Chronic Treatment with the Monoamine Oxidase Inhibitor Phenelzine Increases Hypothalamic-Pituitary-Adrenocortical Activity in Male C57BL/6 Mice: Relevance to Atypical Depression

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Atypical depression has been linked to low hypothalamic-pituitary-adrenocortical axis activity and exhibits physical and affective symptoms resembling those of glucocorticoid deficiency. Because atypical depression has also been defined by preferential responsiveness to monoamine oxidase inhibitors (MAO-I), we hypothesized that MAO-I reverse these abnormalities by interfering with glucocorticoid feedback and increasing hypothalamic-pituitary-adrenocortical activity. To test this hypothesis, we measured plasma hormones and ACTH secretagogue gene expression in male C57BL/6 mice treated chronically with saline vehicle or phenelzine, a representative MAO-I. Changes in glucocorticoid feedback were confirmed by decreased immobility during forced swim testing. Phenelzine significantly increased circadian nadir and postrestraint plasma corticosterone levels in sham-operated mice, an effect that correlated with increased adrenocortical sensitivity to ACTH. Phenelzine increased circadian nadir, but not poststress ACTH in ADX mice, suggesting that phenelzine augmented corticosterone secretion in sham-operated mice by increasing stimulation and decreasing feedback inhibition of hypothalamic-pituitary activity. Consistent with the latter possibility, phenelzine significantly increased plasma ACTH and paraventricular hypothalamus CRH mRNA in ADX, corticosterone-replaced mice. Phenelzine did not increase paraventricular hypothalamus CRH or vasopressin mRNA in ADX mice lacking corticosterone replacement. We conclude that chronic phenelzine treatment induces sustained increases in glucocorticoids by impairing glucocorticoid feedback, increasing adrenocortical responsiveness to ACTH, and increasing glucocorticoid-independent stimulation of hypothalamic-pituitary activity. The resulting drive for adrenocortical activity could account for the ability of MAO-I to reverse endocrine and psychiatric symptoms of glucocorticoid deficiency in atypical depression. (Endocrinology 146: 1338–1347, 2005)
asone suppression, and after CRH stimulation (2–5). Although other studies have shown evidence of normal (14–16) or even increased cortisol production (17), key features of these disorders suggest that glucocorticoid levels may still be inadequate for patient needs. Atypical depression is defined by the presence of lethargy, hypersonnia, weight gain, mood reactivity, and/or rejection sensitivity, with at least two of these defining symptoms indicating severe fatigue; the majority of patients with seasonal affective disorder exhibit similar symptoms (6). Fatigue, both physical and psychological, is also a primary presentation of glucocorticoid deficiency in Addison’s disease (3). Reports of positive mood responses to glucocorticoid infusion (18) support the possibility that inadequate glucocorticoid secretion might account for fatigue symptoms in atypical depression. Elevations in basal ACTH in atypical depression (2) are also consistent with glucocorticoid deficiency. Thus, greater sensitivity to the negative feedback effects than to the mood-elevating effects of glucocorticoids could cause symptoms of glucocorticoid deficiency in depression with atypical features.

To establish physiological models to test these hypotheses, we have exploited therapeutic differences between psychotic and atypical depression, the approximate extremes of HPA dysfunction in depression. Tricyclic antidepressants have been found to be the most effective antidepressants for treating psychotic depression, particularly when combined with antipsychotics (6, 19). In contrast, atypical depression has been partly defined by the superior efficacy of monoamine oxidase inhibitors (MAO-I) over tricyclic antidepressants (6, 20). We hypothesized that because successful treatment usually restores normal HPA function (21), tricyclic and MAO-I antidepressants would have opposing effects on HPA activity. We have recently shown that the tricyclic imipramine can mimic several glucocorticoid actions, including inhibition of circadian HPA activity in adrenalectomized (ADX) mice (22). We therefore predicted that MAO-I would interfere with glucocorticoid feedback action and increase HPA activity. Although other studies have suggested that MAO-I inhibit the HPA axis (23–25), MAO-I have also been found to increase glucocorticoid secretion (26, 27). We included ADX controls to address the possibility that previously reported HPA inhibition was due indirectly to MAO-I-induced glucocorticoid secretion. We have found that chronic phenelzine treatment of male C57BL/6 mice increases hypothalamic-pituitary activity, increases adrenocortical responsiveness to ACTH, and decreases glucocorticoid feedback efficacy. The resulting drive for adrenocortical activity could account for the ability of MAO-I to normalize endocrine, affective, and physical symptoms of glucocorticoid deficiency in atypical depression.

Materials and Methods

Animals and treatments

All procedures were approved by the Institutional Animal Care and Use Committee of Albany Medical College and were consistent with the NIH Guide for the Care and Use of Animals (28). Male C57BL/6 mice (Taconic Farms, Germantown, NY) were housed on a 12-h light, 12-h dark cycle (lights-on at 0700 h) and studied at 2–6 months of age. For experiment 1, mice were ADX or sham-ADX (Sham) under isoflurane anesthesia (2.5% in oxygen). To control for the loss of adrenal steroids other than corticosterone (Cort), ADX mice were replaced with 0.5 μg/ml aldosterone in the drinking fluid, a dose previously shown to approximate circulating levels of aldosterone in mice (29), and were given a choice of 0.5% saline or water to drink. Sham mice were given only water to drink. Drinking fluids for all groups were replaced weekly. After a 1-wk postsurgical recovery period, all mice were injected ip once per day with either 30 mg/kg phenelzine (Sigma-Aldrich Corp., St. Louis, MO) or an equivalent volume of saline vehicle for 4 wk. In the absence of prior evidence for effects of phenelzine on forced swim performance in mice (30), this dose was derived from studies reporting consistent effects of chronic phenelzine treatment on other correlates of emotional behavior in mice (31). At the end of the third week of treatment, all mice underwent forced swim testing to verify antidepressant efficacy (described below). After forced swim testing, mice in experiment 1 were each assigned to one circadian group and one poststress group, with assignments balanced across all drug treatment and adrenalectomy groups. Mice were individually housed for at least 12 h before blood sample collection. During the fourth week of treatment, half of the mice in each group were bled by retroorbital puncture within 1 h of either lights-on [morning (AM)] or lights-off [evening (PM)] to determine circadian nadir and peak levels, respectively, of plasma ACTH and Cort. At the end of the fourth week of treatment (at least 36 h after circadian sampling), mice were subjected to a 10-min restraint stress within 1 h of lights-on to match the timing of the prior AM sample. Half of the mice in each group were killed by decapitation either immediately after release (10 min) or after a 50-min recovery in their home cage (60 min). Blood collected at decapitation was used for analysis of plasma hormones. Brains were frozen in embedding medium (OCT, Sakura Finetek, Torrance, CA) in a dry ice-ethanol bath and were stored at –80 C for in situ hybridization analysis. In all experiments, blood sampling or decapitation occurred within 45 sec of opening the cage.

In experiment 2, intact mice were injected ip with 25 mg/kg phenelzine or saline once per day for 3 wk before ACTH stimulation testing. A subset of the mice was subjected to forced swim testing 2 d before ACTH stimulation to verify antidepressant efficacy. ACTH stimulation was performed within 3 h of lights-on, using doses and sample times derived from the report by Zilz et al. (32); treatment groups were counterbalanced for prior swim testing. Mice were blocked with dexamethasone (500 μg/kg, sc) 2 h before ip injection of 10 μg/kg synthetic ACTH1–24 (Cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga, CA) or vehicle (PBS with 0.3% BSA). Mice were killed by decapitation for analysis of plasma Cort at 30 and 90 min after injection.

In experiment 3, mice were ADX and replaced with both aldosterone in the drinking fluid and with an sc 30-mg pellet containing 10%, 25%, or 50% Cort by weight in cholesterol (33). After postsurgical recovery, mice were injected ip once daily with 25 mg/kg phenelzine or saline. Following confirmation of antidepressant effects by forced swim testing after 3 wk of treatment, mice were subjected to restraint stress as described for experiment 1, except that they were not killed after restraint. All mice were killed basally 48 h later, beginning 1 h after lights-on. Plasma hormones were analyzed as described below; thymus glands were collected and weighed as a measure of cumulative glucocorticoid exposure (34). Brains were collected as described in experiment 1 for in situ hybridization analysis.

Forced swim testing

Mice were subjected to a 5-min forced swim in a 14.5-cm wide × 19.5-cm high beaker of water (25 ± 1 C). Swim testing was performed within 6 h of the daily injection of phenelzine or vehicle and was not affected by the time between injection and testing. Immobility during forced swim testing was defined as time the mouse spent either completely motionless or using minimal, sporadic activity to keep its nose above water. Immobility was scored by an individual blind to the drug and adrenocortical status of the mice.

Plasma hormone assays

Circadian and stress-induced levels of plasma ACTH and Cort were assayed using previously described RIAs (29). ACTH was also assayed using a kit from MP Biomedical (formerly ICM Diagnostics, Irvine, CA), with all reagents and sample volumes reduced by half. The intraassay coefficient of variation for this assay was 14.2% in the 35–53 pg/ml range.
range. ADX mice with plasma Cort greater than 1 μg/dl were excluded from data analysis.

### In situ hybridization

In situ hybridization for hypothalamic CRH and vasopressin mRNA was performed as previously described (35). In brief, brains were sec- tioned at 10 μm on a cryostat, collecting every other section onto Su- perFrost Plus slides (Fisher Scientific, Pittsburgh, PA). Slides from all mice were prehybridized and hybridized simultaneously for a given neuropeptide gene product. Prehybridization treatments consisted of fixation in 4% phosphate-buffered 4% paraformaldehyde, acetylation (0.25%) acetic anhydride/0.1 m triethanolamine), and dehy- dration through graded ethanol, with rinses in 2× SSC (standard saline citrate buffer) in between each step. [35S]UTP-labeled cRNA probes were trans- cribed from appropriately linearized templates using T3 or T7 RNA polymerase (Stratagene, La Jolla, CA). The CRH probe was comple- mentary to the 606-bp Styl-MscI fragment of the rat CRH cDNA (35). The vasopressin probe was complementary to the 251-bp BssH-Dral frag- ment of the 3′ end of rat vasopressin cDNA (29). Slides were hybridized overnight at 60°C with 105 cpm/ml probe in 50% formamidine, 0.3 m NaCl, 10% dextran sulfate, 1× Denhardt’s, 10 mm Tris (pH 7.5), 1 mm EDTA, 500 μg/ml yeast tRNA, and 10 mm diithiothreitol. After hybridization, slides were rinsed in 2× SSC, treated with 20 μg/ml ribonuclease, rinsed in 0.5× SSC, washed 30 min at 60°C, and dehydrated through graded ethanols.

In situ hybridization data were analyzed semiquantitatively using a Typhoon 9210 phosphorimager and ImageQuant 5.0 software (GE Healthcare, Niskayuna, NY; formerly Amersham Biosciences). Slides were exposed concurrently to phosphorimager screens, with mice from every treatment group included on each screen. Comparisons among slides on different screens were performed by normalizing readings to identical sets of 14C standards exposed to each screen (146B, American Radiolabeled Chemicals, St. Louis, MO). Section histology was con- firmed by thionin staining before analysis. CRH and vasopressin ex- pression was analyzed at the level of the paraventricular hypothalamus (PVN) corresponding to Figs. 37 and 38 of the Franklin and Paxinos mouse brain atlas (36). The supraoptic nucleus was also analyzed for vasopressin expression at the level of the optic chiasm [Fig. 36 of the atlas (36)]. Densitometric readings, representing the integrated gray level for pixels within the outline of the PVN or SON, were corrected for the background integrated gray level of the same size area in a nonexpressing part of the same tissue section.

### Data analysis

Data were analyzed by two-way ANOVA for the effects of antide- pressant treatment and adrenalectomy (or, as applicable, Cort replace- ment). The data from experiment 2 were analyzed by two-way ANOVA for the effects of antidepressant treatment and acute (ACTH/vehicle) treatment. Plasma ACTH and CRH mRNA data were log-transformed before analysis to satisfy assumptions of equal variance. As expected, there were significant main effects of adrenalectomy or Cort replacement on all experimental end points. Because these effects were neither novel nor the a priori focus of this investigation, they were not explored by post hoc testing. Differences between vehicle and phenelzine treatments were compared within a given adrenalectomy or Cort replacement group by a single unpaired t test. Multiple comparisons were not performed, except for limited data in experiment 3, where t tests with Bonferroni correction were used to determine whether the various Cort replacement- ments could be distinguished by their effects on plasma Cort levels or thymus weight (see Table 2). Data are presented throughout as the mean ± sem, with ANOVA results in Table 3. Slight differences in residual degrees of freedom within experiments reflect occasional in- ability to measure all end points in every mouse. A few ACTH samples had insufficient plasma for replication. In addition, three mice in high Cort replacement groups in experiment 3 lost their pellets after forced swim testing, but before being killed. These mice are included in the forced swim results (Table 1), but not in any other analyses. Significance was defined as P < 0.05. Where no error bars are visible, the scale of the graph exceeded that of the error.
1.20 ± 0.11 vs. 1.46 ± 0.05 mg/g body weight, respectively; *P = 0.021; n = 8–13/group) that did not occur in ADX mice.

Experiment 2: effects of chronic phenelzine treatment on adrenocortical sensitivity to ACTH

To determine whether the increased glucocorticoid levels observed in phenelzine-treated Sham mice resulted from an increase in adrenal sensitivity to ACTH, we compared Cort responses to ACTH after chronic treatment with phenelzine or vehicle. As in experiment 1, mice tested for forced swim behavior after 3 wk of phenelzine treatment exhibited significant decreases in immobility (vehicle, 63 ± 12 sec; phenelzine, 10 ± 6 sec; *P = 0.0021; n = 6/group). After dexamethasone blockade followed by vehicle injection, mice in both groups had Cort levels near the lower limit of detection (Fig. 4, vehicle). Dexamethasone-blocked mice injected with synthetic ACTH exhibited increases in Cort 30 min later that were significantly greater in phenelzine-treated mice (Fig. 4, ACTH). By 90 min after ACTH injection, plasma Cort levels had returned to baseline and did not differ between saline- and phenelzine-treated mice (not shown).

Experiment 3: effects of chronic phenelzine treatment on feedback inhibition of ACTH

To test whether phenelzine increased glucocorticoid levels in Sham mice in part by interfering with glucocorticoid feedback inhibition, we assessed the ability of glucocorticoid replacement to decrease plasma ACTH in ADX mice. Before phenelzine or vehicle treatment, mice were ADX and replaced with graded levels of Cort via a sc pellet. Three weeks of phenelzine treatment produced similar, significant decreases in immobility during forced swim testing in all Cort replacement groups. Neither the baseline immobility nor the decrease in immobility in response to phenelzine was affected by the level of Cort replacement (Table 1).

Replacement of ADX mice with sc pellets containing 10%, 25%, or 50% Cort progressively increased plasma Cort and decreased thymus weight; these effects were not influenced by phenelzine treatment (Table 2). Plasma Cort in the ADX + 50% Cort group was significantly higher than that in either the ADX + 10% Cort or the ADX + 25% Cort group (Table 2). Differences in plasma Cort between ADX + 10% Cort and the ADX + 25% Cort mice could not be detected statistically in samples collected when mice were killed, approximately 1 month after pellet implantation. However, thymus weight was significantly decreased in the ADX + 25% Cort vs. the ADX + 10% Cort group; thymus weight was not further decreased in the ADX + 50% Cort group compared with the ADX + 25% Cort group (Table 2).

Increasing Cort replacement in ADX mice also progressively decreased circadian nadir plasma ACTH in saline- as well as phenelzine-treated groups (Fig. 5). However, phenel-
zine-treated mice exhibited higher plasma ACTH at similar levels of Cort than did their saline-treated counterparts in the ADX + 10% Cort and ADX + 25% Cort groups. This effect was significant in the 25% Cort replacement group. ACTH levels in ADX + 50% Cort mice were indistinguishable between saline- and phenelzine-treated mice (Fig. 5).

There were similar relative trends in plasma ACTH measured after restraint stress. Immediately after release from a 10-min restraint, plasma ACTH levels tended to be higher in ADX + 10% Cort and ADX + 25% Cort mice treated with phenelzine, although these differences were not significant (Fig. 6, left panel; \( P = 0.381 \) and 0.0631 for ADX + 10% Cort and ADX + 25% Cort mice, respectively). After a 50-min recovery period (60 min after the start of restraint), plasma ACTH still tended to be elevated in phenelzine-treated ADX + 25% Cort mice compared with their saline-treated controls (Fig. 6, right panel). However, although there were significant main effects of Cort replacement, drug treatment, and Cort × drug interaction on plasma ACTH at 60 min, differences between saline- and phenelzine-treated mice did not reach statistical significance in the ADX + 25% Cort group (\( P = 0.0659 \)).

To identify hypothalamic factors potentially contributing to the increased drive for pituitary-adrenal activity in phenelzine-treated mice, we performed in situ hybridization for CRH and vasopressin. Only ADX mouse groups were analyzed so that changes in central activity could be evaluated independently of the marked increases in glucocorticoid secretion observed in phenelzine-treated Sham mice. ADX + 50% Cort mice were not studied because they did not exhibit phenelzine-induced differences in plasma ACTH. In addition, analysis of ADX + 0% Cort mice (experiment 1) was limited to those killed 10 min after restraint to avoid interference from possible stress-induced increases in gene expression (38). Preliminary studies (not shown) indicated that CRH and vasopressin mRNA levels were not increased by 10 min of restraint in ADX mice. Sections from all ADX + 0% Cort, ADX + 10% Cort, and ADX + 25% Cort mice were prehybridized, hybridized, and exposed for densitometric analysis together.

Both glucocorticoid replacement and phenelzine treatment had significant main effects on CRH mRNA levels in the PVN (Fig. 7). Phenelzine treatment significantly increased CRH gene expression in ADX + 10% Cort and ADX + 25% Cort mice, although CRH mRNA levels in ADX + 0% Cort mice were not affected (Fig. 7). Levels of vasopressin mRNA in the PVN tended, although not significantly (\( P = 0.0941 \)), to be inhibited by Cort replacement in ADX mice (Fig. 8). There was no significant main effect of phenelzine treatment on vasopressin expression (Fig. 8). Supraoptic nucleus vasopressin expression exhibited relative changes that were similar to those in the PVN, but also not significant (not shown).

**Discussion**

We have shown that phenelzine, a representative MAO-I antidepressant, increases adrenocortical axis activity. This increased activity results from both decreased glucocorticoid inhibition and glucocorticoid-independent stimulation of hypothalamic-pituitary activity. In addition, possibly because of increased hypothalamic-pituitary drive, phenelzine increases adrenocortical sensitivity to ACTH. The capacity to increase glucocorticoid secretion may account in part for the therapeutic efficacy of MAO-I to improve mood and treat apparent symptoms of glucocorticoid deficiency in atypical depression.

Chronic phenelzine treatment significantly increased basal and stress-induced plasma Cort levels at the circadian nadir. Our results are consistent with reports that MAO-I increase circadian nadir glucocorticoids in rats (26) and humans (27). The differential stimulation of circadian nadir, but not peak, glucocorticoid secretion that we observed also agrees with previous findings in phenelzine-treated rats (26). These increases in glucocorticoids are unlikely to be due to nonspecific stress from drug toxicity. Although phenelzine often, but not consistently, inhibited weight gain (data not shown), weight loss alone does not selectively affect circadian nadir HPA activity, but instead elevates both circadian nadir and peak HPA hormone levels (39).

Phenelzine-induced increases in glucocorticoid secretion could result from increased hypothalamic-pituitary activity, decreased feedback inhibition, or increased adrenocortical responsiveness to stimulation. We have found evidence for all three mechanisms. However, decreased sensitivity to feedback inhibition is fundamental to sustaining elevations.

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**TABLE 1.** Time spent immobile during forced swim testing in mice from experiment 3

<table>
<thead>
<tr>
<th>Immobility (sec)</th>
<th>Saline</th>
<th>Phenelzine</th>
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<tbody>
<tr>
<td>ADX + 10% Cort</td>
<td>86 ± 11</td>
<td>17 ± 3*</td>
</tr>
<tr>
<td>ADX + 25% Cort</td>
<td>74 ± 15</td>
<td>31 ± 9*</td>
</tr>
<tr>
<td>ADX + 50% Cort</td>
<td>92 ± 16</td>
<td>11 ± 3*</td>
</tr>
</tbody>
</table>

Immobility during a 5-min forced swim was scored after 3 wk of treatment with either saline vehicle or phenelzine, as described in Materials and Methods. ANOVA results are shown in Table 3. \( n = 5–8 \)/group.

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**TABLE 2.** Plasma Cort and normalized thymus weight in mice from experiment 3

<table>
<thead>
<tr>
<th>Plasma corticosterone (( \mu g/dl ))</th>
<th>Thymus weight (mg/g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td>ADX + 10% Cort</td>
<td>3.8 ± 0.4*</td>
</tr>
<tr>
<td>ADX + 25% Cort</td>
<td>5.6 ± 0.3*</td>
</tr>
<tr>
<td>ADX + 50% Cort</td>
<td>13.1 ± 2.9*</td>
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<tr>
<td></td>
<td>1.51 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>1.06 ± 0.15*</td>
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<tr>
<td></td>
<td>0.85 ± 0.13*</td>
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Both plasma Cort and thymus weight were significantly affected by Cort replacement, but not antidepressant treatment (see Table 3 for ANOVA results). Different superscripts denote significant differences for each variable by post hoc testing between replacement groups. \( n = 5–8 \)/group.
induced increases in ACTH in ADX activate glucocorticoid feedback; the lack of phenelzine-treated mice. However, phenelzine did not completely inactivate glucocorticoid feedback; the lack of phenelzine-induced increases in ACTH in ADX + 50% Cort mice indicated that inhibition could still occur at sufficiently high glucocorticoid levels. We have shown that phenelzine impairs glucocorticoid inhibition of both plasma ACTH and hypothalamic CRH gene expression. Decreased feedback inhibition of ACTH could account not only for the higher basal and poststress Cort levels in phenelzine-treated Sham mice, but also for the lack of phenelzine effect on ACTH responses to stress in ADX + 0% Cort mice. Effects mediated primarily by changes in feedback would not be evident in the absence of glucocorticoids. Moreover, controlled Cort replacement was less effective in inhibiting circadian nadir ACTH in phenelzine-treated ADX mice, with the strongest effects of phenelzine treatment occurring in ADX + 25% Cort mice. Similar, although not significant, trends occurred in stress-induced ACTH secretion in ADX, Cort-replaced mice. Significant increases in hypothalamic CRH mRNA levels in ADX + 10% Cort and ADX + 25% Cort mice demonstrated that CRH was also less sensitive to glucocorticoid inhibition in phenelzine-treated mice. However, phenelzine did not completely inactivate glucocorticoid feedback; the lack of phenelzine-induced increases in ACTH in ADX + 50% Cort mice indicated that inhibition could still occur at sufficiently high glucocorticoid levels.

Circadian nadir ACTH levels were increased in phenelzine-treated ADX + 0% Cort mice in experiment 1, suggesting that phenelzine also increased hypothalamic-pituitary drive independent of glucocorticoid feedback. However, we did not observe significant increases in either CRH or vasopressin gene expression to account for the elevated basal ACTH levels in phenelzine-treated ADX + 0% Cort mice. Mismatch between CRH and ACTH also occurred in ADX + 10% Cort mice, which exhibited significant elevations in hypothalamic CRH mRNA, but not plasma ACTH. We suspect that this dissociation is most likely attributable to differences in CRH or vasopressin secretion, which, as in other states of chronically increased HPA axis activity, is likely to be regulated by sites proximal to the hypophysiotrophic neurons (40). Norepinephrine, epinephrine, and serotonin release from brainstem afferents to the PVN or its immediate vicinity generally stimulate HPA activity (23, 41), and would probably be enhanced by phenelzine inhibition of monoamine metabolism. Other factors possibly accounting for discrepancies among CRH, vasopressin, and plasma ACTH include changes in pituitary sensitivity or stimulation of ACTH by other secretagogues, such as oxytocin (42). However, most studies indicate that corticotroph responsiveness probably plays a relatively minor role, compared with brain drive or glucocorticoid inhibition, in determining plasma ACTH levels (1, 7, 29, 43). Disagreement also remains as to the requirement for oxytocin in stimulating ACTH secretion (40, 44). Lastly, although ADX, Cort-replaced mice received a slightly lower dose and duration of phenelzine treatment, the highly significant forced swim results in all experiments support the likelihood that the differential effects of phenelzine on CRH and ACTH in ADX + 0% Cort and ADX Cort-replaced mice are relevant to antidepressant action.

The lack of phenelzine effect on vasopressin expression is unlikely to be due to the fact that we measured vasopressin gene expression in the PVN as a whole. Unlike the rat, the mouse PVN does not have an anatomically distinct parvo- and magnocellular division (45). Also, in contrast to the rat, adrenalectomy increases vasopressin expression in the lateral PVN of the mouse, which by anatomical position and neuron size appears to correspond to the magnocellular PVN of the rat (29). Our results are comparable to those of emulsion autoradiography analyses showing modest elevations in total PVN vasopressin after adrenalectomy in the mouse (29).

Two receptors mediate feedback regulation of the HPA axis. The higher affinity, lower capacity mineralocorticoid receptor (MR) regulates basal activity, whereas the lower affinity, higher capacity glucocorticoid receptor (GR) is involved in regulating stimulated HPA activity during stress or the circadian peak (34). In Sham mice, phenelzine-induced Cort levels, which were at least as high as the normal circadian peak levels, would have produced substantial GR occupancy. We have previously shown that the 10% Cort pellet replacement reproduces 24-h mean Cort levels in ADX mice and corresponds to the physiological replacement needed for effective MR-related inhibition of basal ACTH (34, 46). Higher Cort replacements that would progressively occupy GR suppress ACTH and induce atrophy of the thymus, a typical GR target tissue (29, 34, 46). The ability of...
phenelzine to increase both plasma ACTH and PVN CRH mRNA in ADX + 25% Cort mice suggests that phenelzine interferes with at least GR-mediated feedback. Even though plasma Cort levels did not differ between ADX + 10% Cort and ADX + 25% Cort mice when mice were killed, thymus weight was significantly lower, suggesting that GR occupancy was considerably higher, in ADX + 25% Cort mice. Our prior work also indicates that at earlier times after pellet implantation (46), Cort levels in ADX + 25% Cort mice were likely to have been similar to those in phenelzine-treated Sham mice (46). Hypothalamic-pituitary activity at such elevated glucocorticoid levels could only be maintained if GR feedback efficacy were diminished. Phenelzine impairment of MR-mediated feedback, given the significant effects on CRH in ADX + 10% Cort mice, may also occur.

Possibly because of higher basal hypothalamic-pituitary activity, which would increase trophic stimulation of the zona fasciculata, adrenocortical sensitivity to ACTH was increased in phenelzine-treated mice. However, it is also possible that phenelzine could affect adrenocortical activity by inhibiting adrenomedullary or adrenal nerve monoamine metabolism. On balance, both adrenal nerve input and interactions with adrenomedullary chromaffin cells appear to enhance glucocorticoid responses to adrenal stimulation, although the splanchnic nerves also have an inhibitory effect on circadian nadir secretion (reviewed in Refs. 47 and 48). Adrenal nerves contain noradrenergic as well as cholinergic and peptidergic fibers, and rodent chromaffin cells have been found to produce serotonin as well as norepinephrine and epinephrine (47, 48). By inhibiting monoamine metabolism, phenelzine could increase local concentrations of adrenal catecholamines and serotonin, and thus augment the stimulatory effects of these monoamines on glucocorticoid production.

It should be noted that our findings of HPA stimulation by phenelzine differ from other reports of MAO-I effects on HPA activity in rats. Several investigators have found evidence of inhibition of either basal or stress-induced HPA activity by MAO-I, including one report that phenelzine treatment decreased both adrenocortical activity and PVN CRH mRNA in rats (23–25). Although MAO-I have not consistently been found to inhibit HPA activity (25, 49), reports

![Fig. 7. CRH gene expression in the PVN of ADX mice with and without Cort replacement from experiments 1 and 3. ADX + 50% Cort mice were not analyzed because they did not exhibit phenelzine-induced changes in plasma ACTH. A, Representative images of CRH in situ hybridization in the brains of vehicle- and phenelzine-treated ADX + 0% Cort, ADX + 10% Cort, and ADX + 25% Cort mice from experiments 1 and 3. The PVN is indicated by the arrow in the upper left section. B, Results of semiquantitative analysis of PVN CRH gene expression. There were significant main effects of Cort replacement and phenelzine treatment on CRH gene expression, with significant effects of phenelzine treatment at the post hoc level in the ADX + 10% Cort and ADX + 25% Cort groups (see ANOVA results in Table 3). Note the log scale for CRH expression. n = 3–8/group. *, P < 0.05, phenelzine vs. saline in the same Cort replacement group.

Fig. 8. Arginine vasopressin (AVP) hormone gene expression in the PVN of ADX mice from experiments 1 and 3. Analysis and experiment groups are as described in Fig. 7. A, Representative images of vasopressin in situ hybridization in the PVN of vehicle- and phenelzine-treated ADX + 0% Cort, ADX + 10% Cort, and ADX + 25% Cort mice from experiments 1 and 3. The PVN is indicated by the arrow in the upper left section. The supraoptic nuclei are visible ventrolaterally to either side of the PVN, but were analyzed at a more anterior level (not shown). B, Results of semiquantitative analysis of PVN vasopressin gene expression. There was a marginal effect of Cort replacement and no effect of phenelzine treatment on vasopressin gene expression (see ANOVA results in Table 3). n = 3–8/group.
of increased HPA activity (26, 27) after MAO-I treatment are relatively rare. Species-specific differences may account for the potential discrepancies between our results and those in the literature. However, because our data do corroborate the stimulatory effects of MAO-I on circadian nadir HPA activity in both rats (26) and humans (27), the mouse is likely to be a valid and useful model for defining HPA effects of MAO-I.

We also showed that endocrine effects occurred at phenelzine doses that had significant antidepressant activity in the forced swim test. Because forced swim testing is a standard-
ized method of determining antidepressant activity, some of the discrepancies between our results and those in the literature could be due to inadequate dosing in some previous studies. Where reported, antidepressant doses that were insufficient to decrease immobility in the forced swim test were also ineffective on other experimental end points (50). Nevertheless, although the forced swim test is useful to identify HPA effects associated with antidepressant efficacy, it does not necessarily measure the antidepressant effects that may require glucocorticoids. Thus, although our forced swim data in vehicle-treated mice differ from those in rats, in which glucocorticoids have been reported to have depressive-like effects on baseline immobility (51, 52), these differences most likely reflect species-specific factors. Other recent literature indicates that antidepressant effects on forced swim behavior in mice can occur independently of changes in glucocorticoids (53); the glucocorticoid dependence of antidepressant effects on forced swim behavior in rats has not been investigated.

The stimulatory effects of phenelzine that we have observed on HPA activity are of particular relevance to atypical depression, which was defined in part by preferential patient responses to MAO-I (20). In contrast to the more common finding of elevated adrenocortical axis activity in depression, atypical depression is most often associated with low (2, 4) or normal glucocorticoid levels (14–16). Low CRH levels have also been linked to depression with atypical features, although it is unclear whether cerebrospinal fluid CRH accurately reflects the activity of hypothalamic neuroendocrine CRH neurons (16). Because patients with atypical depression are capable of secreting glucocorticoids in response to stimulation (54), the endocrine profile of this depression subtype is more readily explained by increased sensitivity to glucocorticoid feedback than by primary or tertiary adrenocortical failure. This interpretation is supported by the finding that atypical depressed patients exhibit greater sensitivity to dexamethasone suppression (4).

Although there may be other causes of the symptoms of atypical depression, the fatigue (6, 20) and reported elevations in ACTH (2) in these patients are intriguingly similar to symptoms of glucocorticoid deficiency. In combination with reports of glucocorticoid-induced mood improvement (18), these findings suggest that glucocorticoid feedback in atypical depression may keep cortisol at levels too low to inhibit corticotroph activity or elevate mood. Our data indicate that MAO-I are capable of reversing this relative glucocorticoid deficit and possible deficits in CRH (15, 16) by increasing adrenocortical glucocorticoid production and providing a feedback resistant (but not insensitive) drive to increasing adrenocortical glucocorticoid secretion (4). Nevertheless, although the forced swim test is useful to identify HPA effects associated with antidepressant efficacy, it does not necessarily measure the antidepressant effects that may require glucocorticoids. Thus, although our forced swim data in vehicle-treated mice differ from those in rats, in which glucocorticoids have been reported to have depressive-like effects on baseline immobility (51, 52), these differences most likely reflect species-specific factors. Other recent literature indicates that antidepressant effects on forced swim behavior in mice can occur independently of changes in glucocorticoids (53); the glucocorticoid dependence of antidepressant effects on forced swim behavior in rats has not been investigated.

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33. Solano JM, Jacobson L 1999 Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am J Physiol 277:E708–E716


37. Lucki I 1997 The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 8:523–532


45. Whitnall MH 1993 Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. Prog Neurobiol 40:573–629


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