Stress Hormone Responses to Corticotropin-Releasing Hormone in Substance Abusers without Severe Comorbid Psychiatric Disease

Carlo Contoreggi, Ronald I. Herning, Paul Na, Philip W. Gold, George Chrousos, Paulo J. Negro, Warren Better, and Jean L. Cadet

Background: *Preclinical data indicate a crucial role of stress in the acute effects of drugs of abuse, maintenance of self-administration, and susceptibility to relapse. Stress system activation may serve as a marker for a neurochemical dysfunction with prognostic significance in patients with addiction.*

Methods: *We tested pituitary adrenocorticotrophin (ACTH) and adrenal cortisol response to ovine corticotropin-releasing hormone (oCRH) to assess the reactivity of the hypthalamic–pituitary–adrenal (HPA) axis in seven nonsubstance-abusing subjects, 31 polysubstance-abusing subjects without depressive symptoms, and seven subjects with substance abuse and depressive symptoms. No subject met diagnostic criteria for depression or other severe psychiatric disease.*

Results: *Compared with normal control subjects, substance abusers showed significantly lower ACTH and cortisol responses over the course of oCRH stimulation ($p < .0001$). Substance abusers with depressive symptoms showed similarly blunted responses.*

Conclusions: *Polysubstance abusers with no past or current diagnosis of other Axis I disorders show blunted ACTH and cortisol responses to oCRH administration. The finding of an activated HPA axis in this population suggests an overlapping role of central CRH and HPA axis activation in affective disorders and substance abuse, which is likely to constitute an endocrine milieu necessary for the maintenance of addictive behavior. These data support the role of future therapeutic trials with nonpeptide CRH receptor 1 antagonists in these patients.* Biol Psychiatry 2003;54:873–878 © *2003 Society of Biological Psychiatry*

From Brain Imaging (CC) and Molecular Neuropsychiatry Sections (RIH, PN, WB, JLC), Intramural Research Program, National Institute on Drug Abuse; University of Maryland School of Medicine (PJN), Baltimore, Maryland; The Clinical Neuroendocrinology Branch (PWG), National Institute of Mental Health; and the Pediatric and Reproductive Endocrinology Branch (GC), National Institute of Child and Human Development, Bethesda, Maryland.

Address reprint requests to Carlo Contoreggi, M.D., Brain Imaging Branch, 5500 Nathan Shock Drive, Baltimore MD 21224.

Received April 11, 2002; revised September 30, 2002; revised January 7, 2003; accepted January 10, 2003.

Key Words: Corticotropin-releasing hormone, adrenocorticotrophin, cortisol, substance abuse

Introduction

Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis has been described in many psychiatric and somatic disorders. Earlier studies have demonstrated that substance abusers have an altered hormonal stress response to cognitive (Errico et al 1993; Lovallo et al 2000) and environmental stressors (Vescovi et al 1997), as well as to administration of central-acting pharmacologic probes (Coiro et al 1999; Kemper et al 1990; Stine et al 2001). Previously characterized as an epiphenomenon, peripheral alterations in stress hormones were thought to be a consequence rather than a cause of addiction. The discovery of corticotropin-releasing hormone (CRH) as a peptide neurotransmitter in the central nervous system has led to investigation of its role in addiction. The important role of CRH in activation of the central stress system in settings of acute withdrawal from drugs of abuse (Rodriguez de Fonseca et al 1997), as well as its importance in mediating relapse (Shaham et al 1998, 2000), has led to the re-assessment of the peripheral stress hormones as central markers for this and related neuropeptides in addiction.

The discovery of CRH by Vale et al (1981) and its related receptors in the central nervous system by De Sousa and associates (De Souza 1987; De Souza et al 1984; Grigoriadis et al 1989, 1993) opened a line of investigation into how CRH integrates the stress response in both the brain and the endocrine system. In 1986, Gold and associates made a cardinal observation that, in depressed patients, the pituitary showed a blunted response to stimulation with exogenous ovine (o)CRH (Gold et al 1986). This finding was related to elevated concentrations of CRH in the cerebrospinal fluid and pointed to CRH as a central mediator of the peripheral stress response. This finding of a blunted pituitary adrenocorticotrophin (ACTH) response to oCRH demonstrates one component

© 2003 Society of Biological Psychiatry

0006-3223/03/$30.00
doi:10.1016/S0006-3223(03)00167-7

of the integration of the HPA axis. The oCRH test is an important diagnostic tool to differentiate Cushing's disease, Cushing's syndrome, and psuedo-Cushing's syndrome. Complete assessment of the HPA axis has been performed in pseudo-Cushing's syndrome psychiatric patients. Other abnormalities of hormone secretion, including excessive 24-hour cortisol production, loss of normal circadian hormone rhythms, and blunted adrenal responsiveness to exogenously administered cosyntropin (ACTH) and altered pituitary feedback to dexamethasone have all been reported. This area has recently been reviewed (Meier and Biller 1997; Newell-Price et al 1998).

Recently developed nonpeptide antagonists of CRH have been shown to be powerful medications for interrupting maladaptive behaviors in preclinical behavioral models (Habib et al 2000) and for treating depressed patients (Zobel et al 2000). Preclinical data indicate a role for HPA hormones in vulnerability for substance abuse. The reactivity of the HPA axis has been found to predict both the durability of remission for patients with depression and the necessity for continued medical therapy (O'Toole et al 1997). In settings of affective and addictive disorders, the presence of subtle neuroendocrine abnormalities in the regulation of the HPA axis with hypersecretion of cortisol may be a marker for a chemical condition for which therapeutic intervention is possible.

In this study, we tested the pituitary ACTH and adrenal cortisol response to oCRH to assess the reactivity of the HPA axis in substance-abusing and non–substance-abusing volunteers. We screened and tested a cross section of volunteers with substance abuse who did not meet diagnostic criteria for depression or other severe psychiatric disease.

Methods and Materials

Subjects

Both substance-abusing and nonsubstance-abusing volunteers were recruited from the community by paid advertisements, posted flyers, and by word of mouth. All candidate volunteers were screened for study participation over several visits by a third-party contract recruiter. Seven control subjects, 31 substance abusers (SA), and seven substance abusers with depressive symptoms (SA/DEP) were studied. Subjects in this latter group had a Symptom Check List 90–Revised (SCL-90R) depression scale score of 60 or higher. Demographic data and drug use histories were obtained by using the Addiction Severity Index (McLellan et al 1986).

All subjects underwent extensive medical, neurologic, psychological, urine toxicology, and laboratory evaluations before being admitted to the study. This included medical history, physical examination, electrocardiogram, tuberculin skin test, complete blood count, chemistry profile, thyroid panel (including thyrotrophin stimulating hormone), serology for hepatitis B, syphilis, and human immunodeficiency virus type 1 (HIV-1). Pretest counseling was performed and consent was obtained before HIV-1 testing. A minimum of three outpatient visits was necessary for completion of the initial medical and psychological screening. Exclusion criteria were 1) major current or chronic medical or psychiatric illnesses; 2) head injuries with loss of consciousness for longer than 5 min; 3) evidence of any neurologic abnormalities by history or structured examination; 4) current medication usage, including antidepressants, benzodiazepines, or antihypertensives; and 5) HIV positivity. The National Institute on Drug Abuse and Johns Hopkins Bayview Medical Center Institutional Review Boards approved the research protocol for human research. Before study participation, all subjects signed an informed consent form approved by the institutional review boards.

Procedures

Substance abusers were administered the oCRH with sampling after admission to the research unit. Control subjects were tested as outpatients. Substance-abusing subjects underwent psychiatric testing during the screening intake period as outpatients before admission to the inpatient unit. Psychiatric symptoms were measured during screening procedures using the SCL-90R (Derogatis 1983) and the Diagnostic Interview Schedule (DIS) (Robins et al 1988). The DIS is a structured interview that was used to obtain psychiatric diagnoses according to the DSM-III-R. The psychological assessments included in the SCL-90R scales were as follows: somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid, and psychoticism. In addition to the SCL-90R, the Diagnostic Inventory Scale, the Beck Hopelessness questionnaire (Beck et al 1974), and the Ellison Well-Being Measures (Ellison 1983) were administered. The SCL-90R was obtained during screening procedure before the subject was admitted to the study. The Beck and Ellison tools were administered within 5–72 hours of admission, when the subjects were not intoxicated. The DIS was administered after the oCRH session. No subject met either current or past DSM-III-R criteria for Axis I disorders on the DIS interview (Robins et al 1988; Helzer and Robins 1988) except for substance abuse and dependence. Additional analysis was performed on individual DIS items, including tiredness, slow/restless, loss of interest, thoughts of death, worthlessness, trouble thinking, and sleep. The Ellison Well-Being Measures tested for general and spiritual well-being. Substance abusers were not abstinent during screening, and there was no explicit or implicit message given the volunteers to either stop or continue drug use during the screening process. Both continued use of substance and abstinence might alter the HPA as well as the psychological state. The timing of psychological testing was considered to reflect the basal state of the participants' free living in the community, independent of forced abstinence.

To better characterize and stratify the presence and severity of neuroendocrine aberrations of the HPA axis, oCRH stimulation tests were performed in the early afternoon. Ovine CRH was administered at a dose of 1 μg/kg (to the nearest 5 μg). Serial plasma samples were obtained through an indwelling intravenous

Table 1. Demographic and Drug History Measures for the Control Subjects, Drug Abusers, and Drug Abusers with Depression

	Control	Substance Abusers	Substance Abusers/Depressed
Demographic Measures			
Age (years)	28.7 ± 9.1	34.5 ± 5.6	32.7 ± 5.3
Education (years)	13.2 ± 1.8	12.3 ± 1.6	12.9 ± 3.6
Male (%)	85.7	87.1	85.7
African Americans (%)	85.1	96.5	100.0
Drug History			
Cocaine days[a]		17.1 ± 7.3 (97)[b]	18.2 ± 9.5 (86)[b]
Cocaine years		9.7 ± 4.1 (97)[c]	9.3 ± 6.2 (86)[c]
Alcohol days	2.3 ± 2.3 (42)[b]	9.6 ± 6.3 (97)[b]	11.0 ± 9.4 (100)[b]
Alcohol years	6.1 ± 5.3 (86)[c]	13.2 ± 6.7 (100)[c]	13.8 ± 3.5 (100)[c]
Heroin days		11.6 ± 10.2 (64)[b]	17.0 ± 14.7 (42)[b]
Heroin years		4.9 ± 4.9 (71)[c]	8.8 ± 8.4 (42)[c]
Marijuana days		9.2 ± 9.9 (58)[b]	1.5 ± .5 (57)[b]
Marijuana years		12.6 ± 7.8 (94)[c]	16.7 ± 7.4 (86)[c]
Cigarettes/day	5.0 ± 1.4 (28)[b]	18.0 ± 13.2 (87)[b]	19.2 ± 18.0 (71)[b]
Cigarette years	14.0 ± 2.8 (28)[c]	17.3 ± 5.9 (90)[c]	23.0 ± 2.9 (71)[c]

Data presented as mean ± SD (%).
[a]Days indicate the number of days this substance was used during the last 30 days.
[b]Percentage of subjects using this substance in the last 30 days.
[c]Percentage of subjects ever using this substance.

catheter. Eleven pre-heparinized, 10-mL vacutainers were used to collect blood samples at −30, −15, −5, 0, 15, 30, 60, 90, 120, 150, and 180 min. Outpatient control subjects had their oCRH study performed during the first week of participation, after providing consent. Substance abusers who were admitted to the inpatient research unit participated in the oCRH test portion during the first week of stay on the inpatient research unit (SA, 2.8 ± 4 days postadmission; SA/DEP, 3.4 ± 3.8 days). The testing was scheduled to permit inpatient participants to acclimate for several days to the residential unit. Subjects were housed in a locked inpatient unit with no outside peer contact. Periodic urine toxicology was performed to verify abstinence from illicit substances.

Hormone Preparation

Ovine CRH was obtained from Bachem (Torrance, CA). The purified synthetic hormone was supplied as a lyophilized powder compounded by the Pharmaceutical Development Service (PDS) at the Clinical Center, National Institutes of Health. Analysis of purity was performed by the PDS. The hormone was stored in its lyophilized form at room temperature. The hormone was prepared immediately before intravenous administration in pyrogen-free sterile saline as single-dose vials.

Hormone Analyses

All samples were drawn, immediately placed on ice, and subsequently spun in a refrigerated centrifuge at 5°C at 3000 rpm for 10 min. Samples were then immediately frozen and stored at −70°C. until assay. Hormone assays were performed at Hazelton Laboratories (Reston, VA).

Statistical Analyses

A group (control, SA, SA/DEP) × time (four pre- and seven post-stimulation values) analysis of variance (ANOVA) was used to test for differences in ACTH and cortisol levels. Area under the curve (AUC) was calculated for the baseline and postinjection hormone value. A group (control, SA, SA/DEP) × time (pre- and poststimulation) ANOVA was used to test for differences in ACTH and cortisol AUC levels. Post hoc comparisons among group means were made using the least significant difference method.

Results

Means and SDs for demographic characteristics and drug use history for control, SA, and SA/DEP groups are listed in Table 1. The substance abusers listed cocaine as their substance of choice but were in all cases polysubstance abusers. All substance-abusing participants met criteria for polysubstance abuse or dependence. There were no significant differences in any of the demographic data between the three groups and no differences in self-reported substance use history between the SA and SA/DEP groups. Table 2 shows the group psychological profiles, which included items from the SCL-90R, DIS, Beck Hopelessness questionnaires, and Ellison Well-Being Measures. There were no significant differences between the control and SA groups, though the SA/DEP group showed higher scores indicative of current psychiatric symptoms. The SA/DEP group scored significantly worse ($p < .05$) on the SCL-90R Global Severity Index, DIS thoughts of death, Ellison general well-being, and the Beck Hopelessness scale (see Table 2). Table 3 shows the current diagnoses for alcohol, cocaine, and heroin abuse, as well as the listed drugs of choice for the SA and SA/DEP participants and self-reported duration of abstinence.

Table 2. SCL-90R, DIS Items, Beck Hopelessness, and Ellison Well-Being Measures for the Control Subjects, Substance Abusers, and Substance Abusers with Depression

	Control Subjects	Substance Abusers	Substance Abusers/Depressed
SCL-90R			
Global Severity Index	43.3 ± 8.0	44.0 ± 8.8	62.1 ± 3.1[a]
DIS Depression Items			
Tired	.00 ± .00	.06 ± .25	.00 ± .00
Slow/restless	.33 ± .50	.10 ± .54	.11 ± .33
Lost interest	.56 ± .73	.32 ± .65	.89 ± .93
Thoughts of death	.22 ± .22	.29 ± .82	1.33 ± 1.58[a]
Worthlessness	.33 ± .70	.23 ± .50	.67 ± 1.00
Trouble thinking	.11 ± .33	.16 ± .45	.11 ± .33
Sleep	.13 ± .34	.11 ± .34	.20 ± .33
Ellison Well-Being			
General	52.1 ± 6.4	47.9 ± 7.2	42.7 ± 9.6[a]
Spiritual	49.6 ± 7.8	52.2 ± 7.2	49.8 ± 6.9
Beck Hopelessness	2.1 ± 1.6	1.5 ± 1.5	3.7 ± 2.7[a]

Data presented as mean ± SD. SCL-90R, Symptom Check List 90–Revised; DIS, Diagnostic Interview Schedule.
[a]Value significantly higher than others ($p < .05$).

Adrenocorticotrophin stimulation data can be found in Figure 1 (top). Baseline values of ACTH (at −30, −15, −5, and 0 min) in control subjects were not significantly different from values in the SA or SA/DEP groups ($p = .457$ for both groups). Multivariate analysis showed significantly higher ACTH responses over the course of the experiment in the nonsubstance-abusing group (both SA and SA/DEP $p < .0001$).

Cortisol stimulation data can be found in Figure 1 (bottom). Nonsubstance-abusing participants had a trend toward lower basal cortisol than either the SA or SA/DEP groups ($p = .064$ and .056, respectively) after averaging prestimulation cortisol values (at −30, −15, −5, and 0 min). The multivariate analysis showed significantly higher cortisol response over the course of the experiment in the nonsubstance-abusing group (both SA and SA/DEP $p < .0001$).

Table 3. Current Diagnoses for Alcohol, Cocaine, and Heroin Abuse/Dependence as Well as the Listed Drugs of Choice of the Substance-Abusing and Substance-Abusing/Depressed Participants

	Substance Abusers	Substance Abusers Depressed
Current DSM-III-R Diagnoses (*n*)		
Alcohol abuse	1	
Cocaine abuse	10	1
Cocaine/heroin abuse	1	1
Cocaine dependence	12	1
Heroin dependence	2	1
Alcohol/cocaine dependence		2
Alcohol/heroin/cannabis dependence		1
Drug of Choice (*n*)		
Cocaine	27	5
Heroin	2	1
Marijuana	2	
Alcohol		1
Days Abstinent at Time of Testing (mean ± SD)	5.2 ± 3.4	6.0 ± 2.6

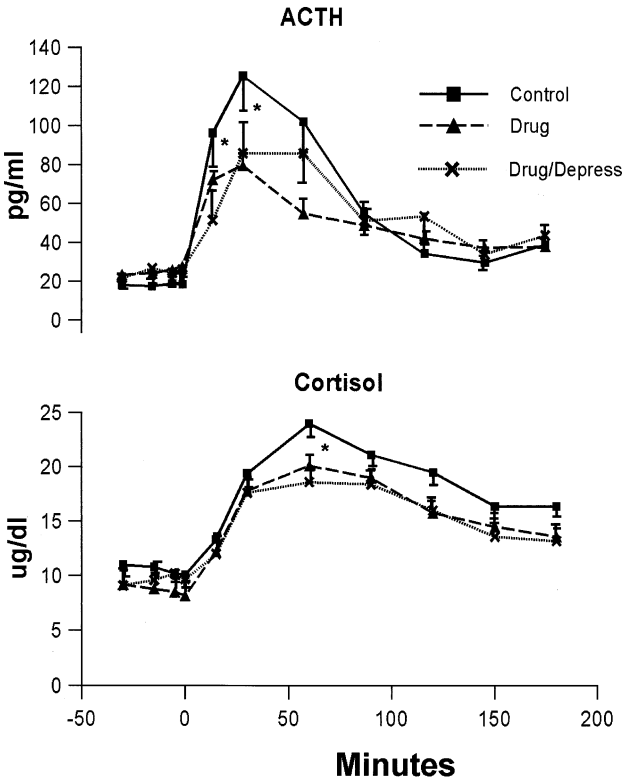

Figure 1. The mean and standard errors for ACTH and cortisol levels for the three groups are plotted at the top for the four baseline and seven post-injection observations. The mean and standard errors for cortisol levels for the three groups are plotted at bottom for the four baseline and seven post-injection observations. An asterisk indicates significant comparisons.

Univariate analyses were performed to compare the nonsubstance abusers with the SA and SA/DEP groups for each time point post-oCRH administration for ACTH. The 15- and 30-min times were the only two points that were significantly different for ACTH ($p < .0001$ and $p = .030$ for SA and SA/DEP, respectively at 15 min and $p < .0001$ and $p = .038$ at 30 min). No other point differences were seen postadministration of oCRH.

Discussion

This study shows that substance abusers with no previous or current diagnosis of affective or other Axis I psychiatric disorders present with a blunted ACTH and cortisol response to oCRH administration. Substance abusers with symptoms of depression, though not meeting diagnostic criteria for depressive disorders, also show blunted stress responses. Blunted stress hormone responsiveness to oCRH is a reliable marker for an activated HPA axis (Meier and Biller 1997; Newell-Price et al 1998).

Previous clinical studies have drawn mixed conclusions about the impact of substance abuse on the HPA axis. Preclinical literature indicates that stress is crucial for the acute effects, maintenance of self-administration, and susceptibility to substance relapse (Cador et al 1993). Chronic maladaptive changes in stress axis function are persistent in substance-abuse models and appear to be mediated through the action of the neuropeptide neurotransmitter, CRH. Central CRH administration has been shown to potentiate locomotor hyperactivity induced by cocaine administration (Sarnyai et al 1992). Susceptibility to stress-induced relapse persists long after detoxification and cessation of substance use (Sarnyai et al 1995; Shaham et al 1998, 2000).

Hypothalamic–pituitary–adrenal axis function has been assessed in a variety of psychiatric and somatic disorders. The stress of chronic disease elevates total cortisol output, breaks down normal circadian diurnal rhythms, and induces the failure to suppress endogenous cortisol secretion after administration of exogenous glucocorticoids, (i.e., dexamethasone). Psychiatric literature has documented a hyperactive HPA axis in major depression and other Axis I disorders, frequent comorbid conditions with substance abuse (Gold et al 1986; O'Toole et al 1997; Newell-Price et al 1998). The finding of chronically activated HPA axis in patients with substance abuse suggests an overlapping role for the CRH system in substance abuse disorders. Drug abuse and dependence activates the HPA, and the associated central activation of the stress system may provide the neuroendocrine milieu necessary for the maintenance of the addictive behavior. Extrapolation of preclinical data suggests that stimulation of the HPA axis in substance abusers is an important component of addictive disorders.

The limits of this study are primarily in the reliability of the self-report information provided by the SA and SA/DEP participants. The participants may have provided inaccurate drug use history and current psychological symptoms to accommodate a perceived bias of the investigators. Second, the timing of the hormone testing was consistently performed in the early afternoon, but the time from admission to testing varied owing to the uncertainties and reliability of the participants to present for inpatient admission. Finally, the activation of the HPA axis in cocaine-preferring substance abusers reported here represented a single time point after about 5 days of abstinence. The time course of this HPA activation requires further study. These substance abusers self-reported preferring cocaine and using cocaine more frequently than other drugs.

It is conceivable that endogenous set point(s) for stress activation and its somatic consequences predispose individuals to develop motivational disorders, such as substance abuse and dependence. After decades of trying to segregate psychiatric illnesses and addiction, our data suggest that they may share similar neuroendocrinologic underpinnings. These data, though limited, point to the commonalties of HPA derangements in these disorders. Corticotropin-releasing hormone released from the hypothalamus activates pituitary release of ACTH subsequent to adrenal cortisol secretion. How the neuropeptide CRH coordinates the central activation to stress is unknown, but such nonpeptide antagonists as antalarmin (Wong et al 1999) should prove valuable as probes, as have pharmacologic probes for serotonin and dopamine. Corticotropin-releasing hormone and its receptors are concentrated in limbic structures and neocortex (especially frontal and prefrontal areas) of the primate brain. Its location in these areas suggests that CRH plays an important role in the cognitive and motivational processes that are disrupted in addiction. These data and basic science studies would indicate that the stress system is as an important factor in substance abuse. Demonstration of the HPA axis abnormalities in substance abusers independent of other psychiatric conditions would suggest that clinical trials of nonpeptide CRH antagonists may be beneficial in treating stress dysfunction when seen in substance abuse, withdrawal, and relapse.

References

Beck AT, Weissman A, Lester D, Trexler L (1974): The measurement of pessimism: The hopelessness scale. *J Consult Clin Psychol* 42:861–865.

Cador M, Cole BJ, Koob GF, Stinus L, Le Moal M (1993): Central administration of corticotropin releasing factor in-

duces long-term sensitization to D-amphetamine. *Brain Res* 606:181–186.

Coiro V, Vescovi PP (1999): Effect of cigarette smoking on ACTH/cortisol secretion in alcoholic after short- and medium-term abstinence. *Alcohol Clin Exp Res* 23:1515–1518.

Derogatis LR (1983): *Administration, Scoring, and Procedures for SCL-90R: Manual II.* Towson, MD: Clinical Psychometric Research.

De Souza EB (1987): Corticotropin-releasing factor receptors in the rat central nervous system: Characterization and regional distribution. *J Neurosci* 7:88–100.

De Souza EB, Perrin MH, Insel TR, Rivier J, Vale WW, Kuhar MJ (1984): Corticotropin-releasing factor receptors in rat forebrain: Autoradiographic identification. *Science* 224: 1449–1451.

Ellison CW (1983): Spiritual well-being: Conceptualization, and measurement. *J Psychol Theol* 11:330–340.

Errico AL, Parsons OA, King AC, Lovallo WR (1993): Attenuated cortisol response to biobehavioral stressors in sober alcoholics. *J Stud Alcohol* 54:393–398.

Gold P, Loriaux D, Roy A, Kling M, Calabrese J, Kellner C, et al (1986): Responses to corticotrophin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *N Engl J Med* 314:1329–1335.

Grigoriadis DE, Heroux JA, De Souza EB (1993): Characterization and regulation of corticotropin-releasing factor receptors in the central nervous, endocrine and immune systems. *Ciba Found Symp* 172:85–101.

Grigoriadis DE, Zaczek R, Pearsall DM, De Souza EB (1989): Solubilization of high affinity corticotropin-releasing factor receptors from rat brain: Characterization of an active digitonin-solubilized receptor complex. *Endocrinology* 125: 3068–3077.

Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, et al (2000): Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. *Proc Natl Acad Sci U S A* 97:6079–6084.

Helzer JE, Robins LN (1988): The diagnostic interview schedule: Its development, evolution, and use. *Soc Psychiatry Psychiatr Epidemiol* 23:6–16.

Kemper A, Koalick F, Thiele H, Retzow A, Rathsack R, Nickel B (1990): Cortisol and beta-endorphin response in alcoholics and alcohol abusers following a high naloxone dosage. *Drug Alcohol Depend* 25:319–326.

Lovallo WR, Dickensheets SL, Myers DA, Thomas TL, Nixon SJ (2000): Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcohol Clin Exp Res* 24:651–658.

McLellan AT, Luborsky L, Cacciola J, Griffith J, McGaham P, O'Brien CP (1986): *Guide to the Addiction Severity Index: Background, Administration, and Field Testing Results.* Rockville, MD: National Institute on Drug Abuse, Treatment Research Reports.

Meier CA, Biller BM (1997): Clinical and biochemical evaluation of Cushing's syndrome. *Endocrinol Metab Clin North Am* 26:741–762.

Newell-Price J, Trainer P, Besser M, Grossman A (1998): The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev* 19:647–672.

O'Toole SM, Sekula LK, Rubin RT (1997): Pituitary-adrenal cortical axis measures as predictors of sustained remission in major depression. *Biol Psychiatry* 42:85–89.

Robins S, Helzer JE, Cuttler L, Golding E (1988): *National Institute of Mental Health Diagnostic Interview Schedule Version III-R.* Washington, DC: U.S. Government Printing Office.

Rodriguez de Fonseca F, Carrera MRA, Navarro M, Koob GF, Weiss F (1997): Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 276:2050–2054.

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1995): Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. *Brain Res* 675:89–97.

Sarnyai Z, Hohn J, Szabo G, Penke B (1992): Critical role of endogenous corticotropin-releasing factor (CRF) in the mediation of the behavioral action of cocaine in rats. *Life Sci* 51:2019–2024.

Shaham Y, Erb S, Leung S, Buczek Y, Stewart J (1998): CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology (Berl)* 137:184–190.

Shaham Y, Erb S, Stewart J (2000): Stress-induced relapse to heroin and cocaine seeking in rats: A review. *Brain Res Brain Res Rev* 33:13–33.

Stine SM, Grillon CG, Morgan CA 3rd, Kosten TR, Charney DS, Krystal JH (2001): Methadone patients exhibit increased startle and cortisol response after intravenous yohimbine. *Psychopharmacology (Berl)* 154:274–281.

Vale W, Spiess J, Rivier C, Rivier J (1981): Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213:1394–1397.

Vescovi PP, DiGennaro C, Coiro V (1997): Hormonal (ACTH, cortisol, beta-endorphin, and met-enkephalin) and cardiovascular responses to hyperthermic stress in chronic alcoholics. *Alcohol Clin Exp Res* 21:1195–1198.

Wong ML, Webster EL, Spokes H, Phu P, Ehrhart-Bornstein M, Bornstein S, et al (1999): Chronic administration of the non-peptide CRH type 1 receptor antagonist antalarmin does not blunt hypothalamic-pituitary-adrenal axis responses to acute immobilization stress. *Life Sci* 65:PL53–PL58.

Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, Holsboer F (2000): Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: The first 20 patients treated. *J Psychiatr Res* 34:171–181.