

Antidepressants and Hypothalamic-Pituitary-Adrenocortical Regulation

FLORIAN HOLSBOER AND NICHOLAS BARDEN

Max Planck Institute of Psychiatry (F.H.), Clinical Institute, D-80804 Munich, Germany; and
Molecular Psychogenetics Laboratory (N.B.), Le Centre Hospitalier de l'Université Laval, Sainte-Foy,
Québec G1V 4G2, Canada

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I. Introduction

PAST studies of antidepressants have focused almost exclusively on their effects on the metabolism and receptors of monoamine neurotransmitters in various brain re-

gions. These studies have been extended to the molecular effects of antidepressants and have led to a profoundly expanded understanding of their actions in the central nervous system. For example, long-term administration of antidepressants decreases the expression of tyrosine hydroxylase, down-regulates cAMP-dependent protein kinase, modulates the mRNA expression of central β -adrenoceptors and serotonin (5-HT) receptors, and alters the functional activity of specific G protein subunits and adenylyl cyclase (1). Taken together, these and many other recent observations clearly indicate that antidepressants interfere not only with the production and release of catecholamines and indolamines but also with the signal transduction of those neurotransmitters that have long been implicated in the pathogenesis and treatment of depression.

More recently, additional target genes of antidepressant treatment have been identified, the most intriguing being those related to hypothalamic-pituitary-adrenocortical (HPA) activity. Altered regulation of this neuroendocrine system has been the subject of investigative efforts in depression research since Gibbons (2) reported more than 30 yr ago that plasma cortisol concentrations are elevated in depression and are normalized after clinical remission. Numerous clinical observations are consistent with the possibility that there is a causal link between HPA dysregulation and psychopathology (3). Recently, a large number of preclinical studies have provided evidence pointing in the same direction.

In this review we demonstrate how findings from the clinical neuroendocrinology of depression have been translated into questions that can be addressed in more detail in preclinical studies, and we also demonstrate how this concerted effort of clinical and preclinical researchers has clarified the interactions of depression, HPA activity, and antidepressant drugs. The data that have emerged from these efforts led us to conclude that disturbed corticosteroid receptor function plays a key role in the development of affective disorders and in their treatment with antidepressants.

A. HPA regulation by neuropeptides and neurotransmitters

The central nervous system is the main controller of the HPA system, whose activity is reflected peripherally in the plasma concentrations of ACTH and corticosteroids (principally cortisol in humans and corticosterone in rats and mice) (4–6). The secretion of ACTH from pituitary corticotropes is stimulated by the release into the portal blood

Address reprint requests to: Florian Holsboer, M.D., Ph.D., Max Planck Institute of Psychiatry, Clinical Institute, Kraepelinstr. 10, D-80804 Munich, Germany.

system of CRH and arginine vasopressin (AVP) from parvocellular neurons in the paraventricular nuclei (PVN) of the hypothalamus. At the pituitary level CRH binds at corticotrophic CRH (type I) receptors, triggering a cascade of enzymatic reactions that begins with stimulation of adenylate cyclase and ultimately regulates POMC gene expression and release of POMC-derived ACTH, lipotropins, and β -endorphin into the circulation.

The receptor-mediated action of CRH is fine-tuned by CRH-binding protein, whose mRNA and immunoreactivity were found to be colocalized with ACTH immunoreactivity in a majority of corticotrophic cells (7). CRH-binding protein is also expressed in the cerebral cortex, subcortical limbic system structures, and brain stem, which suggests that it may also play a role in nonendocrine CRH effects. The effects in the anterior pituitary are modulated not only by AVP but also by other peptidergic factors (8). At the adrenocortical level ACTH induces synthesis and release of corticosteroids, which, in turn, exert negative feedback actions at almost all levels of the HPA system. Over the past several decades the above regulatory principle has been largely confirmed by systemic, cellular, and molecular studies. However, the fine tuning has turned out to be extremely complicated and is still far from being fully understood. In this review we address only those mechanisms that are relevant in the context of depression-associated disturbances of the HPA system and their interactions with antidepressants. For a more detailed description of HPA physiology, the reader is referred to several excellent reviews (4–6, 9–12).

Studies on neurotransmitter regulation now agree that noradrenaline (NA), 5-HT, and acetylcholine (ACh) enhance the secretory activity of the HPA system, whereas γ -aminobutyric acid inhibits it (review in Ref. 10). The role of central catecholamines was long a subject of controversy until Plotsky (13) directly determined the CRH concentration in hypophysial portal plasma after electric stimulation of the ventral noradrenergic ascending bundle arising from the brain stem. This intervention as well as intracerebroventricular administration produced increased release of CRH, supporting a stimulatory action of NA at the hypothalamic level to elicit CRH. CRH, in turn, has a dual role as a hypothalamic neurohormone, not only initiating the neuroendocrine response to stress, but also acting as a neurotransmitter and activating the locus coeruleus (LC), which is composed almost entirely of noradrenergic neurons (14). It is of interest that the LC receives input from the PVN of the hypothalamus from cells that are localized more dorsally than those that receive minor noradrenergic input from the LC through a polysynaptic pathway (15). Whereas this topographical organization argues against a direct reciprocal communication between CRH neurons in the PVN and the LC, central administration of CRH was found to increase the basal firing rate of the LC (14). After restraint-stress the exploratory behavior of mice is altered, and it was shown that such adaptations are mediated by noradrenergic stimulation of CRH release via an α 1-adrenoceptor (16). A stimulatory role for NA via α 1-adrenoceptors has also been suggested by studies employing methoxamine, a selective α 1-adrenoceptor agonist, which released ACTH and cortisol, an effect that could be blocked by thymoxamine, an α 1-adrenoceptor an-

tagonist (17). A series of animal studies explored the effect of 5-HT on the HPA system, and they all support the notion that 5-HT enhances HPA-secretory activity, mainly through 5-HT_{1A}- and 5-HT₂-receptors (18–20). Furthermore, stimulation of muscarinic cholinergic pathways enhances HPA activity, as shown by the ability of arecoline (a muscarinic agonist) to cause ACTH and corticosterone secretion in rats (21). This effect is decreased by coadministration of the muscarinic cholinergic antagonist atropine and also by pituitary stalk transection or anti-CRH administration, suggesting that cholinergic agonist action on the HPA system involves central CRH release.

B. Feedback regulation through corticosteroid receptors

The most important control mechanism of the HPA system is an autoregulatory feedback by corticosteroids that can inhibit ACTH secretion both directly and indirectly by rapid (within minutes) and delayed (more than 2 h) effects (22).

The corticosteroid signal is transduced into cellular actions through two different corticosteroid receptors, the mineralocorticoid receptor (type I, MR) and the glucocorticoid receptor (type II, GR). In the absence of ligand, the GR is maintained in an inactive state, forming a heterooligomer with immunophilins (23) or with heat shock proteins (HSP) such as HSP 90, which, depending on the cellular content, may direct GR-activated gene expression in a tissue-specific way (24, 25). After ligand binding and dissociation from HSPs, this receptor can bind DNA and act as dimeric transcription factor, thereby increasing or decreasing the expression of glucocorticoid-responsive genes. Because HSP bind at the ligand-binding domain, which has a high homology between GR and MR, a similar mechanism most likely applies for both corticosteroid receptors. This dual receptor system for a single class of hormones is advantageous in dealing with the manifold physiological functions of corticosteroids. Under resting conditions, plasma cortisol levels undergo characteristic circadian fluctuations, ranging from about 0.5 nM to 50 nM. When homeostasis is disturbed by cognitive (e.g. death of a partner) or noncognitive (e.g. infection) stressors, the circulating levels of corticosteroids may rapidly exceed 100 nM. Thus, corticosteroid concentrations may fluctuate over a wide range, and a single receptor system would not be capable of translating the hormone signal into an adequate physiological response. With two different types of receptors, a sufficiently flexible dynamic range is available. The MRs, which bind corticosterone with high affinity, are localized particularly in neurons of limbic structures (hippocampus, septum, septohippocampal nucleus, and amygdala) and mediate the tonic influence of corticosterone, which is relevant for the circadian fluctuations, the sensitivity of the stress response, and the organization of the behavioral response to stress (6). The GRs are much more widespread, although they too are found at high concentrations in the limbic system. GRs bind glucocorticoids with a 10-fold lower affinity than MRs, and their main functions in the brain are curtailment of stress-induced HPA hyperactivity through inhibition of CRH, AVP, cytokine and POMC synthesis, and facilitation of information storage in rats (6, 26). The hippocampus has also been implicated as a feedback

site for corticosteroids because fornix transections disrupting neuronal connections between the hippocampus and the PVN reduce the sensitivity of CRH and AVP to corticosteroid feedback (27). Inhibition of GR by RU 486 (28) and of MR by the antimineralocorticoid canrenoate (29) enhances the secretory activity of the HPA system in humans. It is of note, however, that the plasticity of neuronal organization allows a site of feedback action to be shifted to many areas outside the PVN-corticotrope axis. This enables the brain to keep HPA excess under control even if one of the major regulatory systems, e.g. the hippocampus, is ablated (30).

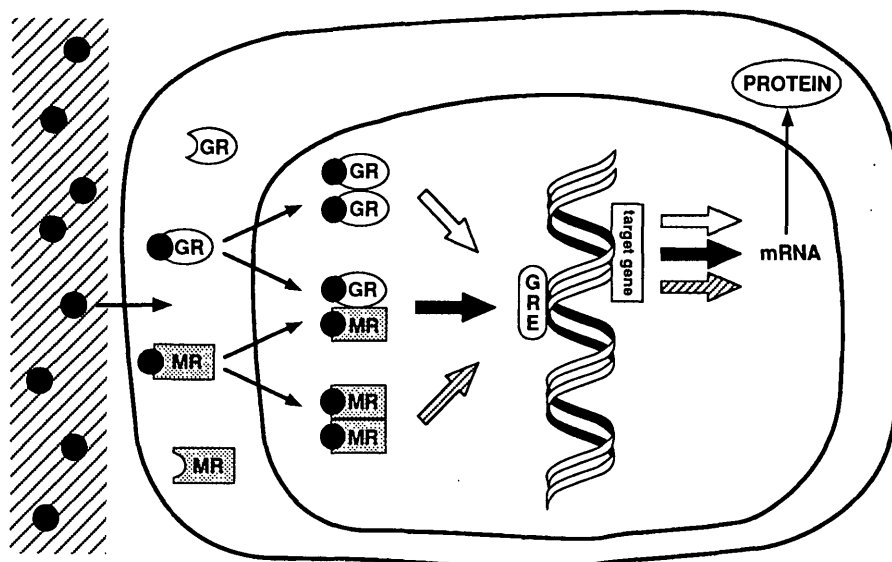
Until recently it was believed that both types of steroid receptors bind to glucocorticoid response elements (GRE) in the regulatory region of target gene promoters as homodimers (31–33). However, Trapp *et al.* (34) have now shown that MRs and GRs are able to form a GRE-binding heterodimeric complex with DNA-binding and transactivation properties different from those of the respective homodimers (Fig. 1). MRs and GRs regulate overlapping sets of genes, but their respective homodimers exert different transcriptional potencies at various GRE-containing promoters (35). Heterodimerization between MRs and GRs extends the possibility of fine-tuned hormone-regulated gene expression in all cells in which the receptors are colocalized (36, 37). In this context the rich endowment of the hippocampus with both receptors colocalized in pyramidal cells (38) is of interest because this brain region is involved not only in HPA regulation but also in information processing, the latter often being disturbed in states of aberrant HPA homeostasis (39, 40).

C. HPA alterations in affective disorders

Prominent HPA abnormalities among patients with depression are: 1) an increased number of ACTH-secretory episodes combined with an increased magnitude of cortisol-secretory episodes (41); 2) elevated urinary "free" cortisol levels (42); and 3) elevated levels of cortisol and CRH in the cerebrospinal fluid (43). In addition to these baseline alterations, disturbed HPA regulation is also seen in several func-

tion tests: a single oral dose of 1–2 mg dexamethasone suppresses ACTH and various corticosteroids much less in patients with depression than in healthy controls (44, 45). Depending on the severity of depression, age, and normative database used, 20–50% of the patients are defined as dexamethasone nonsuppressors. Although this phenomenon is not specific for any clinical diagnosis, it has merit as a prognostic tool (46). Another investigative tool became available after ovine and human CRH were synthesized (47–49). In response to a standard intravenous dose of human or ovine CRH, depressives secrete decreased amounts of ACTH. Despite this blunted ACTH response, cortisol secretion remained unchanged, indicating a functional hyperplasia of the adrenocortical glands secondary to prolonged stimulation by trophic ACTH (50, 51). A straightforward interpretation of the blunted ACTH response to CRH in the presence of hypercortisolism is that elevated baseline cortisol secretion accounts for ACTH blunting via negative feedback. In support of this interpretation are studies in which depressed patients were pretreated with metyrapone, which suppresses cortisol biosynthesis at the adrenocortical level. The subjects had normalized net ACTH output after CRH stimulation despite elevated ACTH levels at baseline (52, 53). Other possible factors that may contribute to decreased ACTH release after CRH infusion are CRH receptor desensitization of corticotropes or altered processing and storage of ACTH precursors leading to dissociation of the ACTH and β -endorphin responses in depression, possibly through differences in posttranslational processing, storing, and release mechanisms (54). Another dissociation between coreleased HPA hormones was observed at the adrenocortical level, where cortisol release after CRH was normal but aldosterone release was blunted (55), confirming different regulation of the zona fasciculata (producing glucocorticoids) and the zona glomerulosa (producing mineralocorticoids). The observed decrease of the ACTH-cortisol ratio with ongoing HPA hyperactivity points to development of adrenocortical hyperplasia. This interpretation was recently confirmed by Nemeroff *et al.* (56), who showed adrenal gland enlargement

FIG. 1. A model of mineralocorticoid (MR) and glucocorticoid (GR) receptor-regulated gene expression. Corticosteroids enter target cells by passive diffusion. In the cytosol they bind to their cognate receptors, which in conjunction with changes in their HSP environment undergo conformational changes. After intranuclear translocation, the MR-MR, GR-GR homodimers or the MR-GR heterodimer are constituted, depending on the relative levels of MR and GR and the concentrations of their ligands. The different receptor dimers bind to GREs in the flanking region of target genes with unique DNA-binding kinetics and induce transcription of hormone-regulated genes. The different transcriptional activities and DNA-binding kinetics of corticosteroid receptor dimers are represented by differently colored arrows. [Derived from T. Trapp *et al.*: *Neuron* 13:1–6, 1994 (34)].



in major depression by computer tomography. In addition, Rubin *et al.* (57) measured adrenal volumes by nuclear magnetic resonance imaging and found that patients with depression had larger adrenals during depression than after successful treatment. The hyperplasia is apparently limited to the zona fasciculata of the adrenal cortex, which produces glucocorticoids and regresses after hypophysectomy, an effect that is counteracted by repeated ACTH infusions. Furthermore, central CRH may enhance sympathetic activity, an effect that causes an increase in the rate of cortisol secretion in response to ACTH (58–60). The possibility of an intra-adrenal CRH-ACTH axis has received considerable interest since several groups showed that CRH can be increased in adrenals in response to hemorrhage in dogs and by sympathetic nerve stimulation in calves (61, 62). Interestingly, the adrenal CRH content is suppressed by cortisol and ACTH, further pointing to an intraadrenal CRH-ACTH axis (63). The major action of CRH on adrenal cortex is its enhancement of ACTH-elicited corticosteroid synthesis and release (64, 65). The intraadrenal CRH system in conjunction with altered sympathetic activity may be altered in depression, resulting in altered ACTH-cortisol ratios. Finally, N-terminal POMC-derived peptides and efferent neural input may also contribute to enhanced sensitivity of the adrenal cortex and to its growth.

D. Combined dexamethasone-CRH test

Recently, a combination of dexamethasone suppression and CRH stimulation has been used in psychiatric patients with depression and other psychiatric diagnoses (66–74). The surprising finding in this series of studies is that dexamethasone-pretreated patients show enhanced ACTH and cortisol responses to CRH, which at first glance would be counter to expectation because an inverse relationship between baseline cortisol and CRH-elicited ACTH had been suggested earlier (75). A comparison of the dose-response curves for depressed patients and normal controls showed that patients need higher dexamethasone dosages to suppress ACTH and cortisol secretion after CRH infusion (S. Modell, A. Yassouridis, and F. Holsboer, unpublished observations). Summarizing broad clinical experience with the dexamethasone-CRH test, Heuser *et al.* (76) concluded that the sensitivity (*i.e.* likelihood to differentiate normal from pathological state) of this test is about 80% and greatly exceeds that of the standard dexamethasone suppression test (20–50%). Endocrine laboratory tests are usually interpreted in relation to the patient's age. If psychiatric patients are clustered into different age groups, the sensitivity of the dexamethasone-CRH test can be increased even further, to above 90%, making this test a prime candidate for laboratory characterization of mentally ill patients (76). Of course, this test was never intended as a diagnostic laboratory test for psychiatric conditions (77). Preliminary data suggest that the combined dexamethasone-CRH test may also be of value in differentiating Cushing's syndrome from pseudo-Cushing's states (78) and in unveiling subtle HPA disturbances in patients with multiple sclerosis (79), in elderly endurance athletes (80), and in patients with Alzheimer's disease, who had decreased cortisol response (81).

II. Pathophysiology of Underlying Altered HPA Regulation in Depression

Our current understanding of the mechanisms involved in depression-related HPA alteration has greatly benefited from preclinical stress research, mainly in rats, and from access to refined laboratory tools. Although there is compelling evidence that CRH hypersecretion drives the HPA system in depression, even very high dosages of the homologous human CRH do not produce dexamethasone nonsuppression in normal men to a degree seen in depression (82). However, when CRH was administered to normal subjects who had been pretreated with dexamethasone and vasopressin, hormone responses were indistinguishable from those of dexamethasone-pretreated depressives stimulated with CRH only (82). This circumstantial evidence led us to postulate that vasopressin, which alone is only a weak secretagogue at the pituitary corticotropes, is elevated in portal blood of patients with depression and may thus potentiate the effect of endogenously released or exogenously administered CRH, or both (67).

Preclinical studies showed that CRH in combination with AVP acts potently to release ACTH and is less sensitive than CRH alone to inhibition by glucocorticoids (83). Chronic stress increases 1) the number of CRH cells that colocalize AVP (84, 85); 2) AVP colocalization in CRH-containing vesicles (86); and 3) AVP content in the zona externa of the median eminence (84, 87, 88). Thus, under chronic stress the action of CRH at corticotropes is enhanced by increased coexpression and release of AVP, rendering the pituitary and higher centers less sensitive to corticosteroid feedback. All the studies that indirectly implicate enhanced AVP release were done in rats, but a recent study by Raadsheer *et al.* (89) provided direct evidence that the preclinical observations are transferable to the clinical condition. These authors found that the hypothalamic PVNs of severely depressed patients contained 4 times as many CRH-expressing neurons and 3 times as many CRH neurons coexpressing vasopressin as the PVNs of healthy controls. The same Dutch group also showed that the number of AVP-immunoreactive neurons in the PVN of depressed patients is increased and that more CRH neurons in this brain area colocalize AVP (90). The observation, that hypercortisolemic depressives secrete less ACTH than healthy controls in response to CRH alone but hypersecrete ACTH in response to CRH if pretreated with dexamethasone, reflects the pharmacological differences between endogenous corticosteroids and synthetic dexamethasone. Dexamethasone efficiently exerts feedback effects in corticotropes but is not as effective as naturally occurring corticosteroids at suprapituitary sites (91, 92). Thus, to a certain extent, the brain feedback sites are deprived of their natural feedback signal, which is similar to what happens after adrenalectomy and may lead to an increase in the expression and release of CRH and AVP from CRH neurons.

These clinical and preclinical studies strongly suggest that impaired glucocorticoid feedback is involved in depression, and this view is amplified by a study of Young *et al.* (93), who administered cortisol intravenously to depressives and found the fast feedback component of pituitary response to be insensitive. Because the HPA system is a highly complex

closed loop, these studies did not reveal whether defunct corticosteroid sensitivity is the primary cause of HPA hyperdrive or whether increased drive of CRH and vasopressin release and exaggerated ACTH secretion increased corticosteroid levels to a degree that subsequently led to corticosteroid receptor down-regulation in the limbic brain.

III. HPA Alteration in Healthy Controls at Genetic Risk for Depression

In recent studies on healthy subjects who had never suffered from minor or major psychiatric illness, but who were members of families highly loaded with depression, several neurobiological signs of depression were found to be present, including the response to the dexamethasone-CRH test (94). In comparison to a control group a much higher proportion of these subjects at genetic risk for depression showed an excessive cortisol response resembling the secretion profiles obtained from depressives (Fig. 2).

Neither these individuals nor those who had already suffered from a depressive episode and were investigated after full remission had abnormal cortisol secretion at baseline (95), making a primary functional defect of corticosteroid signal transduction more likely as the cause of HPA alteration in depression than a primary CRH and/or AVP hyperdrive. In the latter case basal hypercortisolism should be present in a high-risk population as well as in remitted patients with depression. In the presence of hypercortisolism the decreased corticosteroid receptor function would be secondary to a disturbance in the regulation of CRH and CRH/AVP neurons that is different from corticosteroid-mediated inhibition. The relevance of this finding, however, must await clarification of whether those individuals from a high-risk population who present with abnormal dexamethasone-CRH test results are indeed at higher risk for developing the disease than those who have normal test results.

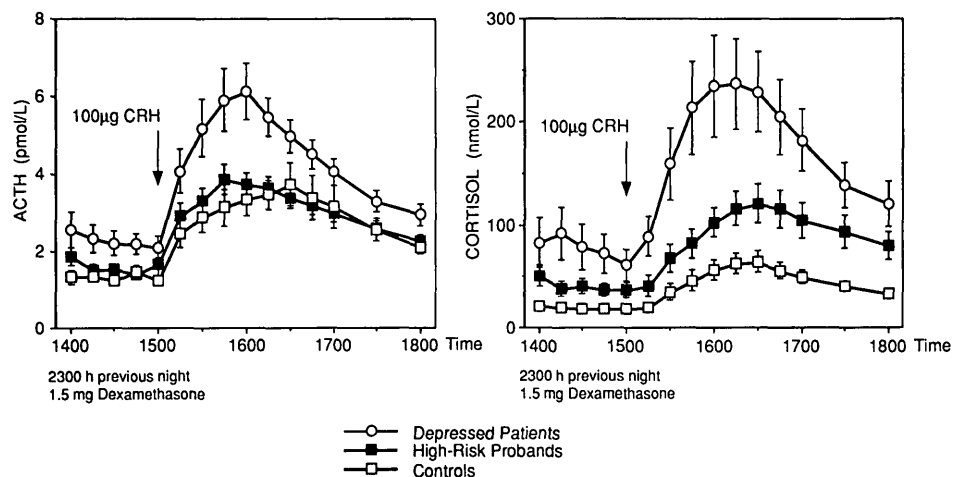
IV. HPA Disturbances in Relation to Causality of Depression

A. The role of natural and synthetic corticosteroids

If corticosteroids are administered for medical reasons, e.g. for arthritis or as adjuvants to chemotherapy, a number of

behavioral symptoms such as mood lability, depression, disturbed sleep, cognitive disturbances, and even psychosis may occur. Likewise, patients with Cushing's syndrome, induced pharmacologically or by pituitary or adrenal tumors, have high psychiatric morbidity (96). Two thirds of these patients have psychopathological features quite similar to those seen in major depression, and approximately 10% attempt suicide. These findings raised the possibility that increased corticosteroid secretion in depression may contribute directly to some of the symptoms. From more specific studies in which synthetic corticosteroids were administered to normal controls, evidence emerged that these hormones may induce memory deficits in humans. Wolkowitz *et al.* (97, 98) administered dexamethasone and prednisone to depressed patients and controls and recorded transient memory deficits involving increased errors of commission (*i.e.* incorrect recall of distracter words along with correct recall of target words). Moreover, Newcomer *et al.* (99) noted an impairment of verbal declarative memory performance in healthy subjects receiving a brief low-dose dexamethasone treatment. Lupien *et al.* (100) also reported that in a subgroup of subjects between 60 and 80 yr of age those with a significant increase in plasma cortisol levels over a period of 3–6 yr were impaired on tasks measuring explicit memory and selective attention compared with elderly subjects with a decrease in plasma cortisol concentrations. Finally, Heuser *et al.* (74, 80) studied elderly marathon runners and found that those who showed exaggerated cortisol secretion in the dexamethasone-CRH test also had cognitive impairment. Taken together, these studies demonstrate that changes in corticosteroid receptor activation do, in fact, induce changes in memory function. It is still unclear whether these changes are direct effects of synthetic steroids on corticosteroid receptors or indirect effects through depriving central (mainly hippocampal) corticosteroid receptors of their naturally occurring ligands (see above), thus creating an imbalance in the MR/GR homeostasis (6). Another intriguing approach to challenging the hypercortisolism of depression as a causative factor is to treat depressed patients with corticosteroid-suppressive drugs (97, 101, 102) or corticosteroid receptor antagonists. Today only preliminary data are available pointing to beneficial effects of such interventions. However, the drugs employed (ketoconazole, aminoglutethimide, me-

FIG. 2. Plasma ACTH and cortisol response to a combined dexamethasone-CRH challenge (expressed as mean ± SE) in 47 probands with high familial risk for major depression (high risk probands), in 20 healthy control probands, and in 18 patients with a current episode of major depression. The plasma cortisol curve shows that dexamethasone-pretreated high risk probands have a higher secretory response to CRH than matched controls, while their ACTH output remains unaltered. [Reproduced with permission from F. Holsboer *et al.*: *Neuroendocrinology*, 62:340–347, 1995 (94). S. Karger AG, Basel.]



tyrapone, mifepristone) induce so many other unspecific effects at various sites, including the central nervous system, that their use to study the direct effects of cortisol suppression on depression is limited. For example, the combined antagonist of progesterone and glucocorticoid receptors, mifepristone (RU 486), produces deterioration of the physiological sleep architecture, thus mimicking one of the cardinal symptoms of depression (103).

B. Preclinical evidence for the CRH hypothesis of depression

After isolation and sequencing of ovine and human CRH (26–28), a large number of studies were conducted exploring the behavioral effects of CRH in rodents and primates after central administration of this neuropeptide. In aggregate, these studies strongly support the view that a central hyperactivity of CRH neurons is involved in the triggering and maintenance of several psychiatric disorders, including depression (reviews in Refs. 12 and 104). When centrally injected, CRH not only activates the secretion of ACTH, but also seems to coordinate the behavioral response to stress. As summarized by Dunn and Berridge (105), almost all behavioral investigations suggest that CRH acts as an anxiogenic and possibly anorectic neuropeptide. These studies have involved neuropeptide administration, usually in high doses, and use of a peptidergic CRH antagonist (α -helical CRH 9–41) or benzodiazepines (106–113). Recently, the suggestion that CRH is anxiogenic has been amplified by administration of CRH antisense oligodeoxynucleotides, which induced anxiolytic effects (114), and a study by Stenzel-Poore *et al.* (115) in which transgenic mice overexpressing CRH were found to exhibit an increase in anxiogenic behavior in conjunction with Cushing's syndrome (116). Because of the close functional interrelationship between the noradrenergic LC and the hypothalamic CRH neurons (14, 15), it is tempting to speculate that stress-induced LC activation leads to increased CRH neuron activity, which in turn feeds back to increased LC firing. Thus, under stressful conditions the behavioral adaptation includes, at least in part, coactivation of CRH and LC neurons through polysynaptic pathways.

Transgenic mice heterozygous for CRH deficiency, as recently described by Muglia *et al.* (117), may provide a tool to further study the role of CRH in stress-related behavior and also the mechanism at the CRH and corticosteroid receptor levels developed to compensate for the genetic defect. The recent cloning and characterization of receptors for CRH revealed the existence of at least two distinct subtypes in the brain (CRH₁ and CRH₂ receptor), the latter existing in two different splice variants (118–120). Both the CRH₁- and CRH₂-receptor have a 70% overall sequence identity and a distinct pharmacology, with CRH having a lower potency for the CRH₂ receptor (121). Whereas the CRH₁ receptor is more widely distributed, the CRH₂ receptor is mainly expressed in the limbic brain, suggesting that the two receptors are differently involved in mediating central effects of CRH. Both receptors are potential targets for a new generation of antidepressants acting directly at CRH receptors by antagonizing the effect of CRH. Suppressing the translation of CRH₁ receptor mRNA by administration of a specific oligodeoxynucleotide antisense probe into the central amygdala or

intracerebroventricularly produced anxiolysis in rats, which is in agreement with a CRH₁ receptor-mediated effect of CRH-elicited anxiety (122, 123). A study by Fuchs and Flügge (124) provided evidence that CRH binding is modulated by psychosocial stress in various brain regions in male tree shrews (*Tupaia belangeri*). These changes in CRH binding occurred also in extrahypothalamic regions thought to be involved in behavioral rather than in neuroendocrine response to stress, *e.g.* frontal and cingulate cortex, amygdala, and choroid plexus.

Whereas animal studies provide good evidence for an involvement of CRH in behavioral states and the results are consistent with an anxiogenic effect of CRH, the evidence that increased CRH secretion is also related to psychiatric morbidity is derived from correlations rather than from direct evidence. Only the availability of specific CRH receptor subtype antagonists that can be administered therapeutically will provide a conclusive answer to the question of whether CRH is causally linked to the development and course of depression. In depressed patients the number of CRH neurons colocalizing AVP is increased (90), which is in agreement with neuroendocrine data (67). Recently, Landgraf *et al.* (125) showed that decreasing the number and function of septal vasopressin receptors (V₁) by local injection of antisense oligodeoxynucleotides to the V₁ receptor subtype mRNA reduces anxiety-related behavior in rats. This finding can be taken as additional evidence that overactive CRH and AVP neurons and those coexpressing both neuropeptides act in concert to produce the signs and symptoms prevalent in depression.

V. Corticosteroid-Induced Modulation of Neurotransmission and Signal Transduction

A. Effects on CRH, AVP, and POMC

One of the most elaborate central actions of corticosteroids is the finely-tuned regulation of their own secretion that involves the limbic system and the pituitary. After activation by a cognate ligand, corticosteroid receptors dimerize and bind to GRE in the regulatory region of target gene promoters activating transcription. The effects of glucocorticoids on the expression of the CRH gene are different in different tissues. In the human placenta, for example, glucocorticoids enhance CRH gene expression (126). The effects of glucocorticoids on CRH expression in the brain are particularly complex. Glucocorticoid administration in the rat increases CRH mRNA levels in the central amygdala, in the magnocellular (oxytocinergic) neurons, and in descending neurons of the PVN (127). CRH expression in the olfactory bulb, midbrain, frontal cortex, and brain stem seems to be less susceptible to corticosteroid fluctuations (128). At the parvocellular neurons that fuel the portal vessels via projections to the median eminence, glucocorticoid administration decreases and adrenalectomy increases CRH mRNA levels (129).

Expression of the CRH gene is apparently different across areas to optimally subserve its various functions. The mechanism governing changes in CRH gene expression is not yet fully understood. Involvement of the protein kinase A pathway is well documented as cAMP increases CRH secretion

from perfused rat hypothalami, and adenylyl cyclase activation by forskolin increases the expression and secretion of CRH from primary hypothalamic cell cultures (130). A study that used AtT-20 cells, transfected with the human CRH promoter, suggested that this promoter contains a cAMP response element because cAMP induced the transcriptional activation of a CRH promoter-driven reporter gene construct (131). Spengler *et al.* (132) identified and characterized a perfect consensus cAMP response element in the CRH 5'-flanking sequence and demonstrated its functional significance.

The mode by which glucocorticoids suppress activation of the CRH gene is not clear and may involve physical protein-protein interactions (133–137). For example, interaction of ligand-activated GR with the *c-jun* component of the AP-1 complex or with RelA protein, a nuclear transcription factor regulating expression of genes relevant during inflammation, has recently been shown to confer suppressive effects of glucocorticoids on gene expression (133, 136).

The expression of AVP is also under glucocorticoid control, as shown by Fink *et al.* (138), who adrenalectomized female rats and measured CRH and AVP in portal blood. Both CRH and AVP were increased, and, in comparison to endogenous corticosteroids, dexamethasone had a much more pronounced effect on AVP under these experimental conditions. Likewise, AVP mRNA in the posterior magnocellular subdivision of the PVN is increased by adrenalectomy and this increase can be prevented by dexamethasone treatment (139). More recently, several studies suggest that CRH and AVP receptor expression are also regulated by corticosteroids (140–143).

At the pituitary level, glucocorticoids decrease secretion of prestored ACTH and POMC gene expression (144). Since glucocorticoid-induced repression of POMC gene expression appears to be primarily at the transcriptional level, it is likely that DNA sequences of the POMC gene mediate this effect. Drouin *et al.* (145) localized POMC promoter sequences responsible for glucocorticoid repression of transcription and identified a negative GRE.

B. Neurotransmitters

Corticosteroids exert a variety of effects on neurotransmission by biogenic amines and amino acids. Early work by Mobley and Sulser (146) showed that in slices from the frontal cortex of rats the NA-induced cAMP accumulation was enhanced by adrenalectomy. Later on it was demonstrated that glucocorticoids regulate the β -adrenoceptor system by controlling the rate of gene expression and may thus modulate actions of catecholamines that act through these receptors (147–149).

Whereas 5-HT has long been known to stimulate the HPA system, more recent studies have shown that corticosteroids may also act at 5-HT receptors. Kuroda *et al.* (150) showed that 5-HT_{1A} receptor binding in rats was increased in the hippocampal CA₂-CA₄ subfields and the dentate gyrus 1 week after adrenalectomy, which was prevented by aldosterone. In a different set of experiments, Chalmers *et al.* (151, 152) adrenalectomized rats and found 5-HT_{1A} receptor mRNA increased in all hippocampal subfields, which agrees

with reported alterations of 5-HT_{1A} receptor binding in response to stress. The effect of adrenalectomy on 5-HT_{1A} receptor gene expression was reversed by dexamethasone, but only in the CA₁ subfield and the dentate gyrus, which contains the highest GR density. The mutual interaction between 5-HT and corticosteroid receptors in the brain was initially studied by de Kloet and co-workers, who first showed that corticosterone controls 5-HT₁ receptor regulation in the raphe hippocampal formation (153, 154). Electrophysiological studies amplified this link by showing that MR agonist administration to brain slices taken from adrenalectomized rats blocks 5-HT-induced hyperpolarization of CA₁ pyramidal neurons, possibly by acting on the coupling of the 5-HT_{1A} receptor to its target G protein (155). Not only 5-HT receptor(s) but also the ligand 5-HT is, at least to some extent, under regulatory control of corticosteroids as the level of tryptophan hydroxylase in serotonergic raphe nuclei is higher in adrenalectomized rats given dexamethasone than in control rats (156).

The possibility that prolonged corticosteroid hypersecretion produces a wide range of changes including pathological adaptation to stress and immune function (157, 158) raised the question of what mechanism may mediate the glucocorticoid-elicited increase in the vulnerability of neurons to noxious insults such as hypoxia or hypoglycemia. A decreased energy supply, *e.g.* ischemia, increases the levels of excitatory amino acids, which activate N-methyl-D-aspartate receptors and increase influx of calcium ions into neurons, thereby promoting subsequent cell death. Glucocorticoids exacerbate this cascade, increasing the vulnerability of the neurons to various insults that are associated with increased levels of excitatory amino acids (159, 160) and decreased intraneural ATP (161).

C. G proteins

Guanine nucleotide-binding proteins (G proteins) play a central role in coupling membrane receptors to various intracellular effector systems. Recently it was shown that G proteins are also targets of glucocorticoids (162, 163). Corticosterone administration to rats increased those G proteins (G_{s α}) that stimulate adenylyl cyclase and decreased those G proteins (G_i) that inhibit this enzyme in response to neurotransmitter binding at G protein-coupled receptors. Adrenalectomy produced opposite effects, suggesting that these G proteins, essential for intracellular signal transduction, are regulated in part by corticosteroids. Interestingly, long-term treatment with antidepressants affects G protein α -subunits in specific ways, which may have implications for their efficacy (164, 165).

D. Neurotrophins

The neurotrophins, a different kind of transmitter effector system, also mutually interact with corticosteroids. Nerve growth factor may act not only as a trophic hormone for nerve fibers, but also as a mediator of stress response. It stimulates the pituitary-adrenocortical system (166, 167) by elevating AVP (168). Lindholm *et al.* (169) demonstrated that nerve growth factor synthesis is differentially regulated in

neurons and astrocytes by dexamethasone. Recently, Kononen *et al.* (170) suggested that brain-derived neurotrophic factor (BDNF) might have a modulatory effect within the rat pituitary. The expression of BDNF in the pituitary seems to depend on adrenocortical steroids in a complex way as both dexamethasone treatment and adrenalectomy produced decreased BDNF mRNA levels pointing to a role of MR. Immobilization stress in rats reduced BDNF mRNA levels in the dentate gyrus and CA₃ and CA₁ hippocampal layers but not its receptor (trkB) (171). Corticosteroids are probably involved in these effects as they can reduce BDNF mRNA levels in the dentate gyrus, but not in CA₃ and CA₁ pyramidal layers, which suggests that stress-elicited corticosterone does not bear sole responsibility for decreased BDNF expression.

These findings suggest that mechanisms involved in stress regulation can induce changes in neurotrophic factor expression. This may have consequences for neuronal survival, particularly in the hippocampus, where both BDNF and corticosteroid receptors are highly concentrated. In this context, it is of note that antidepressants were reported to increase levels of BDNF mRNA in the frontal cortex and in the limbic brain. This could contribute to antidepressant-induced long-term effects on neural plasticity and hence possibly counteracts the stress-elicited BDNF decrease.

E. Nitrogen oxide

Recently, evidence has been found for a possible role of nitrogen oxide (NO) in neuroendocrine mechanisms as NO inhibits the release of CRH and AVP *in vitro* (172, 173). The expression of the enzyme responsible for NO synthesis, NO synthase, is inhibited by glucocorticoids (174).

In summary, these few selected examples demonstrate that glucocorticoids exert a wide range of diverse effects on central nervous system function, all of which may play a role in the development of affective illness. In turn, antidepressants, by interfering with HPA regulation, may have many still unrecognized effects at many levels of brain activity.

VI. Effects of Antidepressants on HPA Function

A. Acute *in vivo* effects

The neurotransmitter/receptor systems involved in HPA regulation are identical to those affected by most antidepressants. The initial action of these serendipitously discovered drugs is either blockade of reuptake transporters that clear released neurotransmitters from the synaptic cleft or inhibition of neurotransmitter degradation in the presynaptic terminal. Because antidepressants act on these neurotransmitters and are clinically effective in treatment of depression, it was long thought that defunct aminergic neurotransmission was the cause of depression. This hypothesis seemed experimentally testable because administration of antidepressants produces changes in hormone secretion, and comparing the endocrine effects in depressives with those in healthy subjects promised to provide insight into the neurotransmitter/receptor disturbance underlying depression (a "window to the brain").

When administered intravenously, intraperitoneally, or orally to healthy human subjects, desipramine, primarily blocking NA uptake, produces activation of the HPA system in a dose-dependent fashion (175). Asnis *et al.* (176) observed that after intraperitoneal administration of 75 mg desipramine, depressives secreted less cortisol than control subjects. Since the cortisol response to desipramine was diminished by prazosin (α 1-adrenoceptor agonist) and unaffected by yohimbine (α 2-adrenoceptor antagonist), propranolol (β -adrenoceptor antagonist), and methysergide (5-HT receptor antagonist), it was concluded that desipramine acts primarily at α 1-adrenoceptors (177). Although Asnis *et al.* (176) did not report plasma ACTH concentrations and did not take the depression-related changes in adrenocortical sensitivity (see above) into account, their finding of a blunted cortisol response to desipramine in depression is also suggestive of an α 1-adrenoceptor deficit. In rats, desipramine and other antidepressant drugs are also capable of activating the HPA system (178, 179).

Another selective NA reuptake inhibitor that increases ACTH and cortisol in men is oxaprotiline. This antidepressant drug exists as a racemic mixture, with only the S(+)-enantiomer being active as an NA-reuptake inhibitor (180). Steiger *et al.* (181) compared the S(+)-oxaprotiline enantiomer with the inactive R(-)-enantiomer, which is devoid of actions at monoamine reuptake transporters and postsynaptic cAMP-binding proteins (182). As illustrated in Fig. 3, the S(+)-enantiomer, but not the R(-)-enantiomer, proved effective as an activator of the HPA system in controls (181). Parenthetically, these authors predicted that only the neuroendocrinologically active compound would also be clinically effective, which ultimately proved true. Furthermore, the mixed 5-HT-NA reuptake inhibitor clomipramine enhances ACTH and cortisol release in men, an effect that was less pronounced in depressives (183). In this case the

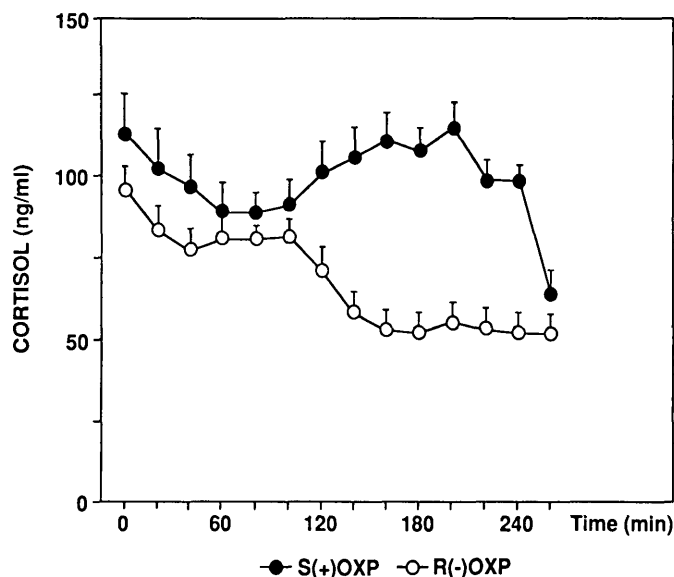


FIG. 3. Plasma cortisol concentration curves after oral administration of 75 mg S(+)- or 75 mg R(-)-oxaprotiline (OXP) to 14 healthy male human controls. Results expressed in mean \pm SE indicate that only the NA reuptake-inhibiting stereoisomer S(+)-OXP enhances cortisol secretion. [Reproduced with permission from A. Steiger *et al.*: *Eur Neuropsychopharmacol* 3:117-126, 1993 (181).]

combined effects of NA and 5-HT reuptake inhibition may have contributed, as is the case with venlafaxine, which along with other actions also inhibits reuptake transporters of both neurotransmitters and activates the HPA system (184).

The role of 5-HT as a stimulator of HPA activities in human controls and psychiatric patients has been elaborated upon by studies that used fenfluramine, which releases 5-HT and also blocks its reuptake. When fenfluramine is administered to healthy men, ACTH and cortisol levels are elevated, and this effect can be suppressed by cyproheptadine, a 5-HT antagonist (185). When rats are treated with fenfluramine they have increased CRH release, which not only activates ACTH and corticosterone but, through its anorexic effect, may also reduce body weight (186). 5-HT has proven to be a potent stimulator of CRH release, and therefore it would be expected that antidepressants acting selectively at 5-HT-reuptake transporters would activate the HPA system. For example, the portal plasma CRH concentration in rats treated with fluoxetine, a 5-HT-reuptake inhibitor, is found to be increased (187). In men, administration of various selective 5-HT-reuptake inhibitors also activates the HPA system, although to varying extents, because metabolites derived from the drugs may counteract the initial effect (188, 189). Lesch *et al.* (190, 191) have administered various dosages of ipsapirone, a 5-HT_{1A} receptor agonist, to men and have found that it can activate the HPA system in humans. This effect is decreased by the nonselective β -adrenoceptor and stereoselective 5-HT_{1A} antagonist pindolol. Because betaxolol, a selective β 1-adrenoceptor antagonist, did not alter HPA activity, these authors concluded that the 5-HT activation of the HPA system mainly involves a 5-HT_{1A} receptor-mediated effect. The ACTH and cortisol response to ipsapirone was suppressed in depression, and therefore the authors also suggested that 5-HT_{1A} receptors or associated postreceptor signaling pathways are subsensitive in these patients.

B. Long-term in vivo effects

Whereas it seems firmly established that antidepressants and the HPA system strongly interact at a regulatory level, much less is known about how these interactions relate to the clinical effectiveness of the drugs. Longitudinal studies in which depressed patients who were dexamethasone non-suppressors were retested weekly suggested that a return to dexamethasone suppressibility of plasma cortisol levels precedes resolution of depressive psychopathology (192–195). In turn, persistence of dexamethasone nonsuppression proved to be associated with a less favorable prognosis (review in Ref. 46). Similarly, serial administration of combined dexamethasone-CRH-test corroborated the finding that normalization of initial HPA alteration is associated with antidepressant-induced clinical improvement (55, 69, 70, 74). In addition, drug-free patients with severe depression have higher rates of HPA abnormality than equally depressed patients under antidepressant treatment. These findings and the finding by De Bellis *et al.* (196) that antidepressants sometimes may reduce CRH levels in human spinal fluid suggest that, in contrast to their acute effects, long-term administration of antidepressants may suppress the HPA system, which

raises the possibility that lowering HPA activity and clinical response are causally related (3).

The observation that trimipramine, which is devoid of actions on the 5-HT and NA systems but potently decreases HPA activity, also is a clinically effective antidepressant is evidence in this direction (70). Furthermore, tianeptine, a tricyclic molecule that enhances 5-HT uptake, thus acting opposite to the new generation of selective 5-HT reuptake inhibitors, reduces HPA activity. Tianeptine's profile in animal models, sensitive to antidepressants, was indicative of antidepressant activity (197, 198), and preliminary clinical studies suggest that it may have beneficial effects in clinical depression (199).

Normally, antidepressants exert acute neuroendocrine effects within minutes or hours, whereas their clinical effect on psychopathology is not usually fully evident for 2 weeks, and it often takes as long as 5–8 weeks. This suggests that the neurotransmitter reuptake inhibition only initiates a cascade of events, the final result being numerous metabolic changes including dampening of the HPA system. In line with this notion are the results of several studies in rats treated with antidepressants at dosages and for time periods that correspond to the clinical condition. Reul *et al.* (200, 201) studied the time course of MR and GR concentrations in selected brain regions and the pituitary of rats treated with amitriptyline, a tricyclic compound that blocks effects at 5-HT- and NA-reuptake transporters and has an anticholinergic action, or with moclobemide, a reversible inhibitor of monoamine oxidase-A. Both drugs increased levels of hippocampal MR transiently by 40–70% between 2 and 5 weeks after the start of treatment. GR levels were also initially increased. In parallel, markedly decreased CRH binding capacity and POMC mRNA content were observed in the anterior pituitary. Moreover, adrenal weight decreased, which is in agreement with lowered ACTH and corticosterone secretion after long-term treatment with amitriptyline or moclobemide. In addition, rats exposed to stress induced by a novel environment secreted more ACTH and corticosterone than rats treated either with amitriptyline or with moclobemide (Fig. 4), which may provide an explanation for the prophylactic effect of antidepressants, possibly buffering stress-elicited HPA hyperactivation. These findings agree with those of Shimoda *et al.* (179), who showed that long-term treatment with various tricyclics (desipramine, clomipramine, or imipramine) can suppress plasma ACTH and corticosterone secretion in rats. Kitayama *et al.* (202) treated rats for 2 weeks with imipramine and found GR immunoactivity to be increased in the LC and the nucleus raphe magnus, and they suggested that antidepressants may help to maintain GR function in NA and 5-HT neuron-containing cell groups. This hypothesis is of considerable interest because the hippocampus, implicated in neuroendocrine and cognitive symptoms of depression, receives ample noradrenergic and 5-HT innervations. In line with these findings is a report by Seckl and Fink (203) showing that, after 2 weeks, tricyclic antidepressant treatment leads to increases in both GR and MR mRNA expression in adult rat hippocampus. No changes in corticosteroid receptor mRNA levels were found in the parietal cortex. As did Reul *et al.* (200), who measured receptor levels, Seckl and Fink (203) also found MR expression to be the earliest change during

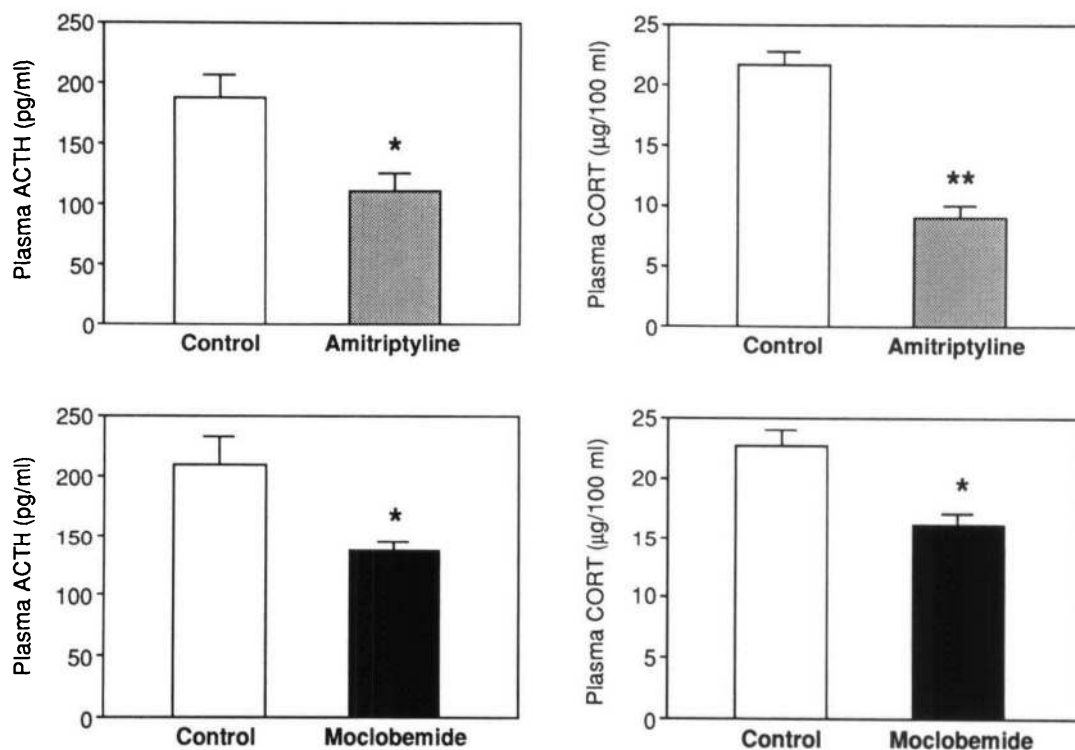


FIG. 4. Effect of long-term treatment with amitriptyline (4.5 mg/kg/day) or moclobemide (4.5 mg/kg/day) for 5 weeks to rats upon their plasma ACTH and corticosterone (CORT) levels after 30-min novel environment stress. Both antidepressant treatments suppress the stress-elicited hormone secretions. [Reproduced with permission from J. M. H. M. Reul *et al.*: *Endocrinology* 133:312–320, 1993 (200) © The Endocrine Society; and J. M. H. M. Reul *et al.*: *Neuroendocrinology* 60:509–519, 1994 (201). S. Karger AG, Basel.]

amitriptyline treatment. Further, Brady *et al.* (204), measuring mRNA levels of CRH in the PVN, of tyrosine hydroxylase (TH) in the LC, of MR in the hippocampus, and of GR in the anterior pituitary in rats treated with desipramine found expression of CRH, TH, POMC, and anterior pituitary GR to be decreased, whereas MR mRNA was increased. These authors suggested that the combined decrease in TH and CRH expression might be involved in the therapeutic efficacy of desipramine. Earlier investigations had also suggested that antidepressant treatment might counteract stress-induced elevations in NA and its metabolites and reduce levels of TH mRNA and protein in the LC (205–207), which would be of interest since the LC cell firing rate can be enhanced by CRH. Furthermore, TH levels that are elevated in rats in response to chronic stress normalize after long-term treatment with imipramine (208). Thus, if it can be shown that the observed antidepressant reduction in CRH mRNA after various antidepressants (209) is translated into reduced functional protein levels and not counteracted by CRH receptor adaptations, some antidepressants may indeed act not only by decreasing noradrenergic cell activity but also, directly or indirectly, by reducing CRH neuronal hyperactivity (3).

McEwen and co-workers (210) showed that repeated restraint stress in rats for 3 weeks causes changes in the hippocampal formation that include suppression of 5-HT_{1A} receptor binding, atrophy of dendrites of CA₃ pyramidal neurons, and impairment of initial learning in the radial arm maze task. Similar effects are induced also by corticosterone. Both the stress-induced changes and those induced by corticosterone are prevented by tianeptine, a drug with reported

effects as enhancer of 5-HT reuptake in the hippocampus (211) and as suppressor of HPA activity (198). Apparently, quite different pharmacological effects can act clinically to improve depression, suggesting that depression can be caused by different etiologies that all ultimately produce impaired HPA function. Correcting this neuroendocrine change through therapeutic interventions seems to be clinically beneficial whatever the primary etiology might have been.

C. *In vitro* studies

After long-term administration, antidepressants are notoriously unspecific and interact with a wide range of systems. In this section we confine ourselves to the hypothesis that antidepressants act by increasing corticosteroid receptor capacity and function, thus optimizing the feedback response to stress-evoked corticosteroid surges.

Pepin *et al.* (212) treated primary cultures of rat brain hypothalamic neurons for 48 h with maprotiline (a tetracyclic antidepressant acting as inhibitor at the NA reuptake transporter), desipramine (an NA reuptake inhibitor), and amitriptyline (an inhibitor of both 5-HT and NA) and found GR mRNA to be increased. This change was also seen in the amygdaloid complex with all three antidepressants. In the cortex only desipramine and amitriptyline, but not maprotiline, increased GR mRNA levels. Measurements of antidepressant-induced changes in glucocorticoid sensitivity were possible in LTK⁻ fibroblast cells (Fig. 5) or mouse Neuro2A neuroblastoma cells

TABLE 1. Increased GR gene and glucocorticoid-sensitive gene activities following treatment of mouse neuroblastoma cells with antidepressant

Gene activity	% Increase (mean \pm SEM)
A. GR-CAT activity	79.1 \pm 12.5 (n = 12)
B. GR mRNA/ β -ACTIN mRNA	72.5 \pm 28.5 (n = 3)
C. MMTV-CAT activity	86.2 \pm 10.5 (n = 14)

The following gene activities were measured in untreated and antidepressant-treated (desipramine, 10^{-6} M, for 24 h before assay) Neuro 2A neuroblastoma cells: A. GR gene expression was determined in cells transfected with CAT under control of a 2.7-kb fragment of the GR gene promoter. B. The ratio of GR mRNA to that of β -ACTIN mRNA was measured by Northern blotting. C. The transcription activity of MMTV-CAT (a glucocorticoid-inducible gene construct that is sensitive to changes in cell GR concentration) was measured in cells transfected with this reporter plasmid and activated with 10^{-6} M dexamethasone. [Derived with permission from M. C. Pepin *et al.*, *Mol Pharmacol* 41:1016-1022, 1992 (215)].

(Table 1), transfected with a plasmid DNA vector consisting of a glucocorticoid-responsive MMTV promoter enhancer element fused to a reporter gene [chloramphenicol acetyl transferase (CAT)] (213-215). Desipramine treatment produced a 2-fold increase in glucocorticoid-stimulated CAT activity. With a chimeric gene construct consisting of GR gene promoter fused to CAT gene (pHGR2.7CAT) a 3-fold desipramine-induced increase in CAT activity was observed. The antidepressant treatment of these cells increased GR mRNA and [3 H]dexamethasone binding after 1-4 days in both LTK⁻ and Neuro2A cells (214). Of particular interest is the desipramine-induced increase in GR promoter element activity in LTK⁻ cells. These fibroblast cells do not secrete catecholamines and therefore the effect of desipramine on GR gene activity involves a mechanism different from that in NA-reuptake inhibition. It is of note that the desipramine concentration needed for maximum effects in these studies was in the range of 10^{-8} M and above, which corresponds to the plasma concentrations needed to achieve clinical efficacy.

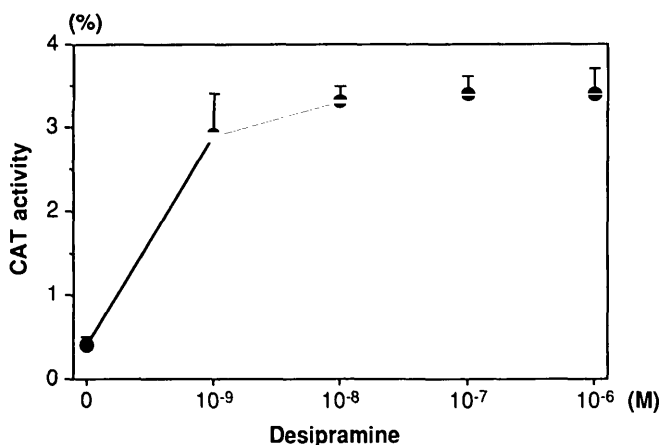


FIG. 5. LTK⁻ cells were incubated with increasing concentrations of desipramine for 24 h before transient transfection with the pHGR 2.7 CAT plasmid. GR promoter activity expressed as percent acetylation of chloramphenicol by CAT increases with increasing doses of desipramine. [Reproduced with permission from M. C. Pepin *et al.*: *Mol Pharmacol* 41:1016-1022, 1992 (215)].

VII. A Transgenic Mouse with a Primary GR Defect as an Animal Model to Study how Antidepressants Affect HPA Hyperactivity

Behavioral phenotypes are created by a complex interplay of genetic and environmental influences, rendering studies of genetic control of behavior extremely difficult. This is particularly true in depression, where the genetic predisposition, biographical influences, and precipitating life events are involved in the causation and course of the illness and where no widely accepted animal model exists. In human depression, a genetic influence is well documented, as is the clinical responsiveness of the disorder to antidepressant drugs. These two robust findings and the high frequency (more than 90% if there is rigid neuroendocrine evaluation) with which major depression is associated with altered HPA regulation (76) have encouraged the development of a transgenic mouse model with which some cardinal symptoms of depression and their response to antidepressant drugs can be studied.

A. Development of the transgenic mouse

A broad clinical data base gathered from depressed patients who were suffering from a depressive episode or who were in remission, as well as data from healthy subjects belonging to families with a high genetic load for depression, converged to create the hypothesis that defunct sensitivity of the HPA system to the negative feedback action of corticosteroids may be causally linked to the pathogenesis of depression and to the therapeutic efficacy of antidepressant treatments. Reverse genetic approaches such as production of a corticosteroid receptor defect by replacement of the GR (216) would be one possibility for testing this hypothesis. Theoretically, such a model would allow analysis of the effect of the steroid hormone receptors on behavior, but adaptive mechanisms throughout development limit the precision by which behavioral changes in such animals can be attributed to a specific gene "knock-out."

An alternative approach is a partial GR gene "knock-down" through incorporation into the mouse genome of a gene fragment directing expression of an antisense RNA complementary to the GR mRNA (217). The precise mechanism by which antisense RNA acts is not yet established. It is believed that a double hybrid RNA once formed is rapidly degraded by RNase. Such mechanisms emerged from studies using oligodeoxynucleotides *in vitro* (218), where RNA-DNA heteroduplexes served as substrates for RNase H (219), although other studies questioned such a possibility (220). The decreased levels of GR mRNA and the neuroendocrine changes in these transgenic mice suggested that impaired GR gene expression would indeed provide an animal model with which this defect can be studied and the findings compared with those in major depression. It is noteworthy, however, that this model does not mimic all aspects of clinical depression because transgenic mice deposit more fat and have higher levels of triglycerides together with a decrease in muscle tissue mass in later life (221). In animals more than 9 months old these differences are accentuated, making them resemble Cushing's syndrome, which is never found among patients with depression and hypercortisolemia.

B. Neuroendocrine changes and their response to antidepressants

Transgenic mice release more ACTH and corticosterone, particularly in the morning, than do normal mice, and the secretion of these hormones is less sensitive to the suppressive action of dexamethasone (222). In fact, a 10-fold higher (20 $\mu\text{g}/100\text{ g}$ body wt) dosage of dexamethasone was required to suppress plasma ACTH and corticosterone levels, which is in accordance with a reduced efficacy of circulating corticosteroids in affecting HPA regulation through GR. The antisense-induced impediment to GR gene expression in the transgenic mouse is documented by decreased GR mRNA in the hypothalamus and the cortex. Pepin *et al.* (215) administered desipramine, an antidepressant with blocking effects on the NA transporter, and observed increased GR mRNA and [^3H]dexamethasone binding in the hypothalamus and cortex. In addition, they found a decrease in initially elevated plasma ACTH and corticosterone levels. Other antidepressants also produced significant reductions in plasma ACTH and corticosterone concentrations after long-term treatment. The changes in receptor binding were less uniform. Whereas the study in which desipramine was administered for 10 days documented an increase in GR capacity of about 30%, no such consistent changes were observed after amitriptyline or moclobemide treatment for 3–4 weeks (200, 201, 223). These discrepancies are best explained by time-dependent adaptive changes. In rats a medium-term exposure to antidepressants was shown to have more pronounced effects on GR capacity than long-term exposure (200, 201). Although not all experiments with transgenic mice show a reduced number of GR-binding sites, the function of these receptors is apparently reduced in these animals, as was documented by elevated plasma and corticosterone levels and the resistance of the receptors to the suppressive effect of dexamethasone (214, 222, 224). The reason for the discrepancy between changes in GR concentration and changes in GR function is still a subject of speculation: it may indicate that intracellular antisense initiates a number of effects other than merely decreasing protein synthesis, and it also points to mechanisms that involve interactions between ligand-activated GR and various other transcription factors, modulating transcriptional efficacy without numerically affecting receptor capacity (136, 137, 225). Interestingly, the number of MRs that are coexpressed in hippocampal pyramidal cells is decreased (N. Barden, J. Stec, F. Holsboer, and J. M. H. M. Reul, unpublished observations). This may also reflect an impaired function of ligand-activated GR, which was found to enhance MR gene expression through an action at the GRE in the MR gene promoter (226). The decrease in MR capacity secondary to impaired GR function may account for HPA hyperactivity in these mice. Elevated plasma levels of corticosterone can further desensitize GR in the hypothalamus and cortex and MR and GR in the hippocampus, counteracting the effects of antidepressants on these steroid receptors. In addition, the possibility that other intracellular feedback loops between transcription, mRNA, and protein formation, including changes in the function of signaling in the presence of unchanged protein quantities, must be considered. The finding that numerous antidepressants all de-

crease HPA hyperdrive in transgenic mice with impaired GR expression is consistent with an action of these drugs on GR synthesis or function, or both.

C. Behavioral changes and their response to antidepressants

Humans with elevated serum corticosteroid titers secondary to endocrine disease or hormone treatment (*e.g.* Cushing's syndrome) as well as patients with major depression exhibit a variety of deficits in cognitive functioning that include problems with storage and retrieval of information. Several behavioral paradigms have been used to study whether the transgenic animal model that mimics neuroendocrine signs of depression is also valid for studies of cognitive and emotional symptoms of depression.

The Morris water maze test (227), which investigates a special form of long-term memory, was first employed. In this test, acquisition of spatial learning is assessed by placing the mice in a circular pool where they are trained to search for a hidden platform in milky water. In order to navigate, the animals can orient themselves by attending to external visual cues. Whereas control mice quickly learn to locate the platform, transgenic mice have profound difficulties developing a search strategy based on learning of the spatial relation between external cues and the escape platform (Fig. 6).

Short-term memory was assessed with the social recognition paradigm, which is based on olfactory discriminative capacity (228). An adult mouse is exposed to a conspecific juvenile and investigates the juvenile for a certain period of time. After reexposure to the same juvenile 60 min later, the investigation time by the adult mouse is much shorter, which is taken as a measure of short-term memory. If the adult is exposed to a different juvenile after 60 min, the exploration lasts as long as the initial exploration. Transgenic mice producing antisense RNA complementary to GR mRNA are

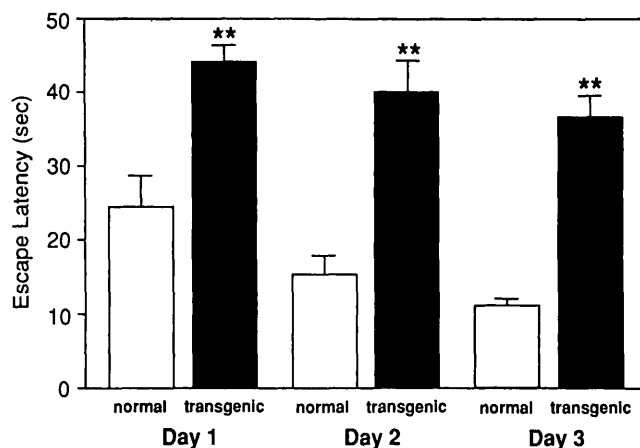


FIG. 6. Spatial learning and memory containment of transgenic mice (3 months old) with impaired GR function and of control mice, tested in the Morris water maze paradigm. Results are expressed as escape latency in seconds (mean \pm SE) that transgenic mice and controls needed to find the platform hidden below the milky water surface in a pool. Transgenic mice needed much longer to learn to locate the platform (**, $P < 0.01$), indicating a reduced ability to learn the spatial relation between external cues and the escape platform (A. Montkowski, N. Barden, C. Wotjak, and F. Holsboer, unpublished observation).

unable to recognize a juvenile they have investigated 60 min earlier, indicating impaired memory.

A third experiment employed the elevated plus maze, a well established animal test of anxiety (229), to assess anxiety-related behavior of the transgenic mice. As outlined above, HPA hyperactivity in depression is believed to be driven by CRH overproduction, possibly involving defective negative feedback through GR. Because overproduction of CRH predictably induces anxiogenic behavior (16, 107, 114, 115), one would assume that the HPA-hyperactive transgenic mice would show increased levels of anxiety. However, these mice made more entries onto, and spent more time on, open arms of the plus maze than the control mice, which would indicate reduced anxiety rather than the anticipated elevated anxiety. Given that normal acquisition, processing, storage, and retrieval of information is necessary for an adequate emotional and behavioral response to a novel fear-inducing situation and considering the behavior in the Morris water maze and in the social recognition paradigm, the most likely explanation is that these transgenic mice have severe cognitive deficits that impede their capacity to evaluate cues indicating danger or safety in a novel environment. Thus, the GR antisense RNA expression produces a kind of HPA disturbance that results in cognitive impairment reducing the animals' capacity to recognize a dangerous situation. These observations suggest profound cognitive deficits in the transgenic mice with HPA defects and support the view that these HPA defects are also causally involved in the cognitive impairment seen in patients with HPA pathology such as Cushing's syndrome or major depression.

When transgenic mice were treated for 7 weeks with moclobemide via drinking water (15 mg/kg body wt) most of the behavioral deficits observed in untreated mice were improved. In the elevated plus maze the cognitive impairment was no longer apparent, and moclobemide-treated transgenic mice were indistinguishable from control mice treated with either moclobemide or placebo. In a similar way memory deficits disappeared in depressives when treated successfully with imipramine (230). Testing these transgenic animals in the social recognition paradigm revealed that moclobemide also improved short-term memory. The deficit of transgenic mice being unable to recognize a juvenile they had met previously was no longer obvious since the investigation time during reexposure was reduced to the same extent as in control mice (224). In control mice, long-term antidepressant treatment produced no behavioral changes, indicating that the drug-induced changes in behavior occur only in the presence of the memory deficit.

Long-term moclobemide treatment also affected behavior in the forced swim test. In this paradigm, mice are placed in a Plexiglas cylinder filled with water, where they are forced to swim (231). After an initial period of escape-directed activity, a normal mouse prefers floating to conserve energy, or because it has given up, or both. When these animals are retested 1 day later their floating time increases. This kind of adaptive behavior most likely reflects the coping strategy of retrieving previous experience from memory stores (232). Originally, this test was used to screen for antidepressant efficacy because most antidepressant drugs increase the latency to immobility. In our normal non-transgenic control

mice, moclobemide had no effect on immobility in this test. When transgenic mice were tested 1 day after the first exposure, their floating time was much shorter than that of the untreated control mice, indicating that they were not able to make use of previous experience. Transgenic mice that had received long-term antidepressant treatment with moclobemide were indistinguishable from normal mice as their floating time increased when retested in the forced swim test (see Fig. 7) (224).

In contrast to the elevated plus maze test, the social recognition paradigm, and the forced swim test, the Morris water maze did not reveal any effect of moclobemide treatment on spatial learning and memory performance in the transgenic mice; perhaps the task involved is too complex to be affected by moclobemide. This suggests that cognitive function is differentially affected by moclobemide, which is in accordance with clinical experience (233). Investigations of mice with targeted gene disruption are a powerful tool for understanding genetic control of behavior. However, the currently available transgenic mice often do not show a behavioral phenotype even though the mutated gene is, according to electrophysiological studies, implicated in the neuronal plasticity of the hippocampus such as reported in the heterozygous BDNF knock-out mouse (234). The absence of a behavioral phenotype in such mutated mice may be the result of adaptations throughout development and calls for conditional targeting allowing time-dependent and tissue-specific triggering of gene disruption (235). Moreover, the approaches where behavioral and neuroendocrine alterations are identified following a mutagenesis screen using the alkylating agent N-ethyl-N-nitrosourea and where the responsible loci are finally mapped may be advantageous in the future (236).

VIII. Conclusions

There is considerable evidence that HPA dysregulation is causally implicated in the onset of depression and that the

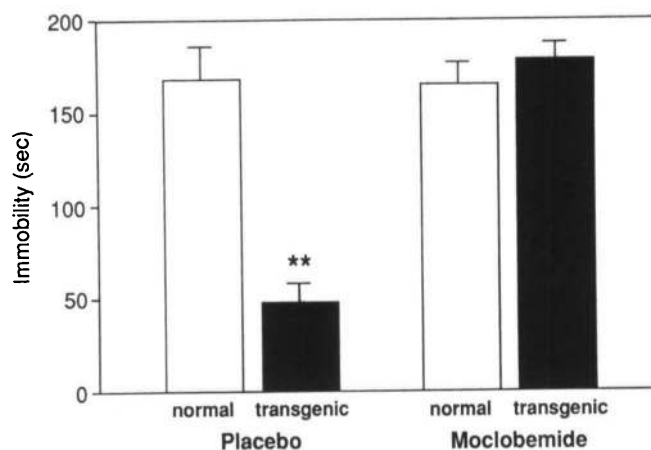


FIG. 7. In the forced swim test, immobility is expressed in seconds (mean \pm SE), in which the animals float. In controls, moclobemide failed to prolong the period in which the animals displayed active escape behavior; moclobemide-treated transgenic mice (3 months old) showed increased immobility (**, $P < 0.01$) [Reproduced with permission from A. Montkowski *et al.*: *J. Neuroendocrinol* 7:841–845, 1995 (224).]

many ways in which antidepressants work include actions on the HPA system. Clinical investigations suggest that psychosocial stressors and life events play a role in triggering depressive episodes among those who have a genetic predisposition to this disorder (237). Stressful environmental challenges are accompanied by central elaboration of catecholamines, emerging from the LC, and by increased levels of glucocorticoids, secreted from the adrenals after hypothalamic activation of CRH and ACTH from the pituitary. In depression, the adaptive response appears to be defective, leaving the HPA system altered until therapeutic interventions begin to be effective.

Antidepressants, known to act mainly on catecholaminergic and serotonergic neurotransmission, also seem to have effects that are independent of their effects on biogenic amine metabolism or receptors and which produce normalization of initial HPA dysregulation. The time course of these neuroendocrine actions on HPA activity and, more specifically, on corticosteroid receptors follows closely that of clinical improvement, supporting the hypothesis that a causal link between HPA activity and antidepressant effects exists.

The evidence, of course, is not entirely consistent because it is difficult to assess clinically relevant effects in laboratory animals. Anxiety, adaptation to environmental stress, and neuroendocrine disturbance are behavioral and hormonal signs of depression that can be studied concomitantly in laboratories for animal behavior, cellular biology, and molecular biology. Specifically, antidepressant regulation of the expression of genes coding for corticosteroid receptors and their genomic function perhaps provides a functional endpoint by which antidepressant-induced effects can be studied. This line of research may open up a lead for the development of new drugs that are more directly targeted to elements of the HPA system and thus more efficient than those currently available.

References

- Nestler EJ, Duman RS 1995 Intracellular messenger pathways as mediators of neural plasticity. In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp 695–704
- Gibbons JL 1964 Cortisol secretion rate in depressive illness. *Arch Gen Psychiatry* 10:572–575
- Barden N, Reul JMHM, Holsboer F 1995 Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? *Trends Neurosci* 18:6–11
- Dallman MF 1993 Stress update. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab* 4:62–69
- Swanson LW 1991 Biochemical switching in hypothalamic circuits mediating responses to stress. *Prog Brain Res* 87:181–200
- de Kloet ER 1991 Brain corticosteroid receptor balance and homeostatic control. *Front Neuroendocrinol* 12:95–164
- Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW 1992 The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc Natl Acad Sci USA* 89:4192–4196
- Antoni FA 1993 Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Front Neuroendocrinol* 14:76–122
- Antoni FA 1986 Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr Rev* 7:351–378
- Tuomisto J, Männistö P 1985 Neurotransmitter regulation of anterior pituitary hormones. *Pharmacol Rev* 37:249–332
- Orth DN 1992 Corticotropin-releasing hormone in humans. *Endocr Rev* 13:164–191
- Owens MJ, Nemeroff CB 1991 Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 43:425–473
- Plotsky PM 1987 Facilitation of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation after activation of catecholaminergic pathways or central norepinephrine injection. *Endocrinology* 121:924–930
- Valentino RJ, Foote SL, Aston-Jones G 1983 Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. *Brain Res* 270:363–367
- Valentino RJ, Foote SL, Page ME 1993 The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann NY Acad Sci* 697:171–187
- Berridge CW, Dunn AJ 1989 Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF. *J Neurosci* 9:3513–3521
- Al-Damluji S, Cunnah D, Grossman A, Perry L, Ross G, Coy D, Rees LH, Besser GM 1987 Effect of adrenaline on basal and ovine corticotropin-releasing factor-stimulated ACTH secretion in man. *J Endocrinol* 112:145–150
- Di Scullo A, Bluet-Pajot MT, Mounier F, Oliver C, Schmidt B, Kordon C 1990 Changes in anterior pituitary hormone levels after serotonin 1A receptor stimulation. *Endocrinology* 127:567–572
- Bagdy G, Calogero AE, Charanjit SA, Szemeredi K, Murphy DL 1989 Long term cortisol treatment impairs behavioral and neuroendocrine responses to 5-HT₁ agonists in rat. *Neuroendocrinology* 50:241–247
- Owens MJ, Knight DL, Ritchie JC, Nemeroff CB 1991 The 5-hydroxytryptamine₂ agonist, (\pm)-1-(2, 5-dimethoxy-4-bromophenyl)-2-aminopropane stimulates the hypothalamic-pituitary-adrenal (HPA) axis. I. Acute effects on HPA axis activity and corticotropin-releasing factor-containing neurons in the rat brain. *J Pharmacol Exp Ther* 256:787–794
- Calogero AE, Kamilaris TC, Gomez T, Johnson EO, Tartaglia ME, Gold PW, Chrousos GP 1989 The muscarinic cholinergic agonist arecoline stimulates the rat hypothalamic-pituitary-adrenal axis through a centrally-mediated corticotropin-releasing hormone-dependent mechanism. *Endocrinology* 125:2445–2453
- Keller-Wood ME, Dallman MF 1984 Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 5:1–24
- Tai PKKT, Albers MW, Chang H, Faber LE, Schreiber SL 1992 Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. *Science* 256:1315–1318
- Pratt WB, Jolly DJ, Pratt DV, Hollenberg SM, Giguere V, Cadepond FM, Schweitzer-Groyer G, Catelli MG, Evans RM, Baulieu EE 1988 A region in the steroid-binding domain determines formation of the non-DNA-binding, 9 S glucocorticoid receptor complex. *J Biol Chem* 263:267–272
- Vamvakopoulos NC, Chrousos GP 1994 Hormonal regulation of human corticotropin-releasing hormone gene expression: implications for the stress response and immune/inflammatory reaction. *Endocr Rev* 15:409–420
- Schöbitz B, de Kloet ER, Holsboer F 1994 Gene expression and function of interleukin 1, interleukin 6 and tumor necrosis factor in the brain. *Prog Neurobiol* 44:397–432
- Sapolsky RM, Armanini MP, Sutton SW, Plotsky PM 1989 Elevation of hypophysial portal concentrations of adrenocorticotropin secretagogues after fornix transection. *Endocrinology* 125:2881–2887
- Lamberts SWJ, Koper JW, de Jong FH 1991 The endocrine effects of long-term treatment with mifepristone (RU 486). *J Clin Endocrinol Metab* 73:187–191
- Dotz C, Kern W, Fehm HL, Born J 1993 Antimineralocorticoid canrenoate enhances secretory activity of the hypothalamus-pituitary-adrenocortical (HPA) axis in humans. *Neuroendocrinology* 58:570–574
- Bradbury MJ, Strack AM, Dallman MF 1993 Lesions of the hippocampal efferent pathway (fimbria-fornix) do not alter sensitivity of adrenocorticotropin to feedback inhibition by corticosterone in rats. *Neuroendocrinology* 58:396–407

31. **Beato M** 1989 Gene regulation by steroid hormones. *Cell* 56:335–344
32. **O'Malley B** 1990 The steroid receptor superfamily: more excitement predicted for the future. *Mol Endocrinol* 4:363–369
33. **Tsai MJ, O'Malley BW** 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* 63:451–486
34. **Trapp T, Rupprecht R, Castrén M, Reul JM, Holsboer F** 1994 Heterodimerization between mineralocorticoid and glucocorticoid receptor: a new principle of glucocorticoid action in the central nervous system. *Neuron* 13:1–6
35. **Rupprecht R, Arriza JL, Spengler D, Reul JM, Evans RM, Holsboer F, Damm K** 1993 Trans-activation and synergistic properties of the mineralocorticoid receptor: relationship to the glucocorticoid receptor. *Mol Endocrinol* 7:597–603
36. **Funder JW** 1994 Corticosteroid receptors and the central nervous system. *J Steroid Biochem Mol Biol* 49:381–384
37. **Funder JW** 1994 The tale of the guinea pig. *Front Neuroendocrinol* 15:384–389
38. **Van Steensel B** 1995 Steroid Receptors and Nuclear Structure. PhD Thesis of the University of Amsterdam, The Netherlands
39. **Van Eekelen JAM, Jiang W, de Kloet ER, Bohn MC** 1988 Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. *J Neurosci* 21:88–94
40. **Herman JP, Schäfer MKH, Young EA, Thompson R, Douglass J, Akil H, Watson SJ** 1989 Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *J Neurosci* 9:3072–3082
41. **Rubin RT, Poland RE, Lesser IM, Winston RA, Blodgett ALN** 1987 Neuroendocrine aspects of primary endogenous depression. *Arch Gen Psychiatry* 44:328–336
42. **Carroll BJ, Curtis GC, Davies BM, Mendels J, Sugerman AA** 1976 Urinary free cortisol excretion in depression. *Psychol Med* 6:43–50
43. **Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W** 1984 Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226:1342–1344
44. **Carroll BJ** 1982 The dexamethasone suppression test for melancholia. *Br J Psychiatry* 140:292–304
45. **Holsboer F, Philipp M, Steiger A, Gerken A** 1986 Multiteroid analysis after DST in depressed patients - A controlled study. *J Affective Disord* 10:241–249
46. **Ribero SCM, Tandon R, Grunhaus L, Greden JF** 1993 The DST as a predictor of outcome in depression: a meta-analysis. *Am J Psychiatry* 150:1618–1629
47. **Vale W, Spiess J, Rivier C, Rivier J** 1981 Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β -endorphin. *Science* 213:1394–1397
48. **Spiess J, Rivier J, Rivier C, Vale W** 1981 Primary structure of corticotropin-releasing factor from ovine hypothalamus. *Proc Natl Acad Sci USA* 78:6517–6521
49. **Shibahara S, Morimoto Y, Furutani Y, Notake M, Takahashi H, Shimizu S, Horikawa S, Numa S** 1983 Isolation and sequence analysis of the human corticotropin-releasing factor precursor gene. *EMBO J* 2:775–779
50. **Holsboer F, Gerken A, von Bardeleben U, Grimm W, Beyer H, Müller OA, Stalla GK** 1986 Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 21:601–611
51. **Gold PW, Loriaux DL, Roy A, Kling MA, Calabrese JR, Kellner CH, Nieman LK, Post RM, Pickar D, Gallucci W, Avgerinos P, Paul S, Oldfield EH, Cutler GB, Chrousos GP** 1986 Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *N Engl J Med* 314:1329–1335
52. **von Bardeleben U, Stalla GK, Müller OA, Holsboer F** 1988 Blunting of ACTH response to human CRH in depressed patients is avoided by metyrapone pretreatment. *Biol Psychiatry* 24:782–786
53. **Lisansky J, Peake GT, Strassman RJ, Qualls C, Meikle AW, Risch SC, Fava GA, Zownir-Brazis M, Hochla P, Britton D** 1989 Augmented pituitary corticotropin response to a threshold dosage of human corticotropin-releasing hormone in depressives pretreated with metyrapone. *Arch Gen Psychiatry* 46:641–649
54. **Rupprecht R, Lesch KP, Müller U, Beck G, Beckmann H, Schulte HM** 1989 Blunted adrenocorticotropin but normal β -endorphin release after depression. *J Clin Endocrinol Metab* 69:600–603
55. **Holsboer F, Gerken A, Stalla GK, Müller OA** 1987 Blunted aldosterone and ACTH release after human CRH administration in depressed patients. *Am J Psychiatry* 144:229–231
56. **Nemeroff CB, Krishnan KRR, Reed D, Leder R, Beam C, Dunnick R** 1992 Adrenal gland enlargement in major depression. *Arch Gen Psychiatry* 49:384–387
57. **Rubin RT, Phillips JJ, Sadow TF, McCracken JT** 1995 Adrenal gland volume in major depression. *Arch Gen Psychiatry* 52:213–218
58. **Dallman MF** 1985 Control of adrenocortical growth *in vivo*. *Endocr Res* 10:213–242
59. **Edwards AV, Jones CT** 1987 The effect of splanchnic nerve stimulation on adrenocortical activity in conscious calves. *J Physiol* 382:385–396
60. **Engeland WC, Gann DS** 1989 Splanchnic nerve stimulation modulates steroid secretion in hypophysectomised dogs. *Neuroendocrinology* 50:124–131
61. **Bruhn TO, Engeland WC, Anthony EL, Gann DS, Jackson IM** 1987 Corticotropin releasing factor in the dog adrenal medulla is secreted in response to hemorrhage. *Endocrinology* 120:25–33
62. **Edwards AV, Jones CT** 1988 Secretion of corticotropin releasing factor from the adrenal during splanchnic nerve stimulation in conscious calves. *J Physiol* 400:89–100
63. **Vinson GP, Hinson JP, Tóth IE** 1994 The neuroendocrinology of the adrenal cortex. *J Neuroendocrinol* 6:235–246
64. **Andreis PG, Neri G, Nussdorfer GG** 1991 Corticotropin-releasing hormone (CRH) directly stimulates corticosterone secretion by the rat adrenal gland. *Endocrinology* 128:1198–1200
65. **van Oers JWAM, Hinson JP, Binnekade R, Tilders FJH** 1992 Physiological role of corticotropin releasing factor in the control of ACTH mediated corticosterone release from the rat adrenal gland. *Endocrinology* 130:282–288
66. **Holsboer F, von Bardeleben U, Wiedemann K, Müller OA, Stalla GK** 1987 Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression - Implications for pathophysiology of DST nonsuppression. *Biol Psychiatry* 22:228–234
67. **von Bardeleben U, Holsboer F** 1989 Cortisol response to a combined dexamethasone-human corticotropin-releasing hormone (CRH) challenge in patients with depression. *J Neuroendocrinol* 1:485–488
68. **von Bardeleben U, Holsboer F** 1991 Effect of age upon the cortisol response to human CRH in depressed patients pretreated with dexamethasone. *Biol Psychiatry* 29:1042–1050
69. **Holsboer-Trachsler E, Hemmeter U, Stohler R, Hatzinger M, Gerhard U, Hobi V** 1991 The dexamethasone-hCRH stimulation test and cognitive performance during antidepressive treatment with trimipramine. *Eur Neuropsychopharmacol* 1:338–340
70. **Holsboer-Trachsler E, Hemmeter U, Hatzinger M, Seifritz E, Gerhard U, Hobi V** 1994 Sleep deprivation and bright light as potential augmenters of antidepressant drug treatment - Neurobiological and psychometric assessment of course. *J Psychiatr Res* 28:381–399
71. **Lammers CH, Garcia-Borreguero D, Schmider J, Gotthardt U, Dettling M, Holsboer F, Heuser IJ** 1995 Combined dexamethasone/corticotropin-releasing hormone test in patients with schizophrenia and in normal controls II. *Biol Psychiatry* 38:803–807
72. **Schmider J, Lammers CH, Gotthardt U, Dettling M, Holsboer F, Heuser IJ** 1995 Combined dexamethasone/corticotropin-releasing hormone test in acute and remitted manic patients, in acute depression and in normal controls I. *Biol Psychiatry* 38:797–802
73. **Schreiber W, Lauer CJ, Krumrey K, Holsboer F, Krieg JC** 1996 Dysregulation of the hypothalamic-pituitary-adrenocortical system in panic disorder. *Neuropsychopharmacology*, in press
74. **Heuser IJE, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Dettling M, Holsboer F** 1996 Pituitary-adrenal system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal controls. *Am J Psychiatry* 153:93–99
75. **Hermus ARM, Pieters GFF, Smals AGH, Benraad TJ, Kloppenborg PWC** 1984 Plasma adrenocorticotropin, cortisol and aldosterone responses to corticotropin-releasing factor: modulatory effect of basal cortisol levels. *J Clin Endocrinol Metab* 58:187–191

76. Heuser I, Yassouridis A, Holsboer F 1994 The combined dexamethasone/CRH-test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res* 28:341–356
77. Krieg JC 1994 Laboratory tests in depression: is it worth the effort? *J Psychiatr Res* 28:337–339
78. Yanovski JA, Cutler Jr GB, Chrousos GP, Nieman LK 1993 Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. *JAMA* 269:2232–2238
79. Grasser A, Möller A, Backmund H, Yassouridis A, Holsboer F 1995 Heterogeneity of hypothalamic-pituitary-adrenal system response to a combined dexamethasone-CRH test in multiple sclerosis. *Exp Clin Endocrinol Diabetes* 104:31–37
80. Heuser I, Wark HJ, Keul J, Holsboer F 1991 Hypothalamic-pituitary-adrenal axis function in elderly endurance athletes. *J Clin Endocrinol Metab* 73:485–488
81. Hatzinger M, Z'Brun A, Hemmeter U, Seifritz E, Baumann F, Holsboer-Trachsler E, Heuser IJ 1995 Hypothalamic-pituitary-adrenal system function in patients with Alzheimer's disease. *Neurobiol Aging* 16:205–210
82. von Bardeleben U, Holsboer F, Stalla GK, Müller OA 1985 Combined administration of human corticotropin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. *Life Sci* 37:1613–1618
83. Levin N, Roberts IL 1991 Positive regulation of proopiomelanocortin gene expression in corticotropes and melanotropes. *Front Neuroendocrinol* 12:1–22
84. De Goeij DCE, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJH 1991 Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 53:150–159
85. Bartanusz V, Jezova D, Bertini LT, Tilders FJH, Aubry JM, Kiss JZ 1993 Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotrophic paraventricular neurons. *Endocrinology* 132:895–902
86. Whitnall MH, Smyth D, Gainer H 1987 Vasopressin coexists in half of the corticotropin-releasing factor axons present in the external zone of the median eminence in normal rats. *Neuroendocrinology* 45:420–424
87. De Goeij DCE, Jezova D, Tilders FJH 1992a Repeated stress enhances vasopressin synthesis in corticotropin releasing factor neurons in the paraventricular nucleus. *Brain Res* 577:165–168
88. De Goeij DCE, Dijkstra H, Tilders FJH 1992b Chronic psychosocial stress enhances vasopressin, but not corticotropin-releasing factor, in the external zone of the median eminence of male rats: relationship to subordinate status. *Endocrinology* 131:847–853
89. Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF 1994 Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Clin Neuroendocrinol* 60:436–444
90. Purba JS, Hoogendijk WJG, Hofman MA, Swaab DF 1996 Increased number of vasopressin and oxytocin expressing neurons in the paraventricular nucleus of the human hypothalamus in depression. *Arch Gen Psychiatry* 53:137–143
91. Sawchenko PE 1987 Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. *Brain Res* 403:213–224
92. Keller-Wood ME, Dallman MF 1984 Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 5:1–24
93. Young EA, Haskett RF, Murphy-Weinberg V, Watson S, Akil H 1991 Loss of glucocorticoid fast feedback in depression. *Arch Gen Psychiatry* 48:693–699
94. Holsboer F, Lauer CJ, Schreiber W, Krieg JC 1995 Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for depression. *Neuroendocrinology* 62:340–347
95. Steiger A, von Bardeleben U, Herth T, Holsboer F 1989 Sleep EEG and nocturnal secretion of cortisol and growth hormone in male patients with endogenous depression before treatment and after recovery. *J Affective Disord* 16:189–195
96. Starkman MN, Scheingart DE, Schork MA 1981 Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels. *Psychosom Med* 43:3–18
97. Wolkowitz OM, Rubinow D, Doran AR, Breier A, Berrettini WH, Kling MA, Pickar D 1990 Prednisone effects on neurochemistry and behavior. *Arch Gen Psychiatry* 47:963–968
98. Wolkowitz OM, Weingartner H, Rubinow DR, Jimerson D, Kling M, Berrettini W, Thompson K, Breier A, Doran A, Reus VI, Pickar D 1993 Steroid modulation of human memory: biochemical correlates. *Biol Psychiatry* 33:744–746
99. Newcomer JW, Craft S, Hershey T, Askins K, Bardgett ME 1994 Glucocorticoid-induced impairment in declarative memory performance in adult humans. *J Neurosci* 14:2047–2053
100. Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NPV, Meaney MJ 1994 Basal cortisol levels and cognitive deficits in human aging. *J Neurosci* 14:2893–2903
101. Murphy BEP 1991 Treatment of major depression with steroid suppressive drugs. *J Steroid Biochem Mol Biol* 39:239–244
102. O'Dwyer AM, Lightman SL, Marks MN, Checkley SA 1995 Treatment of major depression with metyrapone and hydrocortisone. *J Affective Disord* 33:123–128
103. Wiedemank K, Lauer C, Loycke A, Durst P, Machér JP, Holsboer F 1992 Antiglucocorticoid treatment disrupts endocrine cycle and nocturnal sleep pattern. *Eur Arch Psychiatry Clin Neurosci* 241:372–375
104. Holsboer F, Spengler D, Heuser I 1992 The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog Brain Res* 93:385–417
105. Dunn AJ, Berridge CW 1990 Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Rev* 15:71–100
106. Owens MJ, Bissette G, Nemeroff CB 1989 Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in the rat brain. *Synapse* 4:196–202
107. Butler PD, Weiss JM, Stout JC, Nemeroff CB 1990 Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J Neurosci* 10:176–183
108. Swerdlow NR, Britton KT, Koob GF 1989 Potentiation of acoustic startle by corticotropin-releasing factor and by fear are both reversed by alpha-helical CRF (9–41). *Neuropsychopharmacology* 2:285–292
109. Kalin NH, Takahashi LK 1990 Fear-motivated behavior induced by prior shock experience is mediated by corticotropin-releasing hormone system. *Brain Res* 509:80–84
110. Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT 1991 CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharmacology* 103:227–232
111. Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF 1992 Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res* 581:190–197
112. Swiergiel AH, Takahashi LK, Kalin NH 1993 Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Res* 623:229–234
113. Imaki T, Vale W 1993 Chlordiazepoxide attenuates stress-induced accumulation of corticotropin-releasing factor mRNA in the paraventricular nucleus. *Brain Res* 623:223–228
114. Skutella T, Criswell H, Moy S, Probst JC, Breese GR, Jirikowski GF, Holsboer F 1994 Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide induces anxiolytic effects in rat. *Neuroreport* 5:2181–2185
115. Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW 1994 Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J Neurosci* 14:2579–2584
116. Stenzel-Poore MP, Cameron VA, Vaughan J, Sawchenko PE, Vale W 1992 Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 130:3378–3386
117. Muglia L, Jacobson L, Dikkes P, Majzoub JA 1995 Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* 373:427–432
118. Chen R, Lewis KA, Perrin MH, Vale WW 1993 Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 90:8967–8971
119. Chang CP, Pearce II RV, O'Connell S, Rosenfeld MG 1993 Ident-

- tification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 11:1187-1195
120. Vita N, Laurent P, Lefort S, Chalon P, Lelias JM, Kaghad M, Le FG, Caput D, Ferrara P 1993 Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. *FEBS Lett* 335:1-5
 121. Lovenberg TW, Liaw CW, Grigoriadis DE, Cleveneger W, Chalmers DT, De Souza EB, Oltersdorf T 1995 Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 92:836-840
 122. Liebsch G, Landgraf R, Gerstberger R, Probst JC, Wotjak CT, Engelmann M, Holsboer F, Montkowski A 1995 Chronic infusion of a CRH₁ receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regul Pept* 59:229-239
 123. Skutella T, Behl C, Probst JC, Renner U, Nitsch R, Holsboer F 1995 Modulation of corticotropin releasing hormone receptor in cell culture with antisense. *J Mol Med* 73:B25
 124. Fuchs E, Flügge G 1995 Modulation of binding sites for corticotropin-releasing hormone by chronic psychosocial stress. *Psychoneuroendocrinology* 20:33-51
 125. Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M 1995 V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. *J Neurosci* 15:4250-4258
 126. Robinson BG, Emanuel RL, Frim DM, Majzoub JA 1988 Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci USA* 85:5244-5248
 127. Swanson LW, Simmons DM 1989 Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *J Comp Neurol* 285:413-435
 128. Imaki T, Nahan JL, Rivier C, Sawchenko PE, Vale W 1991 Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. *J Neurosci* 11:585-599
 129. Thompson RL, Seasholtz AF, Herbert E 1987 Rat corticotropin-releasing hormone gene: sequence and tissue-specific expression. *Mol Endocrinol* 1:363-370
 130. Emanuel RL, Girard DM, Thull DL, Majzoub JA 1990 Second messengers involved in the regulation of corticotropin-releasing hormone mRNA and peptide in cultured rat fetal hypothalamic primary cultures. *Endocrinology* 126:3016-3021
 131. Phi Van L, Spengler D, Holsboer F 1990 Glucocorticoid repression of cAMP-dependent hCRH gene promoter activity in a transfected mouse anterior pituitary cell line. *Endocrinology* 127:1412-1418
 132. Spengler D, Rupprecht R, Phi Van L, Holsboer F 1992 Identification and characterization of a 3',5'-cyclic adenosine monophosphate-responsive element in the human corticotropin-releasing hormone gene promoter. *Mol Endocrinol* 6:1931-1941
 133. Schüle R, Rangarajan P, Kliever S, Ransone LJ, Bolado J, Yang N, Verma I, Evans RM 1990 Functional antagonism between oncoprotein *c-jun* and the glucocorticoid receptor. *Cell* 62:1217-1226
 134. Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, Herrlich P 1990 Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62:1189-1204
 135. Rangarajan PN, Umesono K, Evans RM 1992 Modulation of glucocorticoid receptor function by protein kinase. *Mol Endocrinol* 6:1451-1457
 136. Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, Koenderman L, Okret S, Gustafsson JÅ, Van der Saag PT 1995 Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* 9:401-412
 137. Ray A, Prefontaine KE 1994 Physical association and functional antagonism between the p65 subunit of transcription factor NF- κ B and the glucocorticoid receptor. *Proc Natl Acad Sci USA* 91:752-756
 138. Fink G, Robinson ICAF, Tannahill LA 1988 Effects of adrenalectomy and glucocorticoids on the peptides CRF-41, AVP and oxytocin in rat hypophysial portal blood. *J Physiol* 401:329-345
 139. Davis LG, Arentzen R, Reid JM, Manning RW, Wolfson B, Lawrence KL, Baldino Jr F 1986 Glucocorticoid sensitivity of vasopressin mRNA levels in the paraventricular nucleus of the rat. *Proc Natl Acad Sci USA* 83:1145-1149
 140. Hauger RL, Millan MA, Catt KJ, Aguilera G 1987 Differential regulation of brain and pituitary corticotropin-releasing factor receptors by corticosterone. *Endocrinology* 120:1527-1533
 141. Hauger RL, Aguilera G 1993 Regulation of pituitary corticotropin releasing hormone (CRH) receptors by CRH: interaction with vasopressin. *Endocrinology* 133:1708-1714
 142. Colson P, Ibarondo J, Devilliers G, Balestre MN, Duvoid A, Guillon G 1992 Upregulation of V_{1a} vasopressin receptors by glucocorticoids. *Am J Physiol* 263:E1054-E1062
 143. Saito R, Ishihara N, Ban Y, Honda K, Takano Y, Kamiya H 1994 Vasopressin V1 receptor in rat hippocampus is regulated by adrenocortical functions. *Brain Res* 646:170-174
 144. Drouin J, Sun YL, Nemer M 1990 Regulatory elements of the pro-opiomelanocortin gene. Pituitary specificity and glucocorticoid repression. *Trends Endocrinol Metab* 1:219-224
 145. Drouin J, Sun YL, Chamberland M, Gauthier Y, De Lean A, Nemer M, Schmidt TJ 1993 Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO J* 12:145-156
 146. Mobley PL, Sulser F 1980 Adrenal corticoids regulate sensitivity of noradrenaline receptor-coupled adenylate cyclase in brain. *Nature* 286:608-609
 147. Collins S, Caron MG, Lefkowitz RJ 1988 β 2-adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. *J Biol Chem* 263:9067-9070
 148. Hadcock JR, Malbon CC 1988 Regulation of β 2-adrenergic receptors by "permissive" hormones: glucocorticoids increase steady-state levels of receptor mRNA. *Proc Natl Acad Sci USA* 85:8415-8419
 149. Harrelson AL, Rostene W, McEwen BS 1987 Adrenocortical steroids modify neurotransmitter-stimulated cyclic AMP accumulation in the hippocampus and limbic brain of the rat. *J Neurochem* 48:1648-1655
 150. Kuroda Y, Watanabe Y, Albeck DS, Hastings NB, McEwen BS 1994 Effects of adrenalectomy and type I or type II glucocorticoid receptor activation on 5-HT_{1A} and 5-HT₂ receptor binding and 5-HT transporter mRNA expression in rat brain. *Brain Res* 648:157-161
 151. Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ 1993 Corticosteroids regulate brain hippocampal 5-HT_{1A} receptor mRNA expression. *J Neurosci* 13:914-923
 152. Chalmers DT, López JF, Vázquez DM, Akil H, Watson SJ 1994 Regulation of hippocampal 5-HT_{1A} receptor gene expression by dexamethasone. *Neuropsychopharmacology* 10:215-222
 153. Joëls M, de Kloet ER 1990 Mineralocorticoid receptor-mediated changes in membrane properties of rat CA₁ pyramidal neurons *in vitro*. *Proc Natl Acad Sci USA* 87:4495-4498
 154. Joëls M, Hesen W, de Kloet ER 1991 Mineralocorticoid hormones suppress serotonin-induced hyperpolarization of rat hippocampal CA₁ neurons. *J Neurosci* 11:2288-2294
 155. Joëls M, de Kloet ER 1992 Control of neuronal excitability by corticosteroid hormones. *Trends Neurosci* 15:25-30
 156. Azmitia EC, Liao B, Chen Y 1993 Increase of tryptophan hydroxylase enzyme protein by dexamethasone in adrenalectomized rat midbrain. *J Neurosci* 13:5041-5055
 157. Munck A, Guyre PM, Holbrook NJ 1984 Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev* 5:25-44
 158. Sapolsky RM, Krey LC, McEwen BS 1986 The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 7:284-301
 159. Stein-Behrens BA, Elliott EM, Miller CA, Schilling JW, Newcombe R, Sapolsky RM 1992 Glucocorticoids exacerbate kainic acid-induced extracellular accumulation of excitatory amino acids in the rat hippocampus. *J Neurochem* 58:1730-1735
 160. Chou YC, Lin WJ, Sapolsky RM 1994 Glucocorticoids increase extracellular [³H]D-aspartate overflow in hippocampal cultures during cyanide-induced ischemia. *Brain Res* 654:8-14
 161. Lawrence MS, Sapolsky RM 1994 Glucocorticoids accelerate ATP

- loss following metabolic insults in cultured hippocampal neurons. *Brain Res* 646:303–306
162. Saito N, Guitart X, Hayward M, Tallman JF, Duman RS, Nestler EJ 1989 Corticosterone differentially regulates the expression of G(s-alpha) and G(i-alpha) messenger RNA and protein in rat cerebral cortex. *Proc Natl Acad Sci USA* 86:3906–3910
 163. Lesch KP, Lerer B 1991 The 5-HT receptor-G-protein-effector system complex in depression. I. Effect of glucocorticoids. *J Neural Transm* 84:3–18
 164. Lesch KP, Aulakh CS, Tolliver TJ, Hill JL, Murphy DL 1991 Regulation of G proteins by chronic antidepressant drug treatment in rat brain: tricyclics but not clorgyline increase G_{oα} subunits. *Eur J Pharmacol* 207:361–364
 165. Manji HK 1992 G proteins: implications for psychiatry. *Am J Psychiatry* 149:746–760
 166. Otten U, Baumann JB, Girard J 1979 Stimulation of the pituitary-adrenocortical axis by nerve growth factor. *Nature* 282:412–414
 167. Tagliatalata G, Angelucci L, Scaccianoce S, Foreman PJ, Perez-Polo JR 1991 Nerve growth factor modulates the activation of the hypothalamo-pituitary-adrenocortical axis during the stress response. *Endocrinology* 129:2212–2218
 168. Scaccianoce S, Cigliana G, Nicolai R, Muscolo LA, Porcu A, Navarra D, Perez-Polo JR, Angelucci L 1993 Hypothalamic involvement in the activation of the pituitary-adrenocortical axis by nerve growth factor. *Neuroendocrinology* 58:202–209
 169. Lindholm D, Castrén E, Hengerer B, Zafa F, Berninger B, Thoenen H 1992 Differential regulation of nerve growth factor (NGF) synthesis in neurons and astrocytes by glucocorticoid hormones. *Eur J Neurosci* 4:404–410
 170. Kononen J, Soynila S, Persson H, Honkaniemi J, Hökfelt T, Pelto-Huikko M 1995 Neurotrophins and their receptors in the rat pituitary gland: regulation of BDNF and trkB mRNA levels by adrenal hormones. *Mol Brain Res* 27:347–354
 171. Smith MA, Makino S, Kvetnansky R, Post RM 1995 Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 15:1768–1777
 172. Costa A, Trainer P, Besser M, Grossman A 1993 Nitric oxide modulates the release of corticotropin-releasing hormone from the rat hypothalamus *in vitro*. *Brain Res* 605:187–192
 173. Rivier C, Shen GH 1994 In the rat, endogenous nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to interleukin-1 beta, vasopressin, and oxytocin. *J Neurosci* 14:1985–1993
 174. Szabó C, Thiemermann C, Wu CC, Perretti M, Vane JR 1994 Attenuation of the induction of nitric oxide synthase by endogenous glucocorticoids accounts for endotoxin tolerance *in vivo*. *Proc Natl Acad Sci USA* 91:271–275
 175. Laakmann G, Schoen HW, Blaschke D, Wittmann M 1985 Dose-dependent growth hormone, prolactin and cortisol stimulation after i.v. administration of desipramine in human subjects. *Psychoneuroendocrinology* 10:83–93
 176. Asnis GM, Sanderson WC, van Praag HM 1992 Cortisol response to intramuscular desipramine in patients with major depression and normal control subjects: a replication study. *Psychiatry Res* 44:237–250
 177. Laakmann GM, Wittmann M, Schoen HW, Zygan K, Weiss A, Meissner R, Müller OA, Stalla GK 1986 Effects of receptor blockers (methysergide, propranolol, phentolamine, yohimbine and prazosin) on desipramine-induced pituitary hormone stimulation in humans. III. Hypothalamo-pituitary-adrenocortical axis. *Psychoneuroendocrinology* 10:83–93
 178. Armario A, Garcia-Marquez C 1987 Tricyclic antidepressants activate the pituitary-adrenal axis in the rat. Tolerance to repeated drug administration. *Eur J Pharmacol* 140:239–244
 179. Shimoda K, Yamada N, Ohi K, Tsujimoto T, Takahashi K, Takahashi S 1988 Chronic administration of tricyclic antidepressants suppresses hypothalamic-pituitary-adrenocortical activity in male rats. *Psychoneuroendocrinology* 13:431–440
 180. Waldmeier PC, Baumann PA, Hauser K, Maitre L, Storni A 1982 Oxaprotiline, a noradrenaline uptake inhibitor with an active and an inactive enantiomer. *Biochem Pharmacol* 31:2169–2176
 181. Steiger A, Gerken A, Benkert O, Holsboer F 1993 Differential effects of the enantiomers R(-) and S(+) oxaprotiline on major endogenous depression, the sleep EEG and neuroendocrine secretion: studies on depressed patients and normal controls. *Eur Neuropsychopharmacol* 3:117–126
 182. Perez J, Tinelli D, Bianchi E, Brunello N, Racagni G 1991 cAMP binding proteins in the rat cerebral cortex after administration of selective 5-HT and NA reuptake blockers with antidepressant activity. *Neuropsychopharmacology* 4:57–64
 183. Golden RN, Ekstrom D, Brown TM, Ruegg R, Evans DL, Haggerty Jr JJ, Garbutt JC, Pedersen CA, Mason GA, Browne J, Carson SW 1992 Neuroendocrine effects on intravenous clomipramine in depressed patients and healthy subjects. *Am J Psychiatry* 149:1168–1175
 184. Daffner BC, Laakmann G, Baghai T, Haag C, Voderholzer U 1993 Influences of venlafaxine on growth hormone (GH) and other hormones in healthy subjects. *Neuropsychopharmacology* 9[Suppl 2]:100S–101S
 185. Lewis D, Sherman BM 1984 Serotonergic stimulation of adrenocorticotropin secretion in man. *J Clin Endocrinol Metab* 58:458–462
 186. Appel NM, Owens MJ, Culp S, Zaczek R, Contrera JF, Bissette G, Nemeroff CB, De Souza EB 1991 Role for brain corticotropin-releasing factor in the weight-reducing effects of chronic fenfluramine treatment in rats. *Endocrinology* 128:3237–3246
 187. Gibbs DM, Vale W 1983 Effects of the serotonin reuptake inhibitor fluoxetine on corticotropin-releasing factor and vasopressin secretion into hypophysial portal blood. *Brain Res* 280:176–179
 188. Walsh AES, Hockney RA, Campling G, Cowen PJ 1993 Neuroendocrine and temperature effects of nefazodone in healthy volunteers. *Biol Psychiatry* 33:115–119
 189. Reist C, Albers L, Rosenzweig B, Chaay H, Helmeste D, Tang S 1994 Neuroendocrine responses to paroxetine. *Neuropsychopharmacology* 10[Suppl 3]:264S
 190. Lesch KP, Söhle K, Poten B, Schoellhammer G, Rupprecht R, Schulte HM 1990 Corticotropin and cortisol secretion after central 5-hydroxytryptamine-1A (5-HT_{1A}) receptor activation: effects of 5-HT receptor and β-adrenoceptor antagonists. *J Clin Endocrinol Metab* 70:670–674
 191. Lesch KP, Mayer S, Disselkamp-Tietze J, Hoh A, Wiesmann M, Osterheider M, Schulte HM 1990 5-HT_{1A} receptor responsivity in unipolar depression. Evaluation of ipsapirone-induced ACTH and cortisol secretion in patients and controls. *Biol Psychiatry* 28:620–628
 192. Holsboer F, Liebl R, Hofschuster E 1982 Repeated dexamethasone suppression test during depressive illness. Normalization of test result compared with clinical improvement. *J Affective Disord* 4:93–101
 193. Greden JF, Gardner R, King D, Grunhaus L, Carroll BJ, Kronfol Z 1983 Dexamethasone suppression test in antidepressant treatment of melancholia. *Arch Gen Psychiatry* 40:493–500
 194. Gerken A, Maier W, Holsboer F 1985 Weekly monitoring of dexamethasone suppression response in depression: its relationship to change of body weight and psychopathology. *Psychoneuroendocrinology* 10:261–271
 195. Holsboer F, Steiger A, Maier W 1983 Four cases of reversion to abnormal dexamethasone suppression test response as indicator of clinical relapse: a preliminary report. *Biol Psychiatry* 18:911–916
 196. De Bellis MD, Gold PW, Geraciotti Jr TD, Listwak SJ, Kling MA 1993 Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am J Psychiatry* 150:656–657
 197. Labrid C, Mocaer E, Kamoun A 1992 Neurochemical and pharmacological properties of tianeptine, a novel antidepressant. *Br J Psychiatry* 160:56–60
 198. Delbende C, Contesse V, Mocaer E, Kamoun A, Vaudry H 1991 The novel antidepressant, tianeptine, reduces stress-evoked stimulation of the hypothalamo-pituitary-adrenal axis. *Eur J Pharmacol* 202:391–396
 199. Defrance R, Marey C, Kamoun A 1988 Antidepressant and anxiolytic activities of tianeptine: an overview of clinical trials. *Clin Neuropharmacol* 11[Suppl]:S74–S82
 200. Reul JMHM, Stec I, Söder M, Holsboer F 1993 Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamo-pituitary-adrenocortical system. *Endocrinology* 133:312–320

201. Reul JM, Labeur MS, Grigoriadis DE, De Souza EB, Holsboer F 1994 Hypothalamic-pituitary-adrenocortical axis changes in the rat after long-term treatment with the reversible monoamine oxidase-A inhibitor moclobemide. *Neuroendocrinology* 60:509-519
202. Kitayama I, Janson AM, Cintra A, Fuxe K, Agnati LF, Ögren SO, Härfstrand A, Eneroth P, Gustafsson JÅ 1988 Effects of chronic imipramine treatment on glucocorticoid receptor immunoreactivity in various regions of the rat brain. Evidence for selective increases of glucocorticoid receptor immunoreactivity in the locus coeruleus and in 5-hydroxytryptamine nerve cell groups of the rostral ventromedial medulla. *J Neural Transm* 73:191-203
203. Seckl JR, Fink G 1992 Antidepressants increase glucocorticoid and mineralocorticoid receptor mRNA expression in rat hippocampus *in vivo*. *Neuroendocrinology* 55:621-626
204. Brady LS, Whitfield Jr HJ, Fox RJ, Gold PW, Herkenham M 1991 Long-term antidepressant administration alters corticotropin-releasing hormone, tyrosine hydroxylase, and mineralocorticoid receptor gene expression in the rat brain. *J Clin Invest* 87:831-837
205. Nestler EJ, McMahon A, Sabban EL, Tallman JF, Duman RS 1990 Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. *Proc Natl Acad Sci USA* 87:7522-7526
206. Roffler-Tarlov S, Schildkraut JJ, Draskoczy 1973 Effects of acute and chronic administration of desmethylimipramine on the content of norepinephrine and other monoamines in the rat brain. *Biochem Pharmacol* 22:2923-2926
207. Huang J, Maas JW, Hu GH 1979 The time course of noradrenergic pre- and postsynaptic activity during chronic desipramine treatment. *Eur J Pharmacol* 68:41-47
208. Melia KR, Nestler EJ, Duman RS 1992 Chronic imipramine treatment normalizes levels of tyrosine hydroxylase in the locus coeruleus of chronically stressed rats. *Psychopharmacology* 108:23-26
209. Brady LS, Gold PW, Herkenham M, Lynn AB, Whitfield Jr HJ 1992 The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-releasing hormone and tyrosine hydroxylase mRNA levels in rat brain: therapeutic implications. *Brain Res* 572:117-125
210. Watanabe Y, Gould E, Cameron H, Daniels D, McEwen BS 1992 Stress and antidepressant effects on hippocampus. *Eur J Pharmacol* 222:157-162
211. Whitton P, Sarna G, O'Connell MT, Curzon G 1991 The effect of the novel antidepressant tianeptine on the concentration of 5-hydroxytryptamine in rat hippocampal dialysates *in vivo*. *Neuropharmacology* 30:1-4
212. Pepin MC, Beaulieu S, Barden N 1989 Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neuronal cultures. *Mol Brain Res* 6:77-83
213. Pepin MC, Barden N 1991 Decreased glucocorticoid receptor activity following glucocorticoid receptor antisense RNA gene fragment transfection. *Mol Cell Biol* 11:1647-1653
214. Pepin MC, Pothier F, Barden N 1992 Antidepressant drug action in a transgenic mouse model of endocrine changes seen in depression. *Mol Pharmacol* 42:991-995
215. Pepin MC, Govindan MV, Barden N 1992 Increased glucocorticoid receptor gene promoter activity after antidepressant treatment. *Mol Pharmacol* 41:1016-1022
216. Cole TJ, Blendy JA, Monaghan P, Kriegstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K, Schütz G 1995 Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. *Genes Dev* 9:1608-1621
217. Pepin MC, Pothier F, Barden N 1992 Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. *Nature* 355:725-728
218. Wagner RW 1994 Gene inhibition using antisense oligodeoxynucleotides. *Nature* 372:333-335
219. Walder RY, Walder JA 1988 Role of RNase H in hybrid-arrested translation by antisense oligonucleotides. *Proc Natl Acad Sci USA* 85:5011-5015
220. Rosolen A, Kyle E, Chavany C, Bergan R, Kalman ET, Crouch R, Neckers L 1993 Effect of over-expression of bacterial ribonuclease H on the utility of antisense MYC oligodeoxynucleotides in the monocytic leukemia cell line U937. *Biochimie* 75:79-87
221. Richard D, Chapdelaine S, Deshaies Y, Pepin MC, Barden N 1993 Energy balance and lipid metabolism in transgenic mice bearing an antisense GCR gene construct. *Am J Physiol* 265:R146-R150
222. Stec I, Barden N, Reul JM, Holsboer F 1994 Dexamethasone nonsuppression in transgenic mice expressing antisense RNA to the glucocorticoid receptor. *J Psychiatr Res* 28:1-5
223. Peiffer A, Veilleux S, Barden N 1991 Antidepressant and other centrally acting drugs regulate glucocorticoid receptor messenger RNA levels in rat brain. *Psychoneuroendocrinology* 16:505-515
224. Montkowski A, Barden N, Wotjak C, Stec I, Ganster J, Meaney M, Engelmann M, Reul JM, Landgraf R, Holsboer F 1995 Long-term antidepressant treatment reduces behavioral deficits in transgenic mice with impaired glucocorticoid receptor function. *J Neuroendocrinol* 7:841-845
225. Truss M, Beato M 1993 Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr Rev* 14:459-479
226. Castrén M, Trapp T, Berninger B, Castrén E, Holsboer F 1995 Transcriptional induction of rat mineralocorticoid receptor gene in neurons by corticosteroids. *J Mol Endocrinol* 14:285-293
227. Morris RGM 1984 Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47-60
228. Bluthé RM, Dantzer R 1990 Social recognition does not involve vasopressinergic neurotransmission in female rats. *Brain Res* 535:301-304
229. Lister RG 1987 The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92:180-185
230. Peselow ED, Corwin J, Fieve RR, Rotrosen J, Cooper TB 1991 Disappearance of memory deficits in outpatient depressives responding to imipramine. *J Affective Disord* 21:173-183
231. Porsolt RD, Bertin A, Jalfre M 1978 "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. *Eur J Pharmacol* 51:291-294
232. West PA 1990 Neurobehavioral studies of forced swimming. The role of learning and memory in the forced swim test. *Prog Neuropsychopharmacol Biol Psychiatry* 14:863-875
233. Allain H, Lieury A, Brunet-Bourgin F, Mirabaud C, Trebon P, Le Coz F, Gandon JM 1992 Antidepressants and cognition: comparative effects of moclobemide, viloxazine and maprotiline. *Psychopharmacology* 106:S56-S61
234. Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T 1995 Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci USA* 92:8856-8860
235. Kühn R, Schwenk F, Aguet M, Rajewsky K 1995 Inducible gene targeting in mice. *Science* 269:1427-1429
236. McInnes IA, Freimer NB 1995 Mapping genes for psychiatric disorders and behavioral traits. *Curr Opin Genet Dev* 5:376-381
237. Post RM 1992 Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. *Am J Psychiatry* 149:999-1010