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Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus

(depression/noradrenergic system/imipramine/electroconvulsive seizures/fluoxetine)

ERIC J. NESTLER*, ANNE MCMAHON†, ESTHER L. SABBAN†, JOHN F. TALLMAN*, AND RONALD S. DUMAN*

*Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine and Connecticut Mental Health Center, 34 Park Street, New Haven, CT 06508; and †Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595

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ABSTRACT **Regulation of tyrosine hydroxylase expression by antidepressant treatments was investigated in the locus coeruleus (LC), the major noradrenergic nucleus in brain. Rats were treated chronically with various antidepressants, and tyrosine hydroxylase levels were measured in the LC by immunoblot analysis. Representatives of all major classes of antidepressant medication—including imipramine, nortriptyline, tranylcypromine, fluvoxamine, fluoxetine, bupropion, iprindole, and electroconvulsive seizures—were found to decrease levels of tyrosine hydroxylase immunoreactivity by 40–70% in the LC. Decreased levels of enzyme immunoreactivity were shown to be associated with equivalent decreases in enzyme mRNA levels. Antidepressant regulation of LC tyrosine hydroxylase appeared specific to these compounds, inasmuch as chronic treatment of rats with representatives of other classes of psychotropic drugs, including haloperidol, diazepam, clonidine, cocaine, and morphine, failed to decrease levels of this protein. The results demonstrate that chronic antidepressants dramatically downregulate the expression of tyrosine hydroxylase in the LC and raise the possibility that such regulation of the enzyme represents an adaptive response of LC neurons to antidepressants that mediates some of their therapeutic actions in depression and/or other psychiatric disturbances.**

Among the best studied actions of antidepressant treatments are their effects on the postsynaptic noradrenergic system. Thus, one of the most consistent adaptive responses to chronic administration of antidepressants, including electroconvulsive seizures (ECS), is a downregulation of the β-adrenergic receptor-coupled cAMP system (1–3). These treatments have also been shown more recently to regulate additional postreceptor sites in this signal-transduction pathway, including specific G protein subunits (4) and cAMP-dependent protein phosphorylation (5, 6). In addition to regulation of the postsynaptic noradrenergic system, adaptive changes also occur in presynaptic noradrenergic elements in response to chronic antidepressant treatment. (*i*) Chronic administration of some antidepressants has been shown to decrease α_2-adrenergic receptor regulation of cAMP production in cerebral cortex (7), an effect presumed to be localized, at least in part, to presynaptic noradrenergic nerve terminals. (*ii*) These treatments have been found to decrease the firing rates of noradrenergic neurons in the locus coeruleus (LC) (8–10), the major noradrenergic nucleus in brain. (*iii*) Chronic drug administration has been shown to alter levels of norepinephrine and its metabolites in cerebral cortex in laboratory animals, as well as in blood, cerebrospinal fluid, and urine in human subjects (see refs. 11, 12). While these findings suggest that chronic antidepressant treatments may alter the synthesis of norepinephrine in the brain, no consistent reproducible effect of these treatments on the biosynthetic pathway for norepinephrine has, to date, been established.

The rate-limiting enzyme in the synthesis of norepinephrine is tyrosine hydroxylase. Tyrosine hydroxylase is an ≈60-kDa protein abundant in noradrenergic and dopaminergic cell bodies and distributed throughout the brain in terminal fields of these catecholaminergic systems. Activity of the enzyme is regulated rapidly by neurotransmitters and neuronal activity through its phosphorylation by several types of protein kinase (for review, see refs. 13 and 14). More long-term regulation of the enzyme is achieved through changes in its expression, apparently at the level of gene transcription, by a variety of chronic perturbations (15–22). Such acute and chronic regulation of tyrosine hydroxylase is thought to play a critical role in modulating the functional activity of catecholaminergic neuronal systems in the brain.

In the present study, the influence of chronic antidepressant treatments on the expression of tyrosine hydroxylase was examined in the LC. We show here that chronic administration of every major class of antidepressant results in a dramatic downregulation of tyrosine hydroxylase expression specifically in the LC. The results raise the possibility that downregulation of the biosynthetic pathway for norepinephrine contributes to the molecular mechanisms through which antidepressant treatments exert at least some of their multiple clinical actions.

METHODS

***In Vivo* Drug Treatments.** Male Sprague–Dawley rats (initial weight 150–200 g) received i.p. injections of imipramine (15 mg/kg; Sigma), nortriptyline (15 mg/kg; Sigma); tranylcypromine (7.5 mg/kg; Sigma), fluvoxamine (15 mg/kg; Duphar, Weesp, Holland), fluoxetine (15 mg/kg; Eli Lilly), bupropion (30 mg/kg; Burroughs Wellcome), iprindole (15 mg/kg; Wyeth-Ayerst), and haloperidol (1 mg/kg; McNeil Laboratories) once daily for 18 days or cocaine hydrochloride (15 mg/kg; Sigma) twice daily for 14 days. Clonidine was given in the drinking water (2 μg/ml) for 14 days with an average consumption of 300 μg/kg per day; morphine was administered by daily s.c. implantation of morphine pellets (containing 75 mg of morphine base; National Institute on Drug Abuse) for 5 days with rats used on day 6; and diazepam was administered by implantation of two sialastic capsules (containing 90 mg of diazepam; Hoffman–La Roche), a third capsule on day 10, with rats used on day 21. Control animals received saline injections or underwent identical surgical procedures but did not receive drug implantations. ECS was administered once daily for 1–10 days through earclip electrodes (35 mA, 0.3 sec); control rats were handled in the same manner, but no current was applied. Unless otherwise spec-

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Abbreviations: LC, locus coeruleus; ECS, electroconvulsive seizures.

ified, all animals were sacrificed by decapitation 18 hr after the last drug treatment or ECS. The above doses, durations of treatment, and routes of administration used for the various treatments were those that have been shown in previous studies to lead to chronic effects of these treatments (see refs. 11, 23–29).

Immunoblotting of Tyrosine Hydroxylase. LC and substantia nigra were excised from 0.75-mm-thick coronal cross-sections of brain by obtaining 15-gauge punches with a syringe needle (30). Isolated brain regions were homogenized (10 mg/ml) in 2% SDS, and aliquots (containing 25–75 μg of protein) were adjusted to contain 50 mM Tris (pH 6.7), 2% SDS, 4% (vol/vol) glycerol, 2% (vol/vol) 2-mercaptoethanol, with bromophenol blue as a marker. The samples were then subjected to one-dimensional SDS/polyacrylamide gel electrophoresis (with 7.5% acrylamide/0.3% bisacrylamide in the resolving gels) and to immunoblotting analysis for tyrosine hydroxylase exactly as described (22) using a commercially available rabbit polyclonal antiserum (1:250; Eugene Tech, Allendale, NJ) and ^{125}I-labeled goat anti-rabbit IgG (500 cpm/μl; New England Nuclear). Resulting blots were dried and autoradiographed with the use of intensifying screens (DuPont). Levels of immunolabeling were quantitated by densitometry or by counting excised bands in a gamma counter. Levels of immunolabeling were normalized to protein levels or "per punch," which contain reproducible levels of protein (see ref. 30). In a typical experiment, six control and treated samples were analyzed on each immunoblot.

Northern Blot Analysis of Tyrosine Hydroxylase mRNA. Total mRNA was extracted from LC and substantia nigra by using published procedures (19). Briefly, brain regions were isolated from control and drug-treated rats and frozen at −70°C until further use. LC from five rats or substantia nigra from two to three rats was pooled; RNA was extracted from the samples (≈5 μg) and then analyzed by Northern blot as described (22, 31) by using a cDNA clone for rat tyrosine hydroxylase provided by Edward Ziff, New York University Medical Center (32). The probe was ^{32}P-labeled by a random-primer method (Amersham) to a specific activity of ≈10^9 cpm/μg. Blots were then rehybridized with a ^{32}P-labeled cDNA clone for 18S ribosomal RNA (provided by I. Wool, University of Chicago) and, in some experiments, with a ^{32}P-labeled cDNA clone for rat dopamine β-hydroxylase (31). Hybridizations were quantitated by densitometric analysis of resulting autoradiograms with exposure conditions within the linear range. In a typical experiment, three control and drug-treated samples (each representing pooled tissue as discussed above) were analyzed on each Northern blot.

RESULTS

Regulation of Tyrosine Hydroxylase Expression in the LC by Imipramine Treatment. Chronic imipramine regulation of levels of tyrosine hydroxylase protein was examined in the LC by immunoblot analysis by using a commercially available antiserum (see *Methods*). It was found that chronic administration of imipramine (at a daily dose of 15 mg/kg) decreased levels of tyrosine hydroxylase immunoreactivity in the LC by 55–60% (Fig. 1; Table 1). Similar results were obtained when imipramine was administered chronically at a dose of 10 mg/kg (data not shown). In contrast to chronic treatment, 1 or 7 days of imipramine treatment did not significantly alter levels of enzyme immunoreactivity (101 ± 1% and 81 ± 9%, respectively, of control ± SEM; n = 4 determinations).

To determine whether imipramine regulation of tyrosine hydroxylase in the LC shows some regional specificity, the effect of chronic imipramine on enzyme levels in other catecholaminergic neurons was examined. In contrast to the LC, chronic imipramine was found to have no effect on levels of enzyme immunoreactivity in the substantia nigra, a midbrain nucleus enriched in dopaminergic cell bodies (Fig. 1 and Table 1).

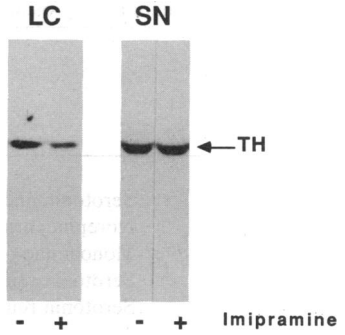

FIG. 1. Autoradiograms showing regulation of tyrosine hydroxylase immunoreactivity by chronic imipramine in the rat LC and substantia nigra (SN). Levels of tyrosine hydroxylase immunoreactivity were quantitated by immunoblot analysis as described. TH, tyrosine hydroxylase.

Next, we studied whether decreased levels of tyrosine hydroxylase immunoreactivity in the LC in response to chronic imipramine are associated with equivalent changes in levels of mRNA for the enzyme, quantitated by Northern blot analysis. Fig. 2 shows that chronic imipramine treatment decreased levels of tyrosine hydroxylase mRNA by ≈45% in the LC. In contrast, chronic imipramine had no effect on levels of enzyme mRNA in the substantia nigra (data not shown). In some experiments, levels of mRNA for dopamine β-hydroxylase, another enzyme in the biosynthetic pathway for norepinephrine, were determined in the LC. It was found (Fig. 2) that chronic imipramine treatment had no effect on levels of dopamine β-hydroxylase mRNA.

Regulation of Tyrosine Hydroxylase Immunoreactivity in the LC by Other Antidepressant Treatments. The influence of a variety of other antidepressant treatments on the expression of tyrosine hydroxylase in the LC was examined to determine whether the downregulation of enzyme expression is a mechanism common to all antidepressants. Indeed, chronic administration of every antidepressant examined in the current study, which included drugs of all major classes of antidepressant medication and ECS (one of the most effective forms of antidepressant treatment), decreased (by 40–70%) levels of tyrosine hydroxylase immunoreactivity in the LC (Fig. 3; Table 1). As observed with imipramine, the effect of these other antidepressant treatments on tyrosine hydroxylase immunoreactivity showed regional specificity, in that no effect on enzyme levels was observed in the substantia nigra (Table 1) and required chronic drug or ECS administration (data not shown).

Regulation of Tyrosine Hydroxylase Immunoreactivity by Other Classes of Psychotropic Drug. A number of other types of psychotropic drug were examined to determine whether regulation of tyrosine hydroxylase is an effect specific to antidepressant treatments. As shown in Table 1 and Fig. 3, chronic treatment of rats with diazepam, haloperidol, clonidine, or cocaine had no significant effect on levels of enzyme immunoreactivity in the LC (Table 1; Fig. 3). Also shown in Table 1 is our earlier observation (22) that chronic morphine treatment increases levels of tyrosine hydroxylase immunoreactivity in this brain region.

DISCUSSION

The major finding of this study is that all major classes of antidepressant treatment decrease the expression of tyrosine hydroxylase in rat LC. Chronic, but not acute, treatment of rats with tricyclic antidepressants, selective serotonin re-

Table 1. Psychotropic drug regulation of tyrosine hydroxylase immunoreactivity in the rat locus coeruleus and substantia nigra

Treatment	Acute mechanism of action	Tyrosine hydroxylase immunoreactivity, % control ± SEM (*n*) LC	SN
Antidepressant drugs			
Imipramine	Serotonin and norepinephrine reuptake blocker	41 ± 3 (6)*	94 ± 10 (6)
Nortriptyline	Norepinephrine reuptake blocker	50 ± 11 (5)*	
Tranylcypromine	Monoamine oxidase inhibitor	36 ± 7 (5)*	93 ± 9 (6)
Fluvoxamine	Serotonin reuptake blocker	31 ± 9 (6)*	114 ± 19 (5)
Fluoxetine	Serotonin reuptake blocker	65 ± 8 (6)*	
Bupropion	?	59 ± 5 (4)*	
Iprindole	?	57 ± 14 (5)†	
ECS	?	39 ± 7 (6)*	97 ± 23 (5)
Nonantidepressant drugs			
Diazepam	Anxiolytic (benzodiazepine agonist)	109 ± 22 (4)	
Haloperidol	Antipsychotic (dopamine and other receptor antagonist)	104 ± 11 (9)	
Clonidine	α_2-Adrenergic receptor agonist	119 ± 11 (10)†	
Cocaine	Monoamine reuptake blocker	102 ± 13 (10)	
Morphine‡	Opiate receptor agonist	158 ± 12 (6)*	93 ± 9 (3)

Rats were treated chronically with the drugs or ECS, and levels of tyrosine hydroxylase were quantitated in the LC and SN (substantia nigra) by immunoblotting analysis, as described. See refs. 11 and 29 for discussion of the acute mechanisms of action of the drugs.
*$P < 0.05$ compared to control by χ^2 test.
†$P < 0.2$ compared to control by χ^2 test.
‡From ref. 22.

uptake blockers, monoamine oxidase inhibitors, certain atypical antidepressants, or ECS resulted in a 40–70% reduction in levels of enzyme immunoreactivity in this brain region but not in the substantia nigra. In contrast to antidepressants, a number of other psychotropic drugs that do not possess clinical antidepressant activity, including diazepam, haloperidol, clonidine, cocaine, and morphine, did not decrease levels of LC tyrosine hydroxylase; in fact, enzyme levels were increased in response to morphine (22) and, possibly, clonidine. Thus, downregulation of tyrosine hydroxylase expression appears to be an effect both common to, and specific for, antidepressant treatments.

The mechanisms by which antidepressants regulate tyrosine hydroxylase in the LC are unclear. Based on studies of adrenal medulla and sympathetic ganglia, levels of tyrosine hydroxylase expression are thought to reflect the physiological activity of the cells and, hence, their requirement for norepinephrine (see 14, 18–20). The same may hold true for the central nervous system, where various forms of behavioral stress or administration of 6-hydroxydopamine increases both LC firing rates and tyrosine hydroxylase expression in this brain region (15–17, 20, 21, 33–35). Moreover, direct depolarization of LC neurons in cultured explants has also been reported to increase expression of the enzyme (see ref. 36). One possibility, then, is that antidepressants downregulate tyrosine hydroxylase expression as a direct consequence of their inhibitory effects on LC neuronal activity. It has been shown that the tricyclic antidepressants and mono-

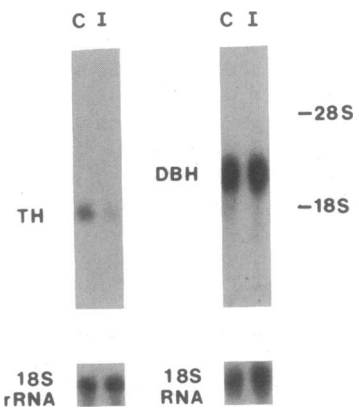

FIG. 2. Autoradiograms showing regulation of tyrosine hydroxylase and dopamine β-hydroxylase mRNA by chronic imipramine in rat LC. Levels of tyrosine hydroxylase and dopamine β-hydroxylase mRNA were quantitated by Northern blot analysis as described. The same blots were reprobed for the 18S ribosomal subunit, which indicated that the lanes contain comparable RNA levels. Levels of LC tyrosine hydroxylase (TH) mRNA, expressed in arbitrary units as a ratio to levels of 18S ribosomal RNA, were 1.0 ± 0.19 vs. 0.58 ± 0.12 for control (lanes C) vs. imipramine-treated (lanes I) rats ($P < 0.05$ by Student's t test), whereas those for LC dopamine β-hydroxylase (DBH) were 1.0 ± 0.27 vs. 1.0 ± 0.21 for control vs. imipramine-treated rats ($n = 3$).

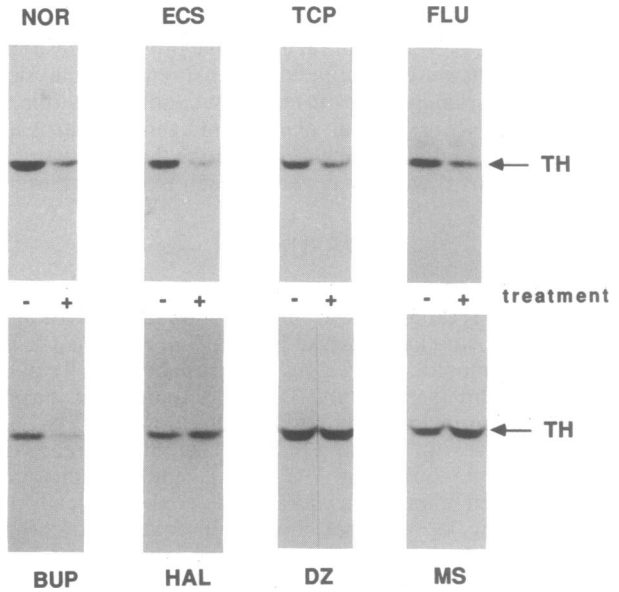

FIG. 3. Autoradiograms showing regulation of tyrosine hydroxylase immunoreactivity by chronic antidepressant and other drug treatments in rat LC. Rats were treated with the drugs or ECS, and levels of tyrosine hydroxylase immunoreactivity were quantitated by immunoblot analysis, as described. NOR, nortriptyline; TCP, tranylcypromine; FLU, fluvoxamine; BUP, bupropion; HAL, haloperidol; DZ, diazepam; MS, morphine; TH, tyrosine hydroxylase.

amine oxidase inhibitors decrease LC firing rates acutely and that such inhibition may persist with chronic drug administration (8, 9). Similarly, chronic administration of sertraline, a serotonin-selective reuptake blocker, has been shown recently to decrease the spontaneous activity of LC neurons compared with vehicle-treated control animals (10); fluoxetine and fluvoxamine might be expected to have analogous actions. A sustained decrease in LC firing rates would decrease the requirement of the neurons for norepinephrine and could trigger the decrease in enzyme expression. Indeed, the failure of some of the other compounds tested in this study to decrease tyrosine hydroxylase expression in the LC is consistent with the view that *sustained* decreases in LC neuronal activity mediate enzyme regulation by the antidepressants. Thus, morphine, clonidine, and cocaine, like the antidepressants, acutely inhibit LC firing, but unlike the antidepressants, substantial tolerance develops to the inhibitory actions of these drugs with LC firing rates returning toward normal levels after chronic drug administration (23, 25, 27). The validity of this hypothesis requires further investigation.

The mechanism(s) by which antidepressants produce sustained decreases in LC neuronal activity and in tyrosine hydroxylase expression is difficult to understand within the framework of known acute actions of these drugs on the brain (see Table 1). Thus, it is generally thought that the tricyclic antidepressants and monoamine oxidase inhibitors decrease LC neuronal activity by increasing synaptic levels of norepinephrine (via inhibition of norepinephrine reuptake and degradation, respectively) and thereby activating inhibitory α_2-adrenergic autoreceptors on LC neurons (see refs. 8, 9, 29). However, not consistent with this view, are the observations that inhibition of norepinephrine reuptake or activation of α_2-adrenergic receptors by cocaine and clonidine, respectively, does not lead to sustained inhibition of LC neuronal activity or to decreased tyrosine hydroxylase expression.

The regulation of tyrosine hydroxylase by the selective serotonin reuptake inhibitors fluoxetine and fluvoxamine is particularly interesting and supports the view that the serotonergic system exerts significant influence on the functional state of noradrenergic neurons (see ref. 10). It is possible that such effects reflect direct actions of the drugs on serotonergic nerve terminals within the LC, where serotonin is known to inhibit LC neurons (37, 38). Alternatively, the drugs could facilitate serotonergic neurotransmission in some other brain region(s) that would then indirectly inhibit LC neuronal activity.

Based on the inability of the known acute actions of classical antidepressants (namely, inhibition of norepinephrine reuptake and α_2-adrenergic receptor activation) to account for chronic antidepressant actions on the LC, it seems likely that these treatments, as well as the atypical antidepressants, have additional acute and chronic actions that mediate their regulation of LC neuronal activity and tyrosine hydroxylase expression. For example, all antidepressant treatments listed in Table 1 have been shown to alter a number of neurotransmitter receptor systems throughout the brain (see refs. 11, 29), indicating that regulation of tyrosine hydroxylase in the LC could be a consequence of complex, polysynaptic actions of many neurotransmitters on LC neurons. It is also conceivable that one or several of the antidepressant drugs regulate tyrosine hydroxylase via direct actions on intracellular messenger pathways and gene expression in LC neuronal cell bodies, actions independent of synaptic inputs. Support for this provocative idea comes from recent studies on C6 glioma cells (see refs. 39, 40).

Antidepressant regulation of tyrosine hydroxylase appears to be mediated at a pretranslational level. Thus, chronic imipramine treatment was found to decrease levels of tyrosine hydroxylase mRNA in the LC. This effect was specific to tyrosine hydroxylase, inasmuch as mRNA levels of dopamine β-hydroxylase, another enzyme in the biosynthesis of norepinephrine, were not influenced by this drug treatment. Decreased levels of tyrosine hydroxylase mRNA and protein observed in response to chronic imipramine indicate that drug treatment may alter levels of the enzyme through the regulation of gene expression, although it is also possible that the effect of the drug occurs through changes in turnover rates of enzyme mRNA and/or protein.

Decreased expression of tyrosine hydroxylase mRNA and protein by chronic antidepressant treatments indicates that the maximal capacity of LC neurons to synthesize norepinephrine is also decreased under these conditions. A separate question concerns what effect antidepressant treatments exert on the average catalytic activity of tyrosine hydroxylase. There is one report of a decrease in tyrosine hydroxylase activity in the LC after chronic imipramine treatment (41). Moreover, chronic administration of certain antidepressants has been reported to decrease norepinephrine levels in cerebral cortex of laboratory animals and levels of norepinephrine metabolites in cerebrospinal fluid and plasma in patients with major depression (see refs. 11, 12). However, chronic ECS has been reported to increase tyrosine hydroxylase activity in the LC (42). The reason for this discrepancy may lie in the fact that, despite lower total enzyme levels, enzyme activity could vary widely depending on the state of activation (i.e., state of phosphorylation) of the enzyme. Such lability in tyrosine hydroxylase activity would particularly complicate analysis of activity in response to *in vivo* drug treatments, where postmortum changes could occur. In contrast, levels of tyrosine hydroxylase mRNA and protein would be expected to be less vulnerable to rapid fluctuations in neuronal activity or postmortum artifacts and, therefore, may provide a more reliable measure of the long-term regulation of the enzyme in response to chronic manipulations.

The findings of the present study, together with earlier evidence for decreased β-adrenergic receptor function in projection areas of noradrenergic neurons (1–3), indicate that chronic antidepressant administration downregulates both presynaptic and postsynaptic noradrenergic function. In fact, it is striking that the same drugs that downregulate tyrosine hydroxylase also downregulate β-adrenergic receptor function. It will be important in future studies in determine whether the two effects are in some way causally related or, rather, whether they reflect complex actions of the drugs on a number of distinct brain regions.

The view that downregulation of the noradrenergic system contributes to the therapeutic actions of antidepressant treatments raises the question as to whether this neurotransmitter system is in some way involved in the etiology or expression of certain forms of clinical depression (see refs. 11, 12). Chronic stress, which may play a role in precipitating episodes of depression, leads to increased levels of tyrosine hydroxylase expression in the LC in rats (see ref. 20), an effect blocked by pretreatment of animals with antidepressants (43). These findings raise the possibility that an overactive noradrenergic system could contribute to depressive symptomatology and that the therapeutic action of antidepressant treatments reverses such overactivity, in part, by decreasing tyrosine hydroxylase expression in the LC. Clinical studies have reported that a subset of depressed patients exhibit increased levels of central and/or peripheral norepinephrine and its metabolites, although the significance of such alterations remains controversial, with other studies reporting decreases or no differences in levels of central norepinephrine in depressed patients (see refs. 11, 12). The results of these various studies also indicate the possibility that, in a similar fashion, the noradrenergic system may be involved in the expression and/or treatment of a number of mental disorders other than depression (e.g., panic disorder,

posttraumatic stress disorder, eating disorders) for which antidepressant medications are known to provide some clinical benefit. Further studies are needed to test these various hypotheses and examine the role played by the regulation of tyrosine hydroxylase expression in the mechanisms by which antidepressant treatments and stress produce their multiple clinical effects.

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