REVIEW

Glucocorticoid Receptors in Major Depression: Relevance to Pathophysiology and Treatment

Carmine M. Pariante and Andrew H. Miller

Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis has been reliably observed in patients with major depression. One of the primary features of this HPA axis hyperactivity is reduced sensitivity to the inhibitory effects of the glucocorticoid dexamethasone on the production of adrenocorticotropic hormone and cortisol during the dexamethasone suppression test and, more recently, the dexamethasone–corticotropin-releasing hormone test. Because the effects of glucocorticoids are mediated by intracellular receptors including, most notably, the glucocorticoid receptor (GR), a number of studies have considered the possibility that the number and/or function of GRs are reduced in depressed patients. Moreover, whether antidepressants act by reversing these putative GR changes has been examined. The extant literature on GR receptors in major depression was reviewed along with studies examining the impact of antidepressants on the GR. The data support the hypothesis that the function of the GR is reduced in major depression in the absence of clear evidence of decreased GR expression. The data also indicate that some antidepressants have direct effects on the GR, leading to enhanced GR function and increased GR expression. Hypotheses regarding the mechanism of these receptor changes involve relevant second messenger pathways that regulate GR function. The findings indicate that the GR is an important molecular target in major depression. Further elucidation of the biochemical and molecular mechanisms involved in GR changes in major depression is an exciting frontier that will no doubt lead to new insights into the pathophysiology and treatment of affective disorders. Biol Psychiatry 2001;49:391–404 © *2001 Society of Biological Psychiatry*

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Introduction

Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis in patients with major depression is one of the most consistent findings in biological psychiatry. Specifically, patients with major depression have been shown to exhibit increased concentrations of cortisol in plasma, urine, and cerebrospinal fluid (CSF); an exaggerated cortisol response to adrenocorticotropic hormone (ACTH); and an enlargement of both the pituitary and the adrenal glands (Gold et al 1988; Holsboer and Barden 1996; Nemeroff 1996; Owens and Nemeroff 1993).

These HPA axis alterations are believed to be secondary to hypersecretion of corticotropin-releasing hormone (CRH), which has behavioral effects in animals that are similar to those seen in depressed patients, including alterations in activity, appetite, and sleep (Owens and Nemeroff 1993). Moreover, depressed patients exhibit increased concentrations of CRH in the CSF, increased CRH messenger RNA (mRNA) and protein in the paraventricular nucleus (PVN) of the hypothalamus (postmortem samples), and a blunted ACTH response to a CRH challenge (likely reflecting downregulation of pituitary CRH receptors) (Gold et al 1988; Nemeroff 1996). Finally, downregulation of CRH receptors in the frontal cortex of victims of suicide (many of whom were presumably depressed) has been described (Nemeroff 1996).

Although the mechanism by which extrahypothalamic CRH is elevated in depression has not been resolved, the increased levels of CRH in the hypothalamus are thought to be related, in part, to altered feedback inhibition by endogenous glucocorticoids. Through binding to their receptors in HPA axis tissues, endogenous glucocorticoids serve as potent negative regulators of HPA axis activity including the synthesis and release of CRH in the PVN (Owens and Nemeroff 1993; Reul and de Kloet 1985). Data supporting the notion that glucocorticoid-mediated feedback inhibition is impaired in major depression come from a multitude of studies demonstrating nonsuppression of cortisol secretion following administration of the synthetic glucocorticoid dexamethasone and more recent studies showing a lack of inhibition of ACTH responses to CRH following dexamethasone pretreatment (Gold et al 1988; Heuser et al 1994; Holsboer and Barden 1996; Nemeroff 1996; Owens and Nemeroff 1993). Although

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nonsuppression to dexamethasone in the dexamethasone suppression test (DST) and the dexamethasone–CRH test likely represent impaired feedback inhibition at the level of the pituitary (de Kloet et al 1998; Miller et al 1992), impaired responsiveness to hydrocortisone challenge in depressed patients suggests these feedback alterations also occur in the brain (Young et al 1991). Furthermore, the existence of reduced HPA axis suppression by dexamethasone in first-degree relatives of depressed individuals suggests that altered feedback inhibition may represent a genetic (trait) vulnerability to the depressive disorders (Modell et al 1998).

Feedback regulation of the HPA axis by glucocorticoids is mediated through two distinct intracellular receptor subtypes referred to as the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (Reul and de Kloet, 1985). The MR has a high affinity for endogenous corticosteroids and is believed to play a role in the regulation of circadian fluctuations in these hormones (especially the regulation of ACTH secretion during the diurnal trough in cortisol secretion). In contrast to the MR, the GR has a high affinity for dexamethasone and a lower affinity for endogenous corticosteroids. The GR is therefore believed to be more important in the regulation of the response to stress when endogenous levels of glucocorticoids are high. Recently, Spencer et al (1998) and de Kloet et al (1998) have clarified that GR activation is necessary for the HPA feedback regulation when levels of glucocorticoids are high (response to stress, circadian peak), but that the MR also plays an important role by modulating GR-dependent regulation. Because patients with major depression exhibit impaired HPA negative feedback in the context of elevated circulating levels of cortisol and because altered HPA axis responsiveness has been characterized with dexamethasone, which selectively binds GR in vivo, studies investigating corticosteroid receptors in major depression have logically focused on the expression and function of GR.

Glucocorticoid Receptors in Depression

Over the past 15 years a number of studies have assessed GRs in patients with major depression. In general, these studies have measured GR numbers directly or have examined the in vitro or in vivo influence of glucocorticoids on functions known to be regulated by the GR. These GR assessments have been made primarily on peripheral cell types including immune cells (mononuclear and polymorphonuclear leukocytes) and fibroblasts (gingival and skin). Limited information exists regarding the number and function of GRs in the central nervous system. Of note, however, are data that have demonstrated similar regulation of GRs in the brain and immune system of laboratory animals. For example, Lowy (1990) has demonstrated that treatment of rats with the amine-depleting drug reserpine (that is known to induce depressive symptoms in humans and to produce dexamethasone nonsuppression in rats) decreases GR levels in the hippocampus, frontal cortex, and pituitary as well as in lymphocytes and the spleen. Similarly, Spencer et al (1991) have found that, both in the brain and in the immune system, the GR upregulates following adrenalectomy and downregulates following chronic treatment with corticosterone. Nevertheless, there are differences in GR regulation among body compartments. For example, the GR in the hippocampus is more sensitive to corticosterone than the GR in the hypothalamus or cerebellum or the GR in the thymus or spleen (Spencer et al 1991). However, the direction of the GR changes induced by a variety of different conditions is similar in the brain and immune tissues, and therefore, given limited access to brain GRs in clinical populations, evaluation of peripheral tissues remains a viable option.

Glucocorticoid Receptor Number and Affinity

STUDIES ON PERIPHERAL CELL TYPES: The number and affinity of GRs in peripheral blood cell types of depressed patients have been measured by a host of investigators using competitive, radioligand binding assays. To evaluate this data, it is helpful to briefly review the steps involved in GR activation by ligand (Figure 1). According to the "nucleocytoplasmic traffic" model of GR action, the GR in its "unactivated" form resides primarily in the cytoplasm in association with a multimeric complex of chaperon proteins including several heat shock proteins (HSPs) (Pratt 1993). After being bound by steroid, the GR undergoes a conformational change ("activation"), dissociates from the chaperon protein complex, and translocates from the cytoplasm to the nucleus, where it either binds to hormone response elements (HREs) on DNA or interacts with other transcription factors (Guiochon-Mantel et al 1996). The activated GR cannot rebind ligand because association with the chaperon protein complex is required for maintaining the receptor in a conformational state receptive to hormone (Pratt 1993).

Two general types of binding assays have been used to assess GRs in depression. In the cytosolic binding assay, cells are lysed rapidly, without a period of incubation in steroid-free media, and only the cytosolic fraction of the lysate is incubated with radiolabeled steroid (Miller et al 1998; Spencer et al 1991). Since activation and translocation of the GR from the cytoplasm to the nucleus is associated with decreased GRs in the cytosolic fraction, a decrease in GR binding in the cytosolic assay can represent either a greater proportion of GRs in the nucleus (activation) or an overall decrease in the total number of

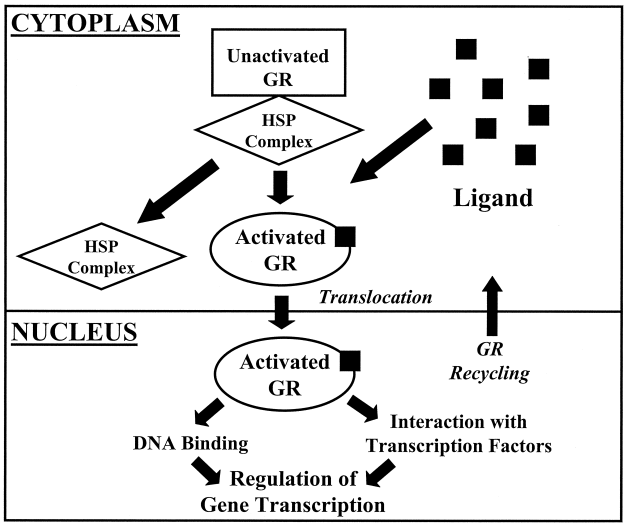

Figure 1. "Nucleocytoplasmic traffic" model of glucocorticoid receptor (GR) activation. The GR in its "unactivated" form resides primarily in the cytoplasm in association with a multimeric complex of chaperon proteins including several heat shock proteins (HSPs). Endogenous glucocorticoids (cortisol in humans, corticosterone in rodents) or synthetic glucocorticoids (e.g., dexamethasone) act as GR ligands (■). After being bound by ligand, the GR undergoes a conformational change ("activation"), dissociates from the chaperon protein complex, and translocates from the cytoplasm to the nucleus, where it regulates gene transcription by either binding to hormone response elements on DNA or by interacting with other transcription factors. The GR then recycles to the cytoplasm and cannot rebind ligand until association with the chaperon protein complex is completed.

available GRs (downregulation). Thus, the cytosolic binding assay cannot distinguish receptor activation from receptor downregulation. In contrast, the whole cell binding assay in most cases includes a period of time (ex vivo) when the cells are incubated in steroid-free conditions to allow dissociation of endogenous hormone from receptor (Steiner and Wittliff 1985). However, it is unclear to what degree ligand dissociation and/or receptor upregulation may occur during this time, both processes being dependent on temperature and pH (Steiner and Wittliff 1985). Aside from the potentially variable ex vivo incubation conditions, the whole cell assay has also suffered from a high degree of nonspecific binding and exaggerated K_d values (Miller et al 1998). Nevertheless, the whole cell assay ostensibly measures total cellular GRs.

As shown in Table 1, although several studies have observed alterations in GR number in depressed subjects, the majority of studies have found no differences between depressives and control subjects. No study has reported alterations in the affinity of GR for ligand (K_d) in depressed patients.

Of the studies showing GR changes, both Gormley et al (1985) and Whalley et al (1986) found a reduction in the number of GRs in depressed patients relative to healthy control subjects. Yehuda et al (1993) also demonstrated that depressed patients had the lowest number of GRs in several populations of psychiatric patients; however, no healthy control group was examined. Finally, Sallee et al (1995) found decreased GR binding in depressed adolescent patients relative to matched control subjects. In this study, reduced cytosolic GR binding at baseline predicted a good clinical response to the antidepressant sertraline, and antidepressant responders exhibited an increased cytosolic GR binding following treatment.

Of the studies finding no GR changes in depression, Schlechte and Sherman (1985), Rupprecht et al (1991aa, 1991b), Wassef et al (1990, 1992), and Maguire et al (1997) found no difference in GR between depressed patients and normal control subjects and/or other psychiatric patients. Rupprecht et al (1991bb) also found no differences between depressives and control subjects during illness or after recovery. Hunter et al (1988) studied

Table 1. Studies of Glucocorticoid Receptor Binding in Major Depression

Source	HDRS (severity)	DST nonsuppressor	Assay type	Cell type	Results
Gormley et al 1985	22.9	54%	Cyt	M	↓
Schlechte and Sherman 1985	—	45%	WC	L	→
Whalley et al 1986	26.9	—	WC	M	↓
Hunter et al 1988	NA	—	WC	M	→
Wassef et al 1990	16.4	36%	WC	M	→
Rupprecht et al 1991a	27.2	—	WC	M	→
Rupprecht et al 1991b	26.9	—	WC	M	→
Wassef et al 1992	22.0	26%	WC	M, P, F	→
Yehuda et al 1993	23.6	—	Cyt	M	↓
Sallee et al 1995	20.5	—	Cyt	L	↓
Maguire et al 1997	24.9	—	WC	L	→

HDRS, Hamilton Depression Rating Scale; DST, dexamethasone suppression test; Cyt, cytosolic binding; M, mononuclear cells (lymphocytes and monocytes); ↓, significant decrease in glucocorticoid receptor of depressed patients vs. control subjects; WC, whole cell binding; L, lymphocytes; →, no difference between depressed patients and control subjects; P, polymorphonuclear cells; F, cultured skin fibroblasts.

Table 2. Studies of Glucocorticoid Receptor Function in Major Depression

Source	HDRS (severity)	DST nonsuppressor	Assay	Steroid manipulation	Resistance
Lowy et al 1984	23.6	67%	Lymphocyte proliferation	DEX (O)	Yes[a]
Gormley et al 1985	22.9	55%	Binding	DEX (O)	Yes[a]
Miller et al 1987	—	—	NK activity	Cortisol (V)	Yes
Lowy et al 1988	28.7	41%	Binding	DEX (O)	Yes[a]
			Lymphocyte proliferation	DEX (O, V)	Yes[a]
Wassef et al 1990	16.4	36%	Binding	DEX (O)	Yes
Rupprecht et al 1991c	29.5	—	Lymphocyte proliferation	Metyrapone[b] (O)	Yes
Wodarz et al 1991	29.5	—	Lymphocyte proliferation	DEX (V)	Yes
Wodarz et al 1992	5.7	—	Lymphocyte proliferation	DEX (V)	No

HDRS, Hamilton Depression Rating Scale; DST, dexamethasone suppression test; DEX, dexamethasone; O, oral administration; NK, natural killer cell; V, in vitro.
[a]Resistance was present only in DST nonsuppressor.
[b]Cortisol synthesis inhibitor.

only recovered patients and found similar results, though the fact that patients had not been previously evaluated during illness limits the significance of the results. Finally, in the only study that has evaluated both immune cells (mononuclear and polymorphonuclear leukocytes) and cultured skin fibroblasts, no differences between depressed patients and a control group of other psychiatric patients were found (Wassef et al 1992).

Several methodological differences differentiate the various studies, including heterogeneity in the patient populations, the control groups, and the endocrine and medication status of the depressed patients. However, the most compelling difference in these studies is the binding techniques that were used to assess GRs (cytosolic binding vs. whole cell binding) (Table 1). All of the three studies that used a cytosolic binding assay (Gormley et al 1985; Sallee et al 1995; Yehuda et al 1993) found a reduced number of GRs in cells from depressed patients. In contrast, seven out of the eight studies using a whole cell assay found no difference between groups (Hunter et al 1988; Maguire et al 1997; Rupprecht et al 1991a,, 1991b,; Schlechte and Sherman 1985; Wassef et al 1990, 1992). As noted, the cytosolic binding assay cannot distinguish downregulation from receptor activation, so the nature of the reduced GR binding in studies using this assay cannot be determined. Nevertheless, the lack of changes in GRs in the whole cell assay (which best measures total GRs) coupled with the decreased GRs found in the cytosolic binding assay (in the absence of differences in receptor affinity) suggests that the GR changes seen in depression are likely secondary to nuclear compartmentalization of the GR or nonassociation of the GR with the chaperon protein complex due to present or recent activation by ligand. Both of these possibilities can be explained by increases in circulating levels of ligand (cortisol).

CNS STUDIES. Two studies have examined GR mRNA in postmortem brain sections. Consistent with data from peripheral blood mononuclear cells, Lopez et al (1998) found no differences in GR mRNA in the hippocampus of six suicide victims with a history of depression relative to a group of six control subjects. Of note, however, MR mRNA levels tended to be lower in the suicide victims and changes in the MR paralleled changes in the serotonin ($5\text{-}HT_{1A}$) receptor. Webster et al (2000) also examined postmortem brain sections and failed to find GR changes specific to depression. In this study, three patient groups—nonpsychotic depression, bipolar disorder, and schizophrenia—all exhibited decreased GR mRNA in the frontal cortex and hippocampus relative to nonpsychiatric control subjects. These latter results suggest that the stress of having a psychiatric disorder may be more relevant to changes in GR (or MR) expression than depression per se.

Glucocorticoid Receptor Function

Two types of studies have explored GR function in depressed patients: 1) those that have used GR binding to evaluate "ligand responsivity" in depressed patients (i.e., the response of GR binding to steroid manipulations that are known to alter the expression or compartmentalization [cytoplasm vs. nucleus] of the GR) and 2) those that have evaluated the impact of glucocorticoids on peripheral cell functions (immune function) known to be inhibited by GR activation.

As shown in Table 2, studies investigating changes in GR binding following in vivo or in vitro treatment with GR agonists have found a remarkable lack of response in depressed patients, especially in those who are nonsuppressors to the DST. Wassef et al (1990) found that control subjects exhibited decreased GR number after oral dexamethasone administration, whereas depressed patients did not. Gormley et al (1985) and Lowy et al (1988) reported that only depressed DST suppressors showed a decrease in GR binding after dexamethasone administration, whereas

nonsuppressors showed no effect of hormone. Since these relatively acute changes in ligand availability failed to alter receptor number in depressed patients, either the pharmacokinetics of the ligand is altered, as has been found with dexamethasone in depressed patients (Carson et al 1988), or there is some alteration in the relative ability of the GR to shuttle from cytoplasm to nucleus. Also of note are two studies in which cortisol secretion was inhibited by metyrapone, a glucocorticoid synthesis inhibitor. In these studies, healthy control subjects exhibited an increase in the number of lymphocyte GRs after metyrapone treatment, whereas depressed patients showed no difference. Since the authors analyzed whole cell GR binding, these findings suggest that GRs from depressed patients may have a reduced ability to respond to acute changes in circulating cortisol concentrations (Rupprecht et al 1991a,a, 1991c,c).

The majority of studies evaluating the impact of glucocorticoids on cellular function have focused on the well-known capacity of dexamethasone to inhibit the ability of peripheral blood mononuclear cells to proliferate in response to polyclonal mitogens, such as concanavalin A (conA), phytohemaglutinin (PHA), and pokeweed mitogen (PWM). Results from these studies have consistently shown reduced responses to dexamethasone in major depression. Lowy et al (1984, 1988) found that patients who were nonsuppressors on the DST showed no decrease in the lymphoproliferative response to PHA and ConA after overnight oral dexamethasone administration, whereas suppressors exhibited significantly decreased lymphocyte proliferation. Moreover, lymphocytes from nonsuppressor subjects were more resistant to the inhibitory effect of dexamethasone administered in vitro. In vitro glucocorticoid resistance in this study was not present when the depressed group was compared to the control group but only emerged when nonsuppressors from the two groups were pooled together and compared with suppressors (Lowy et al 1988). Wodarz et al (1991, 1992) reported that lymphocytes from actively depressed patients showed less dexamethasone-induced inhibition of the proliferative response to PHA in vitro. Interestingly, there was an inverse correlation between plasma cortisol concentration and the dexamethasone-induced inhibition of the proliferative response, suggesting a link between hypercortisolemia and resistance to in vitro GR-mediated responses. After clinical recovery, hypercortisolemia resolved and the sensitivity of lymphocytes to dexamethasone returned to control levels. Miller et al (1987) demonstrated that natural killer cells from depressed patients were "resistant" to the inhibitory effects of cortisol, showing less inhibition of natural killer cell–mediated cytotoxicity after in vitro cortisol treatment when compared with control subjects. Finally, Rupprecht et al (1991c,c) found that healthy control subjects pretreated with metyrapone exhibited a decreased PWM-induced proliferation of B lymphocytes after exposure to dexamethasone in vitro, whereas depressed patients showed no effect. It should be noted that the above in vitro studies overcome the limitations of in vivo studies where hormone bioavailability is potentially affected by altered pharmacokinetics of synthetic or endogenous ligand in depressives.

Consistent with the presence of GR resistance in major depression, Maguire et al (1997) found that despite having higher plasma cortisol concentrations relative to control subjects, melancholic depressed patients exhibited no increase in plasma sialyltransferase levels. Sialytransferases are a family of enzymes that participate in oligosaccharide chain metabolism and are known to be stimulated by glucocorticoids via the GR. No changes in GR binding were found between groups. These findings suggest that impaired GR function and not number may underline the decreased sensitivity of plasma sialyltransferase levels to cortisol in depressed patients.

Although the above data provide strong evidence of glucocorticoid resistance in major depression, there are some data suggesting that glucocorticoid sensitivity in depressed patients remains intact. Specifically, depressed patients have been found to exhibit increased intra-abdominal fat deposition (Thakore et al 1997). Increased intra-abdominal fat deposition is seen in medical illnesses characterized by hypercortisolemia such as Cushing's syndrome and following chronic treatment with glucocorticoids. These findings suggest that intra-abdominal GRs may maintain their sensitivity to glucocorticoids, whereas other tissues/cell types are resistant. In support of this possibility, studies also have shown decreased bone mineral density in depressed patients (Michelson et al 1996; Schweiger et al 1994). Elevated glucocorticoids have been associated with bone loss. Taken together, the results suggest that GRs in the bone compartment, like those in the intra-abdominal fat, may maintain their sensitivity to glucocorticoids. However, in the context of glucocorticoid resistance, increased concentrations of proinflammatory cytokines (including interleukin 6) that are normally negatively regulated by glucocorticoids may in turn disrupt bone formation and lead to bone loss (Manolagas 1998), thus providing an alternate explanation for bone changes in depressed patients. Nevertheless, the ostensible coexistence of glucocorticoid-sensitive and -resistant tissues has given rise to the concept of "localized GR resistance." Such localized resistance also has been described in patients with autoimmune or inflammatory diseases (Lamberts 1996). Recently, work in our lab and others have demonstrated that localized GR resistance may be related to a direct action of cytokines on GR function (see below) (Miller et al 1999; Pariante et al 1999).

Aside from resistant and sensitive cells and tissues within a given individual, it is also possible that there are subpopulations of depressed patients with and without glucocorticoid resistance. Although commonly grouped together, hypercortisolism and glucocorticoid resistance (DST nonsuppression) do not necessarily occur together and may represent distinct states of HPA axis dysfunction or at least different points along an evolution of HPA axis pathology (Asnis et al 1987; Miller et al 1994).

Mechanisms of Glucocorticoid Receptor Resistance

Three major possibilities have been considered regarding the mechanism(s) of GR resistance in depression. These include: 1) GR downregulation secondary to persistent hypercortisolism, 2) a primary alteration in the genetic structure of the GR, and 3) a decrease in GR function secondary to alterations in ligand-independent pathways that regulate the GR (Bamberger et al 1996).

As previously discussed, the cytosolic and whole cell GR binding data do not provide a compelling case for GR downregulation secondary to hypercortisolism in major depression. Nevertheless, it is conceivable that hypercortisolism could overburden the recycling capacity of the GR, with consequent diminished capability of the cell to respond to further stimulation. As for a primary (i.e., genetic) alteration in the GR, data indicate that individuals with a high genetic loading for depression (i.e., euthymic subjects with high familiar risk for affective disorders) may carry a "trait" marker that manifests itself as impaired GR function (impaired HPA negative feedback as measured by the dexamethasone–CRH test) (Modell et al 1998). Nevertheless, although polymorphisms of the GR gene exist, no specific variant has been linked to either glucocorticoid resistance or major depression (Koper et al 1997).

A third consideration is that GR function is altered in major depression via ligand-independent mechanisms. The concept of "ligand-independent" regulation of GR function derives from findings that steroid receptor function is regulated not only by steroid ligand binding, but also by signal transduction pathways driven by compounds unrelated to steroids (O'Malley et al 1995). For example, research has demonstrated that GR function can be influenced by a myriad of nonsteroid compounds including proinflammatory cytokines, such as interleukin 1 (Miller et al 1999; Pariante et al 1999), and participants in the cyclic adenosine monophosphate (cAMP) cascade including protein kinase A (PKA) (Rangarajan et al 1992). Both of these factors have been implicated in the pathophysiology of major depression.

There is considerable evidence that cytokines have a significant impact on GR expression and function. Glucocorticoid resistance has been described in subpopulations of patients with acute or chronic inflammatory diseases such as sepsis, asthma, ulcerative colitis, acquired immunodeficiency syndrome, rheumatoid arthritis, and allogenic organ transplantation, all of which are diseases that have high comorbidity with affective disorders (Lamberts 1996; Norbiato et al 1996; Sher et al 1994; Shimada et al 1993). Moreover, converging evidence suggests that local concentrations of cytokines produced during an inflammatory response may produce an acquired, localized GR resistance (Miller et al 1999). Inhibition of GR translocation from cytoplasm to nucleus by proinflammatory cytokines, such as interleukin 1α, may be a relevant step in this process (Pariante et al 1999). Although the mechanisms of cytokine effects on GR function have yet to be elucidated, activation of mitogen-activated protein kinases, which in turn inhibit GR function, may be involved (Krstic et al 1997; Saklatv et al 1999). Of note for the pathogenesis of GR resistance in major depression is that major depression has been associated with evidence of immune activation, including increased levels of proinflammatory cytokines (including interleukins 1 and 6) and acute phase reactants (Maes et al 1993). Moreover, proinflammatory cytokines are potent inducers of a syndrome of sickness behavior that has many features in common with major depression, including anhedonia, anorexia, fatigue, sleep disturbance, and impaired cognition (Kent et al 1992; Miller et al 1999).

A second possible pathway involved in the pathogenesis of GR resistance is the cAMP/PKA cascade. There is now considerable evidence that phosphorylation of the GR and/or other steroid receptor coactivators by cAMP-dependent protein kinase has a relevant role in the regulation of GR function. For example, adenylate cyclase and PKA activators have been found to increase GR-mediated gene transcription (Rangarajan et al 1992), and β-agonists have been shown to translocate the GR from cytoplasm to nucleus via the cAMP/PKA pathway (Eickelberg et al 1999). These findings are particularly intriguing 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). Moreover, recent work on the mechanism of action of antidepressants suggests that cAMP and PKA play an important role as mediators of the psychotropic effects of these agents (Chen and Rasenick 1995a,a, 1995b,b; Nestler et al 1989; Nibuya et al 1996). Therefore, it is possible that disruption in the cAMP/PKA pathway described in major depression is linked to GR resistance in this disorder and that antidepressants may overcome these receptor alterations via a direct effect on this pathway.

Table 3. Animal Studies on the Effects of Antidepressants on Glucocorticoid Receptor (GR) Expression

Source	Treatment (daily dose)	Duration	Species	Brain region	GR assay	Results
Kitayama et al 1988	IMI (20 μmol/kg, p.o.)	14 days	Rat	HT, HC	ICC	→
				LC, RM, GN		↑
Brady et al 1991	IMI (5 mg/kg, IP)	14 days	Rat	AP, HC	mRNA	→
		8 weeks		AP		↓
		8 weeks		HC		→
Peiffer et al 1991	DMI (20 mg/kg, IP)	10 days	Rat	HC, HT	mRNA	↑
	IMI (20 mg/kg, IP)					↑
	LITH (6 mmol/L/kg, IP)					↑
Brady et al 1992	FLU (5 mg/kg, IP)	14 days	Rat	HC	mRNA	→
	IDA (5 mg/kg, IP)	14 days				→
	PHE (5 mg/kg, IP)	14 days				→
	FLU (5 mg/kg, IP)	8 weeks				→
	IDA (5 mg/kg, IP)	8 weeks				↑
	PHE (5 mg/kg, IP)	8 weeks				→
Pepin et al 1992b	DMI (20 mg/kg, IP)	10 days	Mouse	Brain	mRNA, Cyt	↑
Seckl and Fink 1992	AMI (20 mg/kg, IP)	14 days	Rat	HC	mRNA	↑
	DMI (10 mg/kg, IP)					↑
	CIT (20 mg/kg, IP)					→
Biagini et al 1993	IMI (5 mg/kg, IP)	21 days	Rat	HC	ICC	→
Przegalinski et al 1993	AMI (20 mg/kg, p.o.)	28 days	Rat	HC	Cyt	↑
	IMI (20 mg/kg, p.o.)	28 days				↑
	ECS (1/day)	10 days				↑
Przegalinski and Budziszewska 1993	IMI (20 mg/kg, p.o.)	14 days	Rat	HC	Cyt	↑
	AMI (20 mg/kg, p.o.)					↑
Reul et al 1993	AMI (4.5 mg/kg, DW	3–7 days	Rat	HC	Cyt	↓
		5 weeks		HC, HT	Cyt	↑
Budziszewska et al 1994	DMI (20 mg/day, p.o.)	28 days	Rat	HC	Cyt	↑
	OXA (20 mg/day, p.o.)					→
	CIT (20 mg/day, p.o.)					→
	MIA (20 mg/day, p.o.)					→
Reul et al 1994	MOC (4.5 mg/kg, DW)	5 weeks	Rat	HT, AP	Cyt	↑
Peeters et al 1994	IMI (20 mg/day, IP)	10 days	Rat	HC	Cyt	↓
	AMI (20 mg/day, IP)					↑
	MIR (20 mg/day, IP)					↑
Rossby et al 1995	DMI (10 mg/kg, IP)	10 days	Rat	HC	mRNA	↑
	FLU (10 mg/kg, IP)					→
	DMI (10 mg/kg, IP)		Rat/DSP4	HC		↑
	FLU (10 mg/kg, IP)					→
Eiring and Sulser 1997	DMI (15 mg/kg, IP)	7 days	Rat	HC	mRNA	↑
	OXA (40 mg/kg, IP)					→
Lopez et al 1998	DMI (10 mg/kg, IP)	28 days	Rat	HC	mRNA	→
	ZIM (20 mg/kg, IP)	28 days				→
	FLU (10 mg/kg, IP)	28 days				→

IMI, imipramine; p.o., per os; HT, hypothalamus; HC, hippocampus; LC, locus coeruleus; RM, raphae magnus; GN, gigantocellular nucleus; ICC, immunocytochemistry of GR; →, no difference between antidepressant-treated animals and control subjects; ↑, significant increase in antidepressant-treated animals vs. control subjects; IP, intraperitoneal injections; AP, anterior pituitary; mRNA, GR messenger RNA; ↓, significant decrease in antidepressant-treated animals vs. control subjects; DMI, desipramine; LITH, lithium; FLU, fluoxetine; IDA, idazoxan; PHE, phenelzine; Cyt, cytosolic GR binding; AMI, amitriptyline; CIT, citalopran; ECS, electroconvulsive shock; DW, drinking water; MOC, moclobemide; OXA, oxaprotiline; MIA, mianserin; MIR, mirtazapine; rat/DSP4, rat following neurotoxic lesioning of noradrenergic neurons with DSP4; ZIM, zimelidine.

Impact of Antidepressants on Glucocorticoid Receptor Number and Function

Perhaps the most striking support of the hypothesis that abnormalities in the GR contribute to the pathophysiology of major depression derives from studies suggesting that antidepressants may exert their clinical effects through direct modulation of the GR.

A number of animal studies have examined the impact of long-term in vivo treatment with tricyclic and nontricyclic antidepressants or electroconvulsive therapy on GR expression and glucocorticoid feedback inhibition (Tables 3 and 4). These studies have shown that long-term antidepressant treatment is capable of upregulating GR protein and mRNA in key brain regions including the hippocampus and hypothalamus and decreasing basal

Table 4. Animal Studies on the Effects of Antidepressants on Hypothalamic–Pituitary–Adrenal (HPA) Axis Function

Source	Treatment (daily dose)	Duration	Species	Basal HPA function	Results	Poststress HPA function	Results
Kitayama et al 1988	IMI (20 μmol/kg, p.o.)	14 days	Rat	CORT	→		
Brady et al 1992	FLU (5 mg/kg, IP)	14 days	Rat	CORT	↓		
	IDA (5 mg/kg, IP)	14 days			↓		
	PHE (5 mg/kg, IP)	14 days			↓		
	FLU (5 mg/kg, IP)	8 weeks			→		
	IDA (5 mg/kg, IP)	8 weeks			→		
	PHE (5 mg/kg, IP)	8 weeks			↓		
Biagini et al 1993	IMI (5 mg/kg, IP)	21 days	Rat			CORT	↓
Reul et al 1993	AMI (4.5 mg/kg, DW)	3–7 days	Rat	ADR	↓		
		5 weeks		ADR, ACTH, CORT	↓	ACTH, CORT	↓
Reul et al 1994	MOC (4.5 mg/kg, DW)	5 weeks	Rat	ACTH, CORT	→	ACTH, CORT	↓
				ADR	↓		
Peeters et al 1994	IMI (20 mg/day, IP)	10 days	Rat	CORT	↓		
				ADR	→		
	AMI (20 mg/day, IP)			CORT, ADR	→		
	MIR (20 mg/day, IP)			CORT, ADR	→		
Montkowski et al 1995	MOC (15 mg/kg, DW)	7 weeks	Mouse			ACTH	→
						CORT	↓
Duncan et al 1998	DMI (15 mg/kg, IP)	21 days	Rat			CORT	→
	IMI (15 mg/kg, IP)						→
	AMI (15 mg/kg, IP)						→
	PHE (5 mg/kg, IP)						→
	TRA (7 mg/kg, IP)						→
	FLU (5 mg/kg, IP)						→
Lopez et al 1998	IMI (10 mg/kg, IP)	14 days	Rat			CORT	↓
	DMI (10 mg/kg, IP)	28 days					↓
	ZIM (20 mg/kg, IP)	28 days					→
	FLU (10 mg/kg, IP)	28 days					→

IMI, imipramine; p.o., per os; CORT, corticosterone; →, no difference between antidepressant-treated animals and control subjects; FLU, fluoxetine; IP, intraperitoneal injections; IDA, idazoxan; PHE, phenelzine; ↓, significant decrease in antidepressant-treated animals vs. control subjects; AMI, amitriptyline; TRA, trazodone; DW, drinking water; ADR, adrenal weight; ACTH, arenocorticotropic hormone; MOC, moclobemide; MIR, mirtazapine; DMI, desipramine; ZIM, zimelidine.

and/or stress-induced glucocorticoid secretion. Nevertheless, differences among antidepressants in their ability to influence GR expression and HPA axis function are apparent.

The vast majority of studies using tricyclic antidepressants, such as desipramine, amitriptyline, and imipramine, demonstrate antidepressant-induced GR upregulation in the brain (Budziszewska et al 1994; Eiring and Sulser 1997; Kitayama et al 1998; Petters et al 1994; Peiffer et al 1991; Pepin et al 1992b,b; Przegalinski and Budziszewska 1993; Przegalinski et al 1993; Reul et al 1993; Rossby et al 1995; Seckl and Fink 1992) (Table 3). Negative reports include two studies (Biagini et al 1993; Brady et al 1991) that used lower doses of imipramine (5 mg/kg vs. 10 mg/kg in other studies), one study (Peeters et al 1994) where GR activation (reduced cytosolic GR binding) may have masked GR upregulation (as suggested by reduced basal corticosterone levels), and a final study with desipramine where there is no clear explanation for the negative results (Lopez et al 1998). In contrast, studies examining the selective serotonin reuptake inhibitors, including fluoxetine (Brady et al 1992; Lopez et al 1998; Rossby et al 1995), citalopram (Budziszewska et al 1994; Seckl and Fink 1992), and zimelidine (Lopez et al 1998), have found no effect of these antidepressants on GR expression. Chronic treatment with lithium (Peiffer et al 1991), the selective noradrenaline reuptake inhibitor reboxetine (Ladd et al 1999), the α_2 antagonist idazoxan (Brady et al 1992), the α_2 and 5-HT_2 antagonist mirtazapine (Peeters et al 1994), and electroconvulsive shock (Przegalinski et al 1993) were all found to upregulate the GR, whereas administration of mianserin was not (Budziszewska et al 1994). Studies using monoamine oxidase inhibitors such as phenelzine (Brady et al 1992) and moclobemide (Montkowski et al 1995; Reul et al 1994) have given inconsistent results. Although these studies suggest that activity at the noradrenergic reuptake site may be an important pharmacologic feature of drugs that influence the GR, it should be noted that the noradrenaline reuptake inhibitor oxaprotiline consistently has shown no effects on GR expression (Budziszewska et al 1994; Eiring and Sulser 1997). Moreover, desipramine has been shown to induce GR upregulation even following neurotoxic lesioning of noradrenergic neurons with DSP4 (Rossby et

al 1995). Therefore, the relationship between chemical structure, known pharmacologic mechanisms, and effects on the GR has yet to be clarified.

Regarding the effects of antidepressants on resting and stimulated HPA axis activity, consistent with effects on GR expression, chronic treatment with the tricyclic antidepressants desipramine and imipramine has given the most convincing evidence of enhanced HPA axis feedback inhibition. Nevertheless, despite absent or inconsistent effects on GR expression, fluoxetine, phenelzine, and idazoxan have all been shown to reduce basal corticosterone secretion in at least one study (Brady et al 1992). This lack of a relationship between receptor upregulation and decreased basal and stress-induced HPA axis activity also has been seen with tricyclic antidepressants and indicates that receptor upregulation may not be a prerequisite for enhanced HPA axis feedback inhibition (see below).

Of note, antidepressants have been found to facilitate glucocorticoid-mediated feedback inhibition and upregulate the GR in animal models of HPA axis dysregulation, including a rat model of early maternal deprivation that leads to hypersecretion of CRH in response to stress in adulthood (Ladd et al 1999) and a transgenic mouse model of reduced GR expression (Montkowski et al 1995; Pepin et al 1992b). The latter model has been developed through a genetic manipulation of GR, whose expression is inhibited through a partial "knock out" mechanism (Pepin et al 1992b). These animals have endocrine abnormalities similar to those seen in depression, including increased activity of the HPA axis with elevated concentrations of corticosterone and ACTH, and behavioral deficits indicative of cognitive impairment. Both endocrine and behavioral abnormalities are attenuated by chronic antidepressant treatment (Montkowski et al 1995; Pepin et al 1992b). These findings in laboratory animal models of depression are strikingly consistent with clinical studies demonstrating that chronic antidepressant treatment of patients with major depression is associated with resolution of HPA axis alterations including in vitro glucocorticoid resistance (Linkowski et al 1987; Wodarz et al 1992).

Although this review is not focused on the MR, it is worth mentioning that MR upregulation by chronic treatment with antidepressants and electroconvulsive shock also has been described (Przegalinski et al 1993; Reul et al 1993, 1994; Seckl and Fink 1992; Yau et al 1995; Young et al 1990). Interestingly, fluoxetine and citalopram, which did not induce GR upregulation, upregulated the MR (Brady et al 1992; Seckl and Fink 1992). Lopez et al (1998) reported that chronic stress in rats leads to increased corticosterone levels and to downregulation of 5-HT_{1A} and MR receptors, and that these changes are prevented by treatment with desipramine (but not zimelidine). These data are consistent with the findings in humans, by the same authors, that reduced levels of 5-HT_{1A} and MR receptors were found in hippocampi of suicide victims (Lopez et al 1998). Similar to the GR, MR upregulation may contribute to increased glucocorticoid-mediated negative feedback of the HPA axis (Spencer et al 1998).

Mechanism of Antidepressant Effects on GR Expression and Function

A potent tool for clarifying the mechanisms underlying antidepressant-induced facilitation of GR function and GR upregulation has been the study of antidepressant–GR interactions in in vitro cell culture systems. Pepin et al (1992a) used a fibroblast cell line to show that acute treatment with the tricyclic antidepressant desipramine was capable of enhancing GR function (after 24 hours of treatment), as measured by increased activity of a reporter gene whose regulation is dependent on glucocorticoid response elements located upstream in the mouse mammary tumor virus promoter. Desipramine was also found to induce upregulation of GR protein after 72 hours of treatment. From these data, it was hypothesized that GR upregulation induced by antidepressant treatment may lead to enhanced susceptibility of the HPA axis to negative feedback by glucocorticoids and therefore to normalization of the HPA hyperactivity in depressed patients (Figure 2A).

More recently, we have demonstrated that 24-hour treatment with desipramine enhances GR function in L929 mouse fibroblast cells through facilitation of the translocation of the GR from the cytoplasm to the nucleus. However, no changes in GR binding were found even after incubation with desipramine for 96 hours (Pariante et al 1997). These data suggest that antidepressants exert their primary effect through changes in GR function and not GR expression (Figure 2B). Specifically, acute facilitation of GR activation by antidepressants may lead to increased negative feedback by circulating glucocorticoid hormones on the HPA axis, and then to resolution of glucocorticoid hypersecretion. Once prevailing glucocorticoids are reduced, GR upregulation may occur.

Although there are data indicating negative regulation of the GR by glucocorticoids, it should be noted that upregulation of the GR following in vitro treatment with antidepressants may be secondary to "autoinduction" of GR mRNA and protein by the activated GR. For example, short-term in vitro treatment of several cell lines (Denton et al 1993) as well as primary neuronal cultures (Pepin et al 1990) with GR agonists has been found to upregulate GR mRNA and protein, and these effects are mediated by the activated GR (Denton et al 1993). Of note, in the study discussed above, Pepin et al (1992a) found increased

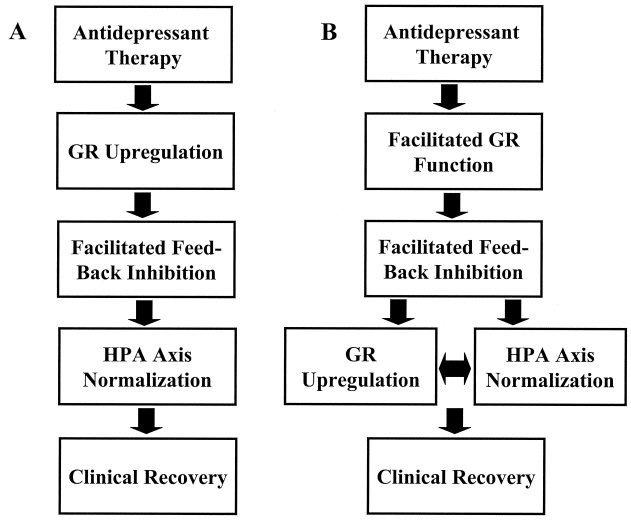

Figure 2. Models of antidepressant effects on the glucocorticoid receptor (GR). Two models have been proposed to explain the effects of antidepressants on the GR and ultimately on the hypothalamic–pituitary–adrenal (HPA) axis. The first model (**A**) is based on studies showing that long-term antidepressant treatment induces upregulation of GR protein and messenger RNA in the brain of laboratory animals and in cell culture. In this model, antidepressant treatment directly induces GR upregulation, which in turn leads to enhanced susceptibility of the HPA axis to negative feedback by glucocorticoids and therefore to normalization of the HPA hyperactivity in depressed patients. An alternative model (**B**) is based on studies showing that antidepressant treatment can enhance GR translocation and function in the absence of GR upregulation. In this second model, acute facilitation of GR activation by antidepressants leads to increased negative feedback by circulating glucocorticoid hormones on the HPA axis, and then to resolution of glucocorticoid hypersecretion. Once prevailing glucocorticoids are reduced, GR upregulation can occur. In addition, the activated GR may itself induce increased GRs (autoinduction).

dexamethasone-induced GR-mediated gene transcription after treatment with desipramine for 24 hours, whereas GR binding was not increased until after 72 hours of desipramine treatment. Moreover, Vedder et al (1999) found that 24-hour in vitro treatment with either amitriptyline or dexamethasone increased GR mRNA in human blood cells, a result that seems to further support that the GR may undergo autoinduction after activation by either antidepressants or agonists. Finally, Okugawa et al (1999) recently provided further evidence of antidepressant-induced GR translocation, demonstrating that 2 days of in vitro treatment of cultured hippocampal neurons with desipramine enhanced nuclear immunoreactivity of the GR. Moreover, desipramine and amitriptyline increased GR binding in these cells after 2–14 days of treatment. Unfortunately, the fact that the hippocampal neurons were cultured in steroid-containing media (from the serum) does not allow clarification of whether antidepressant-induced GR upregulation is a primary effect on GR synthesis or a secondary effect due to GR-mediated autoinduction of receptor expression.

The model depicted in Figure 2B, besides being consistent with our data showing in vitro activation of GR by desipramine (Pariante et al 1997), is supported by studies showing that antidepressants can increase HPA axis negative feedback in animals in the absence of GR upregulation. Brady et al (1992) found that 2 weeks of treatment with fluoxetine, idazoxan, or phenelzine reduces basal corticosterone levels in the absence of GR upregulation. Montkowski et al (1995) demonstrated that long-term antidepressant treatment with moclobemide (a monoamine oxidase inhibitor) induced normalization of the HPA axis and attenuation of the behavioral deficits in GR knockdown animals in the absence of any changes in GR binding. Moreover, in a study by Delbende et al (1991), a single injection of the antidepressant tianeptine given 1–3 hours before a tube restraint stress significantly reduced the stress-induced ACTH and corticosterone release in rats, an acute effect unlikely to be related to GR upregulation. Finally, the notion that facilitated GR function precedes GR upregulation is supported by evidence of increased HPA axis negative feedback in laboratory animals and normalization of HPA axis hyperactivity in depressed patients after as little as 5–7 days of antidepressant treatment. For example, Reul et al (1993) showed that GR binding was initially decreased (possibly reflecting enhanced translocation) in the hippocampus of rats treated with the tricyclic antidepressant amitriptyline for 3 and 7 days, before an increase in GR binding that was seen after 5 weeks of treatment. In these animals, amitriptyline induced a decrease in adrenal weight, likely representing a decrease in HPA axis function, after 5 days of treatment. In addition, two studies have described that HPA hyperactivity in depressed patients, evaluated by means of the dexamethasone–CRH test, began to normalize after 7–9 days of antidepressant treatment, and preceded the therapeutic effects on depressive symptoms (Deuschle et al 1997; Heuser et al 1996). Thus, it is possible that acute facilitation of GR translocation (and function) may represent the molecular mechanism by which antidepressants normalize HPA axis abnormalities in depressed patients, and GR upregulation may be the consequence of facilitated GR function rather than the cause.

In vitro studies have demonstrated that antidepressant effects on GR function are direct and not clearly related to the well-characterized ability of these drugs to inhibit the norepinephrine or serotonin transporter. Possible mechanisms of these direct effects include interaction of antidepressants with HSPS (leading to dissociation of the GR from the HSP complex) and/or stimulation of second messenger pathways known to regulate GR function.

There are intriguing similarities between the effects of desipramine (Pariante et al 1997) and those of heat or chemical shock (Sanchez 1992; Sanchez et al 1994) in L929 cells. Like desipramine, heat and chemical shock induce translocation of the GR in the absence of steroid, have no effect on GR-mediated gene transcription alone, but potentiate dexamethasone-induced GR-mediated gene transcription. Alternatively, activation of cAMP and PKA signal transduction pathways may be involved. Chen and Rasenick (1995b) have found that in vitro treatment of C6 glioma cells with desipramine increases basal levels of cAMP. Activation of PKA, in turn, has been shown to enhance GR function, as previously described (Rangarajan et al 1992).

Finally, as described in a recent article by Budziszewska et al (2000), in vitro pretreatment of L929 mouse fibroblast cells for 5 days with various antidepressants (imipramine, amitriptyline, desipramine, fluoxetine, tianeptine, mianserin, and moclobemide) was found to reduce GR-mediated gene transcription induced by a 2-hour incubation with corticosterone. Although these findings appear to suggest that antidepressants may inhibit GR function, it should be noted that these data are consistent with our findings demonstrating that pretreatment of these same L929 cells with desipramine for 24 hours also leads to reduced dexamethasone-induced GR-mediated gene transcription (Pariante et al 1997). Nevertheless, as noted above, coincubation of desipramine and dexamethasone for 24 hours leads to enhanced GR function. The discrepancy of results between experiments using preincubation and those using coincubation are explained by the fact that desipramine causes GR translocation to the nucleus in the absence of hormone (Pariante et al 1997). Once in the nucleus, the GR is incapable of rebinding ligand (Scherrer et al 1990). Thus, if antidepressants are given prior to steroid exposure, less receptors capable of binding ligand are available in the cytoplasm, and an inhibitory effect is revealed. Since glucocorticoids are reliably available in vivo, the conditions under which antidepressants act clinically are best modeled by those studies using coincubation of drugs and hormones.

Conclusions

Given the importance of glucocorticoids and the GR in the regulation of the HPA axis, it is logical to hypothesize that disruption of glucocorticoid action through altered functioning of the GR may be involved in the pathophysiology of major depression, a disorder that is characterized by HPA axis hyperactivity and CRH hypersecretion. Glucocorticoid resistance in depressed patients provides evidence of GR dysfunction, and data demonstrating GR regulation by steroid-independent factors that are believed to be involved in the pathophysiology of affective disorders (including cytokines and PKA) suggest that second messenger pathways and transcription factors like the GR interact to contribute to disease expression. Finally, findings demonstrating that antidepressants have a direct impact on GR function indicate that antidepressants may resolve HPA axis alterations and treat depression through effects on the GR. Taken together, the findings suggest that further understanding of the GR in major depression is an exciting frontier, which will no doubt lead to new insights into the pathophysiology and treatment of affective disorders.

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