High-Quality Antidepressant Discovery by Understanding Stress Hormone Physiology

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ABSTRACT: Compensating the consequences of impaired corticosteroid receptor signaling is a novel strategy to discover better antidepressants. The prevailing drugs gradually improve stress hormone regulation along with ameliorating psychopathology. The current understanding of how neuropeptides, such as corticotropin-releasing hormone (CRH) and vasopressin (AVP), drive cortisol secretion via corticotrophin has paved the way for CRH- and AVP-receptor antagonists. As alternative strategies, the blockade of corticosteroid receptors or inhibition of cortisol synthesis has emerged. All these strategies are not yet fully clinically developed, but preliminary data from basic and clinical research strongly underscore that such strategies may lead to innovative treatment modalities.

KEYWORDS: antidepressants; corticotropin-releasing hormone; vasopressin

INTRODUCTION

Several facts emerged that call for a paradigmatic shift in antidepressant drug discovery. (1) Depression will soon become the second leading cause for illness-related disability, trailing only cardiovascular disease. The impact of depression on general morbidity is further amplified, because mood disorders are one of the major risk factors for cardiovascular disease. (2) The list of top 10 medicines worldwide based on revenues in the year 2000 contained three antidepressants (Zoloft®, Prozac®, Paxil®). (3) Currently available antidepressants are based on a serendipitous observation in the 1950s, that is, that norepinephrine- and/or serotonin-reuptake inhibitory drugs are efficacious. Since then, all new antidepressants were developed analogous to this pharmacological principle. A common disadvantage of all these drugs is (1) that it takes too long until they work; (2) that they have too many adverse effects; and (3) that only 70–80% of the patients treated are cured. Also, although the scientific fundament on which currently available antidepressants are built is poor, there is growing evidence that the long-known stress hormone dysregulation seen among psychiatric patients is a causative mechanism leading to stress-related psychopathology.

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Ann. N.Y. Acad. Sci. 1007: 394–404 (2003).
doi: 10.1196/annals.1286.038

The pharmaceutical industry has translated the impact of depression on illness-related disability and top revenues in medications into development and aggressive marketing of "more-of-the-same" products. At the same time, pharmaceutical industry and biotech companies embark on the opportunities of systematic, unbiased approaches. These include the search for targets using microarray technology that allows assessment of gene activity under experimentally defined conditions and subsequent identification of drugable targets. Once identified, high throughput screening, which makes possible the testing of every chemical available to see if something interesting happens to the targets, is initiated. The observed effects are further studied functionally in animal models before decisions are made whether or not a clinical development program is to be initiated. Although in principle this approach is very attractive and promises departure from the "usual suspects" approach, an entirely unfocused approach has provided huge, hard to digest, diverse databases. In this article, I delineate how the attrition rate that is currently plaguing high-quality drug discovery may be reduced by preferring precedented targets focusing on existing knowledge of stress hormone physiology.

CORTICOSTEROID RECEPTOR HYPOTHESIS OF DEPRESSION

The observed changes of hypothalamic-pituitary-adrenocortical (HPA) regulation are not specific for the diagnosis of depression or any other past, current, or (most likely) future diagnostic attribution according to manuals released by the World Health Organization or other authorities. Likewise, antidepressants are not specific treatments for any kind of current diagnosis. In contrast, their indication cuts across all syndromes characterized by depressed mood or various forms of anxiety. In all these clinical conditions, perturbed HPA activity either at baseline or in the context of function tests can be found with high frequency.

These changes were long considered as reflections of the stressful experience of affective illness. Several discoveries have challenged this interpretation and also have changed the previous view. The first study monitoring patients during the course of diverse antidepressant treatments revealed that initially abnormal dexamethasone suppression test results (i.e., inappropriately mute suppression of plasma cortisol concentrations by a low dose of the synthetic glucocorticoid dexamethasone [DEX] which acts mainly at the pituitary to suppress ACTH [the main peripheral stimulant for cortisol]) almost always normalizes before clinical remission of depression.[1] Furthermore, once a remitted patient shows high post–dexamethasone plasma cortisol levels, the patient has a much higher risk for relapse. This time grid suggests that HPA normalization is pertinent for recovery while a patient who continues to have or starts again developing HPA abnormality has some ongoing pathology in central neural circuits that leads to psychopathology through a mechanism that opposes antidepressant action.[2] At the same time, Vale's group isolated and characterized the hypothalamic factor that was suggested to be the key neuropeptide centrally governing the hormonal response to stress.[3] This factor, corticotropin-releasing hormone (CRH) was extensively used in animal studies, and it was suggested that CRH not only accounts for stress hormone release but also coordinates a large variety of behavioral adaptations (see FIG. 1).

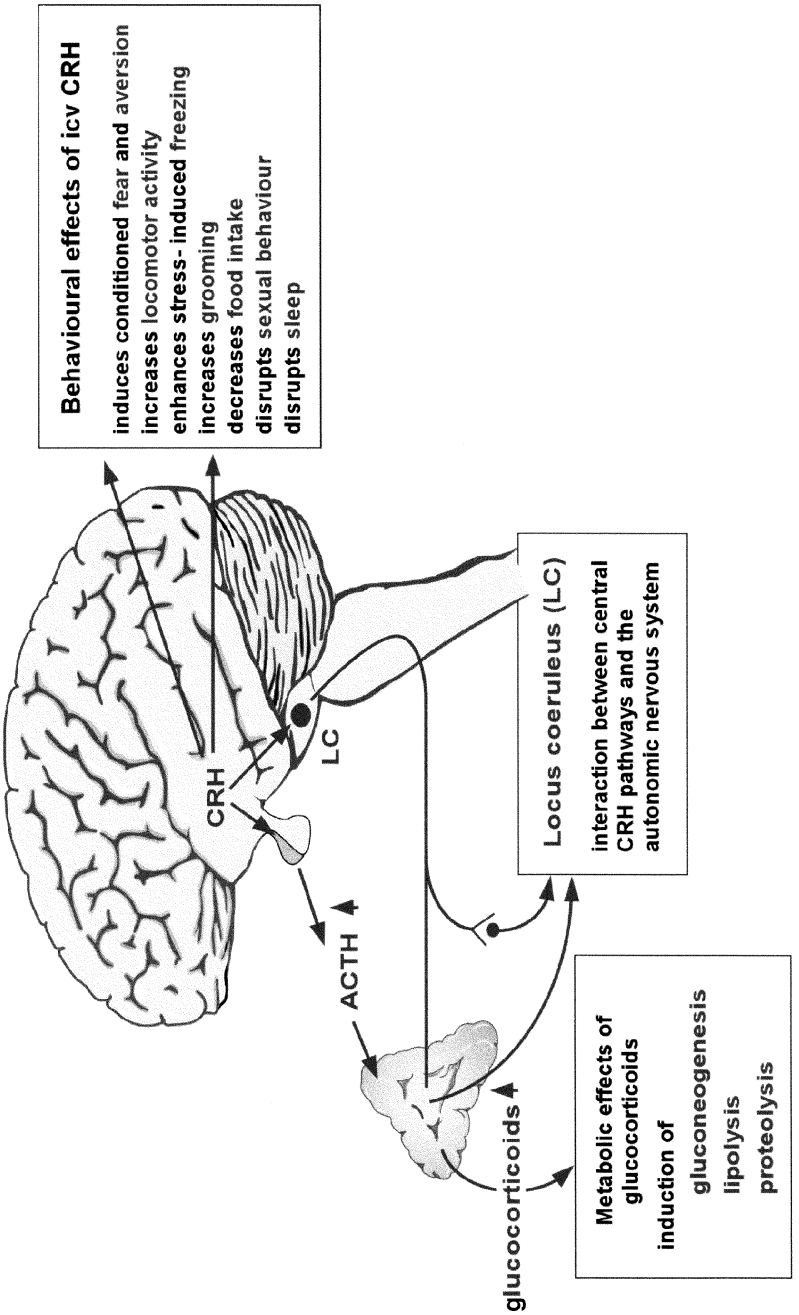

FIGURE 1. Increased levels of corticotropin-releasing hormone induce depresion-like symptoms in animals.

Also the first reports of CRH-induced hormone responses in depressives suggested a causal role for CRH in stress-related psychopathology. This conclusion was substantiated by human studies showing elevated CRH levels in cerebrospinal fluid (CSF) and reduced CRH binding in prefrontal cortex, the latter indicating CRH receptor desensitization by CRH excess. Finally, an increased number of CRH-producing neurons was found in the hypothalami of depressives. From there, nerve fibers project to many circuits in the brain that are implicated in pathophysiology of depression.[4] Of course, CRH does not act alone, and more recently vasopressin (AVP) has been characterized as another neuropeptide being a strong candidate in the development of stress-related disorders. Both neuropeptides, CRH and AVP, have in common that they are regulated by corticosteroid receptors. These receptors are located in the cytosol of nerve cells and kept by other proteins (mostly so-called chaperones) in a three-dimensional configuration, which allows high-affinity binding of cortisol (and other corticosteroids). There are two different corticosteroid receptors in the brain, glucocorticoid receptors (GRs, mainly binding cortisol in humans or corticosterone in rodents) and mineralocorticosteroid receptors (MRs, located mainly in the hippocampus and binding corticosteroids with 10-fold higher affinity, i.e., they are almost always occupied). Once activated by steroid hormones, these receptors dimerize forming homodimers (GR-GR, MR-MR) or heterodimers (GR-MR). These act as transcription factors and bind at specific DNA structures (glucocorticoid response elements [GREs]) which induces activation or repression of gene transcription. Alternatively, activated receptors can interact with other transcription factors and thus can indirectly modulate gene activity.[5]

The corticosteroid receptor hypothesis submits that corticosteroid receptor signaling is impaired in depression.[2] Specifically, if increased CRH and AVP are accounting for a high number of signs and symptoms prevalent in depression, it can be hypothesized that the corticosteroid receptor signaling which represses CRH and AVP gene expression is defunct. A function test was developed, to validate this hypothesis clinically, that probes corticosteroid receptor signaling with high sensitivity. This test combines the suppression of HPA activity by DEX with a CRH-induced ACTH activation and proved to pick up minor HPA changes with high sensitivity. A study by Modell *et al*.[6] used increasing doses of DEX among depressives and controls and showed that the (DEX) dose (ACTH, cortisol)–response curve in the DEX/CRH test was shifted among depressives toward lower dexamethasone sensitivity. Such a change is unlikely reflecting an inherited GR or MR gene defect but rather points to quantitative effects of coregulators, for example, the aforementioned chaperones. This view is supported by the occurrence of major HPA defects only in temporal proximity of affective episodes, but occurrence of GR or MR polymorphisms can not yet be fully excluded. Along this line, note that a sequence in the promoter of CRH was found that confers signals that are conveyed through antidepressants.[7] At this DNA sequence, called CRE (cyclic AMP response element), a transcription factor binds that is called CREB (CRE-binding protein) which activates CRH transcription. To do this, CREB needs to be phosphorylated by a kinase (PKA) which is a key element of a signaling pathway activated by antidepressants. The latter enhance biogenic amine concentrations at specific cell membrane receptors by inhibiting presynaptic reuptake transporters. After endured activation of these aminergic cell membrane receptors, a desensitization develops ultimately leading to decreased CREB phosphorylation and subsequently to decreased CRH gene expression.[8] This

process needs time and may, at least in part, explain the long time to onset of clinical antidepressive effects. Note also that corticosteroids interact with G-protein–coupled receptor–cAMP-PKA pathways. For example, corticosteroids can abolish CREB phosphorylation in CRH neurons.[9] Against this background, it seems justified to investigate the HPA system thoroughly in humans and in appropriate animal models to discover novel targets.

CORTICOTROPIN-RELEASING HORMONE

The aforementioned biological actions of CRH are mediated via two types of G-protein–coupled receptors, CRHR1 and CRHR2, which contain seven transmembrane domains and share considerable sequence homology with one another. CRHR1 and CRHR2 have different expression patterns, and accordingly these receptors play distinct, though overlapping roles both in HPA-axis regulation and in stress-related behavioral effects.[10]

Several lines of evidence point to a key role of CRHR1 in mediating the CRH-elicited effects in depression and anxiety. Infusions of CRH into the rat brain produces anxiety-related behavior in a way similar to what transgenic overexpressing CRH in mouse mutants does.[11] Central administration of CRHR1 antisense probes restrains CRH-evoked and social defeat–evoked anxiety-like behaviors. In contrast, CRHR2 antisense does not produce an anxiolytic effect but does increase immobility in a forced swim test.[12] Because CRHR2 is activated not only by CRH but also by another neuropeptide, called urocortin (Ucn), it seems that a Ucn/CRHR2 system that plays a role in stress-coping behaviors also exists in the brain. Indeed, recently two selective ligands for CRHR2 were discovered (Ucn II, or stresscopin-related peptide, and Ucn III, or stresscopin). The role of CRHR2 is less clear than that of CRHR1, but it appears that CRHR2 has a dual mode of action: in the acute (early) phase CRHR2 is activated mostly through CRH and stresscopin, increasing emotionality (anxiety), whereas in the recovery phase it is also activated, presumably in the amygdala, basal nucleus of the stria terminalis and lateral septum, but now this contributes to reducing emotionality.[13]

One approach to study the effects of a receptor on complex systems such as behavior is to use mouse mutants in which the respective receptor has been deleted by genetic engineering. Mice, in which the CRHR1 gene had been inactivated, showed decreased anxiety-like behavior. The behavioral effect of this gene defect is not limited to anxiety but is also seen in other stress-related conditions such as withdrawal and abuse of alcohol.[14,15] Three lines of CRHR2-deficient mice were studied, but this did not provide a clear answer to the question of whether blockade of CRHR2 would ameliorate anxiety in stressful situations. In two lines of CRHR2 knockout mice increased anxiety-like behavior was found,[16,17] but in the third line no changes were found.[18] In addition, some sex differences in the phenotype were observed. Mouse mutants where both, CRHR1 and CRHR2, were knocked out showed a phenotype that was dominated by the absence of CRHR1.[19] The fact that these mice were viable, as were mutants with a CRH knockout, demonstrates that mammals do not need a functional CRH/CRHR-signaling system to live, making this a preferred system for drug targeting. Because the CRHR1 knockout mice also lacked CRHR1 at the pituitary and elsewhere in the organism, the possibility had to be rejected that

their behavioral phenotype was secondary to the endocrine changes elicited by a CRH-refractory corticotrophic system leading to a diminished hormonal stress response. Therefore, a conditional mouse mutant was generated in which the gene deletion was restricted to the hippocampus and the prefrontal cortex, sparing the HPA axis. These mice also showed decreased anxiety-like behavior, whereas their endocrine system remained largely intact.[20]

These clinical and basic studies led several drug companies to develop specific and selective nonpeptide receptor antagonists with good oral bioavailability and rapid penetration across the blood–brain barrier. The recent advances in biotechnology in combination with optimized behavioral pharmacology techniques have led to the identification of several structural series of compounds that antagonize the effects of CRH at CRHR1.

The only drug that has so far been clinically tested is NBI-30775 (also referred to as R121919). This compound was first tested in a rat line with high innate anxiety and proved to reduce anxiety-like behavior. Interestingly, among those rats selectively bred to produce a low-anxiety phenotype, NBI-30775 did not show any behavioral response which is in accord with the view that neuropeptide receptors are only targets in the presence of pathophysiological mechanisms. Indeed, neuropeptides are usually only hypersecreted under certain physiological demands, such as adaptation to a stressor. A more recent study accords with the view that CRHR1 antagonists suppress stress-elicited behavioral changes in rats. A first clinical study designed as a safety and tolerability study but also rigidly monitoring psychopathological changes supported that CRHR1 antagonists are worthy to be further explored as novel antidepressants.[21] Also, studies investigating the effects of NBI-30775 in animal models and humans with depression underscored the potential of such drugs in the treatment of stress-related sleep disorders.[22,23] Whereas Johnson & Johnson (the licensee of R121919) decided to discontinue the clinical development of this drug, almost all big pharmaceutical companies are searching for new candidates directed against CRHR1 signaling.

VASOPRESSIN

Based on neuroendocrine studies in human and animal models, it was postulated that increased AVP secretion accounts for several signs and symptoms seen in depression.[24] This suggestion was confirmed by Purba *et al.*,[25] who found vasopressin to be increased in paraventricular nucleus neurons of depressives, by Dinan *et al.*,[26] who showed that in depression vasopressinergic responsitivity is enhanced, and by van Londen *et al.*,[27] who found elevated plasma AVP concentrations in the same patients. In contrast with CRH, where elevations in CSF were repeatedly shown, AVP was not found to be elevated in depression.[28] However, it must be recognized that CSF neuropeptide contents do not necessarily reflect hypothalamic secretory activity. In rats, it was observed that cognitive stressors elicit AVP in the supraoptic nucleus.[29] Elevated AVP was also found in the hypothalamus of rats with innate high anxiety and postulated to mediate the increased ACTH and cortisol response in the DEX/CRH test. When treated with an antidepressant, the AVP content in the hypothalamus decreases in these rats along with normalization of initially abnormal DEX/CRH test results.[30] There are two AVP receptors in the brain V1a and V1b (al-

so termed V3) which have locations that clearly favor the view that they also contribute to the behavioral phenotype implicated in stress-related disorders.[31] For example, V1a is expressed in the cortex, suprachiasmatic nucleus, central and medial amygdala, and hypothalamus; V1b is neuroanatomically less well studied but also occurs in cortex and amygdala as well as in supraoptic nucleus. The main physiological role of AVP receptors outside the brain is enhancement of corticosterone secretion, and neuroendocrine studies using a V1b-transgenic mouse overexpressing V1b receptors in corticotrophs confirmed this.[32]

In the light of the above findings, it was postulated that AVP hypersecretion exists in depression and that blocking this mechanism may reduce affective symptoms. A study by Landgraf *et al*.[29] used antisense probes directed against AVPmRNA in the septum and found reductions of anxiety-like behavior in rats. In the same vein are studies by Liebsch *et al*.[33]who injected a mixed V1a/V1b receptor antagonist in the rat septum and also found anxiolytic-like effects. These observations prompted the search for nonpeptide receptor antagonists[34] and recently Griebel *et al*.[35] were able to show that a synthetic V1b receptor antagonist also produces anxiolytic- and antidepressant-like effects and suggested that such drugs are worthy to be considered as novel candidates for antidepressant drug development.

GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS

Corticosteroid receptors constitute the relay between peripheral stress hormone secretion and modulation of behavioral processes in the brain. The hypersecretion of cortisol in depression as well as abnormal HPA function test results may well be secondary to impaired signaling due to inherited or acquired changes in the GR/MR pathways. In case of inherited HPA disturbance, either a polymorphism at the GR or at one or several genes coding for proteins that modulate pharmacological properties of GR (e.g., affinity) may be present.

Several polymorphisms in the glucocorticoid receptor gene had been discovered by Steven Lamberts's group in Rotterdam.[36] One of these polymorphisms consists of two linked point mutations separated by one base pair in codons 22 and 23 in exon 2 of the GR gene. The first mutation is silent, changing codon 22 from GAG to GAA, both coding for glutamic acid (E). The other mutation changes codon 23 from AGG to AAG resulting in an arginine (R) to lysine (K) amino acid exchange. Carriers of this ER22/23 EK allele were found to be less sensitive to the suppressive effect of low dose dexamethasone.[37] Because of the lower effect of cortisol on glucose metabolism, both glucose and insulin were lowered, resulting in a favorable metabolic health profile. Because patients with depression or individuals belonging to families with high genetic load for depression also have glucocorticoid receptor resistance, it would be worthwhile to study whether similar polymorphisms also exist in these patients. Although the most likely location for such a polymorphism would be a mutation in the ligand binding domain of GR,[38] it is yet not fully elucidated through which mechanism ER22/23EK confers glucocorticoid resistance. Alternatively, mutations in the promoter of genes coding for chaperones, for example, BAG-1 or FKBP51, resulting in overexpression of these proteins could lead to hypercortisolism.[39] It needs to be tested at the functional level whether gain or loss of functional activity of these chaperone molecules can be induced by drugs.

A more straightforward approach would be the partial blockade of corticosteroid receptors by low-dose antagonists. This strategy of decreasing cortisol bioavailability originally was advocated by B. E. P. Murphy, but only recently a substantial data base was published by Belanoff *et al*.[40] showing that mifepristone, a GR (and progesterone receptor) antagonist, rapidly ameliorates psychotic depression. The latter clinical condition is almost always associated with elevated cortisol secretion and according to a hypothesis by Piazza *et al*.,[41] this hypercortisolism may lead to increased dopaminergic activity. Notably, drugs that block central dopaminergic receptors are first-line treatments in psychotic states including psychotic depression.

A potential role of MR as a drug target is much less clear. Under conditions of stress, MR capacity increases by a mechanism implicating CRH action.[42] Also, antidepressants increase MR capacity in the rat hippocampus, pointing to a role of MR function in mediating the drug effect.[43] This is further underscored by a clinical study in which the antidepressant effect of amitriptyline was found to be decreased in case of coadministration of spironolactone, an MR antagonist.[44] Still another approach is the decrease of circulating cortisol by agents that block cortisol synthesis. One prominent agent is metyrapone which until recently had been studied only in very small open-label trials with mixed results. However, a study by Klaus Wiedemann's group showed that the effect of antidepressants can be significantly improved by coadministration of metyrapone.[45]

Although all these studies have potential merit for the clinician, it remains unclear by which mode of action antiglucocorticoid strategies may work. In case of GR or MR blockade, a myriad of different molecular events are set in motion, because of the pluripotent actions these ligand-activated nuclear receptors may induce. Similarly unclear are the effects of metyrapone, which results in a substantial increase of so-called neurosteroids. The latter are mainly binding at membrane-located $GABA_A$ receptors modulating their ion conductance which, in turn, translates into behavioral changes, for example, anxiolysis or sleep induction. Interestingly, antidepressants also can change neurosteroid concentrations in the CSF and plasma pointing to a function of these steroid derivatives.[46] There are some activities in pharmaceutical research to explore whether synthetic neurosteroids directed against certain specific $GABA_A$ receptor–mediated functions are possible drug candidates.

CONCLUSION

The currently available antidepressant drugs and anxiolytics have many disadvantages. Therefore, it is very likely that the rich knowledge based on stress hormone pharmacology that has been accumulated over the past decades will soon be exploited to find better drugs. Still, there is yet no CRHR1 antagonist that has proved to be efficacious in large double-blind controlled studies. Similarly, GR antagonists are yet not sufficiently well studied to allow firm predictions. The only exception is psychotic depression in which the data base available seems very promising. Even more in its infancy is the development of V1b antagonists in which only preliminary animal behavioral data exist.

I would not be surprised if the validation of all these "potential targets" that emerge from stress physiology would be validated only if human data from disease genetics are implemented. Maybe HPA-related drugs only work better than the cur-

rently available drugs in such cases in which a central HPA dysregulation exists (irrespective of whether this neuropathology is reflected by peripheral hypercortisolism or not). In any case, the more specific novel drugs get, the better they work among those where the specific neuropathology exists. On the other side, for those patients for whom mechanisms other than specific HPA-related neuropathology are causing the clinical condition, such drugs may not work at all. Therefore, the most important task for human stress hormone research will be precise phenotyping and genotyping of patients, allowing the clinician to choose the right drug at the right moment.

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