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ORIGINAL INVESTIGATION

Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test

Olivia F. O'Leary · Anita J. Bechtholt · James J. Crowley · Tiffany E. Hill · Michelle E. Page · Irwin Lucki

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Abstract
Rationale Few studies have investiga.ted whether the behavioral effects elicited by different types of antidepressant drugs are mediated by either serotonin (5-HT) or the catecholamines norepinephrine (NE) and dopamine (DA).
Objectives By depleting 5-HT, or NE and DA, the present study investigated the contributions of these monoamines to the acute behavioral effects of selective serotonin reuptake inhibitors (SSRIs; fluoxetine and citalopram) and norepinephrine reuptake inhibitors (NRIs; desipramine and reboxetine) in the mouse tail suspension test (TST).
Results Depletion of 5-HT tissue content by *para*-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, completely blocked reductions of immobility by the SSRIs in the TST. In contrast, PCPA did not alter the behavioral effects of the NRIs. Inhibition of catecholamine synthesis by α-methyl-*para*-tyrosine (AMPT) reduced brain NE and DA tissue content, whereas disruption of vesicular storage with reserpine decreased brain NE, DA and 5-HT tissue content. However, neither treatment completely prevented responses to desipramine, fluoxetine, or citalopram in the TST. Depleting both newly synthesized and vesicular components of NE and DA transmission with a combination of reserpine and AMPT completely prevented the behavioral effects of desipramine, reboxetine, and fluoxetine and attenuated those of citalopram. Although PCPA did not alter baseline immobility, AMPT and reserpine increased baseline values in the TST.
Conclusions These studies demonstrated that endogenous 5-HT synthesis mediates the behavioral effects of SSRIs, but not NRIs, in the TST. In contrast, disruption of the behavioral effects of NRI and SSRI antidepressants required disruption of both catecholamine synthesis and vesicular storage and release mechanisms.

Keywords Antidepressant · SSRI · NRI · Serotonin · Norepinephrine · Tail suspension test · Behavior

O. F. O'Leary · J. J. Crowley · I. Lucki
Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA

A. J. Bechtholt · T. E. Hill · I. Lucki (✉)
Department of Psychiatry, University of Pennsylvania, 125 South 31st Street, 2204 TRL, Philadelphia, PA 19104-3403, USA
e-mail: lucki@pharm.med.upenn.edu

O. F. O'Leary
Department of Pharmacology, National University of Ireland, Galway, Galway, Ireland

Present address:
O. F. O'Leary
Neuroscience Center, University of Helsinki, P.O. Box 56, 00014 Helsinki, Finland

Present address:
M. E. Page
Department of Neurosurgery, Farber Institute of Neuroscience, Thomas Jefferson University, 1025 Walnut Street Room 516, Philadelphia, PA 19107, USA

Introduction

Considerable evidence supports a role for the monoamine neurotransmitters serotonin (5-HT), norepinephrine (NE), and dopamine (DA) in both the pathophysiology and treatment of depression (for review see Charney 1998; Delgado 2000, 2004; Frazer 2000; Ressler and Nemeroff 2000). Acute administration of many antidepressants causes

increases in extracellular levels of monoamines because they block presynaptic neurotransmitter transporters or receptors that regulate neurotransmission (Millan et al. 2000). When administered chronically, antidepressants produce changes in receptor regulation, cell signaling, or neuroplasticity, in part, due to persistent overexposure to monoamine neurotransmitters, and these changes may also contribute to their therapeutic effects (Caldecott-Hazard and Schneider 1992; Millan 2006).

Because antidepressants produce their acute pharmacological effects by augmenting the effects of brain monoamines, prior depletion of neurotransmitters would be expected to block their behavioral effects. Thus, studying the effects of monoamine depletions on antidepressant activity is an important strategy for directly assessing the relative contribution of specific neurotransmitters to the behavioral effects of different types of antidepressants. Clinical studies have demonstrated that inhibition of 5-HT synthesis using rapid tryptophan depletion induced temporary clinical relapse in successfully remitted patients treated with selective serotonin reuptake inhibitors (SSRIs), but not with norepinephrine reuptake inhibitors (NRIs; Delgado et al. 1991, 1999). In contrast, inhibition of NE and DA synthesis using α-methyl-*para*-tyrosine (AMPT) induced temporary clinical relapse in successfully remitted patients treated with NRIs, but not with SSRIs (Delgado et al. 1993; Miller et al. 1996). Thus, monoamine depletion studies have provided direct evidence of the roles for these monoamines in the therapeutic effects of antidepressant drugs.

A major issue in basic antidepressant research is to determine whether the pharmacological activity and selectivity of different types of antidepressants is maintained in vivo after acute and chronic administration and is reflected by particular behavioral, neurochemical, or physiological responses to antidepressants. The pharmacological selectivity of many clinically effective antidepressants has been determined by their ability to block either the 5-HT (SSRIs), NE (NRIs), or both the 5-HT and NE transporter proteins [serotonin–norepinephrine reuptake inhibitors (SNRIs)] using in vitro radioligand binding assays. Despite their apparent selectivity for 5-HT or NE transporters in vitro, microdialysis studies have shown that some antidepressants can be nonselective in their effects on forebrain extracellular neurotransmitter levels when administered systemically (Beyer et al. 2002; Hajos-Korcsok et al. 2000; Hughes and Stanford 1998; Shachar et al. 1997; Zocchi et al. 2003). In contrast, other antidepressants (e.g., citalopram) appear to maintain their selectivity in vivo (Millan et al. 2001). Moreover, functional interactions between 5-HT and NE neurons could make antidepressant responses interdependent on both serotonergic and noradrenergic mechanisms. For example, blockade of adrenergic α_1-adrenoceptors decreases serotonergic cell firing and the release of serotonin, whereas blockade of adrenergic α_2-adrenoceptors enhances both 5-HT release and serotonergic cell firing probably by enhancing noradrenergic transmission in the dorsal raphe nucleus (Baraban and Aghajanian 1980; Bortolozzi and Artigas 2003; Garratt et al. 1991; Pudovkina et al. 2003). Noradrenergic tone in the dorsal raphe nucleus has been shown to regulate the acute behavioral effects of the SSRI fluoxetine in the tail suspension test (O'Leary et al. 2007). Substantial evidence also exists for the modulation of noradrenergic transmission by 5-HT. For example, systemic administration of 5-HT_{1A} receptor agonists enhances noradrenergic transmission, whereas systemic administration of 5-HT_{2A} receptor agonists dampens noradrenergic transmission (Chen and Reith 1995; Gobert and Millan 1999; Hajos-Korcsok et al. 1999; Suzuki et al. 1995; Szabo and Blier 2001a,b). If such physiological interactions between these two neurotransmitter systems underlie the behavioral effects of antidepressants targeted selectively at either the 5-HT or NE transporters, then their behavioral effects might not be mediated exclusively by either neurotransmitter.

The tail suspension test (TST) is a behavioral test widely used for measuring antidepressant drug-like activity in drug discovery research with mice (Cryan et al. 2005; Steru et al. 1985). In addition, a variety of genetic studies involving mice have used the TST to measure antidepressant responses or responses to stress (Bechtholt and Lucki 2006; Crowley et al. 2006; Cryan and Mombereau 2004; El Yacoubi et al. 2003). Mice suspended from a heightened support by their tail display immediate episodes of escape-oriented behaviors that subsequently transition to passive immobility. Acute administration of antidepressant drugs typically reduces the time spent immobile, as mice persist in attempting to escape from the bar for longer periods of time. The TST is sensitive to a broad spectrum of antidepressant treatments regardless of their mechanism of action, and the effects of antidepressants are not due to simple changes in locomotor activity. The TST has been evaluated using a broad range of pharmacological and somatic treatments, including: tricyclic antidepressants, monoamine oxidase inhibitors, SSRIs, NRIs, atypical antidepressants such as bupropion, and electroconvulsive therapy (Cryan et al. 2004, 2005; Steru et al. 1985, 1987; Teste et al. 1990).

Consistent with views from the aminergic theories of depression, an earlier and lengthier literature has employed monoamine depletion strategies in animals to show that acute and/or adaptive changes in either serotonergic or noradrenergic transmission mediate the attenuation by antidepressants of many depressive-like behaviors in animals (for review, see Lucki and O'Leary 2004). Despite extensive use of the TST in drug discovery and genetics

research, few studies have been conducted using monoamine depletion strategies to determine the neural substrates underlying the behavioral effects of SSRIs or NRIs in the TST. It may seem obvious to hypothesize that certain neurotransmitters are necessary for corresponding classes of antidepressants to produce their behavioral effects, 5-HT for SSRIs and NE and DA for NRIs. However, as the pharmacological selectivity of individual antidepressants within broad classes can vary and monoamine neurotransmitters have multiple opportunities for functional interactions, monoamine depletion studies also test whether secondary pharmacological effects of SSRIs and NRIs could sustain their antidepressant-like behavioral effects.

The present study examined whether pharmacological selectivity is maintained in vivo after acute administration of prototypical SSRIs (fluoxetine, citalopram) or NRIs (desipramine, reboxetine) in the TST, a behavioral test predictive of antidepressant activity. The behavioral effects of both SSRIs and NRIs were examined in mice after the pharmacological depletion of 5-HT or NE and DA. Depletion of 5-HT was achieved by administration of *para*-chlorophenylalanine (PCPA), a selective inhibitor of tryptophan hydroxylase (Koe and Weissman 1966). Depletion of newly synthesized pools of NE and DA was achieved using the tyrosine hydroxylase inhibitor AMPT (Corrodi and Hanson 1966). Depletion of vesicular NE and DA was accomplished using reserpine. In some experiments, both the vesicular and newly synthesized pools of NE and DA were depleted using a combination of reserpine and AMPT, respectively, although reserpine also depleted 5-HT. The results show that in the TST, the behavioral effects of SSRIs, but not NRIs, are dependent on the presence of endogenous 5-HT synthesis. Furthermore, catecholamine transmitters from both a newly synthesized and vesicular pool were involved in the behavioral effects of NRIs and SSRIs. Depletion of catecholamines, but not 5-HT alone, increased baseline immobility values, and the contribution of altered baseline values to calculation of the antidepressant response was considered.

Materials and methods

Animals

Male C57Bl/6-129Sv F1 hybrid mice, 10–12 weeks old, were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Subjects were housed in groups of five per cage in a temperature and humidity-controlled environment. Mice were maintained on a 12-h light–dark cycle (lights on 0700 hours) in a pathogen-free colony at the University of Pennsylvania, Philadelphia, PA, USA. Food and water were freely available. The care and use of animals was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were kept in the colony 1–2 weeks before the experimental procedures. Behavioral sessions were conducted between 1200 hours and 1800 hours. All experimental procedures were carried out in accordance with protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Drugs and treatment regimens

All drugs were freshly prepared before use and injected in a volume of 10 ml/kg. Drug doses were calculated as the base weight and expressed as milligram per kilogram. Desipramine hydrochloride (Sigma, St Louis, MO, USA), reboxetine hydrobromide (Pharmacia and UpJohn, Kalamazoo, MI, USA), fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN, USA), citalopram hydrobromide (Lundbeck, Copenhagen, Denmark), *para*-chlorophenylalanine methyl ester (PCPA; Sigma), α-methyl-*para*-tyrosine methyl ester (AMPT; Sigma) were dissolved in deionized H_2O and sonicated briefly. A limited supply of reboxetine restricted its use in these studies. Reserpine (Sigma) was first dissolved in 5% glacial acetic acid and then subsequently diluted with deionized H_2O to obtain the appropriate dose. All drugs were administered intraperitoneally (IP) except reserpine, which was administered subcutaneously (SC).

To selectively deplete 5-HT, mice were pretreated with the tryptophan hydroxylase inhibitor PCPA (300 mg/kg, IP) twice daily for three consecutive days with the last dose given 18 h before behavioral testing (Cesana et al. 1993). Behavioral testing of these mice was conducted on the fourth day.

Two different strategies were employed to deplete catecholamines. To deplete newly synthesized pools of NE and DA, mice were treated with a single dose of the tyrosine hydroxylase inhibitor, AMPT (400 mg/kg, IP) 3.5 h before behavioral testing. To deplete vesicular pools of NE and DA, mice were treated with a single dose of reserpine (1 mg/kg, SC) 24 h before behavioral testing. In an effort to deplete both the vesicular and cytoplasmic pools of NE and DA, mice were pretreated with a combination of reserpine (1 mg/kg, SC, 24 h before behavioral testing) and AMPT (200 mg/kg, IP, 3.5 h before behavioral testing), respectively. All control animals received 0.9% saline on the same schedule as the treated groups.

Tail suspension test

The TST was carried out essentially as previously described (Steru et al. 1985; Mayorga et al. 2001). Mice were allowed to acclimatize to the holding room for 3.5–4 h before the

behavioral procedure. Thirty minutes after injection of saline or an antidepressant, mice were individually suspended by the tail from a horizontal bar (distance from floor = 30 cm) using adhesive tape (distance from tip of tail = 2 cm). A 6-min test session was employed, which was videotaped. Videotapes were subsequently analyzed by a trained rater blind to the treatment categorization of the mice. The behavioral parameter recorded was the number of seconds spent in a completely immobile posture, termed immobility.

The doses of antidepressants used in all of the studies was 20 mg/kg of fluoxetine, citalopram, desipramine, and reboxetine. The doses were selected to produce a substantial treatment response (around 75–90% reduction of immobility) of similar magnitude between drugs according to previously published dose-response curves (Cryan et al. 2004).

Tissue analysis of serotonin, norepinephrine and dopamine

Mice were killed by decapitation 30 min after the TST, the brains removed, and a tissue sample from the frontal cortex was dissected on ice to compare tissue content of monoamines after the different pretreatments. Only mice treated with saline were used for analysis of monoamine content. Samples were frozen on dry ice and stored at −80°C until preparation for high performance liquid chromatography (HPLC) analysis.

Tissue samples were homogenized in 0.1 N perchloric acid with 100 μM EDTA (15 μl/mg of tissue) using a tissuemizer (Tekmar, Cleveland, OH, USA). Samples were centrifuged at 15,000 rpm (23,143 g) for 15 min at 2–8°C. The supernatants were filtered using Costar Spin-X™ centrifugal filters (Fisher Scientific, Pittsburgh, PA, USA) and then split into two aliquots. Samples (12 μl) were injected by an autosampler (Sample Sentinel, Bioanalytical Systems, West Lafayette, IN, USA) and analyzed in separate assays for 5-HT, and for NE and DA content using HPLC coupled with electrochemical detection (HPLC-EC).

The HPLC separation for serotonin consisted of a PM80 solvent delivery system and a 10-μl sample loop linked in series to a reversed phase microbore column (ODS 3 μm, 100×1 mm; Bioanalytical Systems). The mobile phase for the separation of 5-HT consisted of 12.42 mM citric acid (Sigma), 39.85 mM $NaPO_4$ monobasic (Fluka, Buchs SG, Switzerland), 0.25 mM EDTA (Fluka), 0.737 mM 1-decanesulfonic acid (Sigma), 10 mM NaCl (Fluka), 0.2% triethylamine (Sigma), 16.5% methanol (Fisher Scientific) adjusted to a pH of 4.1. The flow rate through the system was 60 μl/min, and the detector was set at a potential of +0.60 V relative to a Ag/AgCl reference electrode.

The HPLC separation for norepinephrine and dopamine consisted of a PM80 solvent delivery system and a 10-μl sample loop linked in series to a reversed phase microbore column (ODS 5 μm, 150×1 mm; Bioanalytical Systems). The mobile phase for the separation of norepinephrine and dopamine consisted of 14.5 mM NaH_2PO_4 (Fluka), 30 mM sodium citrate (Fluka), 27 μM disodium EDTA (EMD Chemicals, Gibbstown, NJ, USA), 10 mM diethylamine HCL (Sigma), 1.95 mM 1-decanesulfonic acid (Sigma), 8% acetonitrile (Fisher Scientific), 1% tetrahydrofuran (Fluka) adjusted to pH 3.4. Mobile phase was pumped through the system at 80 μl/min, and the detector was set at a potential of +0.65 V relative to a Ag/AgCl reference electrode.

Standard concentrations of 5-HT or NE and DA were prepared before injection of tissue samples. Tissue concentrations of monoamines were determined using a linear regression analysis of the peak heights obtained from a range of standards. All samples from a single experiment were analyzed at the same time, although samples from different experiments were collected and stored under slightly different conditions over a 3-year period of time.

Statistical analysis

Immobility scores in the TST were analyzed by a two-factor analysis of variance (ANOVA) with pretreatment and antidepressant drug as the main factors. Pairwise follow-up comparisons of individual treatment groups were conducted using Duncan's multiple range test. Monoamine tissue content was analyzed using Student's *t* test. For all comparisons, $P<0.05$ was used as the criterion for statistical significance.

Results

The effects of serotonin depletion on behavioral responses in the TST

Three separate groups of mice were pretreated with PCPA (300 mg/kg b.i.d. × 3 days) before measuring behavioral responses to antidepressants, either the SSRI fluoxetine (Fig. 1, top panel), the SSRI citalopram (Fig. 1, middle panel), or the NRIs desipramine and reboxetine (Fig. 1, bottom panel). For fluoxetine, two-factor ANOVA revealed a significant pretreatment × drug interaction [$F(1,33) = 11.62$, $p<0.005$], although the main effects for drug treatment and pretreatment were not significant, respectively, [$F(1,33) = 0.001$, $p = 0.97$; $F(1,33) = 2.88$, $p = 0.10$]. For citalopram, two-factor ANOVA revealed a significant main effect for drug treatment [$F(1,35) = 25.28$, $p<0.001$], for pretreatment [$F(1,35) = 7.59$, $p<0.01$], and a pretreatment × drug interaction [$F(1,35) = 19.22$, $p<0.001$]. Prior administration of PCPA did not significantly alter baseline immobility in either study. Both fluoxetine and citalopram

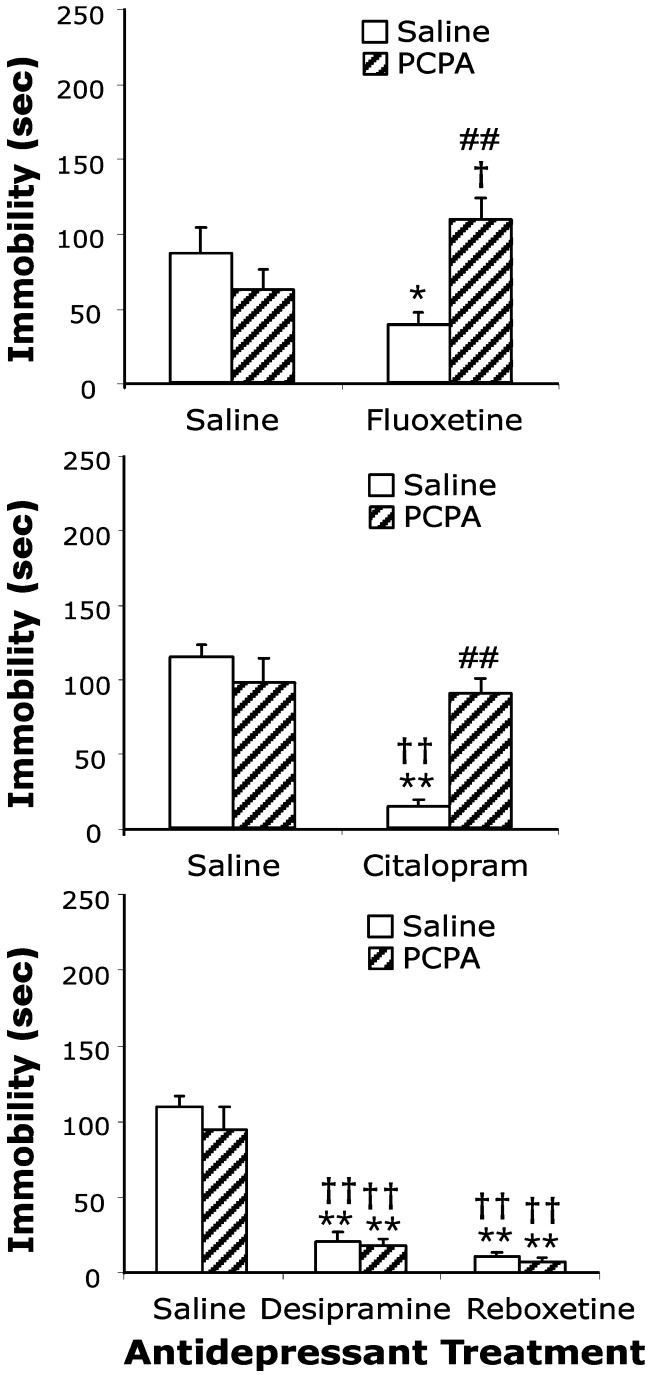

Fig. 1 Blockade of the behavioral response to the SSRIs, fluoxetine (*top panel*), and citalopram (*middle panel*) in the TST after pretreatment with PCPA. The behavioral effects of the NRIs, desipramine and reboxetine, were not affected by PCPA (*bottom panel*). Data are expressed as mean+1 SEM immobility, $n = 7–10$ mice per group. Mice were pretreated with either saline (*solid bars*) or PCPA (*hatched bars*, 300 mg/kg, IP, twice daily) for 3 days before the TST. On the fourth day, mice were injected with either saline, fluoxetine (20 mg/kg, IP), citalopram (20 mg/kg, IP), desipramine (20 mg/kg, IP), or reboxetine (20 mg/kg, IP) 30 min prior to the TST. (*) Indicates significant effects between antidepressant-treated groups and the saline–saline control group, $*p<0.05$, $**p<0.01$; (†) indicates significant differences between antidepressant-treated groups and the PCPA–saline group, $†p<0.05$, $††p<0.01$; (#) indicates significant differences between pretreatment groups given the same antidepressant drug, $\#\#p<0.01$

significantly reduced immobility in saline-pretreated mice. In PCPA-pretreated mice, however, the behavioral response to citalopram was absent. Fluoxetine significantly increased immobility in PCPA-pretreated animals when compared with the corresponding group given saline.

Figure 1 (bottom panel) shows that PCPA pretreatment did not alter the response to the NRIs, desipramine and reboxetine, in the TST. Although the main effect for drug treatment was significant [$F(2,46) = 82.56$, $p<0.001$], neither the main effect for pretreatment nor a pretreatment ×

Table 1 The effect of PCPA pretreatment on monoamine tissue content in the frontal cortex

Study	Monoamine content (pg/mg tissue)				
	Saline	*N*	PCPA	*N*	Percent change
PCPA–Fluoxetine					
5-HT	1084.0±134.5	9	223.4±28.3***	8	−79
NE	457.4±28.7	9	456.2±49.6	8	−0.3
DA	4354.0±81.2	9	4004.9±423.7	8	−8
PCPA–Citalopram					
5-HT	352.1±11.7	9	81.0±6.2***	10	−77
NE	234.6±12.0	9	223.5±12.4	10	−5
DA	206.5±14.3	9	159.7±9.3**	10	−23
PCPA–NRI					
5-HT	539.2±44.2	9	120.5±15.8***	9	−78
NE	400.3±28.0	9	323.9±21.8*	9	−19
DA	957.5±178.0	9	660.3±147.7	9	−31

Only mice tested with saline were included in the analysis. Values are expressed as mean pg/mg tissue±1 SEM. Asterisks indicate groups that differed significantly from the corresponding control group according to Student's *t* test, two-tailed

*$P<0.05$

**$P<0.01$

***$P<0.001$

drug interaction was significant [$F(1,46)=1.15$, $p=0.29$; $F(2,46)=0.37$, $p=0.69$]. In contrast to the SSRIs, desipramine and reboxetine significantly reduced immobility in both the saline-pretreated and PCPA-pretreated animals. Prior administration of PCPA did not alter baseline immobility.

Pretreatment with PCPA significantly inhibited 5-HT synthesis as revealed by the measured reductions in 5-HT tissue content that are summarized in Table 1. PCPA significantly reduced 5-HT content in the frontal cortex by 79% [$t(15)=5.91$, $p<0.001$] in the fluoxetine study, by 77% in the citalopram study [$t(17)=21.08$, $p<0.001$], and by 78% [$t(16)=8.91$, $p<0.001$] in the NRI study. In contrast, PCPA produced small and inconsistent effects on NE and DA tissue content from the saline pretreatment control in the various studies.

The effects of catecholamine depletion on behavioral responses in the TST

Pretreatment with AMPT

The effects of AMPT pretreatment on the behavioral effects of antidepressants in the TST are shown in Fig. 2. Mice were pretreated with saline or AMPT (400 mg/kg, IP) 3.5 h before the TST and received either fluoxetine, citalopram, or desipramine (20 mg/kg, IP) 30 min before the TST. Two-factor ANOVA revealed a significant main effect for pretreatment [$F(1,80)=34.08$, $p<0.001$] and drug treatment [$F(3,80)=89.02$, $p<0.001$], but no significant pretreatment × drug interaction [$F(3,80)=1.63$, $p=0.19$]. Prior administration of AMPT increased baseline immobility values in mice pretreated with saline by approximately 30%. As shown in Fig. 2, all of the antidepressants significantly reduced immobility in both saline-pretreated and AMPT-pretreated animals ($p<0.01$) when compared to the corresponding groups treated with saline.

Pretreatment with AMPT significantly inhibited catecholamine synthesis as revealed by changes in tissue norepinephrine and dopamine content that are summarized

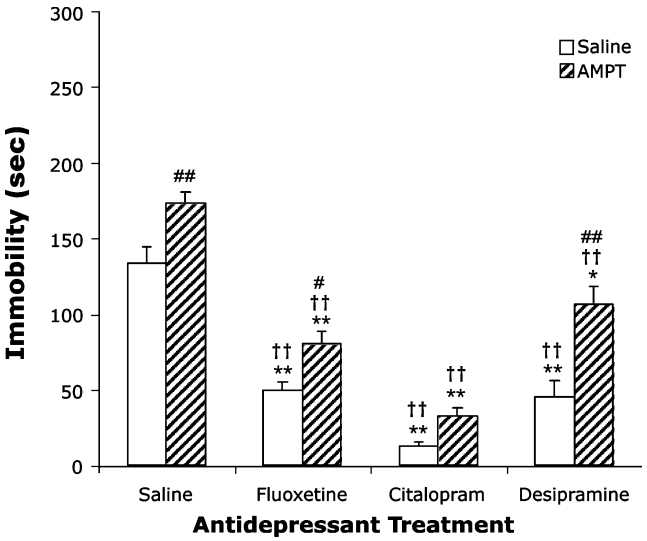

Fig. 2 Behavioral responses to fluoxetine (20 mg/kg, IP), citalopram (20 mg/kg, IP), and desipramine 20 mg/kg, IP), given 30 min before testing, after pretreatment with AMPT (400 mg/kg, IP), given 3.5 h before testing, in the TST. (*) Indicates significant effects between antidepressant-treated groups and the saline–saline control group, *$p<0.05$, **$p<0.01$; (†) indicates significant differences between antidepressant-treated groups and the AMPT–saline group, ††$p<0.01$; (#) indicates significant differences between pretreatment groups given the same antidepressant drug, #$p<0.05$, ##$p<0.01$

Table 2 The effect of drug pretreatments on monoamine tissue content in the frontal cortex

Study	Monoamine content (pg/mg tissue)				
AMPT					
	Saline	*N*	AMPT	*N*	Percent change
5-HT	292.9±32.5	9	277.8±33.9	9	−5
NE	195.3±21.3	9	75.4±6.3***	9	−61
DA	2098.8±254.7	9	697.9±87.8***	9	−67
Reserpine					
	Saline	*N*	Reserpine	*N*	Percent change
5-HT	467.3±14.8	10	101.0±4.8***	9	−78
NE	179.3±13.4	9	13.0±0.6***	9	−93
DA	274.8±94.0	9	14.6±3.5**	9	−95
Reserpine + AMPT					
NRI	Saline	*N*	Reserpine + AMPT	*N*	Percent change
5-HT	186.6±15.3	8	41.4±5.9***	7	−78
NE	187.5±8.7	8	5.8±5.7***	7	−97
DA	999.0±166.3	8	55.0±38.2***	7	−94
SSRI					
5-HT	260.8±30.7	8	59.2±2.7***	9	−77
NE	166.9±12.1	8	3.9±0.8***	9	−98
DA	563.1±111.4	8	24.3±5.9***	9	−95

Separate groups of mice were pretreated with either 400 mg/kg AMPT 3.5 h before testing, 1 mg/kg reserpine 24 h before testing, or 1 mg/kg reserpine (24 h) +200 mg/kg AMPT (3.5 h) before testing. Only mice tested with saline were included in the analysis. Values are expressed as mean pg/mg tissue±1 SEM. Asterisks indicate groups that differed significantly from the corresponding control group according to Student's *t* test, two-tailed

**$P<0.01$

***P<0.001

in Table 2. AMPT significantly reduced norepinephrine by 61% [$t(14)=6.01$, $p<0.001$] and dopamine content by 67% [$t(14)=5.74$, $p<0.001$] without producing significant effects on serotonin content.

Pretreatment with reserpine

The effects of reserpine pretreatment on the behavioral effects of antidepressants in the TST are shown in Fig. 3. Mice were pretreated with saline or reserpine (1 mg/kg, SC) 24 h before the TST and received fluoxetine, citalopram, or desipramine (20 mg/kg, IP) 30 min before the TST. Two-factor ANOVA revealed a significant main effect for pretreatment [$F(1,75)=160.13$, $p<0.001$] and drug treatment [$F(3,75)=30.77$, $p<0.001$], and a significant pretreatment × drug interaction [$F(3,75)=3.26$, $p=0.03$]. Pretreatment of mice with reserpine increased baseline immobility values by 90% ($p<0.01$). As shown in Fig. 3, all of the antidepressants significantly reduced immobility in both saline-pretreated and reserpine-pretreated animals when compared to corresponding groups given saline.

Pretreatment with reserpine produced significant depletion of the biogenic amines as illustrated in Table 2. Reserpine significantly reduced NE [$t(18)=12.41$, $p<0.001$], DA [$t(18)=2.77$, $p=0.013$], and 5-HT content [$t(18)=23.59$, $p<0.001$] in the frontal cortex by 93, 95, and 78%, respectively.

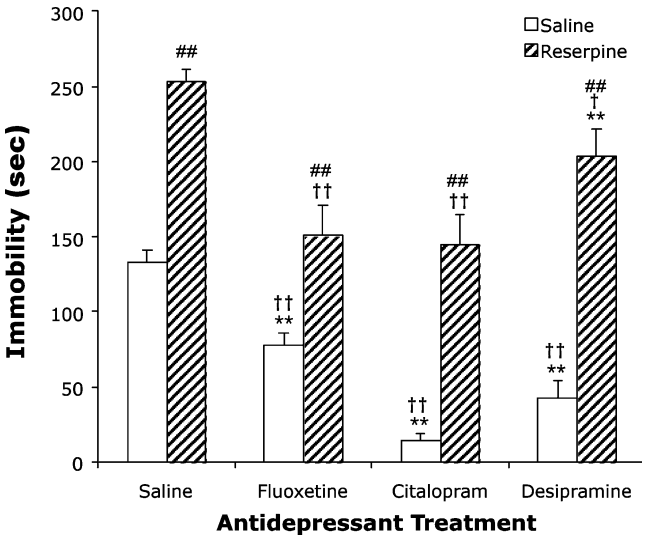

Fig. 3 Behavioral responses to fluoxetine (20 mg/kg, IP), citalopram (20 mg/kg, IP), and desipramine (20 mg/kg, IP), given 30 min before testing, after pretreatment with reserpine (1 mg/kg, IP), given 24 h before testing, in the TST. (*) Indicates significant effects between antidepressant-treated groups and the saline–saline control group, **$p<0.01$; (†) indicates significant differences between antidepressant-treated groups and the reserpine–saline group, †$p<0.05$, ††$p<0.01$; (#) indicates significant differences between pretreatment groups given the same antidepressant drug, ##$p<0.01$

Pretreatment with reserpine + AMPT

Because the behavioral effects of antidepressants could involve catecholamines located in different cellular pools or compartments, the responses to antidepressants were evaluated after reserpine and AMPT were given together. Specifically, vesicular stores of catecholamines were depleted by pretreating mice with reserpine (1 mg/kg, SC) 24 h before the TST, whereas newly formed stores of catecholamines were depleted by pretreatment with AMPT (200 mg/kg, IP) 3.5 h before the TST. Separate groups of mice were then tested with the SSRIs fluoxetine or citalopram (Fig. 4, top panel) or with the NRIs desipramine or reboxetine (Fig. 4, bottom panel).

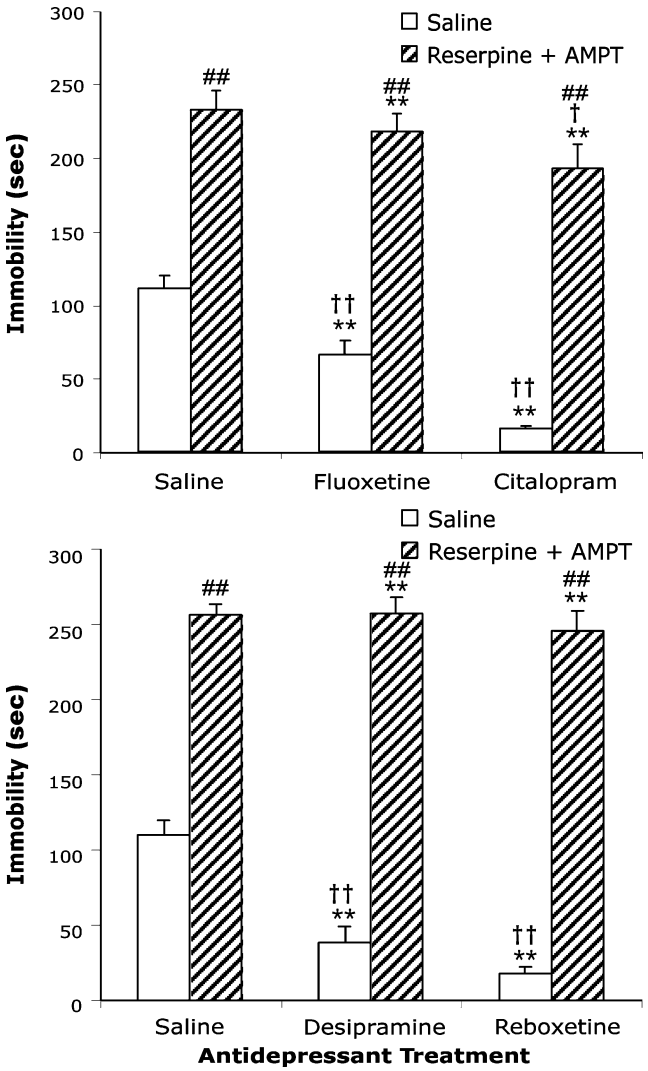

Fig. 4 Behavioral responses to antidepressant drugs in the TST after pretreatment with reserpine (1 mg/kg, SC 24 h) + AMPT (200 mg/kg, IP 3.5 h). The SSRIs fluoxetine (20 mg/kg, IP) and citalopram (20 mg/kg, IP) are shown in the *top panel*. The NRIs desipramine (20 mg/kg, IP) and reboxetine (20 mg/kg, IP) are shown in the *bottom panel*. Antidepressants were given 30 min before testing. (*) Indicates significant effects between antidepressant-treated groups and the saline–saline control group, $^{**}p<0.01$; (†) indicates significant differences between antidepressant-treated groups and the reserpine + AMPT–saline group, $\dagger p<0.05$, $\dagger\dagger p<0.01$; (#) indicates significant differences between pretreatment groups given the same antidepressant drug, $\#\#p<0.01$

In the SSRI study (Fig. 4, top panel), two-factor ANOVA revealed significant main effects for pretreatment [$F(1,50)=264.96$, $p<0.001$] and drug treatment [$F(2,50)=17.59$, $p<0.001$], and a trend for a significant pretreatment × drug interaction [$F(2,50)=2.98$, $p=0.06$]. Prior administration of the reserpine + AMPT combination significantly increased baseline immobility in mice treated with saline by approximately 110% ($p<0.01$). Both fluoxetine and citalopram significantly reduced immobility in saline-pretreated animals. The behavioral effects of fluoxetine were blocked in the reserpine + AMPT group. Although citalopram still significantly reduced immobility after reserpine + AMPT, its response was significantly attenuated when compared to the corresponding saline-treated control group.

In the NRI study (Fig. 4, bottom panel), two-factor ANOVA revealed significant effects for pretreatment [$F(1,45)=636.88$, $p<0.001$], drug treatment [$F(2,45)=14.15$, $p<0.001$], and a pretreatment × drug interaction [$F(2,45)=10.17$, $p<0.001$]. Pretreatment with reserpine + AMPT significantly increased baseline immobility by approximately 130% ($p<0.01$). Although both desipramine and reboxetine significantly reduced immobility in saline-pretreated animals, the responses to both drugs were blocked in mice given the reserpine + AMPT combination.

The effects of the reserpine–AMPT combination on biogenic amine tissue content are summarized in Table 2. In the SSRI study, pretreatment with the reserpine–AMPT combination significantly reduced frontocortical NE [$t(15)=14.35$, $p<0.001$], DA [$t(15)=5.14$, $p<0.001$], and 5-HT [$t(15)=6.97$, $p<0.001$] content by 98, 95, and 77%, respectively. Similarly, in the NRI study, pretreatment with a combination of reserpine + AMPT significantly reduced frontocortical NE [$t(13)=16.83$, $p<0.001$], DA [$t(13)=5.13$, $p<0.001$], and 5-HT [$t(13)=8.39$, $p<0.001$] content by 97, 94 and 78%, respectively.

Effects of monoamine depletion on behavioral responses in the TST

Although PCPA did not alter baseline immobility in the TST, other pretreatments that depleted catecholamines significantly increased scores for baseline immobility time. To evaluate the effect of monoamine depletion on the response of antidepressants in the TST, scores were analyzed in two ways. First, an absolute change score was calculated. The mean value of each control pretreatment (saline, PCPA, AMPT, reserpine, and reserpine + AMPT) was subtracted from test scores obtained from the

corresponding pretreatment groups of animals treated with antidepressant drugs. Second, a percent change score was calculated by dividing each test score after antidepressant treatment with the corresponding mean value for control pretreatment. Values for antidepressants given after either saline or the pretreatment were compared using Student's *t* test, and both transformations are shown in Table 3.

For some of the pretreatments, the method of calculating a drug effect made no difference in interpreting its effect. Pretreatment with PCPA did not alter baseline immobility scores and blocked the effects of the SSRIs fluoxetine and citalopram and failed to block the effects of desipramine and reboxetine irrespective of the method for calculating drug effect. However, pretreatment with reserpine + AMPT produced a 2.1- to 2.3-fold increase in immobility scores. Nevertheless, this pretreatment significantly diminished the effects of each of the antidepressant drugs when calculated either as an absolute difference score or percent change score, with the potential exception of fluoxetine calculated as a change score ($p<0.07$).

In contrast, the type of transformation used made a difference for interpreting whether some pretreatment conditions influenced the effects of particular antidepressants. AMPT, which increased baseline immobility values by approximately 30%, was sufficient to block the effects of desipramine when calculated as a percent change but not as a difference score. More dramatically, however, AMPT appeared to enhance the effects of citalopram because the elevated baseline increased to a near-maximal response. However, the effects of citalopram were actually slightly diminished as a percent change. Although reserpine

Table 3 Summary of antidepressant responses following monoamine depletion

Treatment	Change score			Precent change		
AMPT	*Saline*	*AMPT*	*p*	*Saline*	*AMPT*	*p*
Desipramine	−88.2±10.6	−66.7±12.1	0.203	−66.0±7.9	−38.5±7.0	0.019
Fluoxetine	−84.2±5.5	−93.3±8.8	0.398	−63.0±4.1	−53.9±5.1	0.186
Citalopram	−121.2±2.8	−140.3±5.0	0.004	−90.6±2.1	−81.1±2.9	0.016
Absolute pretreatment baseline values						
Saline	133.8±10.2	173.1±7.0				
Reserpine	*Saline*	*Reserpine*	*p*	*Saline*	*Reserpine*	*p*
Desipramine	−90.0±11.0	−49.5±18.1	0.074	−67.7±8.3	−19.5±7.2	0.001
Fluoxetine	−55.2±8.0	−102.5±20.2	0.056	−41.5±6.0	−40.5±8.0	0.917
Citalopram	−118.3±4.6	−108.4±20.0	0.693	−88.9±3.4	−42.8±7.9	0.001
Absolute pretreatment baseline values						
Saline	133.0±8.4	253.2±7.9				
Reserpine + AMPT	*Saline*	*Reserpine + AMPT*	*p*	*Saline*	*Reserpine + AMPT*	*p*
Desipramine	−71.9±10.7	0.9±10.0	0.001	−65.3±9.7	0.4±3.9	0.001
Reboxetine	−92.0±3.6	−10.9±13.4	0.001	−83.6±3.3	−4.3±5.2	0.001
Fluoxetine	−44.4±9.8	−14.2±12.1	0.072	−40.1±8.8	−6.1±5.2	0.003
Citalopram	−94.8±2.2	−39.3±16.0	0.003	−85.7±2.0	−16.9±6.9	0.001
Absolute pretreatment baseline values						
NRIs	110.6±8.6	232.6±14.5				
SSRIs	110.1±9.1	256.3±6.8				
PCPA	*Saline*	*PCPA*	*p*	*Saline*	*PCPA*	*p*
Desipramine	−89.2±6.8	−76.9±4.9	0.160	−81.8±6.2	−81.5±5.2	0.974
Reboxetine	−98.3±2.4	−87.3±2.4	0.007	−90.1±2.2	−92.6±2.6	0.482
Fluoxetine	−47.6±8.8	46.6±14.2	0.001	−55.1±10.2	74.3±22.7	0.001
Citalopram	−100.7±4.9	−6.9±9.7	0.001	−87.4±4.2	−7.1±9.8	0.001
Absolute pretreatment baseline values						
NRIs	109.1±7.5	94.3±14.7	0.384			
Fluoxetine	86.4±17.8	62.8±13.2	0.313			
Citalopram	115.2±7.6	97.8±16.4	0.272			

Separate groups of mice were pretreated with: AMPT (400 mg/kg, 3.5 h), reserpine (1 mg/kg, 24 h), combined pretreatment of AMPT (200 mg/kg, 3.5 h) + reserpine (1 mg/kg, 24 h), or PCPA (300 mg/kg×3 days). All antidepressants were given 30 min before the TST. Change scores were calculated as the absolute value of drug treatment—baseline. Percent change was calculated as drug treatment/baseline–100. Values are expressed as mean ± SEM. *p* values refer to the comparison between the corresponding saline-drug and the pretreatment-drug groups, according to Student's *t* test. Absolute baseline values are the mean immobility values for the saline–saline control groups run for each experimental series.

appeared to diminish the effects of desipramine irrespective of transformation, the effects of reserpine on responses to fluoxetine and citalopram were dependent on the method of transformation used.

Finally, although treatment with either reserpine or reserpine + AMPT increased baseline immobility to a similar degree, these pretreatments had different effects on the behavioral response to antidepressant drugs. Whereas reserpine + AMPT pretreatment prevented the behavioral effects of desipramine and fluoxetine, reserpine alone was not sufficient to block their behavioral effects. This suggests that synthesis of newly formed pools of catecholamines (inhibited by AMPT), as well as release from stored vesicular pools of monoamines (inhibited by reserpine), mediate the behavioral response to desipramine and fluoxetine in the TST.

Discussion

The goal of the present study was to systematically assess the relative contributions of monoamine neurotransmitters, either 5-HT or NE and DA, to the behavioral effects of both SSRIs and NRIs in the TST. The TST is widely used in the mouse for measuring the behavioral effects of antidepressant drugs with a broad range of acute pharmacological effects (Cryan and Mombereau 2004; Cryan et al. 2005; Steru et al. 1985). This common behavioral response among antidepressants that selectively inhibit 5-HT or NE reuptake provided an advantage in assessing the impact of the depletion of different monoamine neurotransmitters.

The results from the present study demonstrated that a substantial depletion of 5-HT (78–82%) by the tryptophan hydroxylase inhibitor PCPA blocked the ability of SSRIs, but not NRIs, to reduce immobility in the TST. The lack of effect of both fluoxetine and citalopram in PCPA-pretreated mice is consistent with the hypothesis that both compounds elicit their acute behavioral effects by increasing extracellular 5-HT after blockade of the serotonin transporter (Bymaster et al. 2002). The present data are consistent with the one previous study which examined the contribution of endogenous 5-HT to the behavioral effects of fluoxetine in the TST (Rodrigues et al. 2002). The depletion of 5-HT has been used more commonly to study the behavioral effects of SSRIs in other tests sensitive to antidepressant drugs, such as the forced swim test (FST). In the mouse FST, pretreatment with PCPA prevented the behavioral effects of fluoxetine (Eckeli et al. 2000; Gavioli et al. 2004; Zomkowski et al. 2004) and attenuated the effects of paroxetine (Redrobe et al. 1998). Similarly, the behavioral effects of fluoxetine in the FST were blocked or attenuated in rats pretreated with PCPA or methylenedioxyamphetamine to deplete 5-HT (Harkin et al. 2003; Page et al. 1999). Taken together, studies have demonstrated that depletion of 5-HT in rodents effectively prevents the acute behavioral effects of SSRIs in a number of tests for antidepressant activity.

In the present study, pretreatment with PCPA did not prevent behavioral responses to the NRIs, desipramine and reboxetine, in the TST. This observation is consistent with those from previously published studies that examined the role of 5-HT in the behavioral effects of NRIs in other tests sensitive to antidepressant drugs, such as the rat or mouse FST (Gavioli et al. 2004; Lucki et al. 1994; Page et al. 1999) or learned helplessness (Soubrie et al. 1986). Furthermore, selective NRIs did not increase extracellular 5-HT levels (Page and Lucki 2002). Evidence does not currently support a role for 5-HT in the antidepressant-like behavioral effects of NRIs.

Prevention of the behavioral effects of SSRIs by 5-HT depletion was not simply due to an impairment of the animals' behavior, as PCPA did not alter baseline immobility in the TST. These results agree with previous reports which demonstrated that depletion of 5-HT using PCPA does not alter baseline behavior in many antidepressant tests, such as the mouse TST and FST, the modified rat FST, or the learned helplessness paradigm (Borsini and Cesana 2001; Gavioli et al. 2004; Mayorga et al. 2001; Page et al. 1999; Wieland and Lucki 1990). Similarly, destruction of serotonergic neurons with 5,7-DHT did not alter baseline behavior in the rat FST or in the learned helplessness paradigm (Cervo and Samanin 1987; Cervo et al. 1991; Lucki et al. 1994; Soubrie et al. 1986).

The findings of the present PCPA study are also consistent with those of several clinical studies examining the effect of 5-HT depletion on the therapeutic effects of antidepressant drugs. Reduction of 5-HT content in humans, by administering a drink that depletes the substrate tryptophan, induced clinical relapse in successfully remitted patients that had been treated with SSRIs, suggesting critical involvement of 5-HT in mediating therapeutic responses to SSRIs. In contrast, rapid tryptophan depletion did not evoke relapse in those patients who had been treated with the NRI desipramine (Delgado et al. 1991, 1999; Delgado 2004). Some investigators have emphasized a role for 5-HT deficiency in causing depressive behavior (Arango et al. 2002; Maes and Meltzer 1995; Stockmeier 2003). However, the role of 5-HT may be seen more clearly as a mechanism for treating depression than as a precipitatant of depression, and this view is in agreement with clinical studies which have demonstrated that rapid tryptophan depletion did not exacerbate symptoms in unmedicated depressed patients and caused minimal symptoms in healthy subjects with no family history of depression (Benkelfat et al. 1994; Berman et al. 2002; Delgado et al. 1994). Although 5-HT insufficiency may underlie the pathophysiology of

depression or suicide in some vulnerable individuals, evidence clearly supports the recruitment of 5-HT in the mechanisms underlying SSRI-induced remission of depression.

The present experiments also examined the role of NE and DA in the acute behavioral effects of antidepressants in the TST by using drugs that interfere with neurotransmitter synthesis or release. Depletion of NE and DA with the tyrosine hydroxylase inhibitor AMPT (400 mg/kg) increased baseline immobility and appeared to attenuate the effects of desipramine in the TST, but did not completely block its activity. AMPT preferentially inhibits the formation of newly synthesized pools of DA and NE (Weissman et al. 1966), and this dose produced a 61% depletion of NE and a 67% depletion of DA tissue content. A substantially similar level of depletion for NE and DA produced by a lower dose of AMPT (300 mg/kg; data not shown) indicated that the neurochemical impact of AMPT was likely maximized in the mouse under these test conditions. Pretreatment with reserpine (1 mg/kg), which disrupts vesicular storage and release of monoamine neurotransmitters (Corrodi and Hanson 1966), increased baseline immobility and attenuated the effects of desipramine in the TST, but also did not completely block its activity. Reserpine produced a 93 and a 95% depletion of cortical NE and DA content respectively, and a 78% depletion of 5-HT, but this also did not completely block the activity of desipramine.

To both inhibit synthesis and deplete vesicular pools of NE and DA, mice were pretreated with both reserpine (1 mg/kg) and AMPT (200 mg/kg). Other investigators have adopted a similar approach to delineate the combined roles of cytosolic and vesicular pools of DA in the neurotoxic or locomotor effects of psychomotor stimulants (Finn et al. 1990; Simon et al. 1995; Yuan et al. 2001, 2002) or in amphetamine-induced increases in extracellular DA levels (Butcher et al. 1988; Cadoni et al. 1995). Pretreatment with reserpine + AMPT produced a substantial depletion of cortical DA (95%), NE (97%), and 5-HT (78%) content, values that were about the same as when reserpine was given alone, and increased baseline immobility in the TST to levels that were similar to those when reserpine was given alone. Nevertheless, the drug combination was likely critical in completely blocking the effects of the NRI desipramine because reserpine + AMPT completely prevented the behavioral effects of desipramine and reboxetine, whereas desipramine was still active after reserpine given alone. It is also unlikely that depletion of 5-HT contributed to the blockade of the behavioral effects of desipramine, as similar reductions in 5-HT tissue content by PCPA did not prevent the behavioral effects of desipramine and reboxetine in the TST. Taken together, the present studies suggest that depletion of both vesicular and cytoplasmic pools of NE and DA is required to completely prevent the behavioral effects of desipramine.

The behavioral effects of the SSRIs, fluoxetine, and citalopram were also examined after pretreatment with AMPT, reserpine, or their combination. Pretreatment with AMPT did not attenuate the effects of either fluoxetine or citalopram. In addition, pretreatment with reserpine did not completely block the response to fluoxetine or citalopram, despite the substantial depletion of 5-HT, although the magnitude of the drug response depended on the method of calculation (see discussion below). However, the effects of fluoxetine were completely blocked by the reserpine + AMPT combination, whereas the effects of citalopram were attenuated but still present. In contrast to PCPA, measuring the behavioral effects of the SSRIs in catecholamine-depleted animals was complicated by an increase in baseline immobility. Another important observation of these studies was that pretreatment with reserpine did not block the effects of the SSRIs; even though 5-HT tissue content was reduced to a similar extent as when the behavioral effects of both SSRIs were blocked by PCPA. The reason for differences between the effects of pretreatment with reserpine and PCPA may be that inhibiting transmitter synthesis (PCPA) may be more effective than disrupting vesicular storage and release (reserpine) at preventing 5-HT-mediated behaviors.

The findings of the present study with AMPT did not agree with the results of catecholamine depletion studies by Delgado who showed that AMPT caused relapse in depressed patients that were successfully treated with NRIs but not SSRIs (Delgado et al. 1993; Miller et al. 1996). In contrast, the present study found that AMPT increased immobility values in all treatment groups and attenuated, but did not completely prevent, the effects of desipramine. Although the dose of desipramine in the present study was selected to produce a similar magnitude of response as other antidepressants, lower doses of desipramine (producing a smaller response) might be blocked more effectively by AMPT.

The method of calculating drug responses in the TST was considered after catecholamine depletion because many of the drugs used to deplete catecholamine neurotransmitters increased baseline values for immobility before antidepressant treatment (Lucki and O'Leary 2004). Accordingly, drug responses on immobility duration were expressed as either the absolute change or as a percentage change, and could be subject to variations of interpretation when results based on these transformations failed to agree. For example, the responses to citalopram after AMPT and to fluoxetine after reserpine appeared to be enhanced when calculated as an absolute difference score from baseline, but these responses were likely due to the increased baseline level produced by the catecholamine-depleting drugs. On the other hand, the response to citalopram after reserpine was reduced when calculated as a percent change, but not

as a change in absolute value. If the response to citalopram were actually diminished by disruption of vesicular function, this could indicate an important difference between the two SSRIs. Indeed, a role for the biological target of reserpine, the vesicular monoamine transporter 2, was recently suggested in the behavioral effects of citalopram in the TST on the basis of a genetic breeding study (Crowley et al. 2006). By contrast, interpretation of the effects of PCPA pretreatment on antidepressant responses was more straightforward because there was no change in baseline immobility values. Overall, more consistent interpretations appeared to be obtained by considering the percentage reduction of immobility as the best measure of the antidepressant response.

An intrinsic difficulty in working with drugs to produce neurotransmitter depletions in the CNS is that drugs often affect more than a single neurotransmitter. Additionally, pretreatments may be ineffective if they fail to produce a sufficient depletion or blockade of their target, or they may be effective because they produce their effects through a combination of effects rather than the one attributed mechanism. Although guidelines from lesion studies suggest that near-complete depletion of DA or 5-HT may be required to produce evidence of behavioral impairment (Kirby et al. 1995), such benchmarks do not apply when depletion is produced by other methods such as inhibition of synthesis or interruption of vesicular storage. Nevertheless, a surprisingly substantial depletion of catecholamines was required to completely block the response to antidepressants. Another approach to examining antidepressant treatment responses after targeted disruptions of monoaminergic transmission involves the generation of mice with deletion of genes responsible for transmitter synthesis. Although genetic techniques can be more selective than the drugs currently available for studying catecholamine synthesis, animals develop with a long-lasting depletion of the transmitter throughout their life span. For example, deletion of the *dbh* gene, the gene encoding the enzyme responsible for synthesizing NE from DA, caused a complete and selective depletion of NE content. In contrast to the results obtained with AMPT or reserpine in the present study, TST baseline immobility was not altered by the depletion of NE, and the effects of desipramine were blocked completely (Cryan et al. 2004) possibly because NE may be more important for regulating the effect of desipramine and other antidepressants, whereas DA may regulate baseline immobility values in antidepressant tests. Also, genetic depletion of NE completely prevented the effects of fluoxetine, but produced only minor effects on the response to citalopram (Cryan et al. 2004). In the case of 5-HT synthesis, the enzyme responsible for 5-HT synthesis, tryptophan hydroxylase, is expressed in different isoforms and regulated by two distinct genes, *tph1* and *tph2* (Walther and Bader 2003), and their relationship to the antidepressant effects of SSRIs is unknown.

Nevertheless, drugs that inhibit monoamine synthesis or release can be important tools that can characterize the mechanisms underlying an antidepressant-like phenotype of lines of mutant mice, can test an hypothesis concerning a novel drug's mechanism of action, or reveal underlying vulnerability to stress. Mice with gene deletion of NE, DA or 5-HT transporters have been reported to show changes of baseline immobility values in behavioral tests sensitive to antidepressant drugs, as if to mimic the effects of antidepressant treatments (Holmes et al. 2002; Spielewoy et al. 2000; Xu et al. 2000), but the mechanisms underlying these changes are untested. Depletion of 5-HT should also be successful in reversing an antidepressant phenotype in mice lacking the 5-HT transporter if the phenotype is due solely to alterations of 5-HT transmission (Holmes et al. 2002). In another example, female $5{-}HT_{1B}^{-/-}$ mice demonstrated a gender-selective antidepressant behavioral phenotype in the TST and FST associated with increased levels of 5-HT. The decreased immobility was normalized by depleting 5-HT with PCPA (Jones and Lucki 2005). The pattern of these results indicate that higher 5-HT transmission can produce an adjusted "normal" behavioral state in female $5{-}HT_{1B}^{-/-}$ mice that makes them vulnerable to depressive behavior evoked by 5-HT depletion, and this could be a model for an etiological role of 5-HT in depression (Jones and Lucki 2005).

In conclusion, the results of the present study provide evidence that endogenous 5-HT is essential for the acute behavioral effects of SSRIs, but not NRIs, in the TST. The present studies also highlighted some of the difficulties encountered when evaluating the contribution of DA and NE to the behavioral effects of antidepressant drugs. In the present study, the effects of desipramine were attenuated, although not completely blocked, by significant catecholamine depletion with AMPT or reserpine. Complete blockade of desipramine was produced only by the combined treatment with AMPT and reserpine, and may be due to a combination of inhibiting transmitter synthesis and impairing vesicular function. These results agree with a larger body of work in rodents demonstrating alterations of antidepressant behavioral responses after the depletion or destruction of monoamine transmission (for review, see Lucki and O'Leary 2004). Although these data were derived from a limited set of compounds, extrapolation to other SSRI and NRI compounds is likely but not definite. Also, interpretation of the results with some depletion agents could be hampered by a lack of specificity and their effects on baseline immobility. Nevertheless, the development and exploration of selective pharmacological agents for systemic use would provide additional tools for clarifying the role of specific neurotransmitters in the behavioral effects of estab-

lished and putative antidepressant drugs and antidepressant-like behavioral phenotypes associated with genetic deletion. Convergent studies could also use selective lesions, inhibition of transmitter synthesis, and regional inactivation techniques to identify the critical circuitry that supports behavioral responses to antidepressants (Cryan et al. 2002; O'Leary et al. 2007).

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