

The Dissociation of Catecholamine and Hypothalamic-Pituitary-Adrenal Responses to Daily Stressors Using Dexamethasone*

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ABSTRACT

The hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) systems are implicated in the human stress response. One characterization of these systems is that they are nonspecific in their response, but differ in activation threshold and time course. Additionally, they have been found to be affected commonly by strong metabolic stressors and infusions of CRH, and a hypothesis has been developed primarily from animal research that CRH stimulates both the HPA and SAM systems.

To determine whether CRH was significantly involved in tonic as well as psychological stress-induced catecholamine levels in man, we infused 24 normal male undergraduate students with either saline

(n = 12) or dexamethasone (DEX; n = 12) and evaluated their subsequent plasma levels of ACTH, cortisol, epinephrine (EPI), and norepinephrine (NEPI). DEX produced a dramatic decrease in ACTH and cortisol levels, but no significant changes in EPI or NEPI occurred over a 4-h sampling interval. After the administration of math and speech stressors in a controlled laboratory setting, DEX inhibited the ACTH and cortisol release that was noted in the saline group, but stress-induced increases in EPI and NEPI were comparable in both groups.

Thus, our study suggests that there is a difference in the neural pathways for tonic and stress-induced stimulation of the SAM and HPA systems. (*J Clin Endocrinol Metab* 80: 2458-2463, 1995)

STRESS EVOKES a variety of adaptational responses, including stimulation of the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic adrenal medullary (SAM) system. In numerous animal and human studies, metabolic, physical, and psychological stressors activate both of these systems (1, 2). In our work (3-6) and that of others (7, 8), however, a more complicated picture emerges which suggests that the HPA axis and the SAM axis can be differentially stimulated by psychological stress. In our studies using math, speech, examination, and marital conflict, stress stimulation of the SAM axis always occurs, whereas the HPA axis, as evidenced by cortisol levels, was often unchanged (3-6). We have interpreted these data to suggest that the threshold for stimulation of the SAM axis is lower than that for stimulation of the HPA axis and that individual differences in neuroendocrine response to stress are more often expressed as differences in HPA activation.

We also noted that individuals whose sympathetic control of the cardiovascular system was more reactive to stress, as evidenced by a greater decrease in the prejection period, were more likely to show activation of the HPA system, whereas their catecholamine levels were comparable to those in low reactors (3, 5). In these studies changes in cortisol concentration were related to coincident changes in the immune system, whereas catecholamine-immune relationships were less likely to be found (9).

These studies appear to conflict with a variety of animal studies that suggest that brain CRH-secreting neurons may stimulate not only the HPA axis but also the SAM system. CRH-immunoreactive fibers (10) and receptors (11) are localized in the locus ceruleus, a brain nucleus that contains almost half of the brain norepinephrine (NEPI) neurons (12). Also, CRH, when centrally administered, increased the firing rate of locus ceruleus neurons, elevated central and peripheral levels of catecholamines, and increased sympathetic outflow (13-15). Additionally, adrenalectomy promoted immobilization-induced increments in central and peripheral NEPI levels, an effect that was inhibited by glucocorticoids (16, 17). Therefore, a unifying hypothesis has been developed that activation of CRH-containing central nervous system neurons after robust physical and metabolic stressors could be influencing both the HPA axis and catecholamine responses in animals.

Other investigations, however, suggest that CRH in the central nervous system does not constitute a single system, as glucocorticoids may have differential effects on CRH neurons (18-20). Also, in a recent study by Komesaroff and Funder (7), dexamethasone (DEX) given to sheep inhibited the EPI response to hypoglycemia, but did not alter the EPI response to audiovisual stress. This observation is surprising, in that hypoglycemia would appear to be a stronger stress than audiovisual stress for hormonal release and suggested that different pathways were operative in the SAM and HPA responses to metabolic vs. psychological stress.

To investigate whether CRH contributes to tonic or psychological stress-induced catecholamine secretion, we administered iv DEX or saline to normal undergraduate students. After a 4-h baseline period, subjects were administered several brief

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psychological stressors. The study required that the stressors stimulate both the HPA axis as well as the SAM system and that the DEX dose be adequate to suppress tonic and stress-induced HPA secretion. These conditions would allow us to test the hypothesis that if glucocorticoids inhibit CRH secretion, they would also modify catecholamine responses to these daily stressors.

Subjects and Methods

Subjects

Potential male subjects were recruited via the Ohio State University campus newspaper. Interested participants completed a prescreening questionnaire. Inclusion criteria required that subjects 1) be in good health, with neither parent having a history of hypertension; 2) be within 20% of their ideal weight; 3) consume, on the average, less than 15 alcoholic beverages/week; 4) not be math, speech, or needle phobic; 5) not be depressed, as evidenced by scores less than 7 on the short form of the Beck depression scale (21); and 6) not be experiencing a major life trauma (e.g. death of a family member). Twenty-four subjects met this inclusion criteria and agreed to be tested at the Clinical Research Center (CRC) at the Ohio State University Hospitals. The mean age of the subjects was 20.29 yr ($SEM = 0.35$). Twenty-three subjects were Caucasian, and one was African-American. Subjects were paid \$75.00 for 6 h of participation.

Procedure

The day before their arrival at the CRC, subjects were asked to 1) reschedule their appointment if they became ill, experienced a major negative event (e.g. death of a family member), or had a quiz or exam on the day of the study; 2) not consume any alcoholic beverages; 3) refrain from any exercise; and 4) refrain from eating or drinking anything but water from midnight until their scheduled appointment.

All subjects arrived at the CRC at approximately 0700 h. Upon their arrival, the experimental procedures were reviewed, all questions were answered, and informed consent was obtained. Subjects then completed a health background questionnaire that assessed their exercise habits, sleep during the last 3 days, caffeine consumption, experiences of colds, and whether they were taking any medication. They also completed the short form of the Beck depression inventory (21). No subject evidenced depression on the day of the study (Beck depression survey scores, <7), and responses did not differ between subjects who were subsequently randomly assigned to the DEX or saline (SAL) condition.

After completion of these questionnaires, an 18-gauge indwelling catheter was inserted into an antecubital vein on the subject's left arm. After venipuncture, subjects were asked to sit and relax for the next 20 min. A blood sample was collected at the end of 20 min for endocrine assays. Immediately following the baseline blood drawing, subjects received either a 4-mg bolus of DEX or 1 mL SAL, both iv push administered over 3 min using a double blind procedure. Blood for endocrine assays was drawn 0.5, 1, 2, 3, and 4 h after the administration of either SAL or DEX. Subjects remained seated throughout these periods.

Four hours after the DEX infusion, subjects received instructions and performed a 6-min speech and a 6-min math stressor, counterbalanced for order. Ordering of the stressors did not appreciably affect the results and will not be discussed further. Blood was drawn for endocrine assays immediately at the end of each stressor. The second stressor was initiated after the completion of the first stressor blood draw. The endocrine measures obtained at 4 h served as the prestress baseline, the measures obtained after the first of the two stressors served as the midstress response, and the measures obtained after the second stressor served as the poststress response.

Speech stressor

The speech stressor (22) asked subjects to imagine themselves in a department store shopping when a security guard falsely accused them

of shoplifting a belt. Subjects were instructed to prepare a 3-min speech to 1) tell their side of the story, 2) tell why the security guard suspected him of shoplifting, 3) indicate why the guard was wrong, 4) provide proof that he did not steal the belt, 4) suggest what should happen to the security guard for his/her mistake, and 5) summarize the main points. To increase the stressfulness of the situation, subjects were told that their responses would be recorded and compared to the speeches of other subjects. Subjects were given 3 min to prepare and 3 min to present their speeches.

Math stressor

Subjects were instructed to perform six 1-min serial subtraction problems. The subjects were asked to subtract 3 from 2907, 7 from 6828, 13 from 9561, 8 from 5113, 14 from 8318, and 17 from 9994 for trial 1 through 6, respectively. Subjects were told that any mistakes would be corrected by the experimenter, and that they should proceed from the correct number. To maintain maximal task involvement and moderate levels of difficulty while maintaining similar levels of performance across conditions (~10 correct answers/trial), subjects were instructed to go faster and with greater accuracy during the start of trials 3 and 5. Two measures of task performance were calculated: the number attempted and the percentage correct. These math and speech stressors have been used and characterized by others (22) as well as by our laboratory (23-25), and they have demonstrated high intra- and intertask consistency (23-25).

Measures

Assays for EPI, NEPI, ACTH, and cortisol were performed using plasma from the blood samples drawn at baseline, after the first stressor (i.e. 6 min after the onset of the stressors), and after the second of the two stressors (i.e. 12 min after the onset of the stressors). Plasma ACTH levels were assayed using immunoradiometric assays supplied by Nichols Institute (San Juan Capistrano, CA). This assay has intra- and interassay coefficients of variation less than 10%, and the sensitivity is 1 pg/mL. Plasma cortisol levels were assayed using a fluorescent polarization technique (TDX, Abbott Laboratory, Chicago, IL). This assay has intra- and interassay coefficients of variation less than 10%. Plasma catecholamine concentrations (EPI and NEPI) levels were determined by high performance liquid chromatography, using a Waters system with an electrochemical detector (Waters Associates, Milford, MA). The sensitivity of this high performance liquid chromatography system is 10 pg/mL for EPI and 20 pg/mL for NEPI. This assay has intra- and interassay coefficients of variation of 12% for EPI and of 7% for NEPI.

In addition, the subjects' perceptions of the stressors were evaluated to determine if DEX affected task engagement or arousal. Immediately after the task, subjects completed the abbreviated differential emotions scale (26) and an eight-item questionnaire assessing their feelings of uncertainty during the task (three questions), helplessness (two questions), and control (three questions).

Results

Tonic endocrine response

The effects of DEX and SAL on tonic endocrine activity were evaluated by two-way (group: DEX vs. SAL) \times 5 (period: preinfusion, 0.5 h postinfusion, 1 h postinfusion, 2 h postinfusion, 3 h postinfusion, and 4h postinfusion) analyses of variance (ANOVAs). Results using repeated measures ANOVAs were evaluated using an α level of 0.05, and degrees of freedom were corrected using the Hynh-Feldt ϵ . Mean endocrine activity as a function of group and period is summarized in Fig. 1.

Effects of DEX on HPA activity

Analyses of ACTH revealed significant main effects for group [$F(1,22) = 18.66$; $P < 0.0003$] and period [$F(5,110) =$

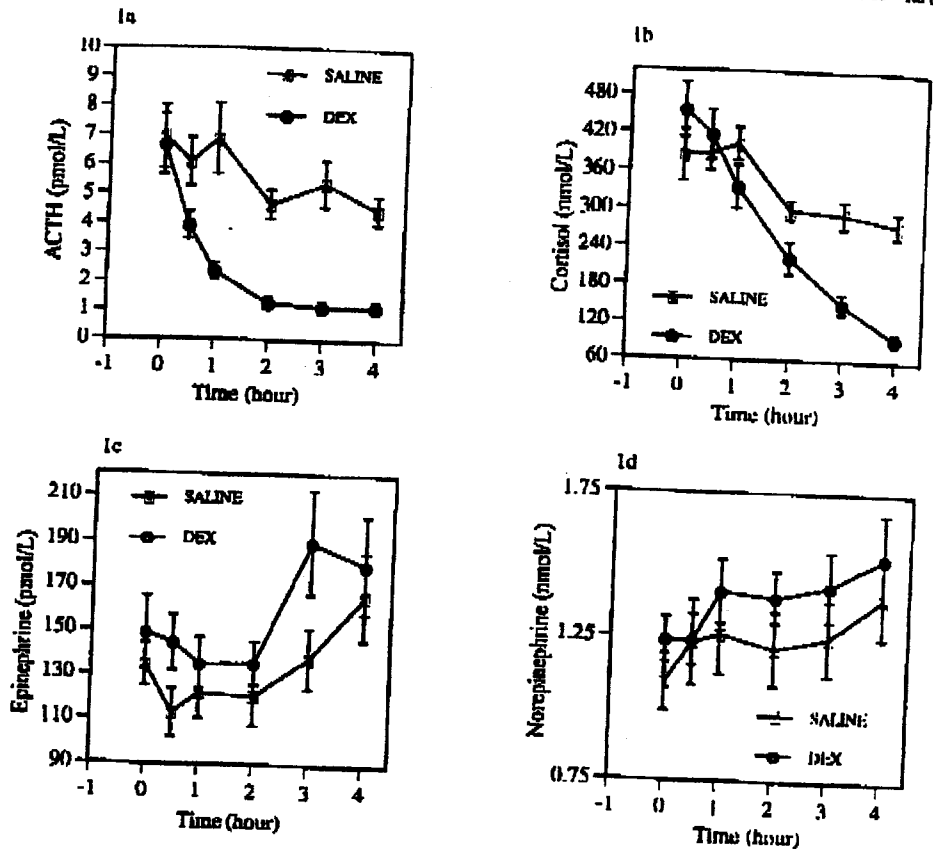


FIG. 1. A 4-mg iv infusion of DEX produced significant inhibition of the HPA axis, but not the SAM axis.

15.94; $P < 0.001$] and a significant group \times period interaction [$F(5,110) = 4.13$; $P < 0.02$; $\epsilon = 0.48$]. As illustrated in Fig. 1a, ACTH did not differ as a function of group at preinfusion, but ACTH was significantly lower at each measurement period after the infusion of DEX compared to SAL ($P < 0.05$). Within-group comparisons further revealed that each postinfusion measure of ACTH was comparable to the preinfusion level after the infusion of SAL ($F \leq 4.25$; $P = \text{NS}$), whereas the postinfusion level of ACTH was lower than the preinfusion level within 30 min of the infusion of DEX and remained significantly lower ($14.64 \leq F \leq 35.95$; $P < 0.003$). Thus, DEX effectively attenuated the release of ACTH.

Results also confirmed the expected effects of DEX on plasma cortisol levels. Analyses of cortisol revealed a significant main effect for period [$F(5,110) = 49.06$; $P < 0.0001$] and a significant group \times period interaction [$F(5,110) = 11.55$; $P < 0.0001$; $\epsilon = 0.46$]. As illustrated in Fig. 1b, cortisol did not differ as a function of group at preinfusion or 30 min postinfusion, but cortisol was significantly lower 1–4 h after the infusion of DEX compared to SAL ($P < 0.05$). Within-group comparisons indicated that the only postinfusion measurement of cortisol lower than the preinfusion level in the SAL group was the final measurement [$F(1,11) = 5.02$; $P < 0.05$]; in the DEX condition, the postinfusion levels of cortisol were lower than the preinfusion level within 30 min and remained significantly lower ($5.53 \leq F \leq 99.90$; $P < 0.05$). Thus, in relation to ACTH, cortisol revealed a parallel, but

slightly lagged, response to DEX, as might be expected given that ACTH stimulates the release of cortisol.

Effects of DEX on SAM activity

Of interest was whether the effects of DEX on ACTH and cortisol would also be observed on plasma catecholamine levels. Analyses of EPI revealed a significant main effect for period [$F(5,110) = 8.46$; $P < 0.001$; $\epsilon = 0.86$], reflecting a rise in EPI across time (see Fig. 1c). Importantly, neither the main effect for group nor the group \times period interaction approached statistical significance. Analyses of NEPI revealed a similar result; the main effect for period was significant [$F(5,110) = 5.69$; $P < 0.01$; $\epsilon = 0.90$; see Fig. 1d], and again, neither the main effect for group nor the group \times period interaction approached statistical significance. Thus, DEX and SAL had comparable effects on plasma catecholamines.

Stress-induced endocrine response

The effects of DEX and SAL on phasic endocrine responses to the brief psychological stressors were evaluated by 2 (group: DEX vs. SAL) \times 3 (period: prestress, midstress, and poststress) ANOVAs. Results using repeated measures ANOVAs were again evaluated using an α level of 0.05, and degrees of freedom were corrected using the Hynh-Feldt ϵ . Mean endocrine activity as a function of group and period is summarized in Fig. 2.

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Effects of DEX on HPA activity

Analyses of ACTH revealed significant main effects for group [$F(1,22) = 57.68$; $P < 0.0001$] and period [$F(2,44) = 5.38$; $P < 0.02$] and a significant group \times period interaction [$F(2,44) = 6.33$; $P < 0.01$; $\epsilon = 0.83$]. Replicating our prior research, the brief stressors elevated ACTH at midstress and poststress periods in the SAL group; however, DEX eliminated the effects of the stressors on ACTH (see Fig. 2a).

The same pattern of results was found in the analyses of cortisol. The ANOVAs yielded a significant main effects for group [$F(1,22) = 95.60$; $P < 0.0001$] and period [$F(2,44) = 4.85$; $P < 0.02$] and a significant group \times period interaction [$F(2,44) = 7.14$; $P < 0.01$; $\epsilon = 0.66$]. As illustrated in Fig. 2b, the brief stressors elevated poststress cortisol levels in the SAL group, but the stressors had no effect on cortisol in the DEX group.

Effects of DEX on SAM activity

Prior research demonstrated that plasma catecholamine levels are elevated by brief psychological stressors. Consistent with this work, analyses revealed significant main effects for period for EPI [$F(2,44) = 16.00$; $P < 0.001$; $\epsilon = 1.0$] and NEPI [$F(2,44) = 46.88$; $P < 0.0001$; $\epsilon = 0.92$]. Importantly, neither the main effect for group nor the group \times period interaction approached statistical significance in the analyses

of EPI or NEPI. As illustrated in Fig. 2, c and d, the brief stressors elevated plasma catecholamine levels, and these responses were unaffected by DEX.

Behavioral effects of DEX

Finally, to evaluate whether the psychological stressors had a different impact on subjects who were infused with DEX vs. SAL, task performance and the poststress ratings of the stressors were subjected to a one-way (DEX vs. SAL) ANOVA. None of these tests approached statistical significance ($P > 0.12$). Thus, the levels of task engagement and stress were equivalent for the DEX and SAL groups.

Discussion

Before we could test the hypothesis that CRH regulates catecholamine release after psychological stress, we had to inhibit the HPA axis before administration of the stressors. Previous work had indicated that maximal inhibition of adrenocortical response occurs between 2–4 h after a single dose of DEX (27); therefore, our stress protocol was initiated at 4 h. Also, as previous work indicated that pituitary ACTH release *in vitro* is not influenced by moderate doses of corticosteroids, the inhibition of ACTH *in vivo* probably results

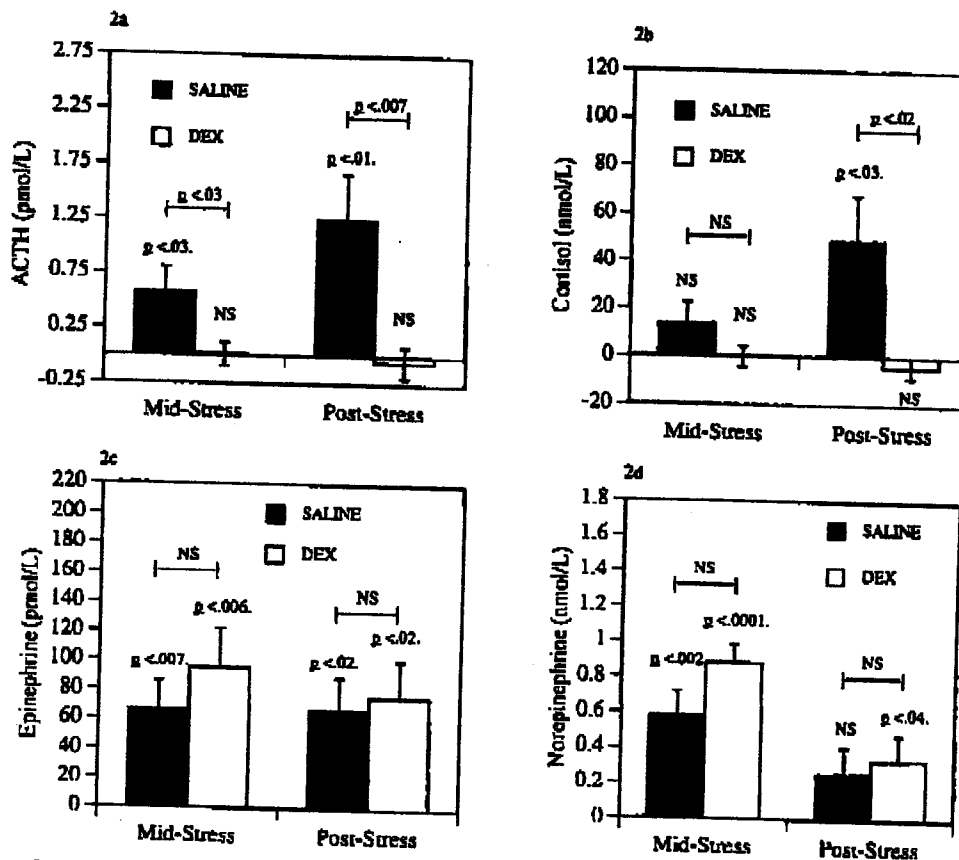


FIG. 2. DEX significantly inhibited the stress-induced increase in ACTH and cortisol, but did not affect the stress-induced increases in EPI and NEPI. The first test of significance is between the basal values (4 h, Fig. 1) and the poststress levels. The second comparison is between the SAL and DEX groups.

from inhibition of CRH neurons or pathways mediating CRH release (27). We observed that a 4-mg DEX infusion acutely inhibited ACTH levels at the first sampling interval of 30 min, and by 2 h, ACTH levels had plateaued. Cortisol levels after DEX administration lagged the fall in ACTH concentrations by 30 min and were continuing to fall at 4 h. By the time that the stressors were given, ACTH and cortisol levels had declined to 85% of their initial levels in the DEX group, and they were approximately 75% lower than levels in the SAL controls, who exhibited the expected diurnal decrease in these levels. These data suggested that the CRH influence on the HPA axis had been attenuated in the DEX-treated subjects.

During this same interval, DEX exhibited no significant effect on plasma NEPI and EPI levels, as both groups began to nonsignificantly increase their catecholamine levels at 3–4 h just preceding the administration of the stressors and possibly in anticipation of that event. This observation would argue against CRH exerting a significant influence on tonic secretion of catecholamines.

As different mechanisms are probably involved in basal vs. stimulated ACTH and cortisol release, we examined the influence of DEX on these hormones after the stressors. The math and speech stress was able to stimulate ACTH and cortisol release in the SAL group, whereas the HPA axis was completely inhibited in the DEX subjects. In contrast, using physical or metabolic stressors in animals that have been given modest concentrations of glucocorticoids, ACTH and cortisol will often be released (28). Most of this work, however, has not dealt with psychological stress, with the exception of a recent study in sheep (7). In this investigation it was noted that DEX inhibited ACTH and cortisol release in response to hypoglycemia and audiovisual stress (exposure to a barking dog for 5 min). In addition, it was noted that DEX inhibited EPI release in response to hypoglycemia, but not to audiovisual stress.

In our study DEX failed to inhibit psychological stress-induced release of EPI and NEPI, as evidenced by similar mid- and poststress levels in the SAL and DEX groups. The stress was equal in both groups, and CRH was presumably activated in the SAL group, as ACTH and cortisol increased appropriately. Therefore, these data suggest that CRH is not involved in stimulating the SAM axis during psychological stress, at least with the speech and math stress paradigm employed in this investigation. This finding is supported by the finding in sheep that audiovisual stress-induced increases in catecholamines were not influenced by glucocorticoid administration (7).

These data, however, conflict with the animal data that describe CRH receptors in locus ceruleus neurons and the ability of glucocorticoids to inhibit plasma catecholamine increases after immobilization stress (16, 17). A threshold effect to explain these differences does not seem plausible, as these metabolic and physical stressors would appear to be stronger stimuli than psychological stress, and the SAM axis was inhibited by glucocorticoids in these reports (16, 17). A more tenable explanation may be that metabolic and physical stressors, as opposed to the psychological stressors employed in this study, use different pathways to stimulate the HPA and SAM systems. For example, the speech and math

stressors that we used probably stimulated the amygdala which mediates learned fear and anxiety responses associated with stressful events (29). Of interest, the amygdala has recently been shown to be a site of extrahypothalamic CRH production (18, 19), as several different nuclei in the amygdala can synthesize CRH (20). Also, restraint stress in rats increases the CRH messenger ribonucleic acid (mRNA) content in the amygdala (18, 20). Of relevance to this discussion is that glucocorticoid administration to rats decreased hypothalamic CRH mRNA synthesis and paradoxically enhanced CRH mRNA synthesis in the amygdala and striatum (20). Hence, one could argue that stressors in humans that involve learned anxiety and fear and are transmitted via the amygdala could stimulate CRH synthesis. The latter effect could also be enhanced by glucocorticoid. Therefore, an increase in amygdala CRH could modify an influence on EPI levels induced by a DEX-induced decrease in hypothalamic CRH levels with diminished input to the locus ceruleus.

The issue of individual differences in stimulation and inhibition of the HPA and SAM axes in humans is evolving from the work in several laboratories and has health implications. The research has shown that individuals differ in their cardiovascular and endocrine reactivity to stress (3–6) and in the ability of glucocorticoids to decrease the HPA response to exercise (30). We have also shown that individuals characterized by high relative to low cardiac sympathetic reactivity have greater stress-related changes in ACTH and cortisol levels, but comparable changes in EPI and NEPI (3). Hence, there is a stronger hormonal influence to down regulate the immune system in those individuals demonstrating greater cardiovascular, endocrine, and immune reactivity to stress. This research has implications for disease processes in which altered HPA axis function has been found, such as depression (1, 31), multiple sclerosis (32), chronic fatigue (33), and premenstrual syndrome (34).

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