a key role in mediating IL-1- as well as IL-2 plus IL-4-induced inhibition of GR function (Goleva et al., 2002;Irusen et al., 2002;Kam et al., 1993;Wang et al., 2004). Indeed, studies

have indicated that IL-1 alpha (and beta) are capable of blocking translocation of the GR from the cytoplasm to the nucleus and reducing dexamethasone-induced GR-DNA binding and GR-mediated, dexamethasone-induced reporter gene activity (Pariante et al., 1999). The effects of IL-1 were reversed using an IL-1 receptor antagonist as well as pharmacologic inhibitors of p38 and p38 antisense oligonucleotides (Wang et al., 2004). Studies have shown that p38 phoshorylates the GR, which may explain its effects on GR translocation/DNA binding (Irusen et al., 2002). Interestingly, IL-1 alpha was associated with increased GR mRNA and protein, possibly secondary to reduced autoregulation of receptor expression (Pariante et al., 1999;Wang et al., 2004). JNK pathways have also been implicated in regulating GR-mediated gene transcription through mutual antagonism/repression with AP1. Activation of JNK results in phosphorylation of c-Jun, which in turn dimerizes with c-Fos to form AP-1, which then interacts with the GR through protein-protein interactions (Figure 1) (Smoak and Cidlowski, 2004). Interestingly, JNK activation has also been shown to increase nuclear export of the GR (Itoh et al., 2002). Recent data from our group indicate that JNK may play an important role in GR regulation under resting conditions likely by way of mutually inhibitory protein-protein interactions between GR and c-Jun (Wang et al., 2005). Inhibition of JNK using a pharmacologic inhibitor resulted in significant increases in dexamethasone-induced GR-GRE binding as well as GR-mediated, dexamethasone-induced reporter gene activity in mouse hippocampal HT22 cells and mouse fibroblasts. Similar effects were also observed after treatment of cells with a JNK-specific antisense oligonucleotide. JNK inhibition was not associated with changes in GR nuclear translocation. Finally, both p38 and JNK pathways have been implicated in the inhibitory effects of TNF-alpha on GR function (Szatmary et al., 2004).

Nuclear Factor-kB—NF-kB is an important nuclear transcription factor that plays a pivotal role in mediating inflammatory and immune responses to proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha (McKay and Cidlowski, 1999). As noted above, inactivated NF-kB is a heterodimer consisting of p65 (Rel A) and p50 subunits associated with the inhibitory factor IkB in cytosol (Figure 1). In response to stress, inflammation or infection, IkB kinase is activated and the phosphorylated IkB dissociates from NF-kB, leading to NF-kB activation. Activated NF-kB translocates to the nucleus and binds to its responsive element, thereby inducing its target genes including proinflammatory cytokines. NF-kB has long been recognized to interact with the GR at multiple levels (McKay and Cidlowski, 1999). NF-kB was found to directly interact with GR in the nucleus through physical association, causing mutual repression of both GR and NF-κB function (McKay and Cidlowski, 1999). In addition, NF-kB and GR have been shown to compete for the coactivators CREB-binding protein (CBP) and steroid receptor coactivator-1 (SRC-1) in the nucleus (Sheppard et al., 1998). Indeed, overexpression of CBP attenuates NF-kB-mediated repression of GR activity (and vice versa), as does overexpression of SRC-1 (Sheppard et al., 1998).

Signal transducers and activators of transcription (STATs)—Stimulation of Type I (e.g. IL-2, IL-4, IL-6, and IL-12) and II (IFN alpha/beta, IFN-gamma, and IL-10) cytokine receptors results in activation of Jak-STAT pathways. Only four mammalian Jaks are known to exist (Jak1, Jak2, Jak3, and Tyk2), while 6 STAT proteins have been identified (STAT1-6). Jaks are protein tyrosine kinases that are integrated within the cytoplasmic portion of Type I and II cytokine receptors, while inactive STATs exist as monomers in the cytoplasm. Upon cytokine binding, either two Type I or two Type II receptors dimerize within the lipid bilayer, permitting transphosphorylation of the receptors to occur. This results in phosphorylation of key tyrosine residues in Jak. These phosphorylated tyrosine moieties are then seen by inactive STAT monomers, which are recruited to the receptors from the cytoplasm. STATs are then phosphorylated by Jaks, leading to STAT dimerization and subsequent nuclear translocation

where these complexes bind to their responsive elements. While various cytokines that bind to Type I and II receptors are known to selectively activate particular Jaks, various cytokines via Jaks have the ability to activate any one of the six STATs. However, most cytokines are known to preferentially activate particular STATs. For example, IL-6 preferentially activates STAT3, while IFN-alpha activates STAT5. For a recent review of Jak-STAT signaling, see (Rogatsky and Ivashkiv, 2006).

GR and the Jak-STAT pathway have been shown to interact through direct GR-STATs protein-protein interactions, and through various cofactors (e.g., CBP/ p300 HATs) (Rogatsky and Ivashkiv, 2006). The most studied interactions between Jak-STAT and GR signaling pathways have been protein-protein interactions between GR and STAT5. Immunoprecipitation studies have demonstrated that STAT5 and GR form complexes. Studies investigating GR-STAT5 interactions have centered more upon how GR synergizes with STAT5. For example, GR has been shown to enhance STAT5 promotion of the milk protein β-casein (Stocklin et al., 1996). While GR enhances STAT5 function, STAT5 induced by IL-2 has been shown to inhibit GR function (Biola et al., 2001). Mechanisms that may be involved in STAT5-induced inhibition of GR function are believed to include disruption of GR translocation (Goleva et al., 2002), disruption of GR-GRE binding and/or disruption of GR egress from the nucleus (Hu et al., submitted). Less is known about how other STAT proteins interact with GR. Selective activation of STAT6 has been shown to inhibit GR function, but it is not known if this involves direct protein-protein interactions (Biola et al., 2000). STAT3 and GR have been coimmunoprecipiated from cell extracts, demonstrating their protein-protein interaction. But unlike other STATs, STAT3 appears to enhance GR function; GR and STAT3 may stabilize each other's interaction with DNA and thus enhance the transcriptional role either normally plays (Lerner et al., 2003). Current evidence suggests that GR and STAT1 do not interact directly, although GR has been shown to enhance the function of STAT1 through an unknown coactivator (Aittomaki et al., 2000). Protein-protein interactions between STAT4 and STAT2 and GR have yet to be explored.

Other Inflammatory Signaling Pathways: The Phospholipase/COX/ Prostaglandin Pathway Another inflammatory signaling pathway that has been reported to interact with and modulate GR function is the phospholipase/COX/prostaglandin pathway. In response to proinflammatory or mitogenic stimuli, cell membrane phospholipids (e.g. phosphotidylcholine) are hydrolyzed by phospholipase A2 (PLA2) to form arachidonic acid (AA) (Tanabe and Tohnai, 2002) (Figure 1). The isozymes cyclooxygenase 1 and 2 (COX-1 and COX-2) are enzymes that synthesize prostaglandins from their AA precursors (Tanabe and Tohnai, 2002). Both COX enzymes exhibit widespread expression in multiple tissue/cell types including the CNS. COX-1 exists constitutively, whereas COX-2 is a rate-limiting enzyme that is readily inducible by many inflammatory factors including IL-1, TNF-alpha and LPS. It is also reported that three MAPK pathways (ERK1/2, JNK and p38) contribute to the induction of COX-2 (Su and Karin, 1996).

COX has been reported to regulate GR function. For example, a recent report showed that nimesulide, a COX-2 inhibitor, induces GR-DNA binding, GR-mediated MMTV-luciferase activity, and GR phosphorylation in cultured human osteoarthritic synovial fibroblast cells (Di Battista et al., 1999). However, the conclusion that this effect was solely a function of COX-2 inhibition is weakened by the fact that nimesulide also inhibits phosphodiesterase (PDE) Type IV. PDE IV inhibitors, like rolipram, have been shown to enhance GR function (Bevilacqua et al., 1994;Miller et al., 2002) (see below). Nevertheless, results from our laboratory have shown that treatment of rat PC12 cells with the widely used non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen (COX-1 and COX-2 inhibitor) or celecoxib (selective COX-2 inhibitor) significantly induced GR-mediated MMTV-luciferase activity, whereas the

selective COX-1 inhibitor, valerylsalicylic acid, had no effects on GR-mediated gene transcription (Hu et al., 2005). Ibuprofen and several other NSAIDs have been shown to inhibit p38 MAPK activity in Jurkat T-cells (Paccani et al., 2002;Paccani et al., 2005). Accordingly, we administered anisomycin, a potent activator of p38, along with celecoxib to rat PC12 cells. Treatment of cells with anisomycin was found to reverse the celecoxib-induced enhancement of GR-mediated gene transcription in a dose-dependent manner, indicating the effects of COX-2 inhibitors on GR may be related to their ability to inhibit p38 (Hu et al., 2005). Recent data also indicate that anisomycin is a potent activator of JNK signaling pathways (Paccani et al., 2005), which in turn have been shown to inhibit GR function (see above). Taken together, these results suggest that MAPK signaling pathways (including both p38 and JNK) may be involved in the effects of COX-2 inhibition on GR function and may represent a final common target for GR regulation as it relates to inflammation and stress-related neuroendocrine pathways.

Therapeutic Implications of Cytokine-GR Interactions

Given the potential impact of cytokines and their signaling pathways on GR function, consideration should be given to therapeutic strategies that might address cytokine-GR interactions and their relevance to glucocorticoid resistance in major depression. Several strategies are immediately apparent and relate to pharmacologic agents and signaling pathways that have been found to influence the GR as well as cytokines and their signaling pathways. These include antidepressants, cyclic AMP and Protein Kinase A (PKA) signaling pathways and COX-2 inhibitors (discussed above).

Antidepressants, GR and Cytokines—A number of studies have established that a variety of antidepressant have the capacity to enhance GR translocation and function both in vivo and in vitro. Indeed, early studies using the antidepressant desipramine (DMI), demonstrated that DMI was capable of both increasing dexamethasone-induced GR-mediated gene transcription and increasing GR translocation (even in the absence of dexamethasone) (Pariante et al., 1997). Other antidepressants, including clomipramine, fluoxetine, paroxetine, and citalopram, have also exhibited these effects on the GR (Pariante and Miller, 2001). It has been demonstrated that the mechanism of antidepressant effects on GR function are related in part to their inhibitory effects on the multi-drug resistance pump (making more hormone available for GR activation), however, other antidepressant-induced signal transduction pathways including c-AMP appear to be involved (see below). Of relevance to interactions between cytokines and GR, data also indicate that antidepressants exhibit the capacity to inhibit cytokine production both in vitro and in vivo (Kenis and Maes, 2002). For example, exposure of mixed glial culture to amitriptyline led to a decrease in LPS-stimulated release of both IL-1beta and TNF-alpha (Obuchowicz et al., 2006). Nevertheless, whether these effects of antidepressants on cytokines are related to their effects on the GR have yet to be established.

cAMP and PKA Regulate GR Function and Interact with Cytokines and their Signaling Pathways—Cyclic AMP-dependent protein kinase A (PKA) signal transduction pathways regulate a wide variety of biological responses. PKA predominately exists in cytosol in the form of a tetramer (two regulatory and two catalytic subunits). Activation of a variety of neurotransmitter receptors (including serotonergic 5-$HT_{4,6,7}$ and adrenergic beta receptors) induces a G protein conformational change and subsequently stimulates adenyl cyclase which catalyzes ATP to cAMP (Nibuya et al., 1996;Wilcox et al., 1998). Intracellular cAMP then binds to the regulatory subunits and triggers a PKA conformation change. The catalytic subunits dissociate from the tetramer and become activated. Activated PKA translocates to different cellular compartments including the nucleus where it phosphorylates cAMP

responsive element binding protein (CREB) and enhances CREB-CRE binding to DNA. This binding triggers the transcription of cAMP target genes.

A large body of evidence indicates that the integrity of the PKA signaling pathway is required for GR function. It has been shown that GR associates both *in vivo* and *in vitro* with the catalytic subunit of PKA in a ligand-independent manner (Doucas et al., 2000). Moreover, PKA has been found to phosphorylate GR independent of the presence of hsp90, and the transformational state of GR and phosphorylation of GR by PKA can be inhibited by H-8, a PKA inhibitor (Haske et al., 1994). Several studies have demonstrated that PKA agonists, including forskolin and 8-Br-cAMP, can increase GR mRNA stability and GR mRNA levels, and enhance GR transcription and function (Dong et al., 1989;Penuelas et al., 1998). Indeed, treatment of primary human lung fibroblasts and vascular smooth muscle cells with the beta 2-adrenergic receptor agonists, salbutamol or salmeterol, was found to translocate the GR to the nucleus, increase GR-GRE binding and increase GRE-driven luciferase reporter gene activity (Eickelberg et al., 1999). In the same study, addition of cAMP alone was shown to induce GR-GRE binding, and a PKA inhibiting peptide was able to reduce this effect. PKA also activates GR function in a ligand-independent manner (Eickelberg et al., 1999). Work in our laboratory also supports a role for the cAMP-PKA pathway in GR regulation. For example, the phosphodiesterase (PDE) type IV inhibitor, rolipram, which antagonizes the breakdown of cAMP, was found to significantly enhance GR-mediated gene transcription, both in the presence and absence of dexamethasone, in LMCAT mouse fibroblast cells and rat C6 glioma cells (Miller et al., 2002). In addition to directly regulating GR function, increased PKA activity has also been found to reverse GR resistance. Medh and coworkers reported that in GR resistant lymphoid cells (CEM-C1), dexamethasone (up to 1 uM) fails to cause cell death. However, when these cells were co-treated with forskolin, dexamethasone (1 uM) caused 90% cell death (Medh et al., 1998). RU486, a GR antagonist, was able to block this effect, demonstrating its dependence on the GR. In further support of the role of cAMP-dependent PKA pathways in GR function, Gruol and colleagues reported that cAMP-resistant cell lines give rise to a significant higher frequency of glucocorticoid-resistant cell variants (10^{-7} vs. 10^{-10} in wild type) (Gruol et al., 1986).

Relevant to cytokine-GR interactions, PKA signaling pathways also interact with both NF-kB and MAPK signaling pathways (Saxena et al., 1999;Takahashi et al., 2002). Indeed, increased PKA activity has been shown to inhibit NF-kB transcription through interaction of the catalytic subunit of PKA with p65, blocking p65 transactivation (Takahashi et al., 2002) (Figure 1). In addition, in a number of cell types, elevated PKA inhibits MAPK pathways by phosphorylation of serine residues on raf-1, which leads to a reduced affinity of raf for Ras (Hafner et al., 1994). PKA can also downregulate the kinase activity of already activated raf. In addition, forskolin, a PKA activator, has been reported to inhibit MAPK-induced raf-1 translocation (Melck et al., 1999). Tamir and coworkers reported that forskolin additionally inhibits T cell activation through the down-regulation of MAPK pathways (Tamir et al., 1996). Taken together, these data suggest that drugs that can activate cAMP-PKA pathways, such as phosphodiesterase type IV inhibitors, may represent an intriguing therapeutic strategy in reversing glucocorticoid resistance. Since PKA pathways can both enhance GR function as well inhibit inflammatory signaling, pharmacologic therapies targeting PKA may represent a “double hit” on relevant mechanisms driving glucocorticoid resistance (Miller et al., 2002).

The interactions of PKA signaling pathways with a) GR signaling and b) signaling pathways of proinflammatory and immunoregulatory cytokines are especially relevant in view of the fact that depressed patients have been found to exhibit reduced G protein function in mononuclear cells (Avissar et al., 1997) and reduced cAMP-dependent protein kinase activity in cultured fibroblasts (Shelton et al., 1996). Cyclic AMP/PKA signal transduction pathways have also

been shown to be reduced in post-mortem brain tissue from depressed patients. Of note, work on the mechanism of action of antidepressants suggests that cAMP and PKA pathways play an important role as mediators of the psychotropic effects of these agents (e.g. (Nibuya et al., 1996). Therefore, it is possible that disruption in the cAMP/PKA pathway in major depression is linked to cytokine-induced GR resistance and antidepressants and other pharmacologic agents that can enhance PKA signaling may overcome GR alterations via a direct effect on crosstalk between these pathways.

COX-2 inhibitors—As noted above, COX-2 inhibitors have shown the capacity to enhance GR function as measured by dexamethasone- and corticosterone-induced GR-mediated reporter gene activity and GR-GRE binding as measured by electrophorectic mobility shift assay. In addition, COX-2 inhibitors led to enhanced GR nuclear translocation. The effects of COX-2 inhibitors were reversed by administration of anisomycin which activates both p38 and JNK signaling pathways. These data suggest that through inhibiting p38 and/or JNK, COX-2 inhibitors may serve to enhance GR function and potentially reverse glucocorticoid resistance. Interestingly, although neuroendocrine parameters were not assessed, COX-2 inhibitors were recently found to augment the effects of the antidepressant, reboxetine, in treating patients with major depression (Muller et al., 2006).

Summary and Conclusions

Glucocorticoids play a fundamental role in modulating the response to a variety of challenges that invoke both neuroendocrine and immune responses, and serve to restore and maintain bodily homeostasis. Glucocorticoid receptors are central to glucocorticoid action, and therefore the integrity of GR signaling is essential to an effective glucocorticoid response. Mounting data suggest that cytokines can significantly influence GR signaling with evidence that induction of cytokine signaling pathways can suppress GR signaling and thereby contribute to glucocorticoid resistance. Glucocorticoid resistance in turn may contribute to over-exuberant neuroendocrine and immune responses to challenge and lead to potentially detrimental consequences including the excessive release of potentially damaging mediators including CRH and proinflammatory cytokines, both of which have been implicated in behavioral alterations. Therapeutic strategies to restore the integrity of GR function may thus involve targeting of relevant inflammatory signaling molecules that have been most implicated in GR disruption including p38, JNK, STAT 5, NF-kB and COX-2. Future studies targeting these molecules represent a natural progression in the evolution of therapeutic strategies that are informed by the notion that immunologic processes are relevant factors in the pathophysiology of behavioral alterations and the development of psychiatric diseases including depression.

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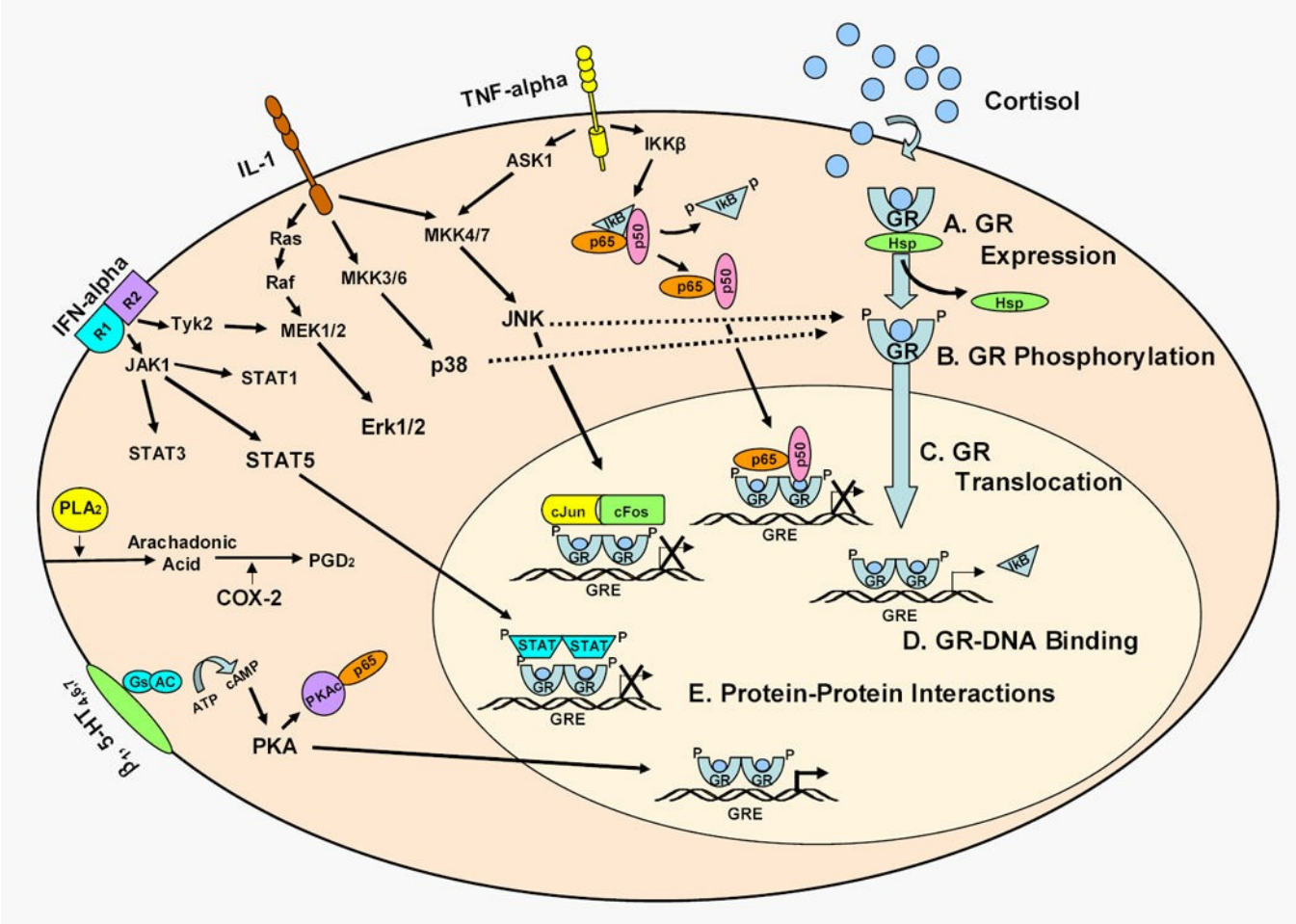

Figure 1. Interactions between Cytokine and Glucocorticoid Receptor Signaling Pathways

Selected cytokines and their signal transduction pathways are depicted in simplified fashion to illustrate representative interactions between cytokine and glucocorticoid receptor (GR) signaling events. Cortisol binds to GR, resulting in dissociation of heat shock protein (HSP) complexes and subsequent phosphorylation. GR then translocates to the nucleus where it dimerizes and either interacts with other transcription factors, or binds to glucocorticoid response elements (GREs) upstream of GR-regulated genes (e.g. inhibitor κ-B or IκB). TNF-alpha binds to its receptor and results in activation of IκB kinase β (IKKβ), which phosphorylates IκB, allowing NF-κB (shown here as p65 and p50 Rel subunits) to translocate to the nucleus. Through protein-protein interactions, activated NF-κB associates with GR, thus interfering with GR-DNA-binding. IL-1 binds to its receptor initiating a) mitogen activated protein kinase (MAPK) kinase (MKK)4/7, which culminates in activation of Jun amino-terminal kinase (JNK), b) MKK3/6, which culminates in activation of p38, and c) Ras, which results in activation of the extracellular signal-related kinase (Erk)1/2. Of note, MKK4/7 activation of JNK can also occur through TNF-alpha receptor binding. As depicted by the dotted lines, both p38 and JNK can phosphorylate key GR residues, thereby disrupting nuclear translocation of GR. Interferon (IFN)-α binds to its receptor resulting in Janus kinase (Jak) phosphorylation, represented as Jak1 and tyrosine kinase (Tyk)2. Jak1 phosphorylates signal transducers and activators of transcription (STAT) proteins, including STAT1, STAT3, and STAT5. Tyk2 can also activate elements of the Ras signaling pathway, resulting in activation of Erk1/2. Activated STATs translocate to the nucleus, where they can interact with GR through protein-protein interactions, thereby interfering with GR DNA-binding. Phospholipids are

hydrolyzed by phospholipase A2 (PLA2) to form arachidonic acid which is metabolized by cyclooxygenase (COX) 2 to produce prostaglandin D2 (PGD2). Stimulation of serotonergic receptors 4, 6, or 7 (5-HT4, 6, 7) and beta adrenergic receptors (β1) induces a conformational change in G stimulatory (Gs) protein, which then activates adenylyl cyclase (AC). AC, in turn, converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). cAMP then induces a conformational changes in protein kinase A (PKA), which translocates to the nucleus where it is able to enhance GR DNA-binding. In addition, the catalytic subunit of PKA (PKAc) interacts with p65, thereby inhibiting NF-κB nuclear translocation.