Published in final edited form as:

Brain Res. 2009 September 8; 1288: 69–78. doi:10.1016/j.brainres.2009.06.082.

Glucocorticoid status affects antidepressant regulation of locus coeruleus tyrosine hydroxylase and dorsal raphe tryptophan hydroxylase gene expression

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Abstract

Brainstem monoaminergic nuclei express glucocorticoid receptors (GR), and glucocorticoids have been shown to inhibit expression of enzymes involved in monoamine synthesis. Monoamine deficits have been implicated in depression pathology. However, it is unknown if antidepressants regulate brainstem GR, and if glucocorticoids might influence antidepressant effects on monoamine-synthesizing enzymes. Our lab has found opposing effects of the monoamine oxidase inhibitor phenelzine and the tricyclic antidepressant imipramine on HPA activity and forebrain GR expression. We therefore hypothesized that phenelzine and imipramine would also affect brainstem GR gene expression differentially, and that antidepressant-induced changes in GR expression would correlate with effects on monoamine-synthesizing enzyme expression. Using *in situ* hybridization, we measured effects of chronic antidepressant treatment on brainstem GR, locus coeruleus and ventral tegmental area (VTA) tyrosine hydroxylase (TH), and dorsal raphe tryptophan hydroxylase (TPH2) gene expression in male C57BL/6 mice that were adrenalectomized and replaced with defined levels of corticosterone. GR expression was decreased by phenelzine in the locus coeruleus and decreased by imipramine in the dorsal raphe. Phenelzine increased locus coeruleus TH and imipramine increased dorsal raphe TPH2 gene expression in a glucocorticoid-dependent manner, suggesting that increases in these enzymes were due to relief of inhibitory glucocorticoid signaling. We did not find antidepressant effects on GR or TH expression in the VTA or on MR expression in any of the nuclei examined. Our findings represent a potential mechanism through which antidepressants and glucocorticoids could alter both HPA activity and mood via effects on brainstem GR, norepinephrine, and serotonin.

Keywords

Glucocorticoid; tricyclic antidepressant; monoamine oxidase inhibitor; HPA axis; glucocorticoid receptor; mineralocorticoid receptor; norepinephrine; serotonin

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Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a hormonal system that is often dysregulated in depression, and therefore has received attention as a potential biological marker for this disease. Elevated HPA activity is the most common form of HPA dysregulation in depression and has been reported to occur in 50-80% of the depressed population (Clayton, 1998; Holsboer, 2001). Successful antidepressant treatment normalizes aberrant HPA activity in depression, and persistent HPA hyperactivity after treatment has been found to be a significant predictor of relapse (Hatzinger et al., 2002), suggesting that circuits that stimulate HPA activity may be involved in depression pathology. However, there are also reports that HPA activity may be lower than normal in some forms of depression (Anisman et al., 1999; Gold et al., 2002; Posener et al., 2000). A more comprehensive theory encompassing these contradictory observations is needed to improve the utility of HPA activity as a biological marker for depression and antidepressant response.

The increased HPA activity that most commonly occurs in depression has been attributed to deficits in glucocorticoid feedback inhibition (Holsboer, 2001; Pariante and Miller, 2001). In support of this theory, dexamethasone suppression of cortisol secretion is impaired in depressed patients and corrected by successful antidepressant treatment (Hatzinger et al., 2002). Animal studies have suggested that antidepressants could correct faulty feedback regulation by increasing the expression or function of the high-affinity mineralocorticoid receptors (MR) or the lower affinity glucocorticoid receptors (GR) that mediate the feedback effects of glucocorticoids in brain (Reul and de Kloet, 1985). Such studies have focused on receptors for glucocorticoids in the forebrain, and almost exclusively on those in the hippocampus (Pariante and Miller, 2001). Notably, however, glucocorticoid receptors are also expressed in brainstem monoaminergic nuclei (Czyrak and Chocyk, 2001; Harfstrand et al., 1986), several of which are thought to be targets of antidepressant action (Nutt, 2002). Remarkably little is known about antidepressant effects on corticosteroid receptor expression in these regions.

The noradrenergic locus ceruleus, the serotonergic dorsal raphe, and the dopaminergic ventral tegmental area all express corticosteroid receptors and have been the focus of antidepressant research. All three of these nuclei have been reported to express GR; the locus coeruleus and dorsal raphe have also been shown to express MR (Arriza et al., 1988; Czyrak and Chocyk, 2001; Harfstrand et al., 1986; Lechner and Valentino, 1999). Depression has often been inferred to result from defects in serotonin and/ or norepinephrine signaling (Delgado, 2006; Hasler et al., 2008; Nutt, 2002), and both the locus coeruleus and the dorsal raphe have been found to exhibit neurochemical and structural changes in depressed suicide victims that have not been described in other noradrenergic or serotonergic nuclei (Bach-Mizrahi et al., 2006; Lowry, 2002). Because ventral tegmental area dopamine neurons are involved in reward and motivated behavior, this circuit has also been linked with the anhedonic symptoms of depression (Harro and Oreland, 2001; Hasler et al., 2008). The locus coeruleus, dorsal raphe, and ventral tegmental area all send projections to forebrain regions implicated in depression pathology such as the prefrontal cortex (Drevets et al., 2002; Loughlin et al., 1986; Moore et al., 1978; Swanson, 1982) and hippocampus (Jay, 2003; Mongeau et al., 1997; Sapolsky, 2000).

Monoaminergic projections from these brainstem nuclei can also influence the activity of the HPA axis (Herman et al., 2003; Lowry, 2002). Locus coeruleus noradrenergic afferents to the hypothalamus have been shown to stimulate HPA activity (Ziegler et al., 1999). Serotonergic projections from the raphe nuclei can both facilitate and inhibit HPA activity, depending on the limbic targets of these projections (Lowry, 2002). Dopaminergic projections from the ventral tegmental area have been reported to affect HPA sensitivity to glucocorticoid feedback inhibition indirectly via the prefrontal cortex (Mizoguchi et al., 2008). Glucocorticoid feedback also modulates the influence of brainstem projections on hypothalamic activity. In the locus

coeruleus and dorsal raphe, glucocorticoids have respectively been reported to inhibit the expression of tyrosine hydroxylase (TH) (Makino et al., 2002), the rate-limiting enzyme for norepinephrine synthesis (Smith et al., 1991), and tryptophan hydroxylase isoform 2 (TPH2), the rate-limiting enzyme for serotonin synthesis in the brain (Clark et al., 2008; Walther and Bader, 2003). This evidence suggests that antidepressants have the potential to affect HPA activity not only via effects on forebrain corticosteroid receptors, but also by regulating GR expression and/ or activity of brainstem monoaminergic neurons.

We have previously shown novel, differential effects of antidepressants on forebrain corticosteroid receptors, and have proposed that theories of antidepressant action should be expanded to include decreases as well as increases in corticosteroid receptor expression (Heydendael and Jacobson, 2008). We have found that chronic treatment with the monoamine oxidase inhibitor (MAOI) phenelzine decreased GR gene expression in feedback-related regions such as the paraventricular hypothalamus and prefrontal cortex and increased plasma corticosterone, plasma ACTH, and hypothalamic CRH mRNA levels in a manner consistent with impaired glucocorticoid feedback. In contrast, chronic treatment with the tricyclic antidepressant (TCA) imipramine increased GR gene expression in the same forebrain regions and exhibited a GR partial agonist-like ability to facilitate HPA feedback inhibition at low glucocorticoid levels (Heydendael and Jacobson, 2008; Kier et al., 2005; Mukherjee et al., 2004). These differential effects on HPA feedback regulation may be relevant to the preferential MAOI responsiveness reported in depression subtypes with enhanced rather than impaired glucocorticoid feedback sensitivity (Levitin et al., 2002; Stewart et al., 2005).

We now suggest that HPA-relevant targets of antidepressants should be expanded to include brainstem GR. Based on evidence for glucocorticoid inhibition of monoamine-synthesizing enzyme expression in specific brainstem nuclei (Clark et al., 2008; Makino et al., 2002), we hypothesized that antidepressant effects on monoamine-synthesizing enzyme expression would correlate with antidepressant-induced changes in corticosteroid receptor expression. To test this hypothesis, we used *in situ* hybridization to measure imipramine and phenelzine effects on brainstem gene expression of GR, MR, TH, and TPH2. Similar to our previous results in forebrain (Heydendael and Jacobson, 2008), we have also found that imipramine and phenelzine have region-specific effects on brainstem GR mRNA levels. Supporting our hypothesis, monoamine-synthesizing enzyme gene expression also correlated with these differential changes in brainstem GR expression.

Results

GR mRNA was strongly expressed in both the locus coeruleus and dorsal raphe but was not markedly expressed in the ventral tegmental area (Figure 1, left column, top to bottom panels). Representative images are also shown in Figure 1 of gene expression of TH in the locus coeruleus (right column, top), TPH2 in the dorsal raphe (right column, middle) and TH in the ventral tegmental area (right column, bottom). GR gene expression was very low in other raphe nuclei such as the median raphe. MR mRNA was also low in all regions examined and did not exhibit any significant antidepressant-induced changes; therefore, these data are not shown.

Figures 2 and 3 depict glucocorticoid and antidepressant effects on GR and either locus coeruleus TH or dorsal raphe TPH2 gene expression. Although the experimental designs differ in certain respects, we have presented the results of 8 weeks of imipramine treatment (Experiment 1; panels A and B) with those of 4 weeks of phenelzine treatment (Experiment 2; panels C and D) because these two treatment paradigms had similar effects to reduce forced-swim test immobility (Heydendael and Jacobson, 2008; Kier et al., 2005; Mukherjee et al., 2004), a behavioral correlate of depression that is associated with significant neurochemical changes and thought to reflect clinically relevant antidepressant actions (Duncan et al.,

1996; Lucki et al., 2001; Shishkina et al., 2007). Effects of imipramine were initially tested in adrenalectomized (**ADX**) as well as sham-adrenalectomized (**Sham**) mice to avoid confounding effects from antidepressant-induced changes in glucocorticoid secretion. When subsequent experiments revealed that 4 weeks of phenelzine treatment had stimulatory effects on HPA activity in Sham mice (Kier et al., 2005) not observed with imipramine, phenelzine-induced increases in corticosterone secretion in intact mice were respectively controlled and mimicked by including additional adrenalectomized mice in Experiment 2 that were replaced with pellets providing physiological (ADX+10% Cort) or elevated levels of corticosterone (ADX+25% Cort; (Kier et al., 2005)). Additional data were collected after 4 weeks of imipramine treatment (Experiment 3) to control for treatment duration and adrenal hormone status, even though this length of imipramine treatment had no significant effects on forced-swim immobility (data not shown); these additional data are presented in Table 1.

Glucocorticoid and antidepressant effects on locus coeruleus GR and TH gene expression

In Experiment 1, adrenalectomy had a significant main effect to increase GR gene expression in the locus coeruleus ($F_{df} = 19.327_{1,29}$, $P < .0001$), but 8 weeks of imipramine treatment had no additional effects (Figure 2A). Adrenalectomy also had significant main effect to increase locus coeruleus TH gene expression ($F_{df} = 23.325_{1,28}$, $P < .0001$, Figure 2B). In addition, locus coeruleus TH gene expression exhibited significant main effects of 8 weeks of imipramine treatment ($F_{df} = 4.397_{1,28}$, $P = .0452$) and a significant interaction between adrenalectomy and imipramine treatment ($F_{df} = 5.75_{1,28}$, $P = .0234$), such that imipramine significantly decreased TH mRNA levels only in the adrenalectomized (ADX+0) group (Figure 2B). Four weeks of imipramine administration (Experiment 3) had similar effects on locus coeruleus gene expression, with no effects on GR but a tendency for imipramine to decrease TH expression in adrenalectomized mice without glucocorticoid replacement ($P = 0.0832$, Table 1). We also regressed locus coeruleus TH against GR gene expression in adrenalectomized mice with no (ADX+0) or fixed (ADX+10% Cort) corticosterone levels from Experiment 3 to determine if there was a correlation between TH and GR at fixed, controlled levels of corticosterone. There was a tendency for a correlation between locus coeruleus TH and GR gene expression in imipramine-treated mice, but neither this nor the relationship in saline-treated mice in Experiment 3 was significant (imipramine: $F_{df} = 3.951_{1,7}$, $P = 0.087$; saline: $F_{df} = 0.400_{1,10}$, $P = 0.541$).

Because imipramine effects in Experiment 1 were most evident in ADX+0 mice, and because resource limitations kept us from analyzing all groups in Experiment 2 simultaneously, we initially focused on adrenalectomized groups with and without corticosterone replacement as being the most specific test of the effects of glucocorticoid manipulation. Separate analyses of Sham mice from Experiment 2 (Table 2) corroborated results in adrenalectomized groups. Consistent with the effect of adrenalectomy to increase locus coeruleus GR in Experiment 1, corticosterone replacement had a significant main effect to decrease GR expression in adrenalectomized mice in Experiment 2 ($F_{df} = 4.548_{2,33}$, $P = .0180$, Figure 2C). However, in contrast to the lack of imipramine effects on locus coeruleus GR expression, 4 weeks of phenelzine treatment had a significant main effect to decrease locus coeruleus GR ($F_{df} = 22.367_{1,33}$, $P < .0001$) that was significant at the post-hoc level in each glucocorticoid replacement group (Figure 2C). Separate analyses confirmed that phenelzine had similar effects to decrease locus coeruleus GR mRNA in Sham mice from Experiment 2 (Table 2).

In agreement with the ability of adrenalectomy to increase locus coeruleus TH in Experiment 1, corticosterone replacement had a significant main effect to decrease locus coeruleus TH expression in Experiment 2 ($F_{df} = 10.940_{2,32}$, $P = .0002$, Figure 2D). TH gene expression in the locus coeruleus exhibited both a significant main effect of phenelzine treatment ($F_{df} = 8.735_{1,32}$, $P = .0058$) and a significant interaction between glucocorticoid replacement and

phenelzine treatment ($F_{df} = 3.71_{2,32}$, $P = .0356$), such that TH gene expression was significantly higher after phenelzine vs. vehicle treatment in the ADX+10% Cort group (Figure 2D). Phenelzine also significantly increased locus coeruleus TH gene expression in Sham mice from Experiment 2 (Table 2). Regression analysis of adrenalectomized mice with no or fixed levels of corticosterone in Experiment 2 revealed a significant correlation between locus coeruleus TH and GR gene expression in phenelzine-treated mice ($F_{df} = 13.318_{1,13}$, $P = 0.0029$) and a marginal ($P < 0.1$) but not significant correlation in saline-treated mice ($F_{df} = 3.844_{1,22}$, $P = 0.0627$).

Glucocorticoid and antidepressant effects on dorsal raphe GR and TPH2 gene expression

Adrenalectomy tended, although not significantly, to increase dorsal raphe GR gene expression in Experiment 1 ($F_{df} = 4.124_{1,29}$, $P = .0515$; Figure 3A). However, there was a significant main effect of eight weeks of imipramine treatment to decrease GR gene expression in the dorsal raphe ($F_{df} = 26.282_{1,29}$, $P < .0001$), which was significant at the post-hoc level in both the Sham and ADX+0 groups (Figure 3A). TPH2 gene expression in the dorsal raphe exhibited significant main effects of adrenalectomy ($F_{df} = 28.558_{1,30}$, $P < .0001$) and 8 weeks of imipramine treatment ($F_{df} = 21.664_{1,30}$, $P < .0001$), with both factors increasing TPH2 gene expression (Figure 3B). Imipramine effects to increase TPH2 expression over vehicle-treated levels were significant at the post-hoc level in the Sham group (Figure 3B). Four weeks of imipramine administration had effects on dorsal raphe GR and TPH2 gene expression that were comparable to those of 8 weeks of treatment, with all groups exhibiting decreases in GR, and only groups with glucocorticoids exhibiting significant increases in TPH2 expression (Sham, ADX+10%; Table 1). Analysis of adrenalectomized mice with no (ADX+0) or fixed corticosterone replacement (ADX+ 10% Cort) in Experiment 3 showed a significant correlation between dorsal raphe TPH2 and GR gene expression only in imipramine-treated mice (imipramine: $F_{df} = 6.477_{1,8}$, $P = 0.034$; saline: $F_{df} = 2.554_{1,10}$, $P = 0.141$).

In Experiment 2, there was a significant main effect of corticosterone replacement to decrease dorsal raphe GR gene expression in adrenalectomized mice ($F_{df} = 9.292_{2,33}$, $P = .0006$; Figure 3C). There was, however, no significant effect of 4 weeks of phenelzine treatment on dorsal raphe GR expression in any of the adrenal hormone groups (Figure 3C and Table 2). TPH2 gene expression in the dorsal raphe displayed a similar pattern, with glucocorticoid replacement having a significant main effect to decrease expression ($F_{df} = 25.05_{3,38}$, $P < .0001$), and phenelzine lacking further effects on TPH2 expression in any adrenalectomized (Figure 3D) or sham-adrenalectomized group (Table 2). There was a significant correlation between dorsal raphe TPH2 and GR gene expression in adrenalectomized mice with fixed corticosterone levels from Experiment 2 after both phenelzine and saline vehicle treatment (phenelzine: $F_{df} = 11.838_{1,12}$, $P = 0.0049$; saline: $F_{df} = 23.516_{1,22}$, $P < 0.0001$).

Glucocorticoid and antidepressant effects on ventral tegmental GR and TH gene expression

Although there were significant main effects of adrenalectomy ($F_{df} = 7.64_{1,30}$, $P < .0097$) and corticosterone replacement ($F_{df} = 10.19_{2,32}$, $P < .0004$) on GR expression in the ventral tegmental area in Experiments 1 and 2, neither imipramine nor phenelzine treatment had any effect on ventral tegmental GR expression (data not shown). Ventral tegmental area TH gene expression was also unaffected by adrenalectomy, glucocorticoid replacement, and antidepressant treatment (data not shown).

Discussion

We have found antidepressant-induced changes in brainstem GR gene expression that are not only drug-specific but also correlate with changes in locus coeruleus tyrosine hydroxylase and dorsal raphe tryptophan hydroxylase mRNA levels. These changes in brainstem monoamine

synthesizing enzyme gene expression suggest a novel role for glucocorticoids in antidepressant-induced changes in serotonin and norepinephrine production. Because serotonin and norepinephrine have been shown to alter HPA activity (Lowry, 2002; Ziegler et al., 1999), and are thought to be abnormal in depression (Nutt, 2002) our data may also reveal GR-related mechanisms for antidepressant effects on HPA activity and mood.

Glucocorticoids inhibit locus coeruleus tyrosine hydroxylase and dorsal raphe tryptophan hydroxylase gene expression

Our data are consistent with prior evidence for glucocorticoid inhibition of enzymes involved in monoamine synthesis. Glucocorticoid treatment can prevent stress-induced increases in locus coeruleus TH gene expression (Makino et al., 2002). Glucocorticoids have also been found to inhibit basal TPH2 gene expression in the dorsal raphe (Clark et al., 2008; Clark and Russo, 1997). In agreement with this literature, we have shown that glucocorticoid replacement of adrenalectomized mice inhibits basal gene expression of TH in the locus coeruleus and TPH2 in the dorsal raphe. Our results are specific to glucocorticoid manipulation because aldosterone was replaced to equivalent, physiological levels in all adrenalectomized groups (Castonguay et al., 2002; Kier et al., 2005; Mukherjee et al., 2004). Glucocorticoid effects on TH and TPH2 expression are also likely to be restricted to specific monoaminergic nuclei, since we did not find ventral tegmental area TH to be inhibited by glucocorticoids, and GR is not expressed by all monoaminergic nuclei (Aronsson et al., 1988).

Glucocorticoid inhibition of monoamine-synthesizing enzyme expression may have a broader significance to both depression pathology and glucocorticoid effects on mood. The ability of clinically effective antidepressants to increase monoamines has led to the suggestion that depression pathology results from monoamine deficiency (Nutt, 2002). Although this idea has been more difficult to substantiate than the possibility that clinical responses to antidepressants depend on increases in monoamines, there is limited evidence that monoamine depletion does increase depressive symptoms in normal individuals, suggesting that sensitivity to monoamine levels could influence depression susceptibility (Delgado, 2006; Hasler et al., 2008). Elevated HPA activity is the most common form of HPA dysregulation in depression (Heuser et al., 1994), implying, along with our data, that elevated glucocorticoids could contribute to the monoamine deficiencies suggested to contribute to depression. These effects could also explain how glucocorticoids can have effects on mood in vulnerable individuals who otherwise lack any history of psychiatric disease (Sirois, 2003).

MAOI and TCA antidepressants differentially affect locus coeruleus and dorsal raphe GR gene expression

We have previously shown that imipramine and phenelzine have brain region-specific effects on forebrain GR expression (Heydendael and Jacobson, 2008); we have now demonstrated differential effects of these antidepressants on brainstem GR expression as well. Phenelzine but not imipramine decreased locus coeruleus GR expression, whereas imipramine but not phenelzine decreased dorsal raphe GR expression. Imipramine-induced decreases in dorsal raphe GR gene expression occurred after both 4 and 8 weeks of treatment, indicating that the differential effects of imipramine and phenelzine on brainstem GR are due to drug-specific differences rather than to the length of antidepressant treatment. Although the antidepressant doses used in these experiments seem high in an absolute sense compared to those used in human patients, these doses are proportional to the higher hepatic capacity to metabolize psychoactive drugs in rodents vs. humans (Liggett, 2004; Lin, 2008). The doses we used were comparable to behaviorally-effective doses used by other investigators (Duncan et al., 1996; Griebel et al., 1998).

The pharmacological basis of the differential effects of imipramine and phenelzine on brainstem GR expression is unknown. Imipramine, a TCA, prevents the re-uptake of both serotonin and norepinephrine (Baldessarini, 2006). Phenelzine, an irreversible, nonselective MAOI, prevents the breakdown of all monoamines by inhibiting monoamine oxidase-A and -B (Baldessarini, 2006) and would be more likely than imipramine to increase dopamine availability. Both locus coeruleus norepinephrine neurons and dorsal raphe serotonin neurons receive dopaminergic projections and express D2 receptors ((Aman et al., 2007; Guiard et al., 2008) and references therein). Since dopamine has been found to alter GR expression and function (Antakly et al., 1987; Casolini et al., 1993), dopamine might also account for the differential effects of phenelzine on GR expression or activity in these nuclei.

The differential effects of MAOI and TCA antidepressants on locus coeruleus TH and dorsal raphe TPH2 gene expression correlate with changes in GR expression

Our data suggest that differential effects of imipramine and phenelzine on GR expression could be relevant to the differing effects of these antidepressants on TH and TPH2 expression. Previous literature has suggested, although not unequivocally, that imipramine and phenelzine have opposing effects on TH gene expression. Imipramine has been reported to decrease locus coeruleus TH (Nestler et al., 1990), and phenelzine has been reported to increase locus coeruleus TH (Brady et al., 1992), although other nonselective MAOI such as tranylcypromine have been reported to decrease locus coeruleus TH expression (Nestler et al., 1990). The correlation between phenelzine-induced decreases in GR and increases in TH gene expression in the locus coeruleus suggests that phenelzine could decrease the inhibitory effect of glucocorticoids on TH by decreasing GR. Supporting this possibility, we observed a significant correlation between locus coeruleus TH and GR gene expression in phenelzine-treated mice. A correlation between locus coeruleus TH and GR gene expression might have been expected in saline-treated mice because of the significant main effects of glucocorticoid treatment on locus coeruleus TH. However, the correlation in saline-treated mice may have been weakened because GR levels are not related to glucocorticoid effects in mice lacking glucocorticoids (ADX+0). The significant effect of phenelzine on TH expression in the Sham and ADX+10% Cort groups but not the ADX+0 group (Figure 2D) would be consistent with the need for glucocorticoids to be present in order to reveal changes in GR activity. Although the mechanisms of phenelzine effects on GR are unknown, several other antidepressants have glucocorticoid-independent effects on GR function (Mukherjee et al., 2004; Pariente and Miller, 2001) that could account for the stronger relationship between locus coeruleus TH and GR after phenelzine treatment. The lack of phenelzine effects on TH expression in the ADX+25% Cort group (Figure 2D) may reflect the inability of phenelzine effects on GR expression or function to overcome the inhibitory effects of high glucocorticoid levels in this group.

Imipramine treatment, on the other hand, failed to affect locus coeruleus GR while decreasing locus coeruleus TH in ADX+0 but not Sham (Figure 2B; Table 1) or ADX +10% mice (Table 1). These findings suggest that imipramine effects on locus coeruleus TH do not require glucocorticoid signaling, and may be obscured by floor effects at the lower levels of TH expressed in the presence of glucocorticoids. Stress-induced increases in locus coeruleus TH have been previously reported in adrenalectomized mice without corticosterone replacement (Makino et al., 2002), indicating that TH expression is not regulated exclusively by glucocorticoids.

In contrast to imipramine effects in the locus coeruleus, imipramine-induced increases in dorsal raphe TPH2 did correlate with imipramine-induced decreases in dorsal raphe GR expression, suggesting that imipramine impaired GR-mediated inhibition of dorsal raphe TPH2. The glucocorticoid dependency of imipramine effects is supported both by the strong correlation between TPH2 and GR expression in imipramine-treated mice and by the lack of imipramine

influence on dorsal raphe TPH2 expression in ADX+0 mice, since changes in GR would not be expected to alter glucocorticoid signaling in the absence of glucocorticoids. Likewise, the lack of phenelzine effects on dorsal raphe TPH2, despite the strong correlation between GR and TPH2 expression, is consistent with the lack of phenelzine effects on dorsal raphe GR. We did not find the strong correlation between dorsal raphe TPH2 and GR in saline-treated mice in Experiment 2 that we observed in Experiment 2, probably because the range of glucocorticoid replacement was more limited in Experiment 3.

It might be argued that changes in receptor and enzyme protein levels, which we did not measure, could differ dramatically from those in mRNA. However, it has been shown that, at least for locus coeruleus TH expression, changes in protein level match the changes in mRNA levels (Nestler et al., 1990). Our data also do not establish that changes in GR affect TH or TPH2 expression within the same neurons, nor whether monoamine levels change as a result. Nevertheless, our results do suggest that changes in gene expression have biological significance, since changes in TH and TPH2, which are inhibited by glucocorticoids, correlated inversely with changes in GR. Our results thus suggest a new mechanism by which antidepressants could alter monoamine availability and HPA activity by regulating brainstem corticosteroid receptor expression.

Relevance to depression

Different forms of depression have been linked with different HPA abnormalities (Gold et al., 2002). Melancholic depression, characterized by a lack of mood reactivity (responsiveness to external events), combined with early morning wakefulness, agitation, anorexia or weight loss (American Psychiatric Association, 2000), is a form of depression that frequently exhibits increased HPA activity (Wong et al., 2000). At the opposite end of the spectrum, atypical depression has been associated with decreased HPA activity and is characterized by psychiatric symptoms that contrast with those of melancholic depression. These symptoms include mood reactivity, lethargy or fatigue, and increased appetite or weight gain (American Psychiatric Association, 2000). Intriguingly, these opposing psychiatric and HPA features are associated with differences in antidepressant response. Melancholic depression has been treated successfully with TCAs (Thase et al., 1995), whereas patients suffering from atypical depression, particularly those with early and chronic disease course, show a poor response to TCA treatment and respond better to monoamine oxidase inhibitors (Stewart et al., 2005; Thase et al., 1995).

Our findings of region- and antidepressant-specific changes in brainstem gene expression may help to explain the differential antidepressant response in melancholic and atypical depression. Imipramine-induced decreases in locus coeruleus tyrosine hydroxylase expression could ultimately reduce locus coeruleus norepinephrine synthesis and reduce noradrenergic stimulation of HPA activity (Ziegler et al., 1999). Such effects could normalize the elevated HPA activity most commonly observed in depression (Wong et al., 2000), while increases in serotonin (via increases in dorsal raphe TPH2 expression) might account for the mood-elevating actions of this class of antidepressants. In contrast, atypical depression has specifically been linked with low norepinephrine as well as low HPA activity (Brady et al., 1991; Gold et al., 2002). The poor response to TCA treatment in atypical depression (Thase et al., 1995) might therefore be attributable to the potential for TCA to exacerbate these reductions in norepinephrine and HPA activity; our and others' evidence (Brady et al., 1992) that MAOI could increase both norepinephrine and HPA activity would be more consistent with the ability to reverse atypical depression deficits.

Our exclusive use of male animals might be criticized for failing to address the higher incidence of depression in females (Marcus et al., 2008). Nevertheless, our data are at least applicable to the approximately 40% of the depressed patients who are male (McGrath et al., 2000), and

suggest novel, GR-related targets of antidepressant action that could be relevant to improving depression treatment in both genders.

Experimental Procedures

Animals and treatments

Brain tissue came from male C57BL/6 mice in previously published experiments (Heydendael and Jacobson, 2008; Kier et al., 2005; Mukherjee et al., 2004). All animal use was approved by the Institutional Animal Care and Use Committee of Albany Medical College and met the standards of the NIH Guide for the Care and Use of Animals (Institute of Laboratory Animal Resources, 1996). Animal housing space was insufficient to allow imipramine and phenelzine to be compared in all adrenal hormone groups contemporaneously, but conditions of treatment and sampling were kept the same across experiments. In Experiment 1, mice were adrenalectomized (ADX+0) or sham-adrenalectomized (Sham) and then treated with 20 mg/kg/d ip imipramine or saline for 8 weeks (Mukherjee et al., 2004). This dose was based on reports of significant effects of chronic imipramine treatment on despair-like immobility in the forced-swim test (Duncan et al., 1996), which we confirmed after eight but not four weeks of treatment (Mukherjee et al., 2004).

For Experiment 2, ADX+0 and Sham mice were treated with 25 mg/kg/d phenelzine or saline ip for 4 weeks (Kier et al., 2005). This dose was based on reports of significant effects of chronic phenelzine treatment on behavior in mice (Griebel et al., 1998), and was verified by forced-swim testing during the last week of treatment (Kier et al., 2005). Because phenelzine increased HPA activity in Sham mice in initial experiments, Experiment 2 was repeated with additional adrenalectomized mice replaced with fixed levels of glucocorticoids (Kier et al., 2005). Glucocorticoid-replaced mice were implanted with 30-mg, sc pellets of 0, 10 or 25% corticosterone by weight (ADX+0, ADX+10% Cort, or ADX+25% Cort, respectively) that respectively provided no, 24 h mean, or circadian peak levels of glucocorticoids (Kier et al., 2005).

Experiment 3. To verify that results in Experiment 1 and Experiment 2 were not attributable to treatment time or corticosterone replacement of adrenalectomized groups, Experiment 3 used Sham, ADX+0, and ADX+10% Cort mice that were treated for 4 weeks with either saline or imipramine to equal the length of Experiment 2 (Heydendael and Jacobson, 2008). ADX+25% Cort mice were not used in Experiment 3 because imipramine did not elevate corticosterone in Sham mice to the same degree as phenelzine (Kier et al., 2005; Mukherjee et al., 2004).

To control for the loss of adrenal steroids other than corticosterone, all adrenalectomized mice were replaced with physiological levels of aldosterone mimicking the lowest diurnal levels of aldosterone in rodents (Castonguay et al., 2002; Kier et al., 2005; Mukherjee et al., 2004) and allowed to select between 0.5% saline or water to drink. Sham-adrenalectomized mice received only water. All mice were killed by decapitation within 3 h of lights-on.

In Situ Hybridization

In situ hybridization for GR and MR was performed with ³⁵S-labeled cRNA probes on 10 µm coronal brain sections from fresh-frozen brains, as previously described (Heydendael and Jacobson, 2008). In situ hybridization for TH and TPH2 was identical to that for GR and MR, with the exception of the hybridization temperature. Slides were incubated with ³⁵S-labeled cRNA probes at 55°C for GR and MR, 50°C for TH, and 60°C for TPH2. A 383 bp Eco RI/ Kpn I fragment of the rat TH cDNA (Dr. Tong Joh, Cornell Medical College in White Plains, NY) was subcloned into pBluescript II SK⁺ and linearized with Bam HI for anti-sense

transcription (Jeong et al., 2000). The mouse cDNA clone for isoform 2 of tryptophan hydroxylase (TPH2) was provided in a pT vector (Promega, Madison, WI) by Michael Bader (Max Delbrück Center for Molecular Medicine, Berlin, Germany), and was linearized with Nco I prior to anti-sense transcription with SP6 (Tenner et al., 2008).

Hybridization signals were detected with a phosphorimager (Typhoon 9210; GE Healthcare, Niskayuna, NY) at 50 μ m resolution after 19–24 h exposure; all images were well within the linear range after this length of exposure. Slides from mice in each treatment and surgery group were hybridized and exposed together except in Experiment 2, where Sham mice were analyzed separately because of limitations in hybridization oven and phosphorimager screen space.

Data analysis

Semi-quantitative densitometric analysis of autoradiographic images was performed with Imagequant 5.0 software (GE Healthcare, Niskayuna, NY), with reference to the mouse brain atlas of Paxinos and Watson (Paxinos and Franklin, 2001). Gray level readings from individual brain regions were corrected for background in a non-expressing area of the same section and normalized to matched ^{14}C standards (146B, American Radiolabeled Chemicals, St. Louis, MO) exposed on each screen. Each gene product was analyzed with the same template and exposure time for all mice in a given experiment, although gray level values may differ between experiments because of differing exposure times. Gene expression values for each mouse were averaged from readings in at least two sections. Mice without two sections from a given brain region were excluded from analysis; consequently, *N* values vary slightly within experiments. ADX+0 mice were only analyzed if their plasma corticosterone was less than 1 $\mu\text{g/dl}$.

Data were analyzed by 2-way ANOVA for the main effects of (1) adrenal hormone status and (2) antidepressant treatment (Statview 5.0, SAS Institute, Cary, NC). ANOVA main effects are reported where they were significant. Post-hoc comparisons were performed by unpaired t-test with Bonferroni correction and are only reported for antidepressant effects because glucocorticoid effects recapitulated those already reported in the literature (Clark et al., 2008; Herman, 1993; Makino et al., 2002). Correlations between either TH or TPH2 and GR gene expression in the locus coeruleus and dorsal raphe, respectively, were analyzed by linear regression (Statview 5.0). Data are presented as mean $\pm$ S.E.M. Significance was defined as $P < 0.05$.

Acknowledgements

This work was supported in part by a Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD), by a bridge grant from Albany Medical College, and by RO1 MH80394 to L.J. The authors gratefully acknowledge the technical expertise of Rebecca Rokow-Kittel.

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Figure 1.

Representative phosphorimager autoradiograms of glucocorticoid receptor (**GR**), tyrosine hydroxylase (**TH**) and tryptophan hydroxylase (**TPH2**) *in situ* hybridization in the locus coeruleus, dorsal raphé nucleus (arrows) and ventral tegmental area (white outline). All hybridization signals were in the linear range of the phosphorimager screens. The template for GR expression in the ventral tegmental area (white outline) was derived from that of TH expression in a neighboring section (arrow), and was distinguished from the substantia nigra with reference to the Franklin and Paxinos Mouse Brain Atlas (Paxinos and Franklin, 2001).

Figure 2.

Effect of chronic imipramine treatment in Experiment 1 (A, B) and chronic phenelzine treatment in Experiment 2 (C, D) on locus coeruleus GR (A, C) and TH (B, D) gene expression. Groups and data analysis are described in Experimental Procedures; *N*s are shown within the respective bars.

*, $P < 0.05$ vs. vehicle in the same adrenal hormone status group

Figure 3.

Effect of chronic imipramine treatment in Experiment 1 (A, B) and chronic phenelzine treatment in Experiment 2 (C, D) on dorsal raphe GR (A, C) and TPH2 (B, D) gene expression. Groups and data analysis are described in Experimental Procedures; *N*s are shown within the respective bars.

*, $P < 0.05$ vs. vehicle in the same adrenal hormone status group

GR, TH and TPH2 mRNA levels in the locus coeruleus (LC) and dorsal raphe (DRN) after 4 weeks of treatment with imipramine or saline vehicle in mice from Experiment 3. Levels are expressed as mean $\pm$ SEM corrected gray level units, as described in Experimental Procedures

Table 1

Brainstem gene expression in mice from Experiment 3						
	Sham		ADX+0		ADX + 10% Cort	
Region / gene	Vehicle n=7	Imipramine n = 6	Vehicle n = 8	Imipramine n = 6	Vehicle n = 4	Imipramine n = 3
LC / GR	0.11 ± 0.01	0.09 ± 0.01	0.29 ± 0.02	0.33 ± 0.03	0.33 ± 0.02	0.32 ± 0.02
LC / TH	0.09 ± 0.01	0.10 ± 0.01	0.20 ± 0.05	0.08 ± 0.01	0.13 ± 0.02	0.13 ± 0.01
DRN / GR	0.38 ± 0.03	0.22 ± .001 [*]	0.70 ± 0.02	0.39 ± 0.02 [*]	0.73 ± 0.02	0.59 ± 0.05 [*]
DRN / TPH2	0.85 ± 0.04	1.26 ± 0.01 [*]	0.49 ± 0.01	0.50 ± 0.02	0.43 ± 0.02	0.61 ± 0.01 [*]

* , P < .05, imipramine vs. vehicle in same adrenal group

Table 2

GR, TH and TPH2 mRNA levels in the locus coeruleus (LC) and dorsal raphe (DRN) after 4 weeks of treatment with phenelzine or saline vehicle in Sham mice from Experiment 2. Levels are expressed as mean $\pm$ SEM corrected gray level units, as described in Experimental Procedures

Brainstem gene expression in Sham mice from Experiment 2		
Region / gene	Vehicle $n = 7$	Phenelzine $n = 6$
LC / GR	0.16 ± 0.01	$0.11 \pm 0.01^*$
LC / TH	0.20 ± 0.02	$0.34 \pm 0.05^*$
DRN / GR	0.12 ± 0.01	0.11 ± 0.01
DRN / TPH2	0.51 ± 0.03	0.59 ± 0.03

^{*}, $P < .05$, phenelzine vs. vehicle