Chronic Imipramine Is Associated with Diminished Hypothalamic-Pituitary-Adrenal Axis Responsivity in Healthy Humans

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ABSTRACT

The hypercortisolism of melancholic depression is thought to reflect hypothalamic hypersecretion of CRH and may be related to the hyperarousal associated with this syndrome. Although chronic administration of imipramine to experimental animals significantly decreases CRH messenger RNA levels in the paraventricular nucleus, it is generally thought that resolution of hypercortisolism following recovery from depression is related to the improvement in mood and decrease in anxiety that accompanies recovery rather than an intrinsic effect of imipramine. The present study was designed to explore whether chronic imipramine administration to healthy, nondepressed volunteers is associated with effects on hypothalamic-pituitary-adrenal (HPA) axis function. We studied basal and provocative measures of HPA axis function in 14 healthy volunteers before and after 6 weeks of imipramine treatment at therapeutic doses. Imipramine was associated with decreased responses in peak ACTH and cortisol to ovine CRH and in peak ACTH to arginine vasopressin (P = 0.02, P = 0.003, and P = 0.02, respectively) without changes in indices of basal HPA axis function. These data are consistent with preclinical findings and support the hypothesis that imipramine has an intrinsic effect on central components of HPA axis function, potentially related to its therapeutic effects. (J Clin Endocrinol Metab 82: 2601–2606, 1997)

ALTHOUGH depression is commonly thought of as an illness of profound emotional and physical slowing, many patients experience it as intense, dysphoric activation and arousal. This is particularly true of melancholic depression, a diagnostic subtype associated with severe anxiety and hopelessness, as well as with physiological indices of severe stress including sustained hypercortisolism and increased sympathetic nervous system activity. This state has been characterized as sustained pathological hyperarousal, occurring either in the absence of an appropriate stressor, or despite the resolution of the triggering stress.

We and others have suggested that the symptoms and signs of melancholia, including hypercortisolism, reflect a generalized, uncontained stress response (1,2). Depression-associated hypercortisolism involves hypersecretion of CRH unresponsive to glucocorticoid-mediated counter-regulation (2). CRH is a principal effector of the generalized stress response, and its central administration to experimental animals initiates a coordinated series of responses characteristic of behavioral and physiologic arousal (3–7). Although adaptive during threatening situations, uncoupled from an appropriate stressor this cluster of responses strongly suggests the pathologic, sustained hyperarousal of melancholic depression.

Tricyclic antidepressants are efficacious agents in the treatment of melancholic depression. We and others have reported that chronic but not short-term administration of imipramine to experimental animals results in a decrease in hypothalamic CRH messenger RNA levels and content (8–10). Antidepressant-induced remission of melancholia is associated with resolution of hypercortisolism (11–14), although this has been thought to reflect the resolution of the distress of the depression rather than a primary medication effect. Based on the preclinical findings, however, we hypothesized that imipramine has an intrinsic effect decreasing activity of the HPA axis, and that this could contribute to its therapeutic effects.

We report here a study designed to test this hypothesis in humans by administering imipramine to healthy humans for 6 weeks at doses comparable to those used clinically and examining its effects on HPA axis function.

Subjects and Methods

Experimental subjects

Subjects were 14 healthy volunteers (10 males, 4 females, mean ± SEM age 37.3 ± 3.4 yr). All subjects underwent complete medical history, physical examination and screening laboratory examinations, a psychiatric interview with a board-certified psychiatrist (D.M.), as well as a structured clinical interview (SCID) for DSM-III-R to exclude current or past psychiatric illness. No subjects were on chronic medication. The study was approved by the National Institute of Mental Health Institutional Review Board, and all subjects gave informed consent to participate.

HPA axis function was assessed in each subject (details below), following which imipramine administration was begun. After 6 weeks of daily imipramine, with doses adjusted to maintain serum imipramine +
Evaluation of HPA axis function

Pituitary-adrenal responses to ovine CRH administration. At 1700 h, subjects were placed at bedrest for the duration of the procedure, and an iv catheter was inserted. From 1800–2000 h, blood was drawn every 15 min for measurement of baseline plasma ACTH and cortisol levels. At 2000 h, subjects received 1 μg/kg ovine CRH by iv injection over 2 min. Blood for ACTH and cortisol determination was sampled at 5, 15, 30, 60, 90, and 120 min after the injection, immediately placed on ice, spun down within 2 h of collection, frozen over dry ice, and stored at −70°C until assayed. One subject (male) was not included in the final analysis of data because of sample loss.

Pituitary-adrenal responses to arginine vasopressin (AVP) administration. A pilot study was conducted in a separate group of eight healthy volunteers (four males, four females, mean age 36.2 ± 1.7 yr) to establish the optimal time of day and dosage for AVP induced pituitary ACTH release. Each subject received a total of eight hour-long infusions of AVP in four graded doses (0, 0.1, 0.3, 1.0 mIU/kg/min) administered in a randomized fashion at both 0900 h and at 2000 h. Plasma ACTH levels were highest in the morning for each dose, and maximal at the 1.0 mIU/kg/min dose (M. Demitrack, data in preparation for publication), and this dose and time was chosen as optimal. At 0730 h subjects were placed at bedrest for the duration of the procedure, and an iv catheter was inserted in each arm. From 0830–0930 h blood was drawn at 15-min intervals for determination of baseline ACTH and cortisol levels. Between 0930–1030 h subjects received a continuous infusion of AVP at a dose of 1 mIU/kg/min. Blood was sampled for ACTH and cortisol every 15 min during the infusion and for 30 min afterwards, then immediately placed on ice, spun down within 2 h of collection, frozen over dry ice, and stored at −70°C until assayed. One subject (female) was not included in the final analysis of results because cramping associated with AVP infusion precluded a second test.

Adrenal responses to maximal and submaximal ACTH stimulation. At 1700 h an iv catheter was inserted and subjects were placed at bedrest for the duration of the procedure. Subjects received on different days stimulation with 0.10 μg/kg (maximal) and 0.003 μg/kg (submaximal) doses of synthetic 1–24 ACTH at 1800 h. These doses were chosen on the basis of a previous dose-finding study (15).

Measurement of 24-h urinary free cortisol excretion. Two or three 24-h urine collections for assessment of urinary free cortisol (UFC) were obtained from 13 subjects before and at the end of the imipramine treatment period. Total creatinine excretion was measured in each specimen to assess completeness of collection, and only collections with at least 0.7 g creatinine/24 h were included in the analysis of data.

Imipramine administration

After completion of the initial HPA axis studies, imipramine was administered to subjects with a gradually increasing dose to 100 mg over 1 week, and after 5 days serum imipramine + desipramine levels were measured and dosage adjusted to maintain levels between 100–300 μg/L. Serum levels were checked biweekly thereafter, and dosage adjusted as necessary. Treatment continued for 6 weeks, at which time the initial series of examinations were repeated while imipramine treatment continued. The mean ± SEM imipramine + desipramine serum level after 6 weeks (μg/L) was 182.0 ± 15.4 (range 103–318; usual therapeutic range 125–275).

Assays

Assays for cortisol for the oCRH and AVP stimulation tests were performed on previously frozen plasma by commercially available radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). For each subject, samples from the pretreatment and post-treatment studies were measured in the same assay. Assays for ACTH were done on previously frozen plasma by commercially available immunoradiometric assay (Nichols, San Juan Capistrano, CA). Assays for cortisol for the ACTH stimulation tests were done on fresh serum by the NIH clinical laboratories using a fluorescence polarization kit (Abbott Laboratories). Urinary free cortisol was measured commercially by a specific radioimmunoassay after extraction with dichloromethane (Smith Kline Bioscience, King of Prussia, PA). The interassay coefficient of variation for cortisol was 9.1% and for ACTH, 11.5%. Intraassay coefficients of variation for both cortisol and ACTH were less than 10%.

Analysis of data

Baseline plasma ACTH and cortisol levels were assessed by determining the mean of samples taken at 15-min intervals during the hour preceding AVP stimulation (morning baselines) and the two hours preceding oCRH stimulation (evening baseline). Responses to AVP, oCRH, and ACTH administration were calculated by determining the peak ACTH and cortisol responses above baseline as well as the net integrated area under the curve using the trapezoidal approximation method. Results of the baseline assessments and the assessments done on imipramine were compared using paired t tests.

Results

All values are mean ± sem. Results are summarized in Figs. 1 and 2 and in Table 1. All ACTH values are pg/mL, and cortisol values are μg/dL.

Evening basal plasma ACTH and cortisol levels and response to oCRH

Initial evening unstimulated plasma ACTH values and cortisol measured before oCRH stimulation were similar before and during imipramine administration (pretreatment ACTH 7.9 ± 1.2, on imipramine 8.8 ± 1.5, P = 0.42; pretreatment cortisol 3.6 ± 0.6, on imipramine 4.4 ± 0.6, P = 0.24).

Peak ACTH and cortisol responses to oCRH were both decreased after imipramine administration (pretreatment peak ACTH 35.1 ± 3.9, on imipramine 28.2 ± 3.6, P = 0.02; pretreatment peak cortisol 16.8 ± 1.4, on imipramine 12.3 ± 1.5, P = 0.003). Net integrated cortisol response (μg/dL/120 min), but not ACTH response (pg/mL/120 min), was significantly decreased (integrated ACTH response pretreatment 2505 ± 248, on imipramine 2109 ± 266, P = 0.10; integrated pretreatment cortisol 1337 ± 131, on imipramine 1045 ± 124, P = 0.02).

Morning basal plasma ACTH and cortisol levels and response to AVP

Morning unstimulated plasma ACTH and cortisol measured before AVP stimulation were similar before and during imipramine administration (ACTH pretreatment 17.2 ± 2.6, on imipramine 17.4 ± 3.0, P = 0.92; pretreatment cortisol 7.4 ± 0.6, on imipramine 7.3 ± 1.1, P = 0.89).

Peak ACTH responses to AVP infusion were decreased on imipramine (pretreatment peak response 30.1 ± 4.6, on imipramine 19.1 ± 3.3, P = 0.02). Cortisol responses after AVP infusion were not significantly different on imipramine (pretreatment peak cortisol 12.2 ± 1.8, on imipramine 9.2 ± 1.7, P = 0.07). Net integrated ACTH response (pg/mL/120 min) approached significance (pretreatment 939 ± 102, on imipramine 642 ± 87, P = 0.08), while net integrated cortisol responses (μg/dL/120 min) did not differ significantly (pretreatment 740 ± 132, on imipramine 634 ± 118, P = 0.33)
Response to maximal and submaximal ACTH

Peak cortisol responses to both maximal and submaximal ACTH administration were similar before and during imipramine administration (cortisol response to maximal ACTH pretreatment $23.2 \pm 0.9$, on imipramine $19.4 \pm 1.7$, $P = 0.11$; cortisol response to submaximal ACTH pretreatment $5.9 \pm 1.4$, on imipramine $6.9 \pm 1.4$, $P = 0.65$).

Urinary free cortisol

Urinary free cortisol (UFC) excretion ($\mu$g/24 h) was similar at baseline and on imipramine (pretreatment $59.6 \pm 5.5$, on imipramine $52.8 \pm 6.0$, $P = 0.20$).

Discussion

Chronic imipramine administration to healthy humans was associated with a significant decrease in peak plasma ACTH and cortisol responses to oCRH and in the peak plasma ACTH response to AVP stimulation. Chronic imipramine treatment did not affect basal plasma ACTH and cortisol levels, UFC excretion, or the plasma cortisol response to ACTH administration.

Several previous studies have failed to demonstrate effects of antidepressants on HPA axis function in healthy humans. Heuser et al. (16) found no effect on basal ACTH and cortisol levels or peak ACTH and cortisol responses after combined CRH dexamethasone administration, nor was there a difference in plasma cortisol following dexamethasone administration. However, animal models have demonstrated decreases in HPA axis function after antidepressant administration (8–10), and the tests used by Heuser et al. were inhibitory and probably poorly suited to demonstrating subtle down-regulation of the HPA axis. Additionally, mean amitriptyline + nortriptyline concentrations were 118 ng/mL, with a range from 32–199 ng/mL, suggesting that many subjects had subtherapeutic plasma drug levels. Kathol et al. (17) reported no effect of imipramine on HPA axis response to insulin-induced hypoglycemia. This study, which involved only 10 days of drug treatment and included only 6 subjects, was too short and of insufficient statistical power to detect modest effects.

Our study did demonstrate a reduction in HPA axis function associated with imipramine administration. These results are likely to be intrinsic medication effects, since all subjects were medication-free at the outset of the study, subjects had plasma drug levels in the therapeutic range for 6 weeks, no subjects had significant medical or psychiatric illness, and each procedure was performed on a separate day under controlled conditions. We note that, because the order
of examinations was not randomized, we cannot absolutely rule out a sequence effect.

The results reported here differ from the attenuated plasma ACTH responses to oCRH that have been consistently demonstrated in patients with major depression and hypercortisolism (13, 18). In depressed patients, blunted ACTH responses to oCRH are associated with adrenal hyperresponsiveness and correlate negatively with basal hypercortisolism (13), likely reflecting an intact pituitary corticotroph responding appropriately to the negative feedback effects of high circulating glucocorticoids. The primary pathophysiologic alteration in these patients appears to be hypothalamic hypersecretion of CRH and the relatively exaggerated plasma cortisol response resulting from sustained hyperstimulation of the adrenal cortex leading, over time, to adrenal hyperresponsiveness.

In contrast, the blunted plasma ACTH responses to oCRH in our study occurred in the context of eucortisolism, not hypercortisolism, and are not attributable to increased glucocorticoid negative feedback. The significant decrease in cortisol secretion after oCRH administration while on imipramine suggests that the adrenal cortex was responding appropriately to the reduction in plasma ACTH responses during oCRH administration or had become relatively hyporesponsive because of a subtle, imipramine-associated down-regulation of HPA axis function.

This pattern of blunted plasma ACTH responses to oCRH in the absence of hypercortisolism may occur in primary pituitary disease or in forms of hypothalamic CRH deficiency. Such conditions include patients with adrenal insufficiency resulting from hypothalamic damage (19), and patients on chronic, alternate-day glucocorticoid therapy given oCRH on the nontreatment day (20), when restraining feedback from exogenous glucocorticoids is absent. The decrease in pituitary response to exogenous CRH in these patients is probably caused by a change in the pituitary corticotroph in which persistent hypostimulation by endogenous CRH (i.e. lack of priming) decreases its capacity to respond, a phenomenon best described in patients with Cushing’s disease (21–23). Thus the blunted pituitary ACTH responses to oCRH observed in the current study, occurring in the context of eucortisolism and following this pattern, are consistent with a decrease in hypothalamic CRH secretion.

Like the responses to oCRH, ACTH responses to exogenous AVP were lower with imipramine. AVP is a relatively weak secretagogue for ACTH alone, but potentiates the effect of CRH (25, 26), and an important determinant of ACTH response to AVP is the concentration of CRH present in

![Graph A: Cortisol Response after AVP Infusion](image1)

![Graph B: ACTH Response to AVP Infusion](image2)

**Fig. 2.** ACTH (2a) and cortisol (2b) responses after AVP administration
hypophyseal blood at the time of study. The imipramine-associated attenuation in plasma ACTH response to sustained AVP infusion likely reflects a relative decrease in hypothalamic CRH secretion following chronic imipramine administration. Alternatively the mechanisms that lead to decreased response to oCRH associated with imipramine administration could also occur with AVP. The reductions in peak cortisol responses after AVP approached but did not reach significance, and it is possible that the greater variability of morning baseline cortisol levels masked a reduction similar to that seen after oCRH infusion. Alternatively, since AVP has a less potent effect on pituitary ACTH release compared with oCRH, reductions in ACTH after AVP may have less pronounced effects at the adrenal.

Adrenal function was relatively unaffected by imipramine. This is consistent with animal studies in which imipramine effects on CRH did not change basal measures of HPA axis function (8) and may be related to several factors. The HPA axis is a dynamic system, and in healthy adults can compensate for small changes in a single component. Decreases in ACTH secretion are counterbalanced by decreases in glucocorticoid negative feedback, preserving the equilibrium of the unstressed system. Under the demanding conditions of a challenge test, however, this equilibrium breaks down because the maximal response of the unprimed pituitary corticotroph is impaired. It is also possible that imipramine has subtle effects on baseline function not detectable in our sample, but that might be seen in a large group of subjects.

The mechanism by which imipramine decreases CRH secretion is uncertain. Imipramine blocks reuptake of norepinephrine into presynaptic neurons. In brainstem regions such as the locus coeruleus, which contain inhibitory α2 autoreceptors (27), accumulation of norepinephrine at the synapse would decrease the firing of noradrenergic neurons. Because norepinephrine is excitatory to CRH release (28), an imipramine-induced decrease in brainstem noradrenergic activation could contribute to the findings reported here. We cannot, however, rule out the possibility that the anticholinergic or antihistaminic effects of imipramine, or an indirect effect, could also account for these findings.

In summary, chronic administration of imipramine to healthy humans results in a significant decrease in plasma ACTH responses to oCRH and AVP in the absence of hypercortisolism. These effects are consistent with an imipramine-associated decrease in hypothalamic CRH release, as well as with data from preclinical studies. Importantly, these data are compatible with studies implicating CRH hypersecretion in the clinical and biochemical manifestations of melancholic depression, and suggest that the resolution of hypercortisolism after successful antidepressant treatment may reflect more than simply a secondary effect related to relief from painful psychological and physiological stressors.

**Table 1.** Measures of HPA axis function before and during chronic imipramine treatment

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>On Imipramine</th>
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<tbody>
<tr>
<td>ACTH (pg/mL)</td>
<td>35.1 ± 3.9</td>
<td>28.2 ± 3.6a</td>
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<tr>
<td>Cortisol (µg/dL)</td>
<td>16.8 ± 1.4</td>
<td>12.3 ± 1.5b</td>
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<tr>
<td>Peak response above baseline to oCRH:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>30.1 ± 4.6</td>
<td>19.1 ± 3.3b</td>
</tr>
<tr>
<td>Morning basal plasma ACTH and cortisol:</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>17.2 ± 2.6</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>Evening basal plasma ACTH and cortisol:</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>7.9 ± 1.2</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>Peak cortisol response above baseline to maximal ACTH stimulation:</td>
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<tr>
<td>Baseline</td>
<td>23.2 ± 0.9</td>
<td>19.4 ± 1.7</td>
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<tr>
<td>Peak cortisol response above baseline to submaximal ACTH stimulation:</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>5.9 ± 1.4</td>
<td>6.9 ± 1.4</td>
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<tr>
<td>Urinary-free cortisol Excretion (µg/24 h):</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>59.6 ± 5.5</td>
<td>52.8 ± 6.0</td>
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<tr>
<td>On Imipramine</td>
<td></td>
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</table>

*P < 0.05.

**References**

9. Brady LS, Gold PW, Herkenham M, Lynn AB, Whitfield HJ. 1992 The an-


