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Psychiatric Implications of Altered Limbic-Hypothalamic-Pituitary-Adrenocortical Activity

Florian Holsboer

Department of Psychiatry, University of Freiburg, Hauptstrasse 5, D-7800 Freiburg, Federal Republic of Germany

Summary. Hormones of the limbic-hypothalamic-pituitary-adrenocortical (LHPA) system are much involved in central nervous system regulation. The major LHPA neuropeptides, corticotropin-releasing hormone (CRH), vasopressin (AVP) and corticotropin (ACTH) do not only coordinate the neuroendocrine response to stress, but also induce behavioral adaptation. Transcription and post-translational processing of these neuropeptides is regulated by corticosteroids secreted from the adrenal cortex after stimulation by ACTH and other pro-opiomelanocortin derived peptides. These steroids play a key role as regulators of cell development, homeostatic maintenance and adaptation to environmental challenges. They execute vitally important actions through genomic effects resulting in altered gene expression and nongenomic effects leading to altered neuronal excitability. Since excessive secretory activity of this particular neuroendocrine system is part of an acute stress response or depressive symptom pattern, there is good reason to suspect that central actions of these steroids and peptides are involved in pathophysiology determining the clinical phenotype, drug response and relapse liability.

This overview summarizes the clinical neuroendocrine investigations of the author and his collaborators, while they worked at the Department of Psychiatry in Mainz. The major conclusions from this work were: (1) aberrant hormonal responses to challenges with dexamethasone, ACTH or CRH are reflecting altered brain physiology in affective illness and related disorders; (2) hormones of the LHPA axis influence also nonendocrine behavioral systems such as sleep EEG; (3) physiologically significant interactions exist between LHPA hormones, the thyroid, growth hormone, gonadal and other neuroendocrine systems; (4) hormones of the LHPA axis constitute a bidirectional link between immunoregulation and brain activity; and (5) future psychiatric research topics such as molecular genetics of affective disorders, familial risk studies, drug response analysis and neurobiology of aging will benefit from extended knowledge of neural corticosteroid effects at a clinical, cellular, and molecular level.

Key words: Limbic system – Hypothalamic-Pituitary-Adrenocortical system – Psychiatry

Introduction

Few neurobiological research areas have received as much attention in psychiatry research as the neuroendocrinology of the limbic-hypothalamic pituitary adrenal cortex (LHPA) system. Three investigative lines addressing the following topics now dominate the field: (1) clinical investigation of the limbic-hypothalamic pathophysiology underlying altered pituitary adrenocortical regulation in affective disorders, which now apply considerably refined techniques; (2) extended pharmacological studies, in the knowledge that each component of the LHPA axis is not only involved in neuroendocrine function but directly modulates neurotransmitter function and behavioral systems; and (3) molecular studies focusing on the fact that glucocorticoids (GC) have genomic effects in the brain and regulate transcription of many genes, including those which code for behaviorally active neuropeptides.

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The present article reviews some of these new developments emphasizing their relevance for understanding pathophysiology of affective disorders and related illnesses.

LHPA Physiology

According to the current model of LHPA regulation, ACTH secretion is under multifactorial control, with CRH being the key hormone (see Fig. 1). Many aspects of ACTH biosynthesis and secretion in response to stimuli can not be fully explained by CRH alone and to date vasopressin (AVP) is considered as the most important peptidergic factor co-regulating the actions of CRH at the pituitary level. The specific receptors to which both peptides (CRH and AVP) bind at the pituitary level are coupled to enzymes on the inner components of the plasma membrane by GTP binding proteins (G-proteins). Binding of CRH causes an increase in the intracellular concentration of cAMP which activates a protein kinase. Reisine et al. (1986) showed that this cAMP-dependent protein kinase is essential for CRH-induced ACTH secretion. While the CRH receptor is coupled to adenylate cyclase the effect of AVP receptor binding at the inner face of the plasma membrane is activation of phospolipase C (Thomas et al. 1984). This enzyme catalyzes the formation of diacyl glycerols and inositol phosphates from phosphatidylinositol polyphosphates. Both hydrolysates act as second messengers, and AVP-mediated enhancement of inositol phosphate biosynthesis has been recently demonstrated (Todd and Lightmann 1987). Of particular interest is the finding by Zatz and Reisine (1985) that lithium, which inhibits the phosphatase necessary to regenerate inositol from its phosphomonoesters, can induce ACTH secretion. Also the cell systems, where CRH and AVP exert their effects, are different, because their responses can be dissociated if corticotrophic cells are sorted out on the basis of their specific peptidergic response (Schwartz and Vale 1988). The synergy between CRH and AVP has long been recognized in basic clinical studies (Gillies and Lowry 1979; Gillies et al. 1982). The physiological significance of AVP as a synergizing co-factor of CRH-mediated effects has also been recently studied by us (von Bardeleben et al. 1985) and by Salata et al. (1988). The latter group applied AVP in the morning, when endogenous CRH secretory activity is high, and compared the ACTH release with that obtained after evening administration of AVP, when endogenous CRH release is low. As expected, the AVP induced effects on ACTH secretion were much higher in the morning, because higher ambient endogenous levels of CRH provide a more powerful synergizing effect. The mechanism underlying the phenomenon is unresolved and the possible intracellular interaction between adenylate cyclase and protein kinase and the role of AVP to release calcium ions from intracellular stores (via inositol phosphate induction) are subject to current active research. The final step of the cascade induced by CRH receptor binding, subsequent transmembrane signal transduction and amplification is an increase in pro-opiomelanocortin (POMC) gene transcription (Eberwine et al. 1987). The precursor POMC contains sequences of several biologically active peptide hormones (e.g. ACTH, N-POMC, β-endorphin, melanotropin) and is present not only in the anterior pituitary, but also in distinct brain areas (e.g. hypothalamus, amygdala and cortex), pointing to its involvement in nonendocrine actions as well. While CRH increases both POMCmRNA and secretion of POMC derived peptides, AVP increases only secretion but not the transcription rate. However, this would not allow one to conclude that the effect of POMCmRNA generation is solely a cAMP-mediated process, because phorbol

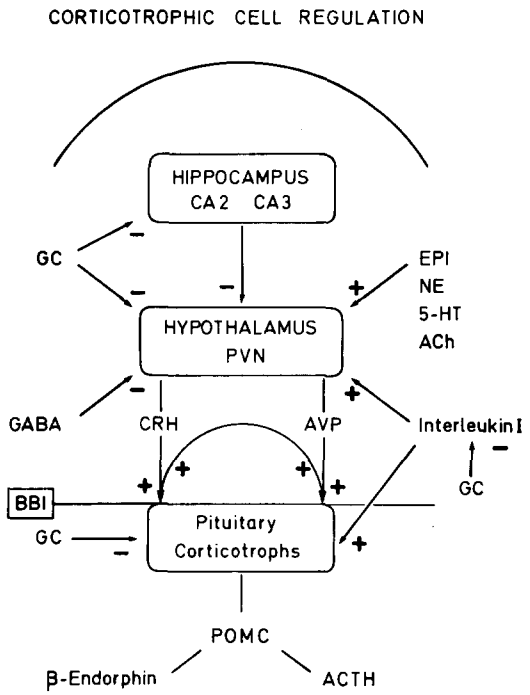

Fig. 1. The current model of pro-opiomelanocortin *(POMC)* derived peptide biosynthesis is based upon studies measuring corticotropin-releasing hormone *(CRH)* in hypophyseal portal blood and the effects of regulators upon mRNA turnover rates in specific brain areas. *GC,* Glucocorticosteroids; *EPI,* epinephrine; *NE,* norepinephrine; *5-HT,* serotonin; *ACh,* acetylcholine; *GABA,* gamma-aminobutyric acid; *AVP,* arginine-vasopressin; *BBI,* blood brain interface; *CRH,* corticotropin releasing hormone; *PVN,* paraventricular nucleus

esters also activate POMC gene expression. Phorbol esters directly activate protein kinase C and mimic the effects of receptor-mediated phosphatidyl inositol hydrolysis, leading to the same second messenger system used by AVP (which does not activate cAMP) and α-adrenergic receptors.

ACTH is the key hormone to enhance biosynthesis and release of adrenal steroids (mineralo- and glucocorticosteroids). Like the mechanisms of CRH at the pituitary level, effects of ACTH at the adrenal cortex (particularly development of hyperplasia secondary to stimulant overexposure) are supported by other peptides derived from the N-terminal part of the ACTH precursor (N-POMC). The precise nature of these concerted actions is not yet clear (Estivariz et al. 1988). Those corticosteroids which are hydroxylated in C-11 position suppress synthesis and secretion of ACTH by inhibiting anterior pituitary POMC gene transcription. In turn, adrenalectomy elevates corticotropic POMCmRNA and AVPmRNA levels in a glucocorticosteroid-sensitive mode. Control of POMC gene transcription by glucocorticosteroids involves interaction of the ligand-activated glucocorticoid receptor with the DNA (Ringold 1985). According to the suggestion by Beato et al. (1987) glucocorticoid receptor binding to the DNA would result in a conformational change of the DNA preventing its nuclear interaction with phosphoproteins and thus blocking CRH-induced gene transcription.

In addition to these effects upon the anterior pituitary brain structures are also subject to corticosteroid feedback. The hypothalamic PVN cells which contain CRH-secreting neurons contain GC receptors and CRHmRNA is decreased in response to corticosteroid exposure. Similarly, glucocorticoids may suppress the mRNA for interleukin-1 (Il-1), another stimulator of CRH and ACTH. Numerous inputs from extrahypothalamic sites influence activity within the LHPA axis. Of particular interest here is the hippocampus with the highest corticosteroid receptor density in the brain, these being mineralocorticoid receptors with tissue specific corticosterone or cortisol preference in this particular limbic area (Reul and de Kloet 1985). Work by Sapolsky et al. (1984) suggests that ligand exposure to GC receptors reduces their capability to shut off excitatory inputs for PVN-located CRH synthesis. Thus, glucocorticoids may regulate their homeostasis and phasic and tonic conditions at different levels through different genomic (slow) and other neurochemical (fast non-genomic) effects.

At this point it should be noted that the feedback mechanisms between adrenocortical hormones and limbic-pituitary peptides are as yet poorly understood; for example, the effect of GCs upon CRH synthesis differs in various brain locations (Beyer et al. 1988) and may be oppositve to the effects in peripheral tissues (Robinson et al. 1988). Various stressors use different mechanisms to elevate ACTH and cortisol; therefore the degree of steroid sensitivity may vary. Neither baseline nor stress-activated ACTH and cortisol can predict corticosteroid suppressibility. The reason for the development of insensitivity to corticosteroid feedback after specific stressors may be their use of neural pathways which are independent of circulating corticosteroid levels.

Release of CRH from the hypothalamus is modulated by a variety of neuronal afferents and the role of specific neurotransmitters has long been a matter of controversy (Tuomisto and Mannisto 1985). In vitro studies suggested that central norepinephrine inhibits hypothalamic CRH release through the β-adrenergic receptor and partially through the α-receptor. At the pituitary level peripheral epinephrine and norepinephrine are thought to stimulate the ACTH release by a β_2-adrenergic and/or by an α_1-adrenergic effect. Electrical stimulation of noradrenergic fibers in the ventral ascending pathway inhibits the ACTH response to surgical stress and this finding by Ganong (1977) supported an inhibitory role for norepinephrine. This concept was embraced because it fitted well into the long favored idea that depression is etiologically linked to central norepinephrine and/or epinephrine deficiency, resulting in weakened inhibition of CRH secretion and consequently in hypercortisolism. However, in vivo studies have now proved convincingly that epinephrine and possibly also norepinephrine enhance CRH by α_1 and α_2-receptors (Guillaume et al. 1987; Plotsky et al. 1987). Also serotonin (5-HT), probably through 5-HT_{1A} or $5HT_2$ receptors, and acetylcholine through muscarinergic receptors are excitatory transmitters at the CRH neuron. The only neurotransmitter which inhibits CRH release into the pituitary portal circulation is γ-aminobutyric acid (GABA), which conforms with ACTH and cortisol suppressing effects of benzodiazepines (BZD) and the cortisol activating effects of inverse BZD agonists, e.g. β-carbolines. At present, it remains unresolved whether production and release of CRH and its effects on POMC gene expression and post-translational processing is resulting from postulated alterations in monoaminergic circuits. Alternatively, changes in monoaminergic systems could be considered as results from altered neuropeptide activity, as for example central CRH administration elevates catecholamines. In essence, the LHPA system is regulated by interactions between non-peptidergic hormones, neuropeptides, neurotransmitters and their receptors in a complex mode that possibly represents a general scheme for other CNS interactions.

Clinical Studies Exploring LHPA Regulation

Basal Secretion of ACTH and Cortisol in Depression

The major change of LHPA activity in depression is an elevated amount of cortisol released per secretory burst. While amplitude and duration of any single cortisol pulse are enhanced, there is no reported increase in pulse frequency or flattening of circadian periodicity (Linkowski et al. 1987; Halbreich et al. 1985). Over a 24-h observation period, Mortola et al. (1987) found an increased number of ACTH bursts, which were temporally related with cortisol secretion. The overall secretion of ACTH is not elevated, indicating development of supersensitive adrenal glands after persistent overexposure to their major trophic stimulator (Gerken and Holsboer 1986; Jaeckle et al. 1987; Amsterdam et al. 1987a, b). To date, all studies exploring the adrenal cortex response to ACTH have been conducted with supramaximal doses and future studies should use physiological doses of this peptide. The parent abnormality of hypercortisolism is the nonsuppression of ACTH and cortisol by the synthetic glucocorticoid dexamethasone (dexamethasone suppression test, DST) which under physiological conditions provides a powerful feedback signal, shutting off POMC generation and thus ACTH and cortisol secretion. Pathology underlying DST nonsuppression of LHPA hormones is discussed separately below.

Pituitary Stimulation with Human Corticotropin-Releasing Hormone in Depression and Panic Disorder

The isolation and sequence analysis of CRH from ovine hypothalami (Spiess et al. 1981), sequence deduction of human CRH from the nucleotide sequence of cloned DNA by Shibahara et al. (1983) and subsequent peptide synthesis have provided a tool to directly explore pituitary function in depression linked with hypercortisolism.

The first series of reports utilizing human CRH in depressives was released from our group and showed that ACTH response is blunted in these patients (Holsboer et al. 1984a, 1986a, 1987a). We further observed that the mean cortisol secretion monitored during 5 h prior to stimulation is inversely related to the stimulated amount of ACTH (Fig. 2). Finally, the normal cortisol release is dissociated from blunted ACTH release after CRH, which points toward a hypersensitive adrenal cortex as result of longterm overstimulation. From these data we concluded that elevated circulating cortisol prevents adequate ACTH response to CRH via an intact negative feedback loop. Therefore, exaggerated secretory activity of corticotrophic and adrenocortical cells must be related to a central alteration of CRH regulation. This interpretation is consistent with our earlier results when applying ovine CRH to depressives, controls and remitted patients (Holsboer 1983; Holsboer et al. 1984b, c, 1985a). Our findings with human CRH have now been replicated by Lesch et al. (1988) and further amplified by similar findings utilizing the long-acting heterologous ovine analogue by Amsterdam et al. (1987a), Gold et al. (1984, 1986), and Risch et al. (1988).

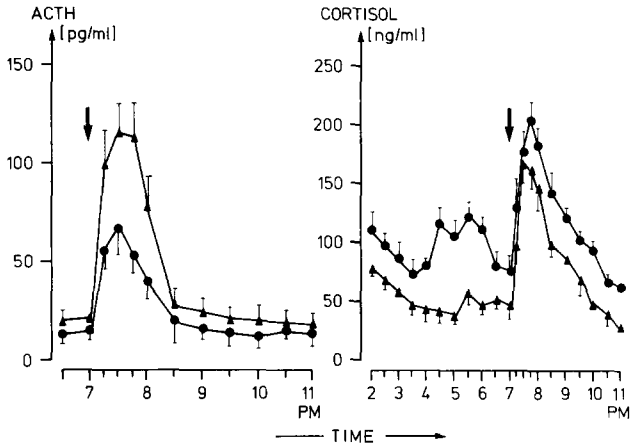

Fig. 2. h-CRH stimulation test in depression. *Left:* ACTH responses to h-CRH in patients with depression and in controls show significantly decreased AUC values (expressed as pg × min/ml × 10^3) in depressives (AUC in depression: 3.0 ± 2.6; in controls: 6.2 ± 3.4, $P < 0.01$); *right:* cortisol secretion (mean ± SE) at baseline (2.00 p.m.–7.00 p.m.) and following a bolus injection of 100 µg h-CRH at 7.00 p.m. in patients with endogenous depression, (●, $n = 11$) and in matched controls (▲, $n = 11$). Mean (± SD) cortisol levels were higher in depressives than in controls (95.3 ± 25.8 ng/ml versus 56.6 ± 14.6 ng/ml, $P < 0.01$). AUC (expressed as ng × min/ml × 10^3) for cortisol following h-CRH were indistinguishable between depressives and controls (11.5 ± 4.2 versus 11.8 ± 4.6, n.s.). Data from Holsboer et al. (1986a)

We extended the hormonal evaluation following CRH administration and observed that corticosterone responds much more vigorously than cortisol and may be a good candidate with which to unravel minor adrenocortical changes (Holsboer et al. 1984b, 1985a). The most interesting finding in the multihormonal analysis after CRH injection is a blunted response of aldosterone in depression, which contrasts with the indistinguishable glucocorticoid (corticosterone, cortisol) responses between patients and controls (Holsboer et al. 1987a). This dissociation between mineralo- and glucocorticoid release after h-CRH treatment adds to earlier reports of disturbed electrolyte homeostasis in patients with affective disorders, which was related to impaired aldosterone production

rates (Hullin et al. 1981), and blunted aldosterone responses to dexamethasone among depressed patients (Holsboer et al. 1982a).

Disturbed LHPA activity at baseline, or following perturbation tests, is not specific for depression. Anxiety states and particularly panic disorder are frequently associated with LHPA alterations. For example, elevated cortisol at baseline or DST nonsuppression have been reported in panic disorder. Anxiety states induced by drugs (e.g. inverse BZD-receptor agonists, e.g. β-carboline), or exposure to phobic stimuli are also associated with hypercortisolism (Insel et al. 1984). We explored LHPA function in these patients and showed that they also have a blunted ACTH response after CRH (Holsboer et al. 1987b). This finding is not contaminated by coincidental depression, because after sorting out pure panic disorder (i.e. without concurrent depression) the blunted ACTH response remained indistinguishable from that in pure depressives and patients with both panic disorder and depression. Just as in depression the cortisol responses were normal despite low ACTH output, indicating that the adrenal cortex had developed hyper-responsiveness to ACTH, probably because each panic attack is accompanied by excessive LHPA activation via release of adrenogenic POMC peptides.

To explain the blunted ACTH response in patients with affective disorders two not mutually exclusive mechanisms have to be considered: (1) elevated levels of cortisol may be responsible for restrained ACTH release and (2) desensitization of pituitary CRH receptors may occur. In any case an excessive LHPA activity in these patients is most likely driven by chronic CRH hypersecretion. The group led by Nemeroff tested the hypothesis that CRH is hypersecreted at the level of the hypothalamus and possibly higher CNS areas in depression. They reported in two studies that cerebrospinal fluid (CSF) concentrations of CRH are elevated in drug-free depressed patients compared with normal controls and nondepressed psychiatric patients (Nemeroff et al. 1984; Banki et al. 1987). While portal concentrations of the peptide cannot be definitely inferred from CSF investigations, this finding supports hyperactive CRH-producing brain nuclei. This notion is further strengthened by a recent study of the same group (Nemeroff et al. 1988), which showed that the number of CRH binding sites in the frontal cortex was markedly reduced in suicide victims compared with controls who died of a variety of causes which are not likely to have been associated with depression (sudden myocardial infarct, homicide etc.). This down-regulation (decreased B_{max}) was not associated with changes in affinity (K_D). Chronic administration of CRH leads to prolonged increases in plasma ACTH levels (Stalla et al. 1986). In a study by Young and Akil (1985) pituitary cultures taken from chronically stressed rats responded the same as normal controls after CRH challenge. Vale et al. (1983) showed that the long-term effects of CRH are associated with exaggerated contents of POMC derived peptides, presumably mediated by an increase in POMC gene transcription (Wand and Eipper 1987). These findings are puzzling and unique for CRH, because overexposure of pituitary CRH receptors due to prolonged CRH treatment results in homologous desensitization and blunted ACTH response of these cells after exposure to CRH (Reisine and Hoffmann 1983). Prolonged exposure to AVP, which acts through protein kinase C, does not increase ACTH synthesis. Probably CRH-induced long-term actions of cAMP dependent kinase are different from second messenger effects as induced by AVP. However, like AVP induced stimulation of ACTH secretion, AVP may also down-regulate CRH receptors through activation of protein kinase C and probably calcium ions. A first attempt to investigate the role of elevated concentration of corticosteroids as a principal factor causing blunted ACTH release after CRH in depression was reported from our laboratory (von Bardeleben et al. 1988a). We partially blocked the synthesis of 11-hydroxylated corticosteroids with a low dose of metapyrone, thus depriving the LHPA system from its major inhibitory feedback signal. As illustrated in Fig. 3, the observed areas under the ACTH response curves in depressives treated with metapyrone were significantly higher as in untreated depressives and indistinguishable from those seen among controls. From this finding we conclude that the hypercortisolism driven by hypersecretion of CRH from the hypothalamus and ACTH (and N-POMC) from the anterior pituitary is the determinant factor for blunted ACTH response to CRH in depression. We are aware that several other effects need to be considered as possible confounders. For example, AVP stimulates prostaglandin production via the cytochrome P-450-dependent pathway and metapyrone, which inhibits this enzyme, prevents AVP-induced ACTH secretion (Okajima and Hertting 1986). If this effect should play a role in the human in vivo experiments, then rather a decreased instead of an increased ACTH response would be observed. Therefore, we surmise that down-regulation of CRH receptors is not primarily responsible for blunted ACTH response to CRH. Even if a significant down-regulation should be present it has not necessarily to be of physiological significance, because in vitro studies have demonstrated a maximal ACTH response to CRH, when only 50% of the receptors ("reserve receptors") were occupied by the ligand (Aguilera et al. 1986).

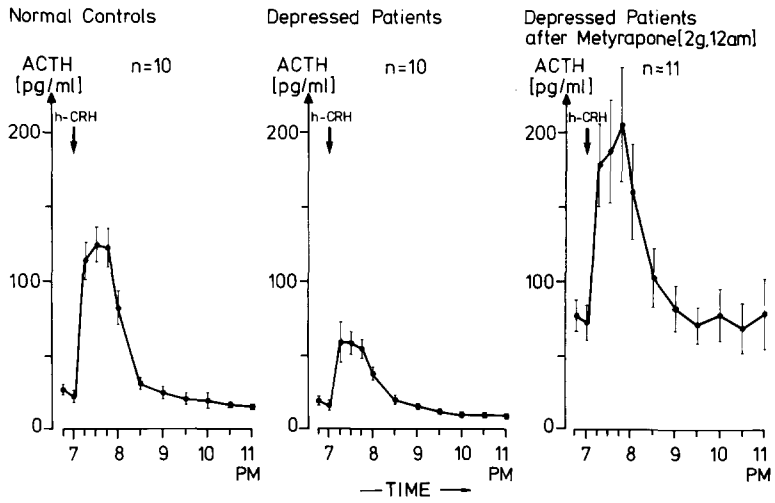

Fig. 3. Effect of 11β-steroid-hydroxylase inhibition upon h-CRH tests in depression. ACTH (mean ± SEM) response curves after h-CRH demonstrate that the reduction of C-11 hydroxylated glucocorticoids in depression by a single metapyrone dose (2 g) leads to increased net ACTH output which is indistinguishable from that in controls. Areas under concentration curves expressed as pg/ml/min × 10^3 are in depressives without metapyrone: 2.6 ± 1.1; depressives with metapyrone: 9.0 ± 6.7; controls 6.8 ± 2.4). Data from von Bardeleben et al. (1988a)

Dexamethasone Suppression Test

About 20 years ago it was first communicated by Carroll et al. (1968, 1981) that certain depressive patients are refractory to the corticosteroid-suppressing effect of a single dose of dexamethasone. This potentially important finding was at first much questioned. While some investigators claimed that DST nonsuppression would indicate a specific diagnostic subclass (Carroll et al. 1981), others considered this finding to be an unspecific epiphenomenon subject to confounding factors such as weight loss, medication or bioavailability of the test drug (review: Arana et al. 1985). In 1980 we initially documented in a controlled study with 102 patients that the DST has only moderate specificity for the clinical diagnosis of endogenous depression (Holsboer et al. 1980). In a later study enrolling 111 consecutively hospitalized depressed patients who were carefully monitored for all known confounding variables (effects of acute admission, weight change, medication status, plasma dexamethasone levels) we applied a standardized polydiagnostic examination and replicated our initial finding (Holsboer et al. 1986b). Now the enthusiasm about the DST as a diagnostic tool has waned (Berger et al. 1988). However, it should be noted that the DST cannot be blamed for this development, as no laboratory test, thought to confirm diagnostic attributions, can be better than the applied diagnostic scheme itself. After our first reports that the DST might successfully be used at a descriptional level as state marker to monitor clinical progress during pharmacotherapy (Holsboer et al. 1982b, 1983; Gerken et al. 1985) and confirmatory studies by other groups (e.g. Greden et al. 1983) we embarked on a series of studies exploring the pathophysiology underlying the DST nonsuppression phenomenon.

First we extended the number of analyzed steroids and measured not only cortisol, but also its precursor, 11-deoxycortisol and its equilibrium steroid cortisone (review: Holsboer 1985). We also examined the mineralocorticosteroid pathway and analyzed 11-deoxycorticosterone, corticosterone and aldosterone. We submitted that the multisteroid analysis provides a more subtle adrenocortical evaluation, allowing for insight into activity patterns of enzymes involved in glucocorticoid biosynthesis and may additionally increase the sensitivity of the test to detect discrete LHPA alterations. Of particular interest was our original finding that DST nonsuppression is associated with low plasma dexamethasone levels (Holsboer 1983; Holsboer et al. 1984b). This led some investigators to speculate that DST nonsuppression might be caused by hampered bioavailability of the test drug (Arana et al. 1984). However, pharmacokinetic studies administering dexamethasone orally and intravenously in depressives and subsequent analysis in the early biophase allowed us to reject this speculation (Holsboer et al. 1986c, d; Wiedemann and Holsboer 1987).

While the hypercortisolism in depression seemed to be driven by hypersecretion of CRH, we were impressed that basal cortisol secretion only moderately predicts the suppressibility of cortisol by dexamethasone (Holsboer et al. 1984e). We therefore further investigated the role of CRH as a causative factor for DST nonsuppression: von Bardeleben et al. (1985) infused CRH, AVP or placebo to normal controls pretreated with 1.5 mg dexamethasone and found that neither treatment could induce an escape of plasma cortisol from suppression. However, if both neuropeptides were administered concurrently they overrode the suppressive effect of dexamethasone. This finding implied that AVP and CRH act in con-

cert to overcome the inhibitory action of dexamethasone. Based on several reports which agreed that the ACTH and cortisol response to ovine CRH is determined by the level of circulating glucocorticoids (Lytras et al. 1984; Hermus et al. 1987) one would expect that in depressives elevated post-DEX cortisol plus the test drug DEX itself would prevent release of cortisol after CRH. However, we found the opposite (Holsboer et al. 1987c; von Bardeleben and Holsboer 1989). As illustrated in Fig. 4 depressives pretreated with DEX released considerably more cortisol after CRH than healthy subjects.

The interpretation of the observed feedback resistance to endogenous (suppressed or nonsuppressed adrenocortical steroids) plus exogenous (DEX) corticosteroids involves altered brain physiology and can not be explained by peripheral (pituitary-adrenocortical) changes. If only pituitary mechanisms on CRH receptors play a role, the opposite effect should be expected. Elevated endogenous CRH secretion into the portal vessels would result in down-regulation and desensitization of corticotrophic CRH receptors. In addition, elevated glucocorticoids selectively down-regulate pituitary CRH receptors without altering brain CRH receptors (Hauger et al. 1987). If elevation of both hormones, CRH and glucocorticoids, results in down-regulated pituitary CRH receptors, a blunted rather than an exaggerated ACTH and cortisol response to an exogenous bolus of CRH should be expected. Therefore suprapituitary sites, particularly the hippocampus and hypothalamus must be inspected as mediators of GC-negative feedback resistance in depressed patients. Recent research has developed the concept that basal activities and homeostatic disturbances in response to stress are regulated by different corticosteroid receptors (Reul and de Kloet 1985). These receptor systems were originally termed type 1 and type 2 GC receptors. Now these receptor types were shown to be structurally identical with the mineralocorticosteroid receptor (MR) and the glucocorticosteroid receptor (GR) as analyzed applying gene technology (Arriza et al. 1987). The limbic area and particularly the hippocampus are of special interest because the latter contains a highest density of MRs which bind in hippocampal neurons corticosterone (rat) or cortisol (hamster, human?) with 6- to 10-fold higher affinity than the GR in the same neuron (Reul and de Kloet 1985). If corticosteroid levels are elevated as is the case in hypercortisolemic depression, GRs get down-regulated. This down-regulation increases the vulnerability of GC-containing neurons toward hypoxemia or nutritional deficits resulting finally in neuronal loss (Sapolsky et al. 1988). According to Reul et al. (1987) the number of MRs increases in parallel with GR down-regulation, and this may occur within the same limbic neuronal cell, capable of expressing both GR and MR genes at the same time (Arriza et al. 1987).

Sapolsky et al. (1984, 1988) have recently published studies where the effect of hippocampal corticosteroid receptor density and ligand occupation was correlated with social and neuroendocrine measures among rodents and primates. The major point of their work is that the hippocampus serves as a negative feedback instrument upon the LHPA axis. If enhanced adrenocortical secretory activity down-regulates tissue specifically corticosteroid receptors in this area, then the hippocampus gradually loses its capacity to shut off LHPA activity. This mechanism would feed forward the deleterious effects upon corticosteroid-containing neurons, making them refractory to negative feedback. This concept is substantiated by observations that hippocampal lesions produce LHPA overactivity and accumulation of CRHmRNA in the hypothalamus. If GRs are down-regulated in a physiologically significant degree then DEX, which binds predominantly at GR, has a diminished potency to suppress ACTH and cortisol. At the same time as DEX suppresses ACTH and thus endogenous adrenal steroid production the excessive number of MRs is devoid of a ligand, because endogenous corticosteroids are not fully substituted by exogenous DEX at this receptor in the hippocampus. This increased MR/GR ratio in the limbic brain may induce a priming (i.e. sensitizing) effect upon pituitary cells toward a specific secretagogue. By which mechanism this priming is achieved remains to be resolved. One attractive explanation emerged from a study by Sapolsky et al. (unpublished observation) where the AVP and CRH concentrations in portal blood were compared with hippocampal corticosteroid receptor occupation. It appeared that decreased GR occupancy has a more pronounced effect upon AVP than upon CRH. Therefore one may speculate that the increased MR/GR ratio secondary to long-term hypercortisolism is accompanied by an increased AVP/CRH ratio. Interestingly, the phenomenon of cortisol release after DEX/CRH administration was reversible and disappeared if depressive symptomatology resolved or switched into mania (Holsboer et al. 1987c). It must be noted that no data have emerged from CSF studies in depressives which point to enhanced AVP secretory activity in brain areas which contribute to CSF composition. However, the direct effects of AVP upon the pituitary gland can be markedly different from those induced by administration of the peptide into the third ventricle (Lumpkin et al. 1987). Therefore the AVP concentrations in the CSF of depressives may be a poor reflection of the actual

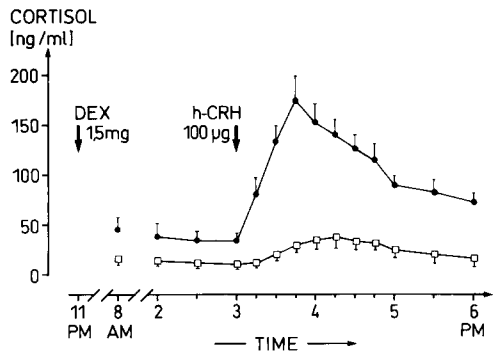

Fig. 4. Mean areas under the cortisol concentration curves (AUC) expressed as ng/ml/min ± SD min after 100 µg CRH administered to dexamethasone (1.5 mg, 11.00 p.m. the day before) pretreated normal controls (NC □) and depressed patients (DEP ●; 5 bipolar, 20 unipolar) show an increase among the latter group (NC: 1767 ± 1565; DEP 7026 ± 4739; $P < 0.01$). The patient group was significantly older than the controls (48.3 ± 12.5 years versus 25.4 ± 3.3 years). Neither the DST nor the CRH test alone are strongly influenced by age and the escape phenomenon in the DEX/CRH test disappeared when patients were monitored longitudinally and became euthymic. Therefore we surmise that the observed effect is primarily determined by the disease process this is corroborated by our recent replication study where we used age matched samples (von Bardeleben and Holsboer 1989)

AVP bioavailability at the pituitary gland. In several cases where cortisol remained releasable during the DEX/CRH paradigm, despite the absence of depressive psychopathology, we noted an increased liability for a clinical relapse (Holsboer et al. 1987c).

LHPA Hormones and Alcoholism

When administered at moderate dosages ethanol has anxiolytic effects, while the neuroendocrine effects are rather opposite. Acute and chronic drinking of ethanol-containing beverages activates the LHPA axis through secretion of CRH into the hypophyseal portal blood vessels (Rivier et al. 1984). In some actively drinking chronic alcoholics the extent of this neuroendocrine abnormality can reach dimensions which are clinically relevant and may account for some of the Cushingoid physical stigmata of alcoholism.

Also, withdrawal from alcohol elevates ACTH and cortisol and the cortisol secretory pattern returns to normalcy only after prolonged enforced abstinence. Studies with the DST also showed nonsuppression during the detoxification process and adequate cortisol suppression after several weeks of sobriety (Swartz and Dunner 1982). In the light of these neuroendocrine similarities between alcoholism and depression and several family studies, pointing to a frequent association between these disorders, a thorough evaluation of aberrant LHPA regulation utilizing CRH seemed warranted.

The first major findings employing CRH among patients with alcoholism were reported from our group when we noted that hypercortisolism in acutely withdrawn patients is associated with blunted response of ACTH after a CRH challenge (Holsboer et al. 1987b; Heuser et al. 1988). Next we studied patients meeting the criteria for medium-term abstinence (no alcohol for 14–42 days) and found that their basal cortisol secretion (mean levels between 2.00 p.m. and 5.00 p.m.) had normalized. Contrary to expectation these patients had blunted ACTH responses to CRH (Holsboer et al. 1987b; von Bardeleben et al. 1989). Even more surprising were our results obtained from strictly observed patients who abstained from alcohol for more than 10 months. As illustrated in Fig. 5 we found cortisol secretory patterns that were indistinguishable from those of matched controls, whereas the ACTH responses to CRH remained blunted (Holsboer et al. 1987b). The findings among acutely withdrawn patients may be properly interpreted on the basis of hypercortisolism, restraining adequate ACTH response to CRH via feedback. However, after medium- and long-term abstinence alternative mechanisms must be considered as these patients were normocortisolemic at baseline. The most likely explanation is based on a study by Dave et al. (1986), who showed a decrease of pituitary CRH binding in rats who were exposed to ethanol over prolonged periods. These changes were accompanied by decreased adenylate cyclase activity and subsequently by reduced production of POMCmRNA. It has been recently demonstrated by Tabakoff et al. (1988) that continuous ethanol exposure leads to reduction in receptor-stimulated cAMP levels in platelets. In fact, these authors were able to classify correctly 75% of alcoholics and 73% of nonalcoholics by biochemical investigation of blood cells even after long-term detoxification. Recently, Mochly-Rosen et al. (1988), utilizing a neural cell line, showed that chronic alcohol causes desensitization of receptors that stimulate adenylyl cyclase, the cAMP synthesizing enzyme. Since it is well established that CRH binding to receptors at corticotrophs leads to an activation of cAMP, the findings of ethanol-induced impairment of cAMP biosynthesis agree with our finding of blunted ACTH response to CRH in normocortisolemic abstinent patients with alcoholism. Whether the neuroendocrine abnormalities found among dry alcoholics are measures of the underlying genetic susceptibility for alcoholism or biological scars of many years of heavy ethanol consumption remains open. Also the question as to what extent persistence of abnormalities contributes to the high risk for relapse in these patients should be the subject of more research if one considers that dependence on

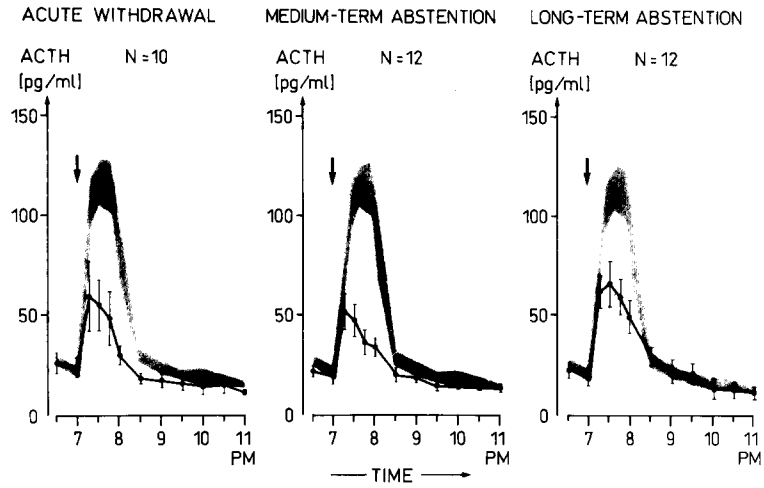

Fig. 5. h-CRH stimulation test response after termination of alcohol absue. ACTH responses were found to be blunted among patients with alcoholism (acute withdrawal 1.5 ± 0.9 pg/ml/min 10^3; medium-term abstention: 1.8 ± 0.5; long-term abstention: 3.5 ± 1.9). This effect is not necessarily explained by concurrent hypercortisolism since baseline (2 p.m.–5 p.m.) plasma cortisol secretion was only significantly elevated after acute withdrawal (acute withdrawal: 102.8 ± 42 ng/ml; medium-term abstention; 64 ± 27.3 ng/ml; long-term abstention: 55.8 ± 22.5 ng/ml). Normal control data (shaded areas): ACTH: 5.8 ± 1.5 pg/ml/min 10^3; cortisol (2 p.m.–5 p.m.): 49.3 ± 15.9 ng/ml. Data from Heuser et al. (1988) and von Bardeleben et al. (1989)

alcohol is extremely common and a deteriorating illness.

LHPA Hormones and Eating Disorders

Eating disorders are associated with a wide range of biochemical changes and many attempts have been undertaken to sort out which findings are causative for the disease and which may result from it. Leibowitz et al. (1983) demonstrated that CRH released from the PVN attenuates the feeding produced by norepinephrine. While norepinephrine enhances feeding through α_2 postsynaptic receptors, most studies support an inhibitory role for serotonin on food intake which is mediated through mechanisms in the PVN (Blundell 1984). CRH is also most likely involved here, because 5-HT is an excitatory transmitter for hypothalamic production and release of this neuropeptide. Benzodiazepines (BZDs), which enhance food intake, suppress LHPA activity, probably by activating the GABA-mediated inhibition of CRH release (Cooper and Estall 1985). On the other hand, inverse BZD-receptor agonists, such as β-carbolines, reduce food consumption (Cooper 1986). These BZD-receptor ligands not only oppose the behavioral complex of BZDs but also their neuroendocrine effects, as they increase cortisol, probably through elevated CRH. This indirect evidence for an anorectic effect of CRH is further supported by studies in which this neuropeptide was administered in animals directly. Morley and Levine (1982) administered CRH into the lateral ventricles of rats and observed decreased food intake. These results were corroborated by other studies (Britton et al. 1982; Ruckebusch and Malbert 1985), which also reported behavioral changes consistent with the hypothesized stress amplification of CRH. Probably CRH induces anorexia via suppressing hunger. For example, Brown et al. (1982) reported that CRH is able to maintain blood glucose levels via increasing sympathetic outflow in several brain areas. Accordingly, CRH concentration was found to be elevated in major brain areas of starving rats (Suemaru et al. 1986). In the same vein are clinical studies which applied CRH stimulation tests to patients with anorexia and found blunted ACTH responses, suggesting increased endogenous CRH release, which attenuates pituitary CRH receptor sensitivity (Hotta et al. 1986; Gold et al. 1986b). This notion is amplified by the observation that the CSF of anorectics contains elevated CRH (Kaye et al. 1987). In the light of these data it is hard to recognize hypercortisolism in anorexia as a phenomenon solely secondary to food abstinence. A primary role of enhanced CRH release, resulting in anorectic behavior and concomitantly in neuroendocrine alteration, must also be considered. Such a viewpoint is not new, as it was debated long ago whether or not Simmonds disease can be differentiated from anorexia as a primarily pituitary triggered disease or a so-called "hypophyseal cachexia" (Escamilla and Visser 1942). Progressive anorexia and starving have metabolic effects of their own, resulting also in endocrine changes which add to the basic neuroendocrine disturbance. Experimental weight loss in humans, for example, results in exaggerated LHPA activity and subsequent suppression of the gonadal axis (Fichter et al. 1986). Elevated CRH resulting from stress, starving or a primary disturbance of CRH regulation also suppresses gonadal activity, impairing reproductive function (see below). Thus, cause and effect are mutually synergizing each other and make discrimination on the basis of the clinical phenotype impossible. However, with the underlying cause for the documented CRH excess still unclear, there remains little doubt of its role as a key mediator of anorectic behavior.

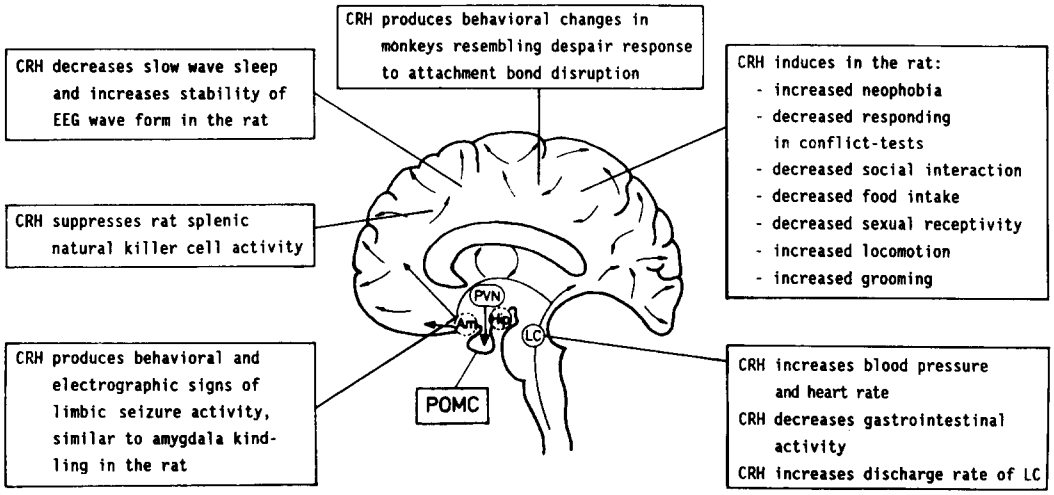

Fig. 6. Behavioral effects of central CRH. Compilation of nonendocrine behavioral data illustrates that CRH induces manifold effects which are characteristic for depression

Behavioral Implications of Altered LHPA Activity

Animal Studies

The profound effects of GCs on behavior in animals were originally demonstrated by Bohus and de Wied (review: 1980). The best known example is that GCs facilitate extinction of an active avoidance response in rats, while passive avoidance retention was suppressed by pharmacological GC doses. Primates living in their natural habitat were studied by Sapolsky (1983), who observed among baboons that their basal adrenocortical function is strongly determined by their social rank within the troop. High-ranking male animals had significantly lower plasma cortisol concentrations than subordinates. After immobilization both reached the same cortisol levels; however after termination of stress high-ranking baboons returned faster to plasma cortisol baseline. When baboons were socially promoted within their troop, their cortisol secretory patterns adjusted to the higher rank.

The increasing awareness that CRH mediates not only endocrine behavior but serves as the key coordinator for stress responses has led to a series of experiments investigating behavioral effects of this novel neuropeptide (Fig. 6). The vast majority of studies agree with the concept that behavioral signs of stress, anxiety and depression can be mimicked by intracerebroventricular injection of CRH. As illustrated in Fig. 6 many of the behavioral changes common among depressives, such as psychomotor retardation, sleep disturbances (loss of slow wave sleep), anxiety, loss of appetite and sexual activity resemble the cardinal symptoms of depression. Moreover, anxious behavior in the rat as induced by CRH can be antagonized by benzodiazepine treatment. Post et al. (1988) recently reviewed the parallels in the phenomenology of recurrent affective illness to kindling and sensitization. Repeated electrical stimulation of the brain with low subthreshold currents may elicit full-blown limbic seizures. This effect is a function of the number of repetitions and not of the amount of applied current, which is initially too low to produce spiking responses in the early treatment phase. After a long period, as a result of effective kindling, seizures may occur even without preceding stimulation. This model is thought to mimic several of the characteristics of depression, where after an increasing number of episodes lesser stimulants are necessary to precipitate another episode. In this regard it is of interest that Ehlers et al. (1983) observed that a single high dose of CRH can produce the late onset of seizures several hours after CRH administration. The seizures which were provoked by CRH involved amygdala spiking and resembled those seen after electrical kindling. The behavioral seizures were similar to those observed in electrical kindling and CRH-induced seizures showed cross-sensitization to amygdala kindling (Weiss et al. 1986). Although highly speculative, these first experiments would support the concept of "neuropeptide kindling", where through repeated sensitization by CRH a similar progressive behavioral responsiveness develops which paves the way for increasing recurrency of affective episodes. Importantly, carbamazepine, which is not only an anticonvulsant but also prophylactic in recurrent affective disorders, can interfere strongly with neuroendocrine responsiveness to CRH (von Bardeleben et al. 1986).

LHPA Hormones and EEG Sleep

Polygraphic recording of sleep is a method for analyzing accurately one aspect of human behavior that is intimately associated with affective disorders. While LHPA hormones show a remarkable inertia toward short-term variation of the sleep-wake schedule there are several demonstrations that a relationship between the cortisol secretory pattern and sleep pattern may exist. Born et al. (1986), for example, suggested that slow wave sleep (SWS) suppresses cortisol because offset of SWS is frequently followed by a steep increase of cortisol. Our group (Steiger et al. 1987) extensively investigated the interaction between cortisol secretion and sleep EEG variables and failed to find a regular inter-relationship between these measures among normals. We also tested the hypothesis of enhanced LHPA activity as one mechanism involved in reduced depth and termination of sleep by stimulating the endocrine system in normal male controls with CRH or placebo in a pulsed mode (Holsboer et al. 1988a). We chose the time before and after sleep onset (10.00 p.m.–1.00 a.m.) for pituitary activation because the endogenous activity of the LHPA system is minimal during this time period. The major change of sleep pattern following active CRH was a drop in SWS and an increase of shallow sleep (stages 1 and 2). The number of stage shifts remained unaltered under both treatments, ruling out the possibility that unspecific sleep disturbances account for the findings. It is unclear how these central effects of CRH are mediated. Direct actions of CRH after passage through leaky sites of the blood brain interface are possible. However, the central effects can also be mediated through afferent pathways to the brain or CRH receptors directly located at the blood brain interface. It is unlikely that the observed effects are mediated through activation of cortisol because von Bardeleben et al. (1988c) found that cortisol infusions during sleep enhance rather than suppress SWS. In line with our interpretation are data by Ehlers et al. (1986), who showed that SWS is decreased after direct administration of CRH to rats. Also our finding of blunted nocturnal growth hormone (GH) during CRH administration (Holsboer et al. 1988a) agrees with the observation made by Rivier and Vale (1984) that CRH acts centrally to inhibit GH secretion in the rat.

In depression, several sleep characteristics, such as shortened REM latency, decreased amount of SWS and augmented REM density, have been frequently reported. We investigated to what degree these abnormalities are state-dependent and studied male drug-free patients during depression and after they were fully recovered (Steiger et al. 1989). The major findings were that most EEG sleep measures being characteristically altered during depression failed to normalize after clinical remission and long-term withdrawal from tricyclics. Also GH, whose nocturnal secretion is blunted in depression, remains unchanged. In contrast, enhanced secretion of cortisol, elevated cortisol nadir and advanced rise of plasma cortisol early in the morning were found to be state-dependent markers of depression, as they normalized when the patients studied were euthymic. The dissociation of changes in cortisol secretory activity and sleep pattern supports the notion that nocturnal hypersecretion of cortisol during depression is not determined by concurrent EEG sleep alterations. Whether or not the observed sleep EEG abnormalities are trait markers indicating vulnerability to upcoming relapses or whether they are "biological scars" resulting from preceding episodes remains open. This question is pertinent for the development of biological markers, which it is hoped will be incorporated into future diagnostic schemes. Delineating high-risk groups is therefore a necessary first step in attaining this important research goal.

Interaction Between LHPA Hormones and Neuroendocrine Regulation of the Gonads, Thyroid and Growth Hormone System

The regulation of pituitary cells secreting thyrotropin (TSH) and GH is subject to activity patterns of the LHPA system. In addition, thyroid hormones are involved in several steps of LHPA hormone and GH secretory activity. These interactions can be confusing when establishing neuroendocrine profiles of patients with affective disorders because not all hormonal systems may disclose abnormalities at the same time when they are cross-sectionally inspected. The following section attempts to highlight the cross-talk between neuroendocrine axes and demonstrates that neuroendocrine alterations can never be recognized as isolated phenomena.

Growth Hormone Regulation by Glucocorticoids, Thyroid Hormones and Gonadal Hormones

Long-term overexposure to GCs results in inhibited secretion of GH at baseline as well as in response to various stimuli. For example, patients with Cushing's disease or Cushing's syndrome due to an autonomous adrenal tumor have extremely low GH response to GHRH (Hotta et al. 1988) and dexamethasone suppresses basal GH levels in patients with acromegaly (Nakagawa et al. 1987). In contrast to these inhibitory effects observed in vivo, GH release is enhanced

by GCs when in vitro system are studied (Nakagawa et al. 1987; Ceda et al. 1987). The molecular mechanism involves binding of the GC-receptor complex on a GC regulatory element within the first intron of the GH gene, causing enhanced gene transcription. Interestingly, this GC mediated effect is synergized by triiodothyronine, which also stabilizes GHmRNA in pituitary tumor cells (Nyborg et al. 1984). The next effect of GCs upon releasable GH in vivo is determined by the magnitude of suprapituitary inhibition via enhanced somatostatin and decreased release of GHRH. The duration of LHPA hyperexposure is also crucial. Short-term treatment with corticosteroids resulted in adequate GH response to GHRH. We recently showed that pulsed administration of CRH and more prominently of ACTH induced an increase of spontaneous GH bursts in number and quantity per pulse during daytime (Wiedemann et al., unpublished). We also found that spontaneous sleep-related nocturnal GH decreases when CRH is administered in pulses during sleep (Holsboer et al. 1988a). Also in rats central administration of CRH inhibits GH secretion, probably by stimulating somatostatin (Rivier and Vale 1984). The consequences of these new findings for the neuroendocrinology of affective disorders are manifold. After the initial report by Matussek et al. (1980) that clonidine and other stimulators of GH exert a diminished GH-releasing effect when applied to depressives, many GH provocation tests were introduced, all suggesting a blunted release of GH in depression. All these centrally acting stimulants exert the effects by enhancing GHRH, which affects specifically GHmRNA in pituitary cells (Gick et al. 1984). The releasable amount of GH after GHRH is controlled by the level of circulating GH. Since Linkowski et al. (1987) showed that during the waking state baseline GH is elevated in depression, the blunting might result from restraint response due to feedback inhibition. Considering the frequency of LHPA alterations in depression hypercortisolism may also account for the net inhibitory effect upon releasable GH. A recent study showing blunted GH response to clonidine in hypercorticoid rats supports such a possibility. Of particular interest are recent studies with GHRH in depression, although they yielded contradictory findings. While Lesch et al. (1987) reported a clearcut attenuation of GH secretion after GHRH administration to depressives, Krishnan et al. (1988) and Eriksson et al. (1988) reported no difference between patients and controls. GH is also releasable after thyrotropin releasing hormone (TRH) secretion, but only under certain experimental and pathophysiological conditions. For example, the GH response to TRH is enhanced in hypothyroid states, which can be explained by increased TRH receptor sensitivity. Growth hormone response to GHRH is blunted in hypothyroidism (Valcavi et al. 1986), while GH response to TRH can be enhanced, probably because thyroid-hormone-sensitive TRH receptors are increased in these patients. The decreased GH response to GHRH and other stimulators, including hypoglycemia, or sleep in hypothyroid patients suggests that GH synthesis and secretion is critically dependent on thyroid hormones, which exert their effects at the transcriptional level. The thyroid receptor becomes activated after ligand binding, resulting in a tighter and more specific interaction with thyroid hormone response elements in the promoter region of the growth hormone gene (Rousseau et al. 1987). Like GCs thyroid hormones also increase the GHmRNA content of rat pituitary tumor cells and both hormones act synergistically at the transcriptional level (Nyborg et al. 1984).

The gonadal system is also critical for GH responses to stimulation. Human females exhibit greater GH response to various stimuli such as clonidine, insulin or the amino acid arginine. With increasing age, the releasable amount of GH after arginine stimulation declines, which points among other factors to an estrogen dependency. The effects of the ovulatory cycle point in the same direction, because at midcycle more GH is secreted after stimuli than in the early follicular phase. The effects of estrogens upon GHRH-stimulated GH are less clear and the majority of studies reject gonadal steroid dependence (e.g. Gelato et al. 1984).

Interaction Between GCs and Thyroid Hormones

Both hormonal systems can act in concert to coordinate neuronal circuits in the brain by activating or deactivating expression of genes. Regulation of GCs and thyroid hormones are also mutually inter-related. GCs may increase TRH receptors at the anterior pituitary level and blunted TSH response to TRH in hypercortisolemic patients is probably due to the suprapituitary effects of GCs. Conversely, the thyroid status might also have an effect on the characteristics of the GC receptors (Leseney et al. 1987). Meaney et al. (1987) showed that thyroid hormones influence the development of GC receptors in pituitary and brain. GC receptors and thus development of a functional LHPA system are incomplete after birth. In the first weeks of postnatal life, GC receptors are developed mainly under the influence of thyroid hormones. Only a few clinical studies have addressed the functional interaction conclusively. Kamilaris et al. (1987) reported that the ACTH responses to CRH in hypothyroid patients are modulated by the amount of circulating thyroxin. During

thyroxin treatment ACTH and cortisol release after CRH was significantly lower than after replacement therapy had been stopped. To what degree thyroxin may interfere with CRH receptors and other factors involved has not yet been clarified. We found that the ACTH response to CRH and the TSH response to TRH were positively correlated in a pooled sample of depressives and matched controls (Holsboer et al. 1985b, 1986a). We also found that ACTH responses to CRH were reciprocally associated with baseline cortisol secretion and it is known that hypercortisolism suppresses releasable TSH. Therefore we surmised that suprapituitary effects of cortisol are the common denominator determining the blunted response of corticotrophic and thyrotrophic pituitary cells to specific secretagogues. In addition AVP is involved in regulation of thyrotrophic cells and most data agree that AVP not only stimulates ACTH but also TSH from pituitary in vitro preparations, although intraventricular administration may suppress basal TSH secretion probably via a negative ultrashort-loop feedback mechanism (Lumpkin et al. 1987).

Interaction of Gonadal Hormones with LHPA Hormones

Sexual receptivity is attenuated in female rats after central administration of CRH (Sirinatsinghji et al. 1982). Accordingly, luteinizing hormone (LH) is suppressed by central administration of CRH in a similar way to that by which exposure to stressors, such as inescapable, intermittent electroshocks, can suppress pulsed LH release (Rivier et al. 1986). The stress-induced effect on gonadotropin can be reversed if a CRH antagonist is coadministered to stress, suggesting that stressful conditions suppress reproductive functions through enhanced secretion of CRH. Almeida et al. (1988) recently suggested that CRH acts mainly by stimulating β-endorphin and dynorphin released from the hypothalamus and proposed that these opioids mediate the CRH-induced suppression of LH and luteinizing hormone releasing hormone (LHRH). In line with this "opioid hypothesis" are findings by Briski et al. (1984), who showed that stress-induced inhibition of LH can be reversed by naloxone.

The decrease in sexual interest is a characteristic symptom in human depression and is probably also mediated through central hypersecretion of CRH. There are only a few studies addressing this topic. We compared nocturnal testosterone and cortisol secretion in male patients with depression (Steiger et al. unpublished data) and observed that elevated secretion and early morning surges of cortisol were associated with lowered mean testosterone secretion. After full clinical remission, cortisol secretory activity decreased while testosterone increased. Since a direct effect of glucocorticoids on LHRH and LH is unlikely (Lopez-Calderon et al. 1987), we suggest that activity of both endocrine axes is altered in the opposite direction by central hypersecretion of CRH.

The role of LHPA Hormones in the Immuno-Neuroendocrine Connection

The interaction between stress, hormones and the immune system has been recognized since Selyes' formulation of the stress adaptation syndrome, which included morphological changes such as adrenocortical enlargement and atrophy of the thymus and other lymphatic structures. In the past few years an upsurge of new data has considerably strengthened the view that humoral and neural pathways link brain function bidirectionally with the peripheral immune system. In the following it is shown that there is now ample reason to believe that the two-way communication takes place by means of LHPA hormones. Classic examples of glucocorticoid involvement are their suppressing effects upon nascent T-cells in the thymus, redistribution of lymphocytes and diminution of the lymphoproliferative response to mitogens. More recently it became clear that the immune system feeds back to the neuroendocrine system; for example, interleukin-1, interleukin-6 (hepatocyte stimulating factor) and thymosin F5 all activate ACTH and corticosteroids. Before focusing on immunoregulation of the LHPA system it is necessary to note that bidirectional communication between the peripheral immune system and the brain is not only established by neuroendocrine hormones but also by a pervasive network of autonomic nerve fibers. Felten et al. (1987) discovered a unique mesh of nerves in the spleen, bone marrow, lymph nodes and thymus. These neural fibers radiate into fields profuse with T-cells, where lymphocyte processes are manipulated through common aminergic and cholinergic neurotransmission. Thus, peripheral lymph tissues are not only humorally connected by neuroendocrine circuits but are also neuroanatomically "hard-wired" with the CNS. The limbic area and particularly the hypothalamus is the primary locus in which to study immuno-endocrine interaction in the brain. Besedovsky et al. (1983) showed that peripheral immune response can evoke neuronal firing in the hypothalamus, which is associated with decreased norepinephrine turnover in this area and the brain stem, but not in the residual brain. This activation is accompanied by elevation of ACTH and cortico-

steroids. It has been frequently shown that in response to virus infection substances are released which may activate each level of the LHPA system. Here a central role is played by interleukin 1 (Il-1), a protein produced mainly by stimulated macrophages and monocytes. The major immunological function of this cytokine is differentiation and activation of lymphocytes and stimulation of lymphokines. In addition, administration of Il-1 to rats increases plasma ACTH and corticosterone concentrations (Besedovsky et al. 1986). The mechanism accounting for this effect is not entirely clear and it appears that different sites are concurrently involved. Intraperitoneal administration of Il-1 to rats elevates ACTH and this effect can be blunted by immunoneutralization of CRH (Berkenbosch et al. 1987). A direct effect of Il-1 upon hypothalamic CRH secretion is supported by the observation that intravenous injection of Il-1 to rats elevates concentrations of CRH in the hypothalamic-hypophyseal vessels (Sapolsky et al. 1987). A recent immunohistochemical approach documented that the hypothalamic areas containing CRH cell bodies are richly innervated by Il-1 immunoreactive fibers (Breder et al. 1988). This supports the notion of a physiologically relevant CRH-releasing potency of Il-1, which is secreted in response to infection, injury or antigenic challenge. A pertinent question remains: how does the 31 kDa polypeptide convey a message from the periphery to the brain? One possibility would be the passage at the organum vasculosum of the lamina terminalis (OVLT), a circumventricular organ without a blood-brain barrier that is located at the anteroventral tip of the third ventricle. Alternatively, Il-1 could exert its effects through activating afferent pathways to the hypothalamus. The studies of Il-1-induced effects at the pituitary level are conflicting. Some authors have rejected a direct stimulatory effect upon the anterior pituitary, from experiments with primary cultures or mouse ACTH-secreting pituitary tumor cells (Berkenbosch et al. 1987; Sapolsky et al. 1987; Uehara et al. 1987). Other investigators, however, showed rather drastic ACTH-releasing effects applying Il-1 in both cell systems (Wolowski et al. 1985; Bernton et al. 1987). The recent observation that Il-1 (and Il-2) enhances the expression of POMCmRNA in both primary cultures and At-T_{20} cell lines in a similar manner to CRH speaks in favor of a direct effect of Il-1 upon corticotrophs, which may add to the indirect effect of Il-1 as a CRH-releasing factor. Further evidence for a physiological significance of Il-1 as a CRH-releasing factor comes from a recent report documenting that GCs also feed back negatively on Il-1 production by inhibiting transcription of the interleukin-1β gene and destabilization of the Il-1 mRNA (Lee et al. 1988).

The release of ACTH in response to immunostimulants is not restricted to the pituitary because Smith et al. (1986) showed that leukocytes produce ACTH after virus or endotoxin exposure as well as after specific stimulation of POMC gene expression by CRH. Moreover, ACTH and cortisol can be elevated in hypophysectomized humans after specific stimulation with CRH (Fehm et al. 1988). We recently studied the effect of a low subpyrogenic dosage of recombinant interferon-gamma (IFN-gamma) upon pituitary-adrenocortical function (Holsboer et al. 1988b). The major finding from this study was a significant increase of cortisol with maxima around 2–3 h after intravenous injection. We found no appreciable increase in ACTH and confirmed this pituitary-independent effect of IFN-gamma by applying a pituitary cell culture which did not respond to IFN-gamma exposure. Since the cortisol elevating response to IFN-gamma was abolished by pretreatment with dexamethasone, we ruled out a direct effect of the immunopeptide upon the adrenal cortex. We postulate that lymphocytes, when activated by IFN-gamma, release an adrenogenic compound which induces cortisol secretion from the adrenal cortex.

The studies referred to here suggest that an interaction exists between the immune system and all the elements of the LHPA axis, serving as a fine-tuned communication circuit. These observations could serve as a starting point for a scientifically based psychosomatic research program. For example, Schleifer et al. (1984) compared lymphocyte stimulation responses in husbands before and after the death of their wives. The lymphocyte function was significantly lower 2 months after bereavement when compared with the prebereavement levels. Another study compared natural killer (NK) cell activity in three groups of women (whose husbands had incurable lung cancer or had died from lung cancer, during the previous 6 months, and controls). It was found that NK activity decreased in women with major life changes and was minimal in depressed bereaved women; this decrease may be related to the observed increased mortality of bereaved spouses. These conditions are associated with intermittent or sustained CRH hyperactivity, which may be pathogenetically significant, because recently Irwin et al. (1987) demonstrated that CRH, when injected into the rat brain, suppresses NK cytotoxicity an effect which does not require peripheral LHPA hormones. It was recently shown that the non-coding region of the type 16 human papilloma virus (HPV-16) contains a glucocorticoid-responsive element, whose activation by a GC-receptor complex results in increased transcription. Pater et al. (1988) showed that GCs are necessary for an oncogenic transformation of primary cells exposed to

HPV-16 DNA and an activated Ha-ras oncogene. The clinical implications are only speculative; however, HPV-16 is associated with a high risk of cervical cancer, which may become clinically relevant among women experiencing conditions where hypercortisolism is part of the overall symptom pattern.

Conclusion and Future Perspectives

Establishing neuroendocrine profiles from psychiatric patients promised delineation of the central neurotransmitter/receptor disturbances underlying psychopathology. This concept had merit and fertilized psychiatry research for a considerable time. Now we are entering a new stage in clinical neuroendocrinology because refined bioanalytical techniques and molecular biology provide new means of studying the brain concurrently as a source and major target for hormones. Regulation of genes can be studied at various steps of their expression, including post-translational processing and in situ hybridization. Here modulation of expression of those genes which code for neuropeptides and receptors involved in mediation of mood and behavior is of particular interest. Steroid hormones, which bind to high-affinity (K_D about 1 n*M*) cytoplasmatic receptors are one major family regulating brain genes. Binding of the GC ligand to its receptor triggers conformational changes enabling the dimer receptor molecule to interact with high-affinity acceptor sites (glucocorticoid regulatory elements, GRE) on chromatin and DNA (Scheidereit and Beato 1984). The physiological response is then induced by activation or deactivation of adjacent promoters influencing transcriptional efficiency. By this route GCs control in a tissue-specific way expression of genes which are directly or indirectly involved in adrenocortical activity, such as genes coding for Il-1, CRH or POMC. At the same time other genes also involved in the mediation of behavior are regulated by GCs. These mechanisms are of general importance as cytosolic GC receptors were recently identified in brain monoaminergic neurons (Härfstrand et al. 1986) and may also be involved in regulatory effects of GCs on β-adrenoceptor-coupled adenylate cyclase (Mobley and Sulser 1980). The neurochemistry of brain amines is influenced by GCs in many ways (McEwen et al. 1986), including the possibility that they also interfere with the synthesis of adrenergic receptors (Fraser and Venter 1980) and second messenger systems (Sorensen 1987). Of particular interest here is the recent finding that β-adrenergic receptors are increased by GCs which increase the rate of receptor gene transcription. After antidepressant drug treatment a decreased number of β-receptors are almost invariably observed. Probably elevated corticosteroid levels counteract the psychotropic drug effect by transcriptionally enhancing β-adrenergic receptor synthesis and thus controlling the responsiveness of the receptor-coupled adenylate cyclase system to agonist stimulation (Collins et al. 1988).

Finally, steroids may affect membrane properties directly. Recent experiments employing voltage clamp techniques showed that several steroids potentiate the chloride currents evoked by GABA in a stereoselective way (Majewska et al. 1986). These steroids are A-ring reduced metabolites of progesterone and deoxycorticosterone and interact with the GABA receptor complex similar to barbiturates and potentiate BZD and muscimol binding. In contrast to BZDs these steroids enhance the chloride conductance by prolonging the mean burst duration of GABA gated chloride channels (Lambert et al. 1987). Other steroids, such as pregnenolone sulfate, which belongs to the 3β-hydroxy-delta-5-steroid family and bears an unsaturated A-ring, have GABA antagonist features which are consistent with its excitatory actions in the brain. The differential roles of GCs on neuronal excitability are particularly interesting, because one class of steroids for which a sole membrane effect has been documented can be synthesized in the rat and monkey brain. The implications of this novel finding, which led to the term "neurosteroid", are as yet poorly understood (Hu et al. 1987; Robel et al. 1987). Future research focusing on regulatory actions will concentrate on the question whether the effects induced by the neurosteroids are restricted to membrane effects, resulting in behavioral changes through modulation of neuronal firing rates. If genomic effects are also involved, then the brain would be able to preserve the whole repertoire of neuronal regulation by steroids even in the absence of a functioning adrenal cortex.

While basic designs for the structure and pattern of neuronal connections are carried in the genes there is no doubt that genetic factors do not always act alone. Experience-related factors account for the actual final clinical phenotype. One language used to communicate stressful experiences is by responding with altered neuroendocrine activity of the LHPA system, which is most vitally involved in adaptation to stress and frequently altered in affective disorders.

Converging efforts in clinical and molecular studies of corticosteroid action in brain function also have profound bearings upon the genetic approach to psychiatric disorders. It remains highly questionable whether psychopathological evaluation alone can define sufficiently reliable clinical phenotypes. Future familial studies will benefit if the search for a genetic basis of psychiatric diseases encompasses neurobio-

logical characteristics, such as baseline measures (e.g. neuroendocrine profiles, sleep EEG) or challenge responses (e.g. suppression or stimulation of hormones or neurophysiological measures). Such studies have to be conducted among recovered patients and unaffected relatives from high-risk pedigrees in order to differentiate between true traits and physiological scars secondary to homeostatic turmoil during preceding affective episodes. The neurobiological characteristics may cut across traditional diagnostic boundaries in so far as they occur most frequently among the syndromes under study. Since measurable aberrancies of the LHPA system are frequent among psychiatric patients and because progress in molecular and cellular neurobiology demonstrates that corticosteroids are potently involved in genomic and nongenomic brain physiology, studying this class of hormones simultaneously on a clinical, cellular and molecular level will significantly promote experimental genetics in psychiatry.

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