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Atomoxetine Increases Extracellular Levels of Norepinephrine and Dopamine in Prefrontal Cortex of Rat: A Potential Mechanism for Efficacy in Attention Deficit/Hyperactivity Disorder

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The selective norepinephrine (NE) transporter inhibitor atomoxetine (formerly called tomoxetine or LY139603) has been shown to alleviate symptoms in Attention Deficit/Hyperactivity Disorder (ADHD). We investigated the mechanism of action of atomoxetine in ADHD by evaluating the interaction of atomoxetine with monoamine transporters, the effects on extracellular levels of monoamines, and the expression of the neuronal activity marker Fos in brain regions. Atomoxetine inhibited binding of radioligands to clonal cell lines transfected with human NE, serotonin (5-HT) and dopamine (DA) transporters with dissociation constants (K_i) values of 5, 77 and 1451 nM, respectively, demonstrating selectivity for NE transporters. In microdialysis studies, atomoxetine increased extracellular (EX) levels of NE in prefrontal cortex (PFC) 3-fold, but did not alter 5-HT_{EX} levels. Atomoxetine also increased DA_{EX} concentrations in PFC 3-fold, but did not alter DA_{EX} in striatum or nucleus accumbens. In contrast, the psychostimulant methylphenidate, which is used in ADHD therapy, increased NE_{EX} and DA_{EX} equally in PFC, but also increased DA_{EX} in the striatum and nucleus accumbens to the same level. The expression of the neuronal activity marker Fos was increased 3.7-fold in PFC by atomoxetine administration, but was not increased in the striatum or nucleus accumbens, consistent with the regional distribution of increased DA_{EX}. We hypothesize that the atomoxetine-induced increase of catecholamines in PFC, a region involved in attention and memory, mediates the therapeutic effects of atomoxetine in ADHD. In contrast to methylphenidate, atomoxetine did not increase DA in striatum or nucleus accumbens, suggesting it would not have motoric or drug abuse liabilities.

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Attention Deficit/Hyperactivity Disorder (ADHD) is a common behavioral disorder found in 3–7% of school age children (American Psychiatric Association 2000; Szatmari 1992; Offord et al. 1987). ADHD is characterized by levels of increased motor activity, impulsive-

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ness, distractibility, restlessness and inattention that is maladaptive and inconsistent with the child's developmental level. Three subtypes have been identified: inattentive, hyperactive/impulsive and combined (inattentive/hyperactive/impulsive). Children with ADHD have increased risk for low educational and vocational attainment, as well as social dysfunction, increased criminality, and drug abuse (Gittelman et al. 1985; Mannuzza et al. 1993). ADHD is frequently associated with oppositional and conduct disorders, depression, and anxiety (Downey et al. 1997; Biederman et al. 1991). ADHD often persists into adult life (Spencer et al. 1996; Gittelman et al. 1985; Barkley et al. 1990).

Dysfunction of catecholamine and particularly dopamine (DA) neuronal systems has been postulated to be involved in ADHD (Castellanos et al. 1996a; Zametkin and Rapoport 1987). Dopamine plays a key role in attentional, psychomotor, reinforcing and rewarding behaviors that are deficient in ADHD. Amphetamine and methylphenidate, which have been widely used to treat ADHD, block DA and norepinephrine (NE) transporters and thereby enhance catecholamine neurotransmission (Barkley 1977; Spencer et al. 1995; Gatley et al. 1996).

Norepinephrine has also been proposed to play a key role in the pathophysiology and pharmacotherapy of ADHD (Zametkin and Rapoport 1987; Pliszka et al. 1996; Arnsten et al. 1996; Biederman and Spencer 1999). The noradrenergic system is involved in attentional processes and has been shown to prime the prefrontal cortex (PFC) for response to sensory stimuli (Segal and Bloom 1976; Aston-Jones et al. 1991; Berridge et al. 1993). Increased basal activity of locus coeruleus noradrenergic cell bodies may decrease the response of the PFC and thus treatments that reduce locus coeruleus activity have been hypothesized to improve attentional, arousal, and cognitive processes (Pliszka et al. 1996).

Pharmacotherapy of ADHD to control the behavioral symptoms has been largely with psychostimulants such as d-amphetamine and methylphenidate (Spencer et al. 1995; Wilens et al. 1998). However, about 10–30% are non-responders or are intolerant to psychostimulant therapy (Barkley 1977; Elia et al. 1991; Greenhill 1995). Adverse reactions associated with stimulant use include insomnia, tics, anorexia, anxiety, and dysphoric mood (Gittelman 1980; Greenhill 1995). The older psychostimulants have short half lives that result in problematic multiple daily doses, particularly with concerns about insomnia after late afternoon and evening administration. In addition, the psychostimulants used in ADHD are controlled substances that have liability for drug abuse and diversion, thus limiting their usefulness (Holman 1994). Concerns about the tolerability and abuse liability of psychostimulants have led to interest in the development of drugs for pharmacotherapy of ADHD with a different mechanism of action.

The tricyclic antidepressants, desipramine and nortriptyline, have emerged as alternative therapies for the treatment of ADHD (Spencer et al. 1996; Wilens et al. 1996). Desipramine and nortriptyline have high affinity for NE transporters relative to DA and serotonin (5-HT) transporters (Wong et al. 1995) and have been shown in clinical evaluations of adults and children to be effective in ADHD (Biederman et al. 1989; Wilens et al. 1996). However, tricyclic antidepressants have significant affinity for α_1-adrenergic, cholinergic and histaminergic receptors potentially resulting in sedation, dry mouth, weight gain and cognitive impairment and also have cardiovascular concerns (Wong et al. 1995; Cookson 1993; Leonard et al. 1995; Walsh et al. 1994). Therefore, the tricyclic antidepressants are also limited in usefulness.

Recently, atomoxetine (tomoxetine, LY139603) was found to be efficacious in the treatment of ADHD in a double-blind, placebo-controlled crossover study in adults (Spencer et al. 1998). In children and adolescents, atomoxetine had superior outcomes in reducing ADHD symptoms compared with placebo and had a graded dose response (Michelson et al. 2001). Atomoxetine significantly reduced core symptoms of ADHD in an open-label study in children (Spencer et al. 2001). Overall, it was well tolerated in the child and adolescent age group (Michelson et al. 2001; Spencer et al. 2001). Atomoxetine has also been shown in a 6-week open label study to produce clinically significant improvement in the symptoms in patients with major depression (Chouinard et al. 1984).

Atomoxetine is a potent NE uptake inhibitor in vitro and in vivo with relatively low affinity for 5-HT and DA uptake processes (Wong et al.1982; Bolden-Watson and Richelson 1993). Furthermore, atomoxetine is a potent inhibitor of the presynaptic NE transporter and has minimal affinity for other neurotransmitter transporters and neuronal receptors (Gehlert et al. 1995; Tatsumi et al. 1997; Wong et al. 1982). In this study, we have further investigated the pharmacology of atomoxetine to understand its therapeutic actions in ADHD. The affinity of atomoxetine and methylphenidate for human monoamine uptake transporters and the potential interaction of atomoxetine with neuronal receptors was evaluated. To evaluate the potency and selectivity of uptake inhibitors of NE and 5-HT transporters in vivo, the ability of uptake inhibitors to block neurotransmitter depletion in rat brain induced by monoamine transporter-dependent neurotoxins was investigated. The activation of expression of the neuronal activity marker, Fos, by atomoxetine was determined in several brain regions. Finally, we compared the effects of atomoxetine administration with another NE uptake inhibitor, reboxetine, and with methylphenidate on extracellular levels of monoamines in rat brain regions potentially involved in ADHD including PFC.

MATERIALS AND METHODS

Transporter Binding Studies

Membranes from HEK 293, MDCK, and HEK293 cell lines transfected with human 5-HT, NE and DA transporters, respectively, were obtained from Receptor Biology, Inc. (Beltsville, MD). All assays were performed in triplicate in a final volume of 0.8 ml containing either a buffer consisting of 50 mM Tris Cl, pH 7.4, 150 mM NaCl, and 5 mM KCl for 5-HT and NE transporters or 50 mM Tris Cl, pH 7.4, and 100 mM NaCl for the DA transporter. The radioligands for 5-HT, NE and DA human transporters were [^{3}H]-paroxetine (0.2 nM, 25 Ci/mmol, Dupont NEN products, Boston, MA), [^{3}H]-nisoxetine (1.0 nM, 86 Ci/mmol, New England Nuclear) and [^{3}H]-WIN35,428 (1.0 nM, 86 Ci/mmol, New England Nuclear), respectively. Membranes equivalent to protein in amounts of 10.3 μg, 16.9 μg or 6.2 μg, respectively, were used in the assays. After incubation at 37°C for 40 min for the 5-HT transporter and 25°C for 30 min for NE and DA transporters, the binding was terminated by rapid vacuum filtration over Whatman GF/B filters (presoaked in 0.5% polyethylenimine) and the filters were washed four times with cold 50 mM Tris Cl buffer, pH 7.4. The filters were then placed in vials containing liquid scintillation fluid and radioactivity was measured by liquid scintillation spectrometry. Non-specific binding was determined in separate samples with 1 μM duloxetine, 10 μM desipramine or 10 μM nomifensine, for 5-HT, NE and DA transporters, respectively.

Determination of Binding to Neuronal Receptors

Inhibition of binding to neuronal receptors was provided by NovaScreen (Hanover, MD) using proprietary receptor binding assays.

Blockade of p-Chloramphetamine and DSP-4 Effects

The 5-HT selective neurotoxin p-chloramphetamine hydrochloride (p-CA) was dissolved in sterile water for intraperitoneal (i.p.) administration. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) in groups of five weighing 140–170 grams were injected with p-CA (10 mg/kg i.p.) 2 h before cervical dislocation. Vehicle or test drugs were dissolved in sterile water and injected 1 h prior to p-CA. The brains were quickly removed, frozen on dry ice and stored at −70°C until assayed. Whole brain 5-HT concentrations were measured using high pressure liquid chromatography with electrochemical detection (HPLC-EC) as previously described (Fuller and Perry 1989).

DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride), a noradrenergic neurotoxin (Jonsson et al. 1981; Grzanna et al. 1989), was dissolved in 0.01 N HCl and injected at 30 mg/kg i.p. one hour after administration of drugs. Cortical NE concentrations were measured 6 h after administration of DSP-4 according to a modification of the method of Fuller and Perry (1977). Cortical tissue was homogenized in 2 ml 0.1 N trichloroacetic acid with 3,4-dihydrobenzylamine hydrobromide added as an internal standard. After centrifugation, 1 ml of the supernatant was added to 150 mg alumina, and the pH adjusted to 8.6 with 0.5 M Tris containing 0.1 M EDTA. Samples were mixed for 10 min, centrifuged and the supernatant decanted. The alumina was washed with 1 ml 50 mM Tris/10 mM EDTA. One ml of 0.1 N formic acid was added to the alumina, and the samples were mixed for 10 min and centrifuged. Cortical NE was measured using a HPLC-EC technique by injecting a 20 μl aliquot of the supernatant onto a BDS Hypersil C-18 analytical column (Keystone Scientific, Inc.). The elution buffer contained 75 mM sodium phosphate, 0.5 mM EDTA, 350 mg/l 1-octanesulfonate sodium, 4% acetonitrile (v/v) and 0.8% tetrahydrofuran, pH 3.0 and the flow rate was 1.2 ml/minute. Dihydrobenzylamine and NE peaks were measured at 600 mV with 10 nA sensitivity using an electrochemical method and compared with samples containing known amounts of NE.

Fos Immunohistochemistry

Two hours after administration of atomoxetine (3 mg/kg, i.p.), the rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and transcardially perfused with 100 ml of phosphate buffered saline (PBS) followed by 100 ml of 4% paraformaldehyde in PBS. The brain was rapidly removed and postfixed for 90 min in 4% paraformaldehyde, and then was transferred to 30% sucrose at 4°C until saturated. After quick freezing, serial 30 μm sections were cut and placed in PBS until processed for immunohistochemistry. In brief, sections were incubated in PBS containing blocking serum and 0.5% Triton-X 100 for 1 h. Sections were then incubated with anti-Fos antibody (Santa Cruz Biotechnology, Inc.) at 4°C overnight. Visualization of the Fos-like immunoreactivity was performed with a Vectastain ABC Elite Kit (Vector Labs, Burlingame, CA) using the standard protocol supplied with the kit. Nickel-intensified diaminobenzidine (DAB) was used as the chromagen to yield a gray-black precipitation product. Following visualization of the Fos immunoreactivity, the sections were mounted on gelatin-coated glass slides and allowed to dry. The sections were then dehydrated and cover slipped. Fos expressing cells were quantitated using the MCID M2 imaging system (Imaging Research, St. Catherines, Ontario).

Microdialysis Studies

Male Sprague-Dawley rats from Harlan Industries (Indianapolis, IN) were used for all studies. For the microdial-

ysis studies, rats weighing 260–300 mg were anesthetized with chloral hydrate/pentobarbital (170 mg/kg and 36 mg/kg, respectively, in 30% propylene glycol and 14% ethanol) for implantation of the dialysis probes.

The microdialysis technique used here has been described previously (Li et al. 1998; Zhang et al. 2000). In brief, a homemade loop type probe with a regenerated cellulose dialysis fiber of 3 mm tip length (6 mm total) fused into PE-10 tubing was used. Coordinates for the PFC were: A (anterior to bregma), 3.2 mm; L (lateral from the midsagittal suture), 0.8 mm; and V (ventral from the dura surface), 4 mm (Paxinos and Watson 1986). The coordinates used for the nucleus accumbens were A, 2.0 mm; L, 1.5 mm; and V, 8.0 mm. The coordinates used for the striatum (caudate putamen) were A, 0.2 mm; L, 3.0 mm; and V, 6.5 mm. At the end of experiments, the probe position was histologically verified by perfusing a dye (2,3,5 triphenyltetrazolium chloride, 5 mg/ml in water) through the dialysis probe and then sectioning the frozen brain to observe the probe location. Rats with improper probe location were not included in the statistical analysis.

Microdialysis experiments were performed two days after surgery to allow the rats to fully recover from the operation and resume normal food intake. The rat was placed in a plastic bowl and connected to a fraction collection system for freely moving animals (Raturn, Bio-Analytical Systems (BAS), West Lafayette, IN). The input tube of the dialysis probe was connected to a syringe pump (BeeHive and BabyBee, BAS) which delivered an artificial cerebrospinal fluid containing 150 mM NaCl, 3 mM KCl, 1.7 mM $CaCl_2$ and 0.9 mM $MgCl_2$ (pH 6.0) to the probe at a rate of 1.0 μl/min. The output tubes from the rats were attached to a refrigerated fraction collector (820 microsampler, Scipro, North Tonawanda, NY). After acclimatization of the rat to the apparatus and establishment of stable monoamine baseline levels, the drugs were administered in a volume of 1 ml/kg i.p. in sterile water. Monoamines in dialysates were measured off-line by the analytical method described in Li et al. (1998). The sensitivity for DA, NE and 5-HT was 0.1 pmol/ml dialysate or 2 fmol/sample (20 μl). All microdialysis data were calculated as percent change from dialysate basal concentrations with 100% defined as the average of the final three drug preinjection values and each group had 5–6 rats.

Drugs

Atomoxetine, reboxetine, and fluoxetine were provided by the Lilly Research Laboratories, Indianapolis, IN. Desipramine, buproprion, imipramine, nomifensine, p-CA, and DSP-4 were purchased from Sigma Chemical Company, St. Louis, MO. Methylphenidate was purchased from Mallinckrodt, St. Louis, MO. All other chemicals were reagent grade quality.

Statistics

Inhibition curves for in vitro studies were analyzed by nonlinear least-squares curve fitting to obtain IC_{50} values. The inhibition constant (K_i) values were calculated from IC_{50} and K_d values according to the method of Cheng and Prusoff (1973). The K_d values of the radioligands used for the 5-HT, NE and DA transporters were 0.18, 2.5 and 22.8 nM, respectively. All values for microdialysis studies were calculated as percentage change at each time point compared with the average of three baseline values. Significant differences for the time course of vehicle control injection on NE, DA or 5-HT were determined by a 1-way analysis of variance (ANOVA) for repeated measures with respect to time. Differences between treatment groups, including control, were determined by a 2-way ANOVA with treatment as the independent variable and time as the repeated measure. If significant, the ANOVA was followed by a post-hoc Duncan's multiple range test on the overall effect of treatment using the Statistica program (StatSoft, Tulsa, OK).

RESULTS

Transporter and Receptor Binding

Atomoxetine inhibited radioligand binding in cells transfected with human NE, 5-HT, and DA transporters with K_i values of 5, 77 and 1451 nM, respectively. (Table 1). The selective NE uptake inhibitors reboxetine (Wong et al. 2000) and desipramine also had high affinity and selectivity for NE transporters. In contrast, methylphenidate had higher affinity for human DA transporters than NE transporters and low affinity for 5-HT transporters. Nomifensine had high affinity for DA and NE transporters and buproprion had moderate affinity for DA transporters. Imipramine inhibited binding to NE and 5-HT transporters, whereas fluoxetine had appreciable affinity for 5-HT transporters only. The Hill coef-

Table 1. Affinity of Atomoxetine and Other Uptake Inhibitors for Human Monoamine Transporters.

Compound	Norepinephrine	Serotonin K_i, nM	Dopamine
Atomoxetine	5	77	1451
Reboxetine	11	440	>10000
Desipramine	3.8	179	>10000
Methylphenidate	339	>10000	34
Bupropion	>10000	>10000	562
Nomifensine	29	4872	53
Imipramine	98	19	>10000
Fluoxetine	1022	7	4752

K_i values were determined from at least six concentrations in triplicate in three separate assays.

ficients for binding of the compounds to the transporters were not significantly different from 1. The affinity of atomoxetine for over 60 other neuronal receptors, transporters and binding sites was investigated. Atomoxetine did not have appreciable affinity for the receptors and binding sites investigated (Table 2).

The ability of atomoxetine to block 5-HT and NE uptake processes in vivo was determined using the transporter specific neurotoxins p-CA and DSP-4 which deplete 5-HT and NE concentrations in rat brain regions, respectively. Atomoxetine blocked depletion of rat cortical NE concentrations by DSP-4 in a dose-dependent manner with an ED_{50} of 2.5 mg/kg p.o. (Table 3 and Figure 1, panel A). However, atomoxetine did not significantly block p-CA–induced depletion of rat brain 5-HT (Figure 1, panel B). Like atomoxetine, the selective NE uptake inhibitors desipramine and reboxetine also blocked depletion of cortical NE by DSP-4 and had modest effects on p-CA–induced depletion of brain 5-HT at doses up to 30 mg/kg (Table 3). Methylphenidate did not inhibit either DSP-4–induced NE depletion or p-CA–induced 5-HT depletion, but the selective 5-HT uptake inhibitor fluoxetine potently inhibited p-CA–induced 5-HT depletion (Table 3).

Table 2. Atomoxetine Has Low Affinity (>1 μM) for Neuronal Receptors, Transporters and Other Binding Sites

Receptor
Neurotransmitter receptors
Adenosine, A1, A2
Adenosine, A2
Adrenergic, α1A, α1B, hα2A, α2B
Adrenergic, β1, β2
Dopamine, D1, D2
GABA A, Agonist Site
GABA A, Benzodiazepine, Central
Glutamate, AMPA Site
Glutamate, Kainate Site
Glutamate, NMDA Agonist Site
Glutamate, NMDA,Glycine(Stry-insens.)
Glycine, Strychnine-sensitive
Histamine, H1, H2
Melatonin
Muscarinic, hM1, hM2 (Human)
Nicotinic (α-bungarotoxin insens)
Opiate, Delta 1, Kappa 1, Mu
Sigma 1*, 2
5-HT, h1A, 1B, 1D, h2A, 2C, 3, 4, h6, h7
Ion channels
Calcium Channel, Type L, N
GABA, Chloride, TBOB Site
Glutamate, Chloride Site
Glutamate, NMDA, phencyclidine
Potassium Channel, ATP-Sens.
Potassium Channel, Ca^{++} Act., Volt Sens.
Sodium, Site 1
Sodium, Site 2
Second messengers
Adenylate Cyclase, Forskolin
Inositol Triphosphate
Protein Kinase C, PDBu
Transporters
Choline transporter
GABA transporter
Adenosine transporter
Brain/gut peptides
Cholecystokinin, CCKA, CCKB
Neurokinin, NK1, hNK2, NK3
Neuropeptide, hNPY1
Neurotensin
Somatostatin, Non-selective
NOS, Constitutive-Neuronal

*Atomoxetine inhibited binding 51% to the Sigma1 binding site; remainder were less than < 50% inhibition at 1μM concentration. h denotes human receptors.

Microdialysis Studies

Basal Levels of Monoamines in Dialysates The basal extracellular (EX) concentrations for 5-HT, NE, DA were 0.24 ± 0.03, 0.93 ± 0.05 and 0.41 ± 0.03, pmol/ml of PFC dialysate, respectively, in freely moving rats. The basal extracellular concentrations of DA in nucleus accumbens and striatum were 3.60 ± 0.50 and 4.54 ± 0.36 pmol/ml of dialysate, respectively.

Effect of Atomoxetine on Monoamine Extracellular Concentrations in Brain Regions

Vehicle injection produced no significant changes in monoamine levels compared with baseline concentrations with respect to time as shown by a 1-way ANOVA with repeated measures ($F_{8,40}$ = 0.399, 2.090, and 0.681 for NE, DA, and 5-HT respectively). These vehicle values were used as controls in all statistical comparisons with the treatment groups mentioned below.

Atomoxetine (0.3 to 3mg/kg i.p.) increased NE_{EX} concentrations in PFC up to a peak level of 290 ± 33% of basal concentrations and the higher doses had longer duration of increase (Figure 2, panel A). The 2-way ANOVA revealed a significant effect of treatment ($F_{3,20}$ = 5.840)

Table 3. Blockade of the p-Chloramphetamine (p-CA)-induced Rat Brain Serotonin Depletion and DSP-4-induced Depletion Of Cortical Norepinephrine Concentrations by Atomoxetine and Other Monoamine Reuptake Inhibitors

Compound	Blockade of p-CA ED_{50}, mg/kg, i.p.	Blockade of DSP-4 ED_{50}, mg/kg, p.o.
Atomoxetine	>30	2.5
Reboxetine	>30	3.2
Desipramine	22.9	1.1
Methylphenidate	>30	>30
Fluoxetine	3.9	>30

Rats were pretreated one h with reuptake inhibitors and sacrificed two h after p-CA (10 mg/kg i.p.) or six h after 30 mg/kg, i.p. DSP-4. ED_{50} values were calculated by probit analysis (log^{10}) at 95% confidence limits.

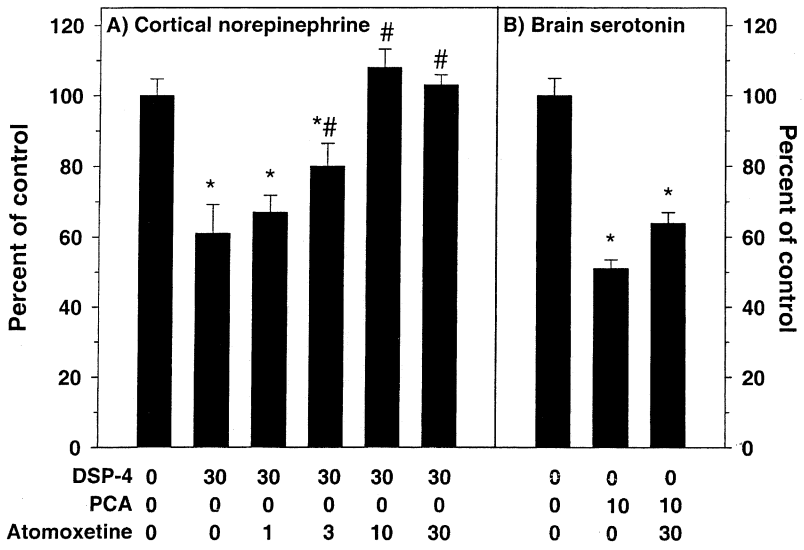

Figure 1. Effect of atomoxetine on the depletion of rat cortical norepinephrine (NE) concentrations produced by DSP-4 (30 mg/kg i.p.) (panel A) and brain serotonin (5-HT) concentrations produced by p-chloroamphetamine (p-CA, 10 mg/kg i.p.) (panel B). Atomoxetine was administered to rats in groups of five 1 h prior to DSP-4 and the rats were sacrificed 6 h after DSP-4 administration. B. Atomoxetine was administered to rats in groups of five 1 h prior to p-CA and the rats were sacrificed 2 h after p-CA administration. Control concentrations of NE in cortex were 1.92 ± 0.10 nmol/g and 5-HT concentrations in whole brain were 2.43 ± 0.12 nmol/g. Means and standard errors of the percent of control are shown. *$p < .05$ as compared with control group; #$p < .05$ as compared with DSP-4 or p-CA alone groups.

and time ($F_{8,160} = 22.615$) and a significant interaction of treatment and time ($F_{24,160} = 3.063$). The 4-h average for NE_{EX} at the 3-mg/kg i.p. dose was 243 ± 12% of basal concentrations ($p < .0022$). Atomoxetine (0.3 to 3 mg/kg i.p.) also increased DA_{EX} up to a peak level of 323 ± 17% ($F_{3,19} = 28.500$, $F_{8,152} = 37.538$, $F_{24,152} = 9.444$ for the effects of treatment, time and interaction, respectively) and a 4-h average of 251 ± 18% of basal concentrations at 3 mg/kg i.p. (Figure 2, panel B) ($p < .001$). The extracellular levels of 5-HT in PFC were not significantly increased by atomoxetine at doses up to 3 mg/kg i.p. (Figure 2, panel C) ($F_{3,18} = 1.255$, $F_{8,144} = 1.609$, $F_{24,144} = 1.263$ for the effects of treatment, time and interaction, respectively). The effects of atomoxetine on DA_{EX} in PFC, nucleus accumbens and striatum were compared (Figure 3). The levels of DA_{EX} in PFC were increased by atomoxetine (3 mg/kg i.p.) to a peak level of 323 ± 17% of basal, but were not significantly altered in nucleus accumbens (3 mg/kg i.p.) or striatum (10 mg/kg i.p.). $Norepinephrine_{EX}$ levels were not quantified in the nucleus accumbens or striatum, but $5\text{-}HT_{EX}$ was not altered by atomoxetine treatment at doses of 3 mg/kg or less (data not shown).

Local perfusion of 0.34 μM atomoxetine through the dialysis probe into the PFC significantly increased NE_{EX} and DA_{EX} to the maximal effect of 175 ± 33 and 190 ± 15% of baseline concentrations, respectively, by 1 h (Figure 4). After 2.5 h the concentration of atomoxetine was increased to 1.03 μM, but the extracellular levels were not appreciably changed from the 0.34 μM concentration. A 1-way ANOVA with repeated measures showed significant effects for NE_{EX} and DA_{EX} ($F_{10,50} = 3.380$ for NE and $F_{10,50} = 6.927$ for DA). The concentration of $5\text{-}HT_{EX}$ was not increased by local perfusion with atomoxetine at either concentration.

Effect of Reboxetine and Methylphenidate on Monoamine Levels in Brain Regions Reboxetine (3 mg/kg i.p.) significantly increased PFC NE_{EX} and DA_{EX} to peak levels of 344 ± 73 and 341 ± 28% of baseline concentrations, respectively, and had a 4 h average percentage increase of 282 ± 18 and 268 ± 21%, respectively (Figure 5, panel A). (NE 2-way ANOVA: $F_{1,10} = 13.257$, $F_{8,80} = 6.492$, $F_{8,80} = 6.040$ for treatment, time and interaction, respectively; DA 2-way ANOVA: $F_{1,10} = 57.777$, $F_{8,80} = 15.947$, $F_{8,80} = 11.241$ for treatment, time and interaction, respectively). Reboxetine did not significantly increase $5\text{-}HT_{EX}$ ($F_{1,7} = 2.113$, $F_{8,56} = 0.845$, $F_{8,56} = 1.624$). Methylphenidate (3 mg/kg i.p.) significantly increased NE_{EX} and DA_{EX} to a peak concentration of 199 ± 16 and 253 ± 25% of PFC basal levels ($p < .05$) and a 4-h average of 145 ± 14 and 168 ± 17% of basal levels ($p < .05$), respectively (Figure 5, panel B). (1-way ANOVA for NE: $F_{8,40} = 21.714$, and for DA: $F_{8,40} = 28.045$).

Methylphenidate significantly increased DA_{EX} concentrations up to 253 ± 25, 209 ± 14 and 267 ± 37% of baseline in PFC, striatum and nucleus accumbens, respectively (Figure 6). The 4-h average increases in DA_{EX} were 168 ± 17, 138 ± 14 and 169 ± 16% of baseline concentrations, respectively. A 2-way ANOVA comparing the three different areas showed that although there was a significant increase in DA_{EX} there was no significant difference between the areas over the 4-h period. The F values were $F_{2,15} = 1.599$ for area comparison (not significant), $F_{8,120} = 75.082$ for effect over time (highly significant for all areas) and $F_{16,120} = 1.644$ for interaction between areas over time (not significant).

Effect of Atomoxetine on Fos Expression in Brain Regions Immunohistochemical localization of the neuronal activity marker Fos was determined 2 h after atomoxetine

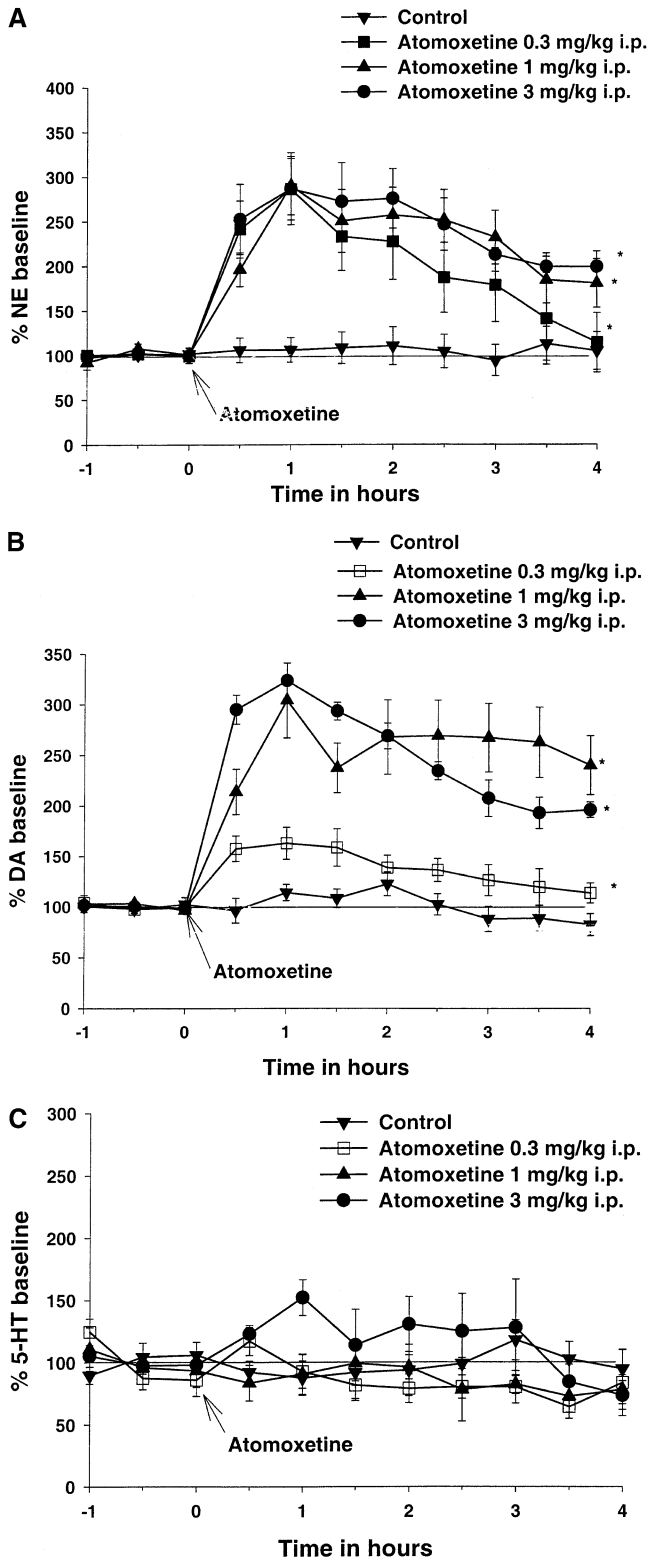

Figure 2. Time course of the effects of control (vehicle) or atomoxetine (0.3, 1, 3 mg/kg i.p.) administration on extracellular concentrations of norepinephrine (NE) (A), dopamine (DA) (B) and serotonin (5-HT) (C) in prefrontal cortex of freely moving rat. Values are the mean ± SEM of the % of pre-drug baseline determined at −1, −0.5 and 0 h. Administration of vehicle or atomoxetine at time 0 h is indicated by the arrow. Atomoxetine significantly increased extracellular NE and DA concentrations throughout the 4-h period ($^{*}p <$ .025, Duncan's post hoc test).

administration (3 mg/kg i.p.) in the same brain areas as in the microdialysis studies. Atomoxetine increased the number of cells expressing Fos-like immunoreactivity in PFC 3.7-fold, but did not significantly increase the number of Fos positive cells in striatum and nucleus accumbens (Table 4, Figure 7).

DISCUSSION

Atomoxetine inhibited binding of [^{3}H]-nisoxetine to human NE transporters with a K_i value of 5 nM and with 15- and 290-fold lower affinity for human 5-HT and DA transporters, respectively. This is consistent with previously reported selectivity of atomoxetine for rat NE uptake processes (Wong et al. 1982; Bolden-Watson and Richelson 1993; Gehlert et al. 1995). Desipramine and reboxetine also were potent and selective inhibitors of binding to human NE transporters, consistent with previous reports (Tatsumi et al. 1997; Wong et al. 2000). In contrast, the psychostimulant methylphenidate had higher affinity for the human DA transporter than the NE transporter. Atomoxetine had low affinity for a number of other radioligand binding sites, suggesting a high degree of selectivity for NE transporters.

Atomoxetine potently blocked in vivo depletion of NE in rats by DSP-4 in a dose-dependent manner, thus demonstrating in vivo blockade of NE transporters. Previous studies have shown DSP-4 depletes NE by a carrier dependent mechanism (Grzanna et al. 1989; Jonsson et al. 1981). Like atomoxetine, desipramine and reboxetine blocked depletion of NE by DSP-4, but fluoxetine and methylphenidate were not effective. Atomoxetine did not appreciably block p-CA–induced depletion of 5-HT, indicating that it does not significantly block 5-HT transporters in vivo at the doses evaluated.

The extracellular concentrations of NE in PFC were increased by atomoxetine, whereas 5-HT_{EX} was not significantly altered by atomoxetine up to 3 mg/kg i.p. in the brain regions examined. Atomoxetine increased DA_{EX} to about the same magnitude as NE_{EX} in the PFC. The higher doses of atomoxetine produced longer lasting increases in NE_{EX} and greater increases in DA_{EX} than the low dose. However, atomoxetine did not increase DA_{EX} in the DA and DA transporter-rich areas—nucleus accumbens and striatum (Soucy et al. 1997; Coulter et al. 1995). This report is in agreement with

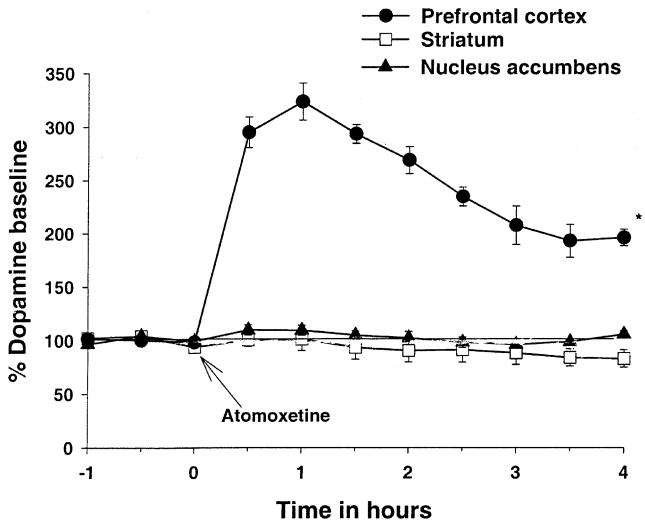

Figure 3. Time course of the effects of atomoxetine administration on extracellular dopamine levels in prefrontal cortex (PFC), striatum and nucleus accumbens of freely moving rat. Values are the mean ± SEM of the % of pre-drug baseline determined at −1, −0.5 and 0 h. Administration of vehicle or atomoxetine (3 mg/kg i.p. in PFC and nucleus accumbens and 10 mg/kg i.p. in striatum) at time 0 h is indicated by the arrow. Atomoxetine significantly increased extracellular norepinephrine and dopamine concentrations throughout the 4-h period only in the PFC (* $p < .05$, Duncan's post hoc test).

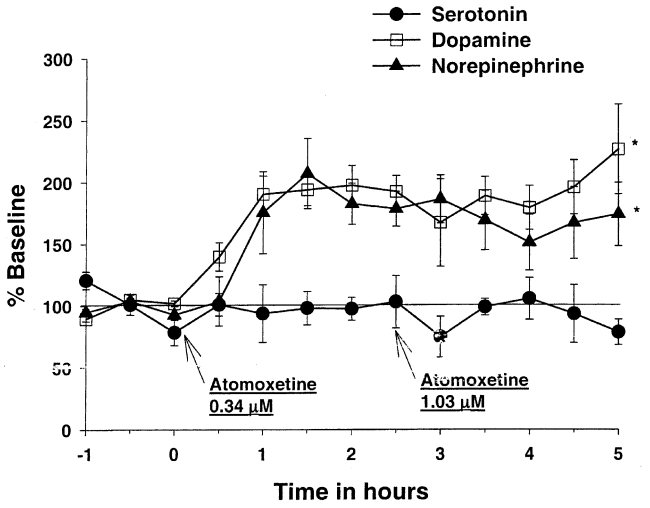

Figure 4. The effects of local perfusion of atomoxetine on extracellular monoamine levels in prefrontal cortex of freely moving rat. Values are the mean ± SEM of the % of pre-drug baseline determined at −1, −0.5 and 0 h. Atomoxetine (0.34 μM) was perfused through the dialysis probe at time 0 to 2.5 h and the concentration was increased to 1.03 μM from 2.5–5 h. Atomoxetine significantly increased extracellular norepinephrine and dopamine concentrations throughout the 5-h period (* $p < .05$, Duncan's post hoc test).

studies using reboxetine and desipramine that have shown selective NE uptake inhibitors increase DA_{EX} as well as NE_{EX} in PFC, but not in the nucleus accumbens (Tanda et al. 1994; Linnèr et al. 2001) or striatum (Carboni et al. 1990; Di Chiara et al. 1992). The transporters for NE are relatively abundant compared with DA transporters in the PFC (Gehlert et al. 1993; Soucy et al. 1997; Coulter et al. 1995; Sesack et al. 1998) and it has been determined that DA is taken up non-selectively by NE transporters in the PFC (Carboni et al. 1990; Di Chiara et al. 1992; Tanda et al. 1997; Yamamoto and Novotney 1998) as well as co-released (Devoto et al. 2001). The NE transporter has similar affinities for NE and DA (Raiteri et al. 1977) and presumably extracellularly-released DA may diffuse transsynaptically to NE transporters (Yamamoto and Novotney 1998). Consistent with this hypothesis, locally administered atomoxetine increased both NE_{EX} and DA_{EX} in the PFC, but not to the magnitude seen with systemic administration. Thus, the increase of the catecholamines is due, at least in part, to a local effect. The onset of increase in catecholamines after local infusion was rapid, suggesting that this is not due to diffusion of the drug to other brain areas.

However, other interactions may also be involved in the increase of DA induced by atomoxetine in PFC. The selective NE transporter inhibitors nisoxetine (Wong et al. 1975) and reboxetine (Wong et al. 2000), as well as α_2-adrenergic antagonists that enhance release of NE (Dennis et al. 1987; Thomas and Holman 1991), increase burst firing of ventral tegmental DA neurons which would result in increased DA release in their terminal fields (Linnèr et al. 2001; Shi et al. 2000; Grenhoff and Svensson 1993).

The immediate-early gene c-fos and its protein products have been increasingly utilized as markers for neuronal activation (Dragunow and Faull 1989; Morgan and Curran 1990; Robertson et al. 1994). In the present study, immunohistochemical localization of Fos protein allowed the quantitation of activated cells in specific forebrain nuclei following vehicle or atomoxetine administration. Atomoxetine significantly and robustly increased the number of Fos-positive cells in the PFC, but not in the nucleus accumbens or the striatum. These findings are in direct correlation with the pattern of increased concentrations of DA induced by atomoxetine in the same forebrain structures. Therefore, these studies suggest that elevations in DA_{EX} activate postsynaptic neurons and Fos expression in the PFC, but the involvement of NE cannot be excluded. In contrast, a study by Lin et al. (1996), found that administration of methylphenidate to cats induced Fos expression in the striatum. Thus, the lack of Fos induction in the nucleus accumbens and striatum may indicate a unique method of action for atomoxetine as compared with methylphenidate.

Reboxetine increased NE_{EX} (Sacchetti et al. 1999) and DA_{EX} in the PFC to similar levels and to about the same

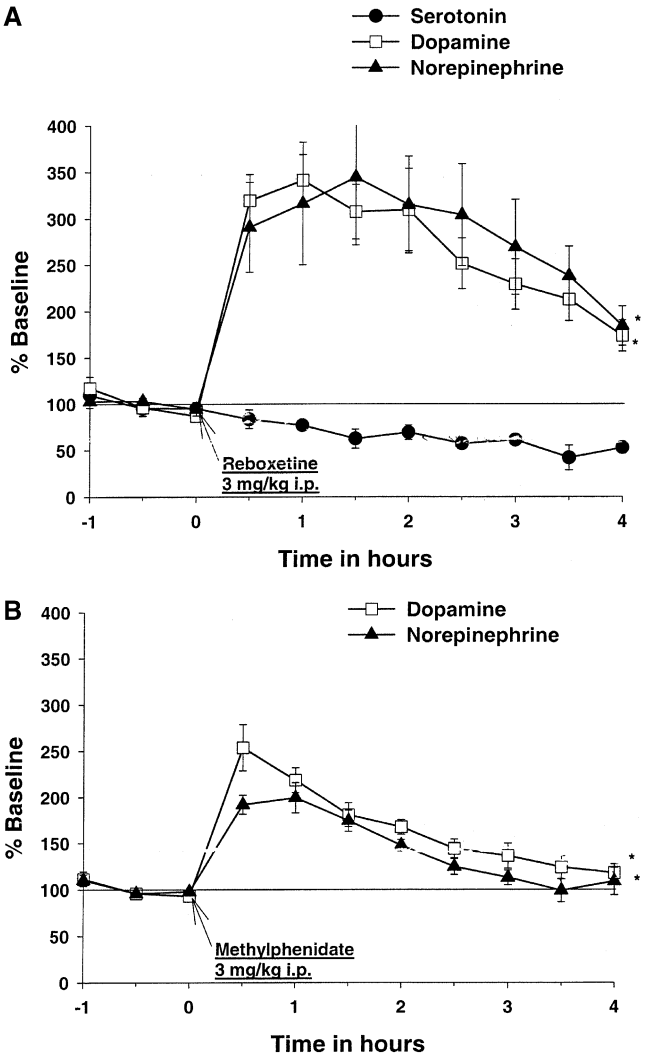

Figure 5. Time course of the effects of reboxetine (3 mg/kg i.p.) (A) and methylphenidate (3 mg/kg i.p.) administration (B) on extracellular monoamine levels in prefrontal cortex of freely moving rat. Values are the mean ± SEM of the % of pre-drug baseline determined at −1, −0.5 and 0 h. Administration of reboxetine or methylphenidate at time 0 h is indicated by the arrow. Reboxetine significantly increased extracellular norepinephrine (NE) and dopamine (DA) concentrations throughout the 4-h period ($^{*}\ p < .05$, Duncan's post hoc test) whereas methylphenidate significantly increased NE and DA for the first 2.5 h ($^{*}\ p < .05$, Duncan's post hoc test).

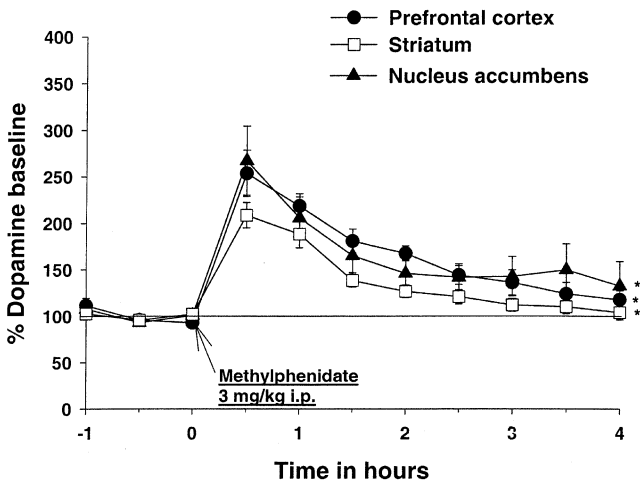

Figure 6. Time course of the effects of methylphenidate (3 mg/kg i.p.) administration on extracellular dopamine levels in prefrontal cortex, striatum, and nucleus accumbens of freely moving rat. Values are the mean± SEM of the % of pre-drug baseline determined at −1, −0.5 and 0 h. Administration of methylphenidate at time 0 h is indicated by the arrow. Methylphenidate significantly increased extracellular dopamine concentrations through the 2.5-h period in all brain regions ($^{*}\ p < .05$, Duncan's post hoc test).

magnitude as found with atomoxetine, consistent with previous reports (Linnèr et al. 2001). In the PFC, methylphenidate caused large increases in NE_{EX} and DA_{EX}, but the increases were of short duration in agreement with previous studies (During et al. 1992), and consistent with a short duration of action in humans. However, methylphenidate, unlike atomoxetine, also increased DA_{EX} in nucleus accumbens which may mediate the methylphenidate-induced increases in locomotor activity and may also be involved in the rewarding aspects of the drug (Kuczenski and Segal 1997, 1999). Furthermore, methylphenidate-induced increases in DA_{EX} in striatum may stimulate motor tracts in that area causing stereotyped behaviors and motor disturbances such as tics in humans. Similarly, imaging studies in humans have shown that methylphenidate increases extracellular levels of DA in the striatum (Volkow et al. 2001).

Atomoxetine is a development candidate for pharmacotherapy of ADHD and has been shown in early studies to be efficacious and well-tolerated for use in this disorder (Spencer et al. 1998, 2001; Michelson et al. 2001). We propose that the atomoxetine-induced increase of DA_{EX} and NE_{EX} in the PFC, and presumably other cortical areas, enhances catecholaminergic neurotransmission in the cortex and this may be a mechanism of action of atomoxetine in the pharmacotherapy

Table 4. Expression of Fos-like Immunoreactivity in the Prefrontal Cortex, Nucleus Accumbens and Striatum of Rat Following Vehicle or Atomoxetine (3 mg/kg i.p.) Administration

Treatment	Prefrontal Cortex	Nucleus Accumbens	Striatum
	Fos positive cells/mm^2		
Vehicle	80 ± 28	129 ± 33	118 ± 26
Atomoxetine	296 ± 26*	152 ± 44	102 ± 35

Rats were treated with vehicle or atomoxetine (3 mg/kg i.p.) for two h prior to determination of Fos expression as described in Methods.
*$p < .001$

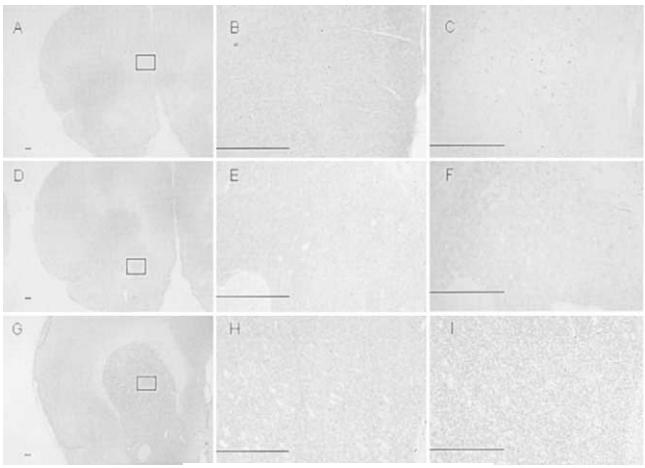

Figure 7. Photomicrographs showing localization of Fos expression following vehicle or atomoxetine (3 mg/kg i.p.) administration to rats in groups of five. Boxed areas in panels A, D, and G indicate the relative area selected for quantitation for the prefrontal cortex (panels A–C), nucleus accumbens (panels D–F) and striatum (panels G–I). Higher magnification of a representative section of vehicle-treated (panels B, E, H) or atomoxetine-treated animals (panels C, F, I) for each respective area are presented. Scale bar = 1 mm.

of ADHD. Catecholamines, particularly DA, are highly involved in ADHD and enhancement of DA and NE neurotransmission in the PFC by psychostimulants and NE uptake inhibitors may play a pivotal role in the efficacy of these drugs in ADHD (Spencer et al. 1995; Biederman and Spencer 1999; Arnsten et al. 1996). The observed increase in DA transporters in brains of ADHD patients (Dougherty et al. 1999; Krause et al. 2000; Dresel et al. 2000) presumably results in decreased DA neurotransmission which may be offset by drugs that enhance dopamine neurotransmission. However, DA transporters in ADHD patients have not been shown to be increased in cortical areas due to the difficulty of imaging the low levels of DA transporters in those regions. If DA transporters are increased in cortical areas, then this may be offset by blockade of NE transporters by atomoxetine.

Enhanced catecholaminergic neurotransmission in the PFC by atomoxetine may affect attentional processes possibly by activation of DA_1 receptors known to mediate working memory in the PFC (Sawaguchi and Goldman-Rakic 1994; Arnsten 1998). Activation of prefrontal cortical areas may also impact neuronal tracts projecting to subcortical areas involved in regulation of DA neurotransmission (Taber and Fibiger 1993; Taber et al. 1995). In support of the role for frontal cortex in ADHD-like behaviors, lesions of the frontal lobes cause a breakdown of goal directed activity, executive function, attention and produce hyperactivity (Benson and Stuss 1982; Petrides and Milner 1982). In imaging studies, the right frontal lobes including PFC (Hynd et al. 1990; Castellanos et al. 1996b) and the caudate nucleus of children with ADHD were smaller in volume than controls, possibly suggesting a neurodevelopmental lag in the maturation of the associated neuronal pathways and their connectivity (Castellanos et al. 1996b). Thus, increasing catecholaminergic neurotransmission in cortical areas may be involved in the efficacy of psychostimulants and atomoxetine in ADHD.

The use of selective inhibitors of NE transporters such as atomoxetine would be expected to have several advantages over the psychostimulants now commonly used for therapy of ADHD. Psychostimulants may induce dose-limiting and intolerable anxiety and dysphoric mood in some patients and do not satisfactorily alleviate symptoms of comorbid conditions such as depression and anxiety (Wilens and Spencer 2000) and may exacerbate those symptoms. Atomoxetine blocks NE uptake in brain and the antidepressant activities of NE transporter inhibitors such as desipramine have been clearly demonstrated (Delgado et al. 1993). Thus, atomoxetine may alleviate symptoms of comorbid depression and anxiety due to enhanced NE neurotransmission. In regard to adverse events compared with the psychostimulants, atomoxetine would be expected to have decreased motor effects such as induction of tics due to its lack of effect on DA_{EX} in the striatal motor areas. Furthermore, since atomoxetine does not increase DA_{EX} in the nucleus accumbens, a region associated with psychostimulation and rewarding behaviors, atomoxetine would not be expected to have drug abuse liability as found with the psychostimulants (Kuczenski and Segal 1997). In fact, atomoxetine did not substitute appreciably for methamphetamine in drug discrimination studies in monkeys (Tidey and Bergman 1998).

In conclusion, the selective inhibitor of NE transporters, atomoxetine, increases NE_{EX} and DA_{EX} in the PFC, but does not alter DA_{EX} in nucleus accumbens and striatum. We have proposed the efficacy of atomoxetine in ADHD is related to its enhancement of cortical NE and DA neurotransmission, particularly in PFC.

REFERENCES

American Psychiatric Association (2000): Diagnostic and Statistical manual of Mental Disorders. 4th ed. Washington, DC, American Psychiatric Association

Arnsten AF (1998): Catecholamine modulation of prefrontal cortical cognitive function. Trends Cogn Sci 2:436–447

Arnsten AF, Steere JC, Hunt RD (1996): The contribution of alpha-2 noradrenergic mechanisms of prefrontal cortical cognitive function: potential significance for attention deficit hyperactivity disorder. Arch Gen Psychiatry 53:448–455

Aston-Jones G, Chiang C, Alexinsky T (1991): Discharge of

noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. Prog Brain Res 88:501–520

Barkley RA (1977): A review of stimulant drug research with hyperactive children. J Child Psychol Psychiatry 18: 137–165

Barkley RA, Fischer M, Edelbrock CS, Smallish L (1990): The adolescent outcome of hyperactive children diagnosed by research criteria. I. An 8-year prospective follow-up study. J Am Acad Adolesc Psychiatry 29:546–557

Benson DF, Stuss DT (1982): Motor abilities after frontal leukotomy. Neurol 32:1353–1357

Berridge CW, Arnsten AF, Foote SL (1993): Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models. Psychol Med 23:557–564

Biederman J, Baldessarini RJ, Wright V, Knee D, Harmatz JS (1989): A double-blind placebo controlled study of desipramine in the treatment of ADD I. Efficacy. J Am Acad Child Adolesc Psychiatry 28:777–784

Biederman J, Newcorn J, Sprich S (1991): Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry 148:564–577

Biederman J, Spencer T (1999): Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. Biol Psychiatry 46:1234–1242

Bolden-Watson C, Richelson E (1993): Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. Life Sci 52:1023–1029

Carboni E, Tanda GL, Frau R, Di Chiara G (1990): Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. J Neurochem 55:1067–1070

Castellanos FX, Elia J, Kruesi MJ, Marsh WL, Gulotta CS, Potter WZ, Ritchie GF, Hamburger SD, Rapoport JL (1996a): Cerebrospinal fluid homovanillic acid predicts behavioral response to stimulants in 45 boys with attention deficit/hyperactivity disorder. Neuropsychopharmacol 14:125–137

Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Vaituzis AC, Dickstein DP, Sarfatti SE, Vauss YC, Snell JW, Lange N, Kaysen D, Krain AL, Ritchie GF, Rajapakse JC, Rapoport JL (1996b): Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry 53:607–616

Cheng YC, Prusoff WH (1973): Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes a 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108

Chouinard G, Annable L, Bardwejn J (1984): An early phase II clinical trial of tomoxetine (LY139603) in the treatment of newly admitted depressed patients. Psychopharmacol 83:126–128

Cookson J (1993): Side effects of antidepressants. Br J Psychiatry 163(suppl 20):20–24

Coulter CL, Happe HK, Bergman DA, Murrin LC (1995): Localization and quantification of the dopamine transporter: comparison of [3H]WIN 35,428 and [125I]RTI-55. Brain Res 690:217–224

Delgado PL, Miller HL, Salomon RM, Licinio J, Heninger GR, Gelenberg AJ, Charney DS (1993): Monoamines and the mechanism of antidepressant action: effects of catecholamine depletion on mood of patients treated with antidepressants. Psychopharmacol Bull 29:389–396

Dennis T, L'Heureux R, Carter C, Scatton B (1987): Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis) in the rat. J Pharmacol Exp Ther 241:642–649

Devoto P, Flore G, Gessa GL (2001): Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. Mol Psychiatry 6:657–664

Di Chiara G, Tanda GL, Frau R, Carboni E (1992): Heterologous monoamine reuptake: lack of transmitter specificity of neuron-specific carriers. Neurochem Int 20:231S–235S

Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ (1999): Dopamine transport density in patients with attention deficit hyperactivity disorder. Lancet 354:2132–2133

Downey KK, Stelson FW, Pomerleau OF, Giordani B (1997): Adult attention deficit hyperactivity disorder: psychological test profiles in a clinical population. J Nerv Ment Dis 185:32–38

Dragunow M, Faull R (1989): The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Methods 29:261–265

Dresel S, Krause J, Krause K-H, LaFougere C, Brinkbaumer K, Kung HF, Hahn K, Tatsch K (2000): Attention deficit hyperactivity disorder: binding of [^{99m}Tc]TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. Eur J Nucl Med 27:1518–1524

During MJ, Bean AJ, Roth RH (1992): Effect of CNS stimulants on the in vivo release of the colocalized transmitters, dopamine and neurotensin, from rat prefrontal cortex. Neurosci Lett 140:129–133

Elia J, Borcherding BG, Rapoport JL, Keysor CS (1991): Methylphenidate and dextroamphetamine treatments of hyperactivity: Are there true responders? Psychiatry Res 36:141–155

Fuller RW, Perry KW (1977): Lowering of epinephrine concentration in rat brain by 2,3-dichloro-alpha-methylbenzylamine, an inhibitor of norepinephrine N-methyltransferase. Biochem Pharmacol 26:2087–2090

Fuller RW, Perry KW (1989): Effects of buspirone and its metabolite, 1-(2-pyrimidinyl)piperazine, on brain monoamines and their metabolites in rats. J Pharmacol Exp Ther 248:50–56

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding Y-S (1996): Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 58: 231–239

Gehlert DR, Gackenheimer SL, Robertson DW (1993): Localization of rat brain binding sites for [^{3}H]-tomoxetine, an enantiomerically pure ligand for norepinephrine reuptake sites. Neurosci Lett 157:203–206

Gehlert DR, Schober DA, Hemrick-Luecke SK, Krushinski J, Howbert JJ, Robertson DW, Fuller RW, Wong DT (1995): Novel halogenated analogs of tomoxetine that are potent and selective inhibitors of norepinephrine uptake in brain. Neurochem Int 26:47–52

Gittelman R (1980): Childhood disorders. In Klein D, Quitkin F, Rifkin A, Gittleman R (eds), Drug Treatment of Adult and Childhood Disorders. Baltimore, Williams and Wilkins, pp 576–756

Gittelman R, Mannuzza S, Shenker R, Bonagura N (1985): Hyperactive boys almost grown up. I. Psychiatric status. Arch Gen Psychiatry 42:937–947

Greenhill L (1995): Attention-deficit/hyperactivity disorder: the stimulants. Child Adolesc Psychiatric Clin North Am 4(1):123–168

Grenhoff J, Svensson TH (1993): Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. Eur J Pharmacol 233:79–84

Grzanna R, Berger U, Fritschy JM, Geffard M (1989): Acute action of DSP-4 on central norepinephrine axons: biochemical and immunohistochemical evidence for differential effects. J Histochem Cytochem 37:1435–1442

Holman RB (1994): Biological effects of central nervous system stimulants. Addiction 89:1435–1441

Hynd GW, Semrud-Clikeman M, Lorys AR, Novey ES, Eliopulos D (1990): Brain morphology in developmental dyslexia and attention deficit disorder/hyperactivity. Arch Neurol 47:919–926

Jonsson G, Hallman H, Ponzio F, Ross S (1981): DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)–a useful denervation tool for central and peripheral noradrenaline neurons. Eur J Pharmacol 72:173–188

Krause K-H, Dresel SH, Krause J, Kung HF, Tatsch K (2000): Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. Neurosci Lett 285: 107–110

Kuczenski R, Segal DS (1997): Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem 68:2032–2037

Kuczenski R, Segal DS (1999): Dynamic changes in sensitivity occur during the acute response to cocaine and methylphenidate. Psychopharmacol 147:96–103

Leonard HL, Meyer MC, Swedo SE, Richter D, Hamburger SD, Allen AJ, Rapoport JL, Tucker E (1995): Electrocardiographic changes during desipramine and clomipramine treatment in children and adolescents. J Am Acad Child Adolesc Psychiatry 34:1460–1468

Li XM, Perry KW, Wong DT, Bymaster FP (1998): Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. Psychopharmacol 136:153–161

Lin JS, Hou Y, Jouvet M (1996): Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-fos immunocytochemistry in the cat. Proc Natl Acad Sci USA 93: 14128–14133

Linnèr L, Endersz H, Öhman D, Bengtsson F, Schalling M, Svensson T (2001): Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. J Pharmacol Exp Ther 297:540–546

Mannuzza S, Klein RG, Bessler A, Malloy P, LaPadula M (1993): Adult outcome of hyperactive boys. Educational achievement, occupational rank, and psychiatric status. Arch Gen Psychiatry 50:565–576

Michelson D, Faries D, Wernicke J, Kelsey D, Kendrick K, Sallee FR, Spencer T (2001): Atomoxetine in the treatment of children and adolescents with Attention-Deficit/Hyperactivity Disorder: a randomized, placebo-controlled, dose-response study. Pediatrics 108:e83

Morgan JI, Curran T (1990): Inducible proto-oncogenes of the nervous system: their contribution to transcription factors and neuroplasticity. Prog Brain Res 86:287–294

Offord DR, Boyle MH, Szatmari P, Rae-Grant NI, Links PS, Cadman DT, Byles JA, Crawford JW, Blum HM, Byrne C (1987): Ontario child health study. II. Six month prevalence of disorder and rates of service utilization. Arch Gen Psychiatry 44:832–836

Paxinos G, Watson C (1986): The Rat Brain in Stereotaxic Coordinates, 2nd ed. San Diego, Academic Press

Petrides M, Milner B (1982): Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man. Neuropsychologia 20:249–262

Pliszka SR, McCracken JT, Maas JW (1996): Catecholamines in attention-deficit hyperactivity disorder: current perspectives. J Am Acad Child Adolesc Psychiatry 35:264–272

Raiteri M, Del Carmine R, Bertollini A, Levi G (1977): Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine and 5-hydroxytryptamine. Eur J Pharmacol 41:133–143

Robertson GS, Matsumara H, Fibiger HC (1994): Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 271:1058–1066

Sacchetti G, Bernini M, Bianchetti A, Parini S, Invernizzi RW, Saminin R (1999): Studies on the acute and chronic effects of reboxetine on extracellular noradrenaline and other monoamines in the rat brain. Br J Pharmacol 128: 1332–1338

Sawaguchi T, Goldman-Rakic PS (1994): The role of D1-dopamine receptor in working memory: Local injections of dopamine antagonists into the prefrontal cortex of Rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 71:515–528

Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998): Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J Neurosci 18:2697–2708

Segal M, Bloom FE (1976): The action of norepinephrine in the rat hippocampus. IV. The effects of locus coeruleus stimulation on evoked hippocampal unit activity. Brain Res 107:513–525

Shi WX, Pun CL, Zhang XX, Jones MD, Bunney BS (2000): Dual effects of D-amphetamine on dopamine neurons mediated by dopamine and nondopamine receptors. J Neurosci 20:3504–3511

Soucy JP, Mrini A, Lafaille F, Doucet G, Descarries L (1997): Comparative evaluation of [3H]WIN 35428 and [3H]GBR 12935 as markers of dopamine innervation density in brain. Synapse 25:163–175

Spencer T, Biederman J, Heiligenstein J, Wilens T, Faries D, Prince J, Faraone SV, Rea J, Witcher J, Zervas S (2001):

An open-label, dose-ranging study of atomoxetine in children with attention deficit hyperactivity disorder. J Child Adolesc Psychopharmacol 11:251–265

Spencer T, Biederman J, Wilens T, Prince J, Hatch M, Jones J, Harding M, Faraone SV, Seidman L (1998): Effectiveness and tolerability of tomoxetine in adults with attention deficit hyperactivity disorder. Am J Psychiatry 155: 693–695

Spencer T, Biederman J, Wilens T, Harding M, O'Donnell D, Griffin S (1996): Pharmacotherapy of attention-deficit hyperactivity disorder across the life cycle. J Am Acad Child Adolesc Psychiatry 35:409–432

Spencer T, Wilens T, Biederman J, Faraone SV, Ablon S, Lapey K (1995): A double-blind, crossover comparison of methylphenidate and placebo in adults with childhood-onset attention deficit hyperactivity disorder. Arch Gen Psychiatry 52:434–443

Szatmari P (1992): The epidemiology of attention-deficit hyperactivity disorder. Child Adolesc Psychiatry Clin North Am 1:361–371

Taber MT, Das S, Fibiger HC (1995): Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. J Neurochem 65:1407–1410

Taber MT, Fibiger HC (1993): Electrical stimulation of the medial prefrontal cortex increases dopamine release in the striatum. Neuropsychopharmacol 9:271–275

Tanda G, Carboni E, Frau R, Di Chiara G (1994): Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? Psychopharmacol 115:285–288

Tanda G, Pontieri FE, Frau R, Di Chiara G (1997): Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. Eur J Neurosci 9:2077–2085

Tatsumi M, Groshan K, Blakely RD, Richelson E (1997): Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 340:249–258

Thomas DN, Holman RB (1991): A microdialysis study of the regulation of endogenous noradrenaline release in the rat hippocampus. J Neurochem 56:1741–1746

Tidey JW, Bergman J (1998): Drug discrimination in methamphetamine-trained monkeys: agonist and antagonist effects of dopaminergic drugs. J Pharmacol Exp Ther 285:1163–1174

Volkow ND, Wang, G, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding Y-S, Gatley SJ, Gifford A, Franceschi D (2001): Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 21, RC121:1–5

Walsh BT, Giardina EG, Sloan RP, Greenhill L, Goldfein J (1994): Effects of desipramine on autonomic control of the heart. J Am Acad Child Adolesc Psychiatry 33:191–197

Wilens TE, Biederman J, Prince J, Spencer TJ, Faraone SV, Warburton R, Schleifer D, Harding M, Linehan C, Geller D (1996): Six-week, double-blind, placebo-controlled study of desipramine for adult attention deficit hyperactivity disorder. Am J Psychiatry 153:1147–1153

Wilens TE, Biederman J, Spencer TJ (1998): Pharmacotherapy of attention deficit hyperactivity disorder in adults. CNS Drugs 9:347–356

Wilens TE, Spencer TJ (2000): The stimulants revisited. Child Adolesc Clin North Am 9:573–603

Wong DT, Threlkeld PG, Best KL, Bymaster FP (1982): A new inhibitor of norepinephrine uptake devoid of affinity for receptors in rat brain. J Pharmacol Exp Ther 222: 61–65

Wong DT, Bymaster FP, Engleman EE (1995): Prozac (fluoxetine, Lilly 110140), the first selective uptake inhibitor and an antidepressant drug: twenty years since its first publication. Life Sci 57:411–441

Wong DT, Horng JS, Bymaster FP (1975): dl-N-Methyl-3-(o-methoxyphenoxy)-3-phenylpropylamine hydrochloride, Lilly 94939, a potent inhibitor for uptake of norepinephrine into rat brain synaptosomes and heart. Life Sci 17: 755–760

Wong EHF, Sonders MS, Amara SG, Tinholt PM, Piercey MFP, Hoffman WP, Hyslop DK, Franklin S, Porsolt RD, Bonsignori A, Carfagna N, McArthur RA (2000): Reboxetine: A pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. Biol Psychiatry 47:818–829

Yamamoto BK, Novotney S (1998): Regulation of extracellular dopamine by the norepinephrine transporter. J Neurochem 71:274–280

Zametkin AJ, Rapoport JL (1987): Noradrenergic hypothesis of attention deficit disorder with hyperactivity: a critical review. In Meltzer HY (ed), Psychopharmacology: the Third Generation of Progress. New York, Raven Press, pp 837–847

Zhang W, Perry KW, Wong DT, Potts B, Tollefson GD, Bymaster FP (2000): Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. Neuropsychopharmacology 23:250–262