

Partial Glucocorticoid Agonist-Like Effects of Imipramine on Hypothalamic-Pituitary-Adrenocortical Activity, Thymus Weight, and Hippocampal Glucocorticoid Receptors in Male C57BL/6 Mice

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Abnormal hypothalamic-pituitary-adrenocortical (HPA) activity may provide clues to the neurochemistry of depression. Psychotic depression has one of the highest rates of elevated HPA activity and is most often responsive to the tricyclic class of antidepressants. Because successful treatment resolves HPA as well as psychiatric symptoms, we hypothesized, in light of evidence that tricyclic antidepressants can affect glucocorticoid receptor function, that these drugs would mimic glucocorticoid feedback inhibition of HPA activity. To test this hypothesis, we measured circadian nadir (morning) and peak (evening) as well as restraint stress-induced levels of plasma ACTH and corticosterone in adrenalectomized (ADX) and sham-ADX (Sham) male C57BL/6 mice after 8 wk of imipramine (20 mg/kg/d, ip) or saline treatment. Antidepressant efficacy was confirmed by decreased immobility in forced-swim testing. When glucocorticoids were low or absent, imipramine mimicked glucocorticoid action in inhibiting evening ACTH in ADX mice and tending to inhibit morning corticosterone in Sham. However, when glucocorticoid levels were high, imipramine appeared to interfere with feedback inhibition by increasing post-stress ACTH and tending to increase evening corticosterone in Sham mice. Imipramine also decreased thymus weight in ADX and increased thymus weight in Sham mice. Imipramine stimulated morning ACTH in ADX mice, possibly by mimicking facilitative effects of high glucocorticoids. Short-term imipramine treatment was capable of inducing nuclear translocation of hippocampal glucocorticoid receptors in ADX mice. We conclude that imipramine effects on glucocorticoid-sensitive endpoints *in vivo* resemble those of a glucocorticoid partial agonist. (*Endocrinology* 145: 4185–4191, 2004)

AS A STRESS-RESPONSIVE SYSTEM, the hypothalamic-pituitary-adrenocortical (HPA) axis has received considerable attention as a prognostic indicator in depression therapy. Sustained elevations in baseline HPA activity occur in a high percentage of patients with major depression (1), and stressful life events have been implicated in precipitating depressive episodes (2). Because HPA hyperactivity often resolves with successful treatment of depression (1, 3), it has been widely assumed that antidepressants inhibit the HPA axis. However, animal studies of antidepressant effects on HPA activity have yielded inconsistent results (reviewed in Ref. 1). There is also controversy in the clinical literature as to whether the failure of HPA activity to normalize with psychiatric improvement in some patients indicates a risk of relapse or a medication-specific effect, independent of treatment success (3, 4). We reasoned that the complexity of this issue might be due in part to the multiple subtypes of depression and that correlating HPA function with specific subtypes might provide more insight into the relationship between HPA activity and depression.

Elevated HPA activity is particularly prevalent in psychotic depression, which has one of the highest rates of

Abbreviations: ADX, Adrenalectomized; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenocortical; MR, mineralocorticoid receptor; TBS, Tris-buffered saline.

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dexamethasone nonsuppression (5, 6). These findings suggest that glucocorticoid feedback inhibition of HPA activity is impaired. However, other central nervous system-related actions of glucocorticoids appear to be intact or even enhanced. Schatzberg and co-workers (6) have proposed that elevated glucocorticoids actually contribute to the pathology of psychotic depression and have shown in a recent open-label clinical trial that the glucocorticoid antagonist RU 486 (Mifepristone) can produce significant psychiatric improvement in patients with this disorder.

Consistent with intrinsic effects of glucocorticoids on mood, psychiatric symptoms occur in other diseases associated with elevated glucocorticoids. These symptoms are independent of the severity of the primary disease, indicating they are not simply a response to illness, and resolve once glucocorticoids are removed. Over two thirds of patients with Cushing's syndrome and up to 30% of patients treated with systemic glucocorticoids present with depression, even though many of these patients lack any history of psychiatric illness. Both Cushing's and glucocorticoid-treated patients may also exhibit psychosis and other symptoms observed in depression, including anxiety, mania, sleep and appetite disturbances, and cognitive impairment (7–9). Analogous to the localized resistance posited by Pariante and Miller (1) to reconcile the evidence for normal peripheral glucocorticoid action with impaired feedback in depression, we hypothesized that brain region-specific deficits in glucocorticoid signaling could account for defective feedback combined with

the apparent effects of glucocorticoid excess on mood in psychotic and possibly other subtypes of depression.

To facilitate localization of brain regions where altered glucocorticoid signaling might contribute to depression pathology, we have established physiological models to identify glucocorticoid-sensitive targets of antidepressants. We specifically hypothesized that glucocorticoid signaling would be altered by antidepressants that are most effective for treating depression subtypes associated with HPA dysregulation. Tricyclics have been found to be the most efficacious antidepressants for treating psychotic depression (5). There is also evidence that tricyclic antidepressants can enhance glucocorticoid receptor (GR) function *in vitro*. These effects include nuclear translocation in the absence of glucocorticoids and, in certain conditions, augmentation of glucocorticoid-induced gene transcription (reviewed in Ref. 1). Based on these observations, we reasoned that the clinical efficacy of tricyclic antidepressants to normalize HPA activity in depression was due to their ability to mimic glucocorticoid feedback action. Consistent with this possibility, tricyclics have often been reported to inhibit hypothalamic-pituitary activity (1). However, because these drugs have also been reported to increase adrenal weight and circadian nadir glucocorticoid levels (10, 11), it is unclear whether the reported inhibition was a direct effect of treatment or an indirect effect of elevated glucocorticoids. We have therefore measured circadian and stress-induced levels of plasma ACTH and corticosterone in adrenalectomized as well as intact mice after chronic treatment with the typical tricyclic antidepressant imipramine. We have also tested the ability of imipramine to induce nuclear localization of GRs in the brains of adrenalectomized (ADX) mice. We have found that imipramine influences glucocorticoid-sensitive endpoints and GR dynamics *in vivo* in a manner consistent with partial agonist action at the GR. This heterogeneous action could serve to limit glucocorticoid levels and their potential impact on emotion in depressed patients.

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 (12). Male C57BL/6 mice (Taconic Farms, Germantown, NY) were 2.5–4 months of age at the time of study and kept on a 12:12 light cycle (lights on at 0700 h). Until the last week of the experiment, mice were housed up to five per cage. Food and drinking fluid were available *ad libitum*. Mice were ADX or sham-ADX (Sham) under inhaled isoflurane anesthesia (2.5% in oxygen) using aseptic technique. To control for loss of mineralocorticoids, ADX mice were replaced at surgery with a 30-mg, sc wax pellet containing 1% aldosterone by weight. In preliminary experiments (not shown), we determined that this level of aldosterone replacement normalized salt preference relative to Sham mice and minimized mortality in long-term ADX mice. To ensure appropriate salt balance, ADX mice were given a choice of 0.5% saline or water to drink. Sham mice were implanted with a wax sc pellet at surgery and given water to drink. After 1 wk of postoperative recovery, mice were injected ip once per day within 5 h of lights on with either imipramine (20 mg/kg) or sterile 0.9% saline vehicle for 8 wk. Mice were weighed weekly to assess potential metabolic effects of imipramine treatment.

Forced swim testing

At the beginning of the eighth week of treatment, immobility was timed during a single forced swim (3 min in a 14.5-cm-wide × 19.5-cm-high beaker of 25 ± 1 C water) to evaluate antidepressant efficacy of imipramine treatment. These conditions are consistent with recently reported parameters for forced-swim testing in mice (13, 14). Swim test performance was evaluated within 2–6 h of imipramine or vehicle injection. Results were not affected by the interval between injection and testing, use of longer swim times up to 15 min, or comparison of immobility between two swims on consecutive days. A single trained observer unaware of the mouse's treatment group scored immobility. Immobility was defined as time the mouse spent either completely motionless or using minimal, sporadic activity to keep its nose above water. Any rhythmic paddling movements, even if only by one foot, were not scored as immobility.

Plasma hormone and tissue analysis

Mice were individually housed for at least 12 h before blood sample collection. Samples were obtained by retroorbital puncture within 30 sec of touching the cage. Blood samples for circadian trough (morning) and peak (afternoon) hormone levels were obtained 2 d after swim testing. Half of the mice in each group were sampled within 1 h of lights on (morning); the other half was sampled within 1 h of lights off (evening). Stress-induced hormone levels were assessed within 3 h of lights on in a terminal experiment at least 36 h after the last circadian sample, at the end of the eighth week of treatment. Mice were killed by decapitation either immediately or 30 min after the end of a 10-min tube restraint, beginning within 1 h of lights on. Thymus glands were collected on saline-moistened filter paper and weighed as an index of peripheral glucocorticoid action. Circadian and stress-induced levels of plasma ACTH and corticosterone were assayed using previously described RIAs (15). ADX mice with plasma corticosterone greater than 1 µg/dl were excluded from data analysis.

Glucocorticoid receptor localization

ADX mice prepared as above were injected with imipramine or saline once per day on the 2 d preceding killing, with the third injection occurring 1 h before mice were killed. Imipramine-treated mice were injected with 30 mg/kg imipramine, the dose originally defined by Porsolt *et al.* (16) to have acute effects on immobility in mice. As a positive control, some mice were injected once with corticosterone (1 mg/kg, sc) 1 h before they were killed. Brains were removed and fixed according to a modification (Wotus, C., and W. C. Engeland, personal communication) of a technique by Khan and Watts (17). In brief, brains were immersed in ice-cold 0.1 M acetate-buffered 4% paraformaldehyde (pH 6.0), followed by 48 h at 4 C in 0.1 M borate-buffered 4% paraformaldehyde (pH 9.5). Brains were cryoprotected in 30% sucrose in Tris-buffered saline (TBS), frozen, and sectioned at 30 µm. Floating sections were stored in 30% ethylene glycol, 20% glycerol in TBS at –20 C and rinsed four times for 15 min each in TBS before use for immunocytochemistry.

Glucocorticoid receptor immunocytochemistry was performed by incubating floating sections for 24 h at 4 C with a rabbit polyclonal primary antibody to the GR (Affinity Bioreagents, Golden, CO) diluted 1:1000 in TBS with 3% BSA (IgG-free fraction V) and 0.5% Triton X-100 (American Bioanalytical, Natick, MA). Sections were then rinsed and incubated 1 h at room temperature with a Cy3-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) diluted 1:400. Sections were shielded from light as much as possible for this and all subsequent manipulations. Sections were then washed four times in TBS, mounted on slides (SuperFrost Plus, Fisher Scientific, Pittsburgh, PA), and coverslipped with *n*-propyl gallate (50 mg/ml in Tris-buffered 90% glycerol, pH 7.5).

Reagents and supplies

Except as noted, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Data analysis

Hormone and thymus weight data were analyzed for main effects of adrenal status and drug treatment by two-way ANOVA. As expected, adrenalectomy had significant main effects on body weight gain, plasma hormones, and thymus weight. Differences between Sham and ADX mice were not subjected to *post hoc* testing except for forced-swim behavior, which was analyzed by *t* test with Bonferroni correction for multiple comparisons. Effects of imipramine on all experimental endpoints were analyzed within Sham or ADX groups by unpaired *t* test without Bonferroni correction. Data are presented throughout as mean \pm SEM; significance was defined as $P < 0.05$.

Glucocorticoid receptor localization was evaluated using epifluorescence microscopy with an Olympus BX50 microscope equipped with an Optronics model S99808 CCD camera and Pictureframe 2.0 software (Optronics, Goleta, CA). Digital images of the CA1 pyramidal cell layer of the hippocampus captured at $\times 600$ magnification under oil immersion, using filters for tetramethyl rhodamine isothiocyanate/Cy3 fluorescence (excitation, 510–550 nm; emission, ≥ 590 nm), were scored qualitatively for nuclear *vs.* cytoplasmic localization of immunoreactivity by an observer blinded to the identity of the samples.

Results

Eight weeks of imipramine treatment had minimal effects on body weight (Table 1). Sham mice treated with imipramine exhibited slightly but significantly less weight gain than their vehicle-treated counterparts by the end of the experiment. This difference in weight gain was not observed at earlier times in the experiment (not shown), nor was a significant effect on weight gain detected in ADX mice at any time during treatment.

To verify that the dose and duration of imipramine treatment was sufficient for antidepressant effects, we measured immobility during forced-swim testing after 8 wk of treatment. As originally developed by Porsolt *et al.* (16), this test measures the tendency of rodents to become immobile and float after being placed in a container of water in which they can neither stand up nor escape. The ability of drugs to increase swimming and struggling behavior under these conditions has proven to be a good predictor of clinical antidepressant efficacy (18). We found a significant main effect ($P = 0.0018$) of imipramine to decrease time spent immobile during forced-swim testing (Fig. 1), with no significant effect of adrenalectomy or adrenalectomy \times drug treatment interaction. There was no difference in immobility between vehicle-treated Sham and ADX mice. Despite the significant overall effect of imipramine on immobility, immobility within adrenal groups did not differ significantly at the *post hoc* level, in part because of corrections for multiple comparisons (Sham mice, vehicle *vs.* imipramine treatment, $P = 0.036$ before correction and 0.072 after correction; ADX mice, vehicle *vs.* imipramine treatment, $P = 0.090$ before correction and 0.18 after correction).

We evaluated imipramine effects on unstressed HPA ac-

TABLE 1. Change in body weight over 2 months of daily ip injections with either saline vehicle or 20 mg/kg imipramine, expressed as a percentage of body weight on the first day of treatment

	Vehicle (%)	Imipramine (%)
Sham	16.52 \pm 1.14	13.08 \pm 1.14 ^a
ADX	10.09 \pm 1.39	8.30 \pm 1.09

n = 14–24/group.

^a $P < 0.05$ *vs.* vehicle in the same adrenal group.

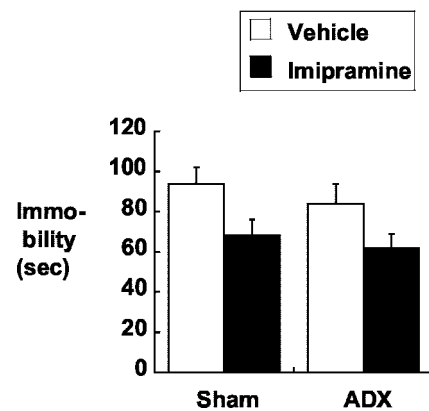


FIG. 1. Time spent immobile during forced swim testing in Sham and ADX mice after 8 wk of daily injections with saline vehicle (white bars) or imipramine (20 mg/kg, ip; black bars). Imipramine had a significant main effect on immobility ($P = 0.0018$). n = 14–24/group.

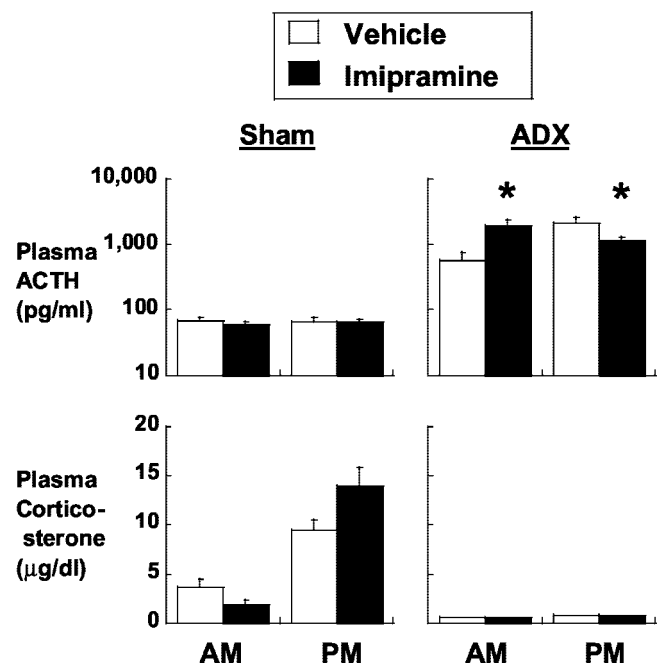


FIG. 2. Circadian nadir (morning; AM) and peak (evening; PM) plasma levels of ACTH (top) and corticosterone (bottom) in Sham (left) and ADX (right) mice after 2 months of vehicle or imipramine treatment. Pairs of white and black bars depict data from vehicle- and imipramine-treated mice, respectively, at the time of day indicated at the bottom of the figure. Groups are as in Fig. 1; note log scale for plasma ACTH graphs. n = 6–12/group (AM) and 8–13/group (PM); *, $P < 0.05$, imipramine *vs.* vehicle in the same adrenal group.

tivity by measuring circadian trough (morning) and peak (early evening) levels of ACTH and corticosterone. ADX mice displayed significant increases in morning plasma ACTH and significant decreases in evening plasma ACTH after imipramine treatment, whereas Sham mice showed no treatment effects on plasma ACTH (Fig. 2, top). However, imipramine did tend to decrease morning and increase evening plasma corticosterone in Sham mice (Fig. 2, lower left), although these effects were not significant ($P = 0.086$ in both cases). Plasma corticosterone in ADX mice was near the

lower limit of detection and unaffected by imipramine (Fig. 2, lower right).

We also tested the ability of the HPA axis to respond to and recover from stress by measuring plasma ACTH and corticosterone levels immediately or 30 min after a 10-min restraint. Restraint was performed within 1 h of lights on to match the timing of the basal morning sample. Peak plasma corticosterone in Sham mice and plasma ACTH in ADX mice at the end of restraint were approximately 2- to 4-fold higher relative to basal morning levels measured within the previous 36 h. There was no significant effect of imipramine on the absolute levels of plasma corticosterone and ACTH measured immediately after the end of restraint (Fig. 3, 10' bars). However, Sham mice treated with imipramine exhibited significantly higher levels of ACTH than their vehicle-treated counterparts after the 30-min recovery period (Fig. 3, upper left, 40' bars). There was no effect of imipramine on plasma corticosterone at 40 min in Sham mice (Fig. 3, lower left) or on plasma hormones at any time in ADX mice (Fig. 3, right).

To assess glucocorticoid-like effects of imipramine on other glucocorticoid target tissues, we measured normalized thymus weight, a standard index of integrated glucocorticoid exposure (19). Imipramine treatment significantly increased normalized thymus weight in Sham mice, whereas it significantly decreased normalized thymus weight in ADX mice (Fig. 4).

To determine whether the ability of imipramine to influence HPA activity correlated with effects on GR function in brain, we tested the ability of imipramine to cause GR trans-

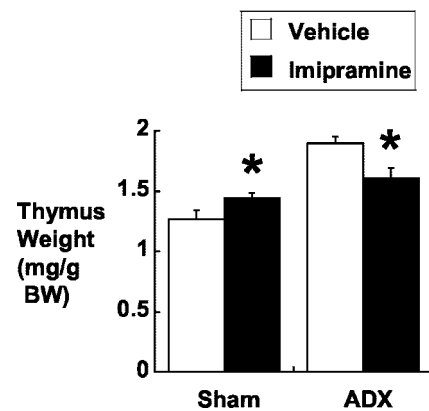


FIG. 4. Normalized thymus weight (milligrams wet weight of thymus per gram of final body weight) in Sham and ADX mice after 2 months of vehicle or imipramine treatment. Groups are as in Fig. 1. $n = 14-22/\text{group}$; *, $P < 0.05$, imipramine *vs.* vehicle in the same adrenal group.

location from the cytoplasm to the nucleus, an early step in GR activation (20), in the hippocampus of ADX mice. For these experiments, we chose a high dose of imipramine (30 mg/kg) specifically to compare maximal effects on GR translocation with that of the endogenous ligand, corticosterone. This dose of imipramine significantly decreased swim test immobility in a parallel set of mice treated similarly to those for evaluating GR localization (imipramine *vs.* saline, 17 ± 14 *vs.* 81 ± 13 sec, respectively; $P = 0.011$; $n = 5/\text{group}$). However, chronic administration of this higher dose did not produce any greater differences in HPA axis activity than those depicted in Figs. 2 and 3 (not shown).

In ADX mice injected with saline vehicle, GR immunoreactivity in CA1 pyramidal cells 1 h after injection was largely cytoplasmic, as distinguished by the absence of staining in cell nuclei (Fig. 5, vehicle). In ADX mice injected with corticosterone 1 h before sacrifice (plasma corticosterone, 19.1 ± 0.6 $\mu\text{g}/\text{dl}$; $n = 3$), GR immunoreactivity was concentrated in nuclei in distinct, round, strongly stained profiles (Fig. 5, corticosterone). ADX mice injected with imipramine exhibited a similar pattern of staining, with GR immunoreactivity no longer excluded from the nucleus (Fig. 5, imipramine). No staining occurred in sections from any treatment group if the primary antibody was omitted (Fig. 5, no primary Ab). Evaluation of GR localization in mice killed 30 or 120 min after injection yielded similar results, as did assessment of mice receiving only a single injection of imipramine (not shown).

Discussion

In support of our hypothesis, we have found that imipramine, a representative tricyclic antidepressant, exerts significant effects on HPA activity independent of glucocorticoid secretion and suggestive of partial glucocorticoid agonist activity. These *in vivo* pharmacological effects were mirrored by the ability of imipramine to induce at least partial nuclear localization of GRs. The extent to which imipramine has weak glucocorticoid agonist or antagonist effects on HPA activity may depend on neuron- or pathway-specific factors.

Two receptor systems mediate the effects of endogenous

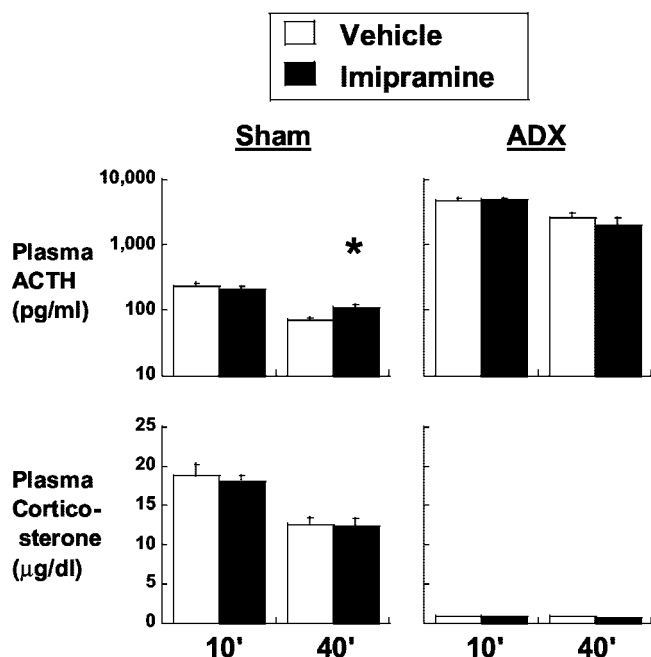


FIG. 3. Stress-induced plasma levels of ACTH (top) and corticosterone (bottom) in vehicle- and imipramine-treated Sham and ADX mice (left and right panels, respectively). Pairs of white and black bars depict data from vehicle- and imipramine-treated mice, respectively, that were restrained for 10 min and then either sampled immediately (10') or allowed to recover for 30 min before sampling (40'). Groups and symbols are as in Fig. 1; note log scale for plasma ACTH graphs. $n = 8-11/\text{group}$ (ACTH) and $8-13/\text{group}$ (corticosterone); *, $P < 0.05$, imipramine *vs.* vehicle in the same adrenal group.

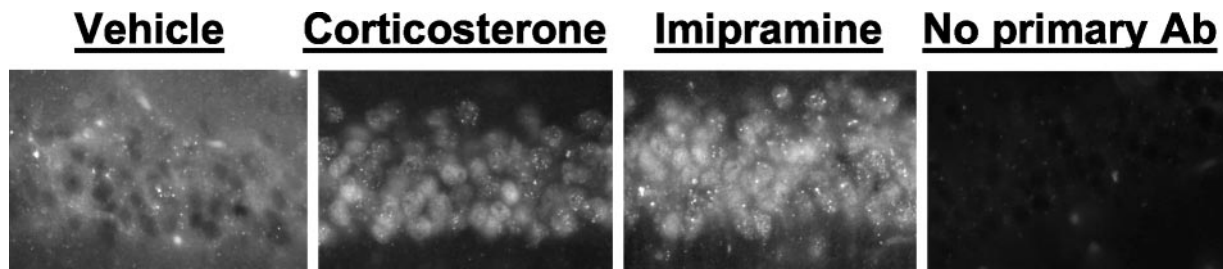


FIG. 5. From left to right, GR immunoreactivity in the CA1 pyramidal cell layer of the hippocampus in ADX mice treated with saline vehicle, corticosterone, or imipramine, respectively, as described in *Materials and Methods*. The far right panel is representative of results when the primary antibody (Ab) was omitted from the procedure. Pictures are representative of staining in at least three mice per treatment.

glucocorticoids on HPA activity. Occupancy of the high-affinity, corticosterone-preferring mineralocorticoid receptor (MR) is thought to set basal activity of the HPA axis in rodents during the circadian nadir, whereas occupancy of both the MR and the lower-affinity, higher-capacity GR controls elevated HPA activity during stress or the circadian peak (1, 19). We have found that when central pathways dictating high glucocorticoid levels are activated, as during stress or the circadian peak, imipramine appears to exhibit partial agonist activity in GR-mediated glucocorticoid negative feedback. In the absence of competition with corticosterone, imipramine exhibited weak GR agonist-like effects to inhibit evening ACTH in ADX mice without fully reducing ACTH to levels observed in Sham mice. In Sham mice, which have high glucocorticoids during the circadian peak or stress recovery, imipramine appeared to antagonize negative feedback, increasing post-stress ACTH levels and tending, albeit not significantly, to increase evening corticosterone.

We also found imipramine-induced decreases in ADX thymus weight and increases in Sham thymus weight. These effects would also be consistent with respective mimicry or antagonism of GR-mediated thymic involution, a classic index of glucocorticoid action (19). It is unlikely that Sham mice exhibited increases in normalized thymus weight solely because of their lower final body weight, because weight loss alone decreases, rather than increases, normalized thymus weight (21).

In agreement with the pharmacological effects of imipramine *in vivo*, we found that imipramine promotes nuclear localization of hippocampal GRs in ADX rats. Several studies have shown that tricyclic antidepressants induce nuclear translocation of GR in cultured cells and neurons (1, 22, 23). We have now shown that antidepressant doses of imipramine can induce GR translocation in neurons *in vivo*, in the context of their neuroanatomical location and synaptic circuitry within the brain.

The hippocampus has long been a focus of depression research. Hippocampal volume reductions are thought to predict vulnerability to or progression of depression, whereas antidepressant stimulation of dentate gyrus neurogenesis has been suggested to contribute to their clinical efficacy by preventing hippocampal atrophy (24). High levels of GR and MR expression make the hippocampus an attractive candidate to participate in glucocorticoid feedback control of HPA activity (25). This abundance of GR and MR has also been proposed to make the hippocampus vulnerable to potential detrimental effects of glucocorticoids during de-

pression-related increases in HPA activity, which could in turn exacerbate depression-associated hippocampal atrophy (26). Although not all studies are in agreement on this issue (27, 28), our experiments provide an *in vivo* correlate with which to investigate glucocorticoid-relevant mechanisms of antidepressant action, and are consistent with the pharmacological effects we have observed on plasma ACTH regulation. Further work will be necessary to determine the extent to which this ligand-independent nuclear localization occurs in other brain regions and correlates with changes in HPA activity during chronic antidepressant administration.

Collectively, our physiological data agree with the recent demonstration in a hippocampal cell line that some tricyclic antidepressants enhance GR transcriptional activity *in vitro* at low glucocorticoid levels but inhibit this activity at higher glucocorticoid concentrations (23). The glucocorticoid-independent nuclear translocation that we demonstrated in hippocampal neurons *in vivo* could contribute to these effects. Facilitated entry of receptors into the nucleus could accelerate or enhance the effects of low glucocorticoid levels, whereas nuclear retention of unliganded receptors is thought to make them unavailable for binding additional hormone (1). Whereas the precise mechanisms by which antidepressants such as imipramine regulate GR activity remain to be defined, it seems unlikely that the agonist-like actions we and others have observed result solely from intermolecular interaction between GR and imipramine. A growing body of literature links the cAMP and protein kinase A pathways to glucocorticoid-independent actions of antidepressants and other compounds on GR function (1, 29); at least some of the enhancing effects of increased phosphorylation on GR activity may be independent of GR itself (30). Antidepressant induction of GR and MR expression, which has been reported by several investigators (1, 22), could also be involved in imipramine effects on HPA function; it remains to be determined whether similar receptor regulation is evident *in vivo* when drug-induced changes in glucocorticoid secretion are controlled.

The dual, partial glucocorticoid agonist-like action that we have observed for imipramine may also account for discrepancies in the literature. Several investigators have shown that tricyclic antidepressants alter GR function in cultured cells, but there has been disagreement as to whether these drugs facilitate or impair glucocorticoid signaling (1). Because these studies used a variety of cell lines ranging from fibroblasts to hippocampal neurons, the relative agonist or antagonist action of tricyclic antidepressants may depend in part on

cell-specific factors. Our finding that adrenal status influences imipramine effects on the HPA axis may also account for contradictions among reports of inhibition (10, 11, 31), stimulation (11), or no effect (32) of tricyclic antidepressants on circadian or stress-induced HPA activity (1). Particularly because tricyclic antidepressants have recently been reported to alter intracellular corticosteroid uptake (33), our use of ADX mice more directly tested the ability of these drugs to alter HPA activity.

Inconsistencies in the reported HPA actions of imipramine may also be due to uncertainty that drug doses were sufficient for antidepressant effects. We verified that HPA effects occurred at a chronic imipramine dose that produced a significant main effect ($P = 0.0018$) of imipramine treatment on immobility in the forced-swim test, an accepted predictor of antidepressant efficacy (18). The daily dose in our studies was also the same as that reported by other investigators to produce significant antidepressant effects in mice within a shorter time (14). We did not find significant imipramine effects on immobility in Sham mice only because we took a very conservative approach to include *post hoc* tests for effects of adrenalectomy on immobility. In contrast to findings in rats (34), we did not find that adrenalectomy increased swimming in vehicle-treated mice, probably because of species differences in forced-swim behavior (35). Although the effect of imipramine on forced-swim immobility was not significant in ADX mice, it seems unlikely that adrenalectomy altered sensitivity to imipramine, because hormonal effects of imipramine were more pronounced in ADX mice. Chronic administration of 30 mg/kg/d imipramine, a dose we and others (16) found to have rapid effects on immobility in mice, did not have any more dramatic endocrine effects than did the 20-mg/kg dose (not shown). Therefore, the HPA axis effects we report are unlikely to be changed by higher doses of imipramine.

Our data did not uniformly demonstrate a pattern of inhibitory (GR agonist-like) effects in ADX mice and stimulatory (GR antagonist-like) effects in Sham mice. Imipramine effects in Sham mice were modest and did not involve parallel changes in ACTH and corticosterone. However, because repeated blood sampling is required to detect changes in circadian ACTH or maximal adrenocortical output (36, 37), it is possible that more frequent blood samples would reveal a bigger impact of imipramine treatment in Sham mice. Similarly, although the inhibition of evening ACTH in imipramine-treated ADX mice would predict that stress-induced stimulation of ACTH in ADX mice should also have been inhibited, our sampling time course after stress may have been too limited to detect this effect.

The increased morning ACTH in imipramine-treated ADX mice also may not seem to fit a partial glucocorticoid agonist model of imipramine action. However, positive adrenal steroid feedback on hypothalamic-pituitary activity has been demonstrated (38–40). In particular, work by Laugero *et al.* (40) has shown that central corticosteroid infusion increases hypothalamic-pituitary activity in ADX rats at the circadian nadir. Thus, increased morning ACTH in our ADX mice could be attributed to imipramine mimicking the facilitative effects of GR occupancy at a time when glucocorticoid levels should normally be low. We doubt that our results are at-

tributable to imipramine antagonism of the effects of aldosterone replacement in ADX mice, because aldosterone exerts little inhibition of hypothalamic-pituitary activity (15, 19, 41).

We cannot exclude the possibility that our results are due to pharmacological actions of imipramine unrelated to the GR. Imipramine increases serotonin and norepinephrine levels and blocks cholinergic, histaminergic, and adrenergic receptors (42, 43). All of these actions can potentially affect HPA activity, either via central effects on hypothalamic function or via direct effects at the pituitary or adrenal. Analogous to dopaminergic activation of ovarian steroid receptors (44), these neurochemical effects could also account for changes in intracellular GR localization without direct regulation of GR translocation by imipramine. The thymus gland, in addition to being a classic glucocorticoid target organ, receives neural input (45); altered thymus weight in the current studies could be due to neurally mediated effects of imipramine. These diverse alternatives notwithstanding, partial glucocorticoid agonist activity of imipramine is a more parsimonious interpretation of our data, particularly in the context of growing *in vitro* evidence for imipramine effects on GR function.

Because elevated HPA activity is usually observed at the circadian nadir in depression (46), the glucocorticoid-like activity of tricyclic antidepressants at low glucocorticoid levels could account for their clinical efficacy to reduce abnormal HPA activity along with psychiatric symptoms. This interpretation can be reconciled with two seemingly contradictory observations. Our findings that imipramine tended to increase post-stress and circadian peak HPA activity in imipramine-treated Sham mice imply that tricyclic antidepressants could adversely augment HPA activity under other conditions. Nonetheless, interference of these drugs with the action of higher glucocorticoid levels could also attenuate potential deleterious effects of these increases on mood. Secondly, tricyclic antidepressants are reportedly ineffective in glucocorticoid-induced mood disorders (7). However, these reports do not negate the significance of tricyclic effects on GR signaling in depression, because even in untreated depression GR occupancy is probably much lower, and thus more likely susceptible to tricyclic antidepressant modulation, than that produced by pharmacological levels of synthetic glucocorticoids. Further *in vivo* work is necessary to elucidate the neuroanatomical targets for the agonist and antagonist effects of tricyclic and other antidepressants on corticosteroid signaling.

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