Effects of Norepinephrine and Serotonin Transporter Inhibitors on Hyperactivity Induced by Neonatal 6-Hydroxydopamine Lesioning in Rats

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ABSTRACT

Consistent with their clinical effects in attention deficit-hyperactivity disorder (ADHD), the stimulants methylphenidate and amphetamine reduce motor hyperactivity in juvenile male rats with neonatal 6-hydroxydopamine (6-OHDA) lesions of the forebrain dopamine (DA) system. Since stimulants act on several aminergic neurotransmission systems, we investigated underlying mechanisms involved by comparing behavioral actions of d-methylphenidate, selective inhibitors of the neuronal transport of DA [GBR-12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride), amfonelic acid], serotonin [5-hydroxytryptamine (5-HT), citalopram, fluvoxamine], and norepinephrine (NE; desipramine, nisoxetine) in 6-OHDA lesioned rats. Selective dopamine lesions were made using 6-OHDA (100 μg, intracisternal) on postnatal day (PD) 5 after desipramine pretreatment (25 mg/kg, s.c.) to protect noradrenergic neurons. Rats were given test agents or vehicle, intraperitoneally, before recording motor activity for 90 min at PD 25 in a novel environment. d-Methylphenidate stimulated motor activity in sham controls and antagonized hyperactivity in lesioned rats. Selective DA transport inhibitors GBR-12909 and amfonelic acid greatly stimulated motor activity in sham control subjects, too, but did not antagonize hyperactivity in lesioned rats. In contrast, all selective 5-HT and NE transporter antagonists tested greatly reduced motor hyperactivity in 6-OHDA lesioned rats but did not alter motor activity in sham controls. The findings indicate that behavioral effects of stimulants in young rats with neonatal 6-OHDA lesions may be mediated by release of NE or 5-HT and support interest in using drugs that increase activity of norepinephrine or serotonin to treat ADHD.

Attention deficit-hyperactivity disorder (ADHD) is a complex developmental behavioral and cognitive disorder that affects approximately 3 to 5% of school-aged boys (Barkley, 1997). For more than 50 years, the most widely prescribed pharmacological treatment for patients with ADHD has been psychostimulant drugs including methylphenidate and amphetamine. Stimulants facilitate the release and synaptic availability of dopamine (DA) (West et al., 1995; Pliszka et al., 1996; Barkley, 1997; Solanto, 1998; Fleckenstein et al., 2000). Stimulants also facilitate the release of other monoamine neurotransmitters, notably norepinephrine (NE) and serotonin (5-HT), which may also contribute to therapeutic mechanisms including improvements in response-delay, working memory, and attention, as well as modulation of motor activity (Pliszka et al., 1996; Jacobs and Fornal, 1997; Solanto, 1998; Biederman and Spencer, 1999). This view is strongly supported by the reported clinical usefulness of selective inhibitors of NE transport, including desipramine, nortriptyline, and atomoxetine in ADHD (Biederman and Spencer, 1999; Popper, 2000). Furthermore, venlafaxine and other even more selective serotonin reuptake inhibitors (SRIs) may also yield limited benefits in ADHD (Biederman and Spencer, 1999; Popper, 2000). Salient features of ADHD can be simulated in juvenile rats with neonatal 6-hydroxydopamine (6-OHDA) lesions that selectively destroy DA projections to forebrain when combined with desipramine pretreatment to prevent loss of NE (Shaywitz et al., 1978; Zhang et al., 2001; Davids et al., 2002). The severe depletion of DA yields increased motor activity and learning deficits (Shaywitz et al., 1978; Takasuna and Iwasaki, 1996). Hyperactivity resulting from neonatal 6-OHDA lesions is dose dependently reversed by stimulants

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ABBREVIATIONS: ADHD, attention deficit-hyperactivity disorder; β-CIT, 2-β-carbomethoxy-3-β-[4’-iodophenyl]tropane; CPu, caudate-putamen; DA, dopamine; DAT, dopamine transporter; 5-HT, 5-hydroxytryptamine (serotonin); NAc, nucleus accumbens septi; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine; PD, postnatal day; SRI, serotonin reuptake inhibitor.
including \textit{d}-amphetamine and both \textit{dl}- and \textit{d}-methylphenidate (Shaywitz et al., 1978; Zhang et al., 2001; Davids et al., 2002). Learning deficits exhibited by 6-OHDA lesioned rats also respond favorably to stimulants (Shaywitz et al., 1978). These actions parallel the effects of stimulants in patients with ADHD (Kostrzewska et al., 1994; Biederman and Spencer, 1999).

The hypothesis that stimulants act simply by activating forebrain dopaminergic systems is inconsistent with the typically severe depletion of cerebral DA in such lesioned rats (Joyce et al., 1996; Schwarting and Huston, 1996). On the other hand, support for contributions of NE to 5-HT to the actions of stimulants in 6-OHDA lesioned rats includes the following: 1) NE neurons are typically preserved in the lesioning model (Luthman et al., 1990; Ordway, 1995); 2) 5-HT neurons may actually overgrow in response to early removal of DA neurons (Towle et al., 1989; Kostrzewska et al., 1998); and 3) selective inhibitors of NE and 5-HT reuptake can exert inhibitory effects on behavioral activity in rats under some conditions (O’Connor and Leonard, 1988; Geyer, 1996). Remarkably, however, direct testing of the behavioral effects of selective NE or 5-HT inhibitors in 6-OHDA lesioned hyperactive rats and comparisons with selective inhibitors of DA reuptake remain to be carried out.

Accordingly, we tested the hypothesis that selective inhibitors of the inactivation of NE or 5-HT by neuronal transport would inhibit motor hyperactivity in juvenile male rats with selective neonatal lesions of the forebrain DA system, but that selective inhibitors of DA transport would not. We employed pairs of chemically dissimilar agents, including selective inhibitors of the transport of: 1) NE (desipramine, nisoxetine); 2) 5-HT (citalopram, fluvoxamine); and 3) DA \textit{amfonelic acid, GBR-12909 (1-[2-bis[4-fluorophenyl]methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride) for comparison with \textit{d}-methylphenidate.

### Experimental Procedures

#### Materials and Animal Subjects

\textit{Amfonelic acid, d-amphetamine sulfate, desipramine hydrochloride, GBR-12909 dihydrochloride, nisoxetine hydrochloride, and 6-hydroxydopamine hydrobromide} were obtained from Sigma/RBI (Natick, MA). Donated drugs included \textit{d}-methylphenidate (Celgene, Warren, NJ), \textit{H} \textit{citalopram hydrobromide} (Lundbeck, Copenhagen, Denmark), and fluvoxamine maleate (Duphar, Amsterdam, Netherlands). We recently tested these drugs for potency and selectivity at monoamine transporters, using homogenates of rat forebrain and selective radioligands (Kula et al., 1999), as is summarized in Table 1. \textit{d}-Methylphenidate and \textit{d}-amphetamine showed >10-fold selectivity for DA and NE over 5-HT transporters. \textit{Amfonelic acid and GBR-12909 were highly selective for DA transporters (DATs) (≥350-fold compared with NE and 5-HT transporters). Desipramine and nisoxetine were highly selective for the NE transporter (≥340-fold compared with DA and 5-HT transporters), and citalopram and fluvoxamine were highly selective for the 5-HT transporter (≥1800-fold compared with DA and NE transporters; Table 1).

#### Behavioral Testing of Responses to Selective Transport Inhibitors

Motor activity was recorded with an infrared photoemitter activity-monitoring system (San Diego Instruments, San Diego, CA). Tests were conducted in a novel environment (45.3 cm × 21.8 cm × 20.3 cm transparent plastic cages within 4 × 8 horizontal infrared beams at 3.3 cm elevation), between 10:00 AM and 4:00 PM in the absence of food and water (Zhang et al., 2001; Davids et al., 2002). Locomotor activity was scored as breaking of consecutive photobeams. Data were collected at 5-min intervals for 90 min.

Experiments included a total of 54 sham and 80 of the 6-OHDA lesioned rats. All subjects underwent a 90-min pretest session on PD 22 to verify the presence of hyperactivity, and effects of test drugs all were examined on PD 25. Test agents were given at PD 25 using a random stratified regimen to balance levels of locomotor activity in the pretest test session rated as modest (90-min cumulative score 900-1800), moderate (1801-2700 counts), or high (>2700 counts). Test agents included \textit{d}-methylphenidate, GBR-12909, desipramine, nisoxetine, citalopram, and fluvoxamine (all 10 mg/kg), and am-

### Table 1

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Dopamine [3H]CIT</th>
<th>Norepinephrine [3H]Nisoxetine</th>
<th>Serotonin [3H]Paroxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{d}-Methylphenidate</td>
<td>125 ± 10</td>
<td>126 ± 7.0</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>\textit{d}-Amphetamine</td>
<td>1,000 ± 150</td>
<td>1,000 ± 150</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>GBR-12909</td>
<td>0.15 ± 0.05</td>
<td>&gt;10,000</td>
<td>52.8 ± 4.4</td>
</tr>
<tr>
<td>Amfonelic acid</td>
<td>18.7 ± 1.3</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Desipramine</td>
<td>&gt;10,000</td>
<td>0.06 ± 0.04</td>
<td>228 ± 20</td>
</tr>
<tr>
<td>Nisoxetine</td>
<td>505 ± 50</td>
<td>0.46 ± 0.20</td>
<td>158 ± 29</td>
</tr>
<tr>
<td>Citalopram</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>&gt;10,000</td>
<td>5,000 ± 600</td>
<td>2.77 ± 0.17</td>
</tr>
</tbody>
</table>

Data adapted from Kula et al., 1999; reprinted with permission from Elsevier Science.
fonelic acid (3 mg/kg), or vehicle, given i.p. (5 ml/kg) immediately before behavioral testing. Drug doses chosen were previously found to be behaviorally active, with respect to d-methylphenidate (Davids et al., 2002), amfonelic acid (Mueller, 1993), GBR-12909 (Rothman and Glowa, 1995), desipramine (O’Connor and Leonard, 1988), nisoxetine (West et al., 1995), and citalopram and fluvoxamine (Sanchez and Meier, 1997) in intact rats or animal models of antidepressant, anxiolytic, or antiaggressive activity. All drugs were dissolved in water, except for amfonelic acid, which was dissolved in aqueous sodium hydroxide with pH adjusted to 8.0 with diluted HCI

Verification of Neonatal 6-Hydroxydopamine Lesioning.

Lesions were verified by autoradiographic analysis of DATs as an index for DA terminals in rat forebrain using the selective radioligand [3H]β-CIT (Kula et al., 1999). Rats were sacrificed by decapitation at 72 h (PD 28) after behavioral testing on PD 25, and brains were quickly removed and frozen. Coronal sections (10 μm) of brain tissue were prepared in a cryostat at −17°C, thaw-mounted on gelatin-coated microscopic slides, and stored at −80°C until autoradiographic analysis. For this analysis, brain sections were preincubated for 60 min at room temperature in 50 mM Tris-citrate buffer (pH 7.4) containing 120 mM NaCl and 4 mM MgCl2. Incubation continued for another 60 min in fresh buffer containing 2 nM [3H]β-CIT. Specific binding was defined with excess GBR-12909 (1 μM). After incubation, slides were washed twice in ice-cold buffer for 5 min, rinsed in cold deionized water, and air-dried. Slides were exposed to [3H]-sensitive Hyperfilm radiographic film at 4°C for 14 days with [3H]-standards and developed using standard photographic procedures. Density of radioligand binding to DATs was quantified with a computerized MCID-M4 image analyzer (Imaging Research, Inc.). Density of radioligand binding to DATs was quantified in lateral and medial caudate-putamen (CPu) expressed as femtomoles per milligram of tissue. Radioligand density was quantified in lateral and medial caudate-putamen (CPu) and nucleus accumbens septi (NAC), all as detailed previously (Tarazi et al., 2001).

Data Analysis. Lesion effects on DAT binding were analyzed by two-way analysis of variance for overall changes across treatments and brain regions, followed by post hoc Dunnett’s t tests for planned comparisons. Data are presented as means ± S.E.M. Since motor activity scores involved repeated measurements over 90 min, behavioral data were analyzed using a population-averaged panel-data model based on random-effects general estimating equations with Stata software for the Macintosh microcomputer (Stata Corp., College Station, TX) (Li and Zeger, 1986). Differences between treatment groups are considered statistically significant at p ≤ 0.05 in two-tailed tests.

Results

Effects of Lesioning on Dopamine Terminals. Neonatal 6-OHDA lesioning yielded major reductions in DAT binding in CPu as well as in NAc when tested at PD 28, following pharmacological assessments (Table 2). Average losses of specific DAT binding were 78% in both lateral and medial CPus and 70% in NAc in lesioned animals compared with sham controls.

Lesion-Induced Hyperactivity. Neonatal 6-OHDA lesioning resulted in a marked overall increase in locomotor activity over 90-min testing sessions in a novel environment at PD 25 (p < 0.01 versus sham controls; Fig. 1). Motor activity within the initial 10 min was similar in lesioned (n = 12) and sham control subjects (n = 8), but in contrast to the controls in which locomotion declined rapidly to a stable level within 30 min, activity in lesioned rats failed to decline within the 90-min session. Cumulative locomotor scores for the entire session in lesioned versus sham subjects averaged 3109 ± 876 versus 285 ± 82 counts (an 11-fold difference; p < 0.01).

Behavioral Effects of Test Agents. Motor activity was markedly increased by d-methylphenidate (10 mg/kg, i.p.) in sham control subjects (n = 6, p < 0.001; Fig. 1). At the same dose, hyperactivity in lesioned rats was significantly antagonized by d-methylphenidate (n = 10, p < 0.01; Fig. 1).

The selective DAT inhibitors amfonelic acid (3 mg/kg, i.p.; n = 6) and GBR-12909 (10 mg/kg, i.p.; n = 6) markedly increased locomotor activity in sham control rats (both p < 0.001; Fig. 2A). However, at the same doses, neither drug significantly altered motor activity in 6-OHDA-lesioned rats (GBR-12909, n = 10, p = 0.69; amfonelic acid, n = 9, p = 0.28) (Fig. 2B).

The SRIs citalopram (n = 7, p = 0.88) and fluvoxamine (n = 8, p = 0.49), both tested at 10 mg/kg, i.p., had no effect on locomotor activity in sham controls (Fig. 3A), but at the

**Table 2**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Density of Tissue DAT Labeling</th>
<th>Sham Controls</th>
<th>6-OHDA Lesioned</th>
<th>% Loss</th>
<th>fnmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral caudate-putamen</td>
<td>204.5 ± 5.0</td>
<td>44.7 ± 2.9 a</td>
<td>78.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial caudate-putamen</td>
<td>180.2 ± 5.2</td>
<td>40.4 ± 4.0 a</td>
<td>77.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens septi</td>
<td>126.3 ± 6.3</td>
<td>38.2 ± 3.3 a</td>
<td>69.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.001 vs. corresponding sham lesioned controls.

![Fig. 1. Effects of neonatal 6-OHDA lesioning on mean ± S.E.M. locomotor activity counts per 5 min versus time during testing at PD 25 in sham controls (open circles; n = 8) and neonatally 6-OHDA-lesioned male rats (filled circles; n = 12) given vehicle i.p. immediately before testing. Locomotion increased markedly in lesioned rats (p < 0.01). Further demonstrated effects of d-methylphenidate, 10 mg/kg, i.p., on sham (open triangles; n = 6) and lesioned rats (filled triangles; n = 10) are shown. Locomotion in sham rats increased markedly (p < 0.001) and decreased significantly in lesioned rats (p < 0.01) after treatment with d-methylphenidate.](image-url)
same dose, both SRIs markedly decreased locomotor activity in 6-OHDA lesioned rats (citalopram, \(n = 9\), \(p < 0.01\); fluvoxamine, \(n = 11\), \(p < 0.05\); Fig. 3B). These decreases with citalopram and fluvoxamine, respectively, were by 80.9\% ± 4.3% and 69.9\% ± 10.3%.

In addition, the selective NE transport inhibitors desipramine (\(n = 7\), \(p = 0.69\)) and nisoxetine (\(n = 7\), \(p = 0.82\); both given at 10 mg/kg, i.p.) did not affect locomotor activity in sham rats (Fig. 4A), but both drugs greatly decreased locomotor activity at the same dose in 6-OHDA lesioned rats (desipramine, \(n = 10\), \(p < 0.05\); nisoxetine, \(n = 9\), \(p < 0.01\); Fig. 4B). Desipramine and nisoxetine, respectively, decreased motor hyperactivity in the lesioned rats by 79.3\% ± 11.1% and 83.8\% ± 4.0%.

A summary comparison of results for overall changes in locomotor activity in 6-OHDA lesioned rats across 90-min testing sessions with all test agents is provided in Fig. 5.

**Discussion**

In accord with previous studies (Shaywitz et al., 1978; Zhang et al., 2001; Davids et al., 2002), neonatal 6-OHDA lesioning of developing DA projections in rat forebrain resulted in robust hyperactivity when lesioned male rats were tested in a novel environment at a later developmental stage, PD 25 (Fig. 1). Autoradiographic analysis of the binding of a potent and selective radioligand to DAT sites in forebrain indicated major losses of DA innervation in the DA-rich CPu and NAc of lesioned subjects compared with sham controls (Table 2). In line with previous studies, 3-methylphenidate increased locomotor activity in sham control subjects (Patrick et al., 1987; Davids et al., 2002) and antagonized hyperactivity in 6-OHDA lesioned rats (Davids et al., 2002) (Fig. 1).

**Behavioral Effects of Inhibitors of Dopamine Transport**. Selective inhibitors of DA reuptake, amfonelic acid and GBR-12909, both greatly increased locomotor activity in
sham control rats (Fig. 2), as expected from previous studies (Mueller, 1993; Rothman and Glowa, 1995). Behavioral effects of stimulants are believed to be mediated, at least in part, by increased effects of DA, particularly in the behaviorally critical mixed limbic-motor region, NAc (Solanto, 1998). Infusions of DA or amphetamine within NAc can increase locomotion (Staton and Solomon, 1984), whereas destruction of DA terminals in NAc prevented motor hyperactivity in response to d-amphetamine (Kelly and Iversen, 1976). Stimulants enhance the extracellular, and presumed synaptic, concentrations of DA in rats in correlation with their behavioral effects (Kuczenski and Segal, 1997, 2001), as well as in brain tissue of human subjects diagnosed with ADHD and visualized with positron-emission tomography (Volkow et al., 2001).

A particularly noteworthy finding is that the DAT-selective agents amfonelic acid and GBR-12909 (Table 1) did not diminish hyperactivity in 6-OHDA lesioned rats (Fig. 2), in contrast to the actions of the less amine-selective stimulants methylphenidate and amphetamine (Shaywitz et al., 1978; Zhang et al., 2001). Indeed, amfonelic acid tended to increase the post lesioning motor activity, although nonsignificantly (Figs. 2B and 5), perhaps reflecting an effect of incompletely lesioned forebrain DA terminals. Despite major losses of DA neurons and stores, in vivo studies using microdialysis and voltammetry indicate that some extracellular DA can still be detected after 6-OHDA lesioning (Joyce et al., 1996; Schwartz and Huston, 1996). Nonetheless, the lack of hyperactivity-reducing effects of the DAT-selective stimulants probably reflects removal of DA projections to NAc and other forebrain regions in the neonatally 6-OHDA lesioned rats to limit the actions of these DA-selective agents (Table 2). Moreover, the findings support our hypothesis that the hyperactivity-reducing effects of the less DAT-selective stimulants, methylphenidate and amphetamine, in this experimental model of ADHD are not mediated by release of DA. The findings thus encourage further consideration of the relative contributions of NE and 5-HT to the antihyperactivity effects of stimulants in the lesioning model.

Behavioral Effects of Inhibitors of Serotonin Transport. The selective SRIs citalopram and fluvoxamine (Table 1) did not affect motor activity in sham control rats, but both drugs strongly antagonized motor hyperactivity induced by neonatal 6-OHDA lesions (Figs. 3 and 5). These results are in accord with the reported ability of the 5-HT releasing agent fenfluramine and mixed 5-HT receptor agonist quipazine to reduce motor hyperactivity in 6-OHDA-treated rats (Heffner and Seiden, 1982). Furthermore, antihyperactivity effects of psychostimulants in 6-OHDA lesioned rats can be antagonized by the 5-HT antagonist methysergide (Heffner and Seiden, 1982). These results suggest that the locomotor-reducing effect of methylphenidate and amphetamine in 6-OHDA lesioned rats may reflect increased serotonin neurotransmission.

Neonatal destruction of nigrostriatal DA neurons by intracerebral administration of 6-OHDA leads to striking changes in the rat 5-HT system. Previous studies have demonstrated apparent 5-HT hyperinnervation of the striatum, with increased 5-HT content and 5-HT uptake as well as increased expression of 5-HT transporters and various 5-HT receptor subtypes, and increased electrophysiological responsiveness to 5-HT agonists (Towle et al., 1989; Descarrries et al., 1992; Kostrzewa et al., 1998). These changes may contribute to the antihyperactivity effects of selective SRIs found in this study (Figs. 3 and 5). However, serotoninergic systems have complex effects on behavioral arousal and motor responses. 5-HT can inhibit motor output in intact animals, often in reciprocity with catecholaminergic systems, including activation induced by stimulants (Gerson and Baldessarini, 1980; Geyer, 1996; Yeghiayan et al., 1997). However, 5-HT can also activate motor responses, in part at the spinal cord level (Gerson and Baldessarini, 1980; Geyer, 1996).

Behavioral Effects of Inhibitors of Norepinephrine Transport. The selective NE reuptake inhibitors desipramine and nisoxetine (Table 1) strongly antagonized motor hyperactivity in lesioned rats without affecting activity in sham control subjects (Figs. 4 and 5). The NE system, including tissue concentrations of NE and expression of various adrenergic receptor types in forebrain tissue, appears to undergo more or less normal development in the neonatally DA-selective lesioned rat (Luthman et al., 1990; Ordway, 1995), making NE available for actions of stimulants as well as selective inhibitors of NE transport.

In addition to their prominent effects on the DA system, stimulants including methylphenidate and amphetamines also release NE (Kuczenski and Segal, 1997, 2001). Interestingly, amphetamine-like stimulants can exert even greater effects on the release of NE than of either DA or 5-HT (Rothman et al., 2001). These observations support the hypothesis that enhanced NE activity can contribute to the antihyperactivity effect of stimulants in 6-OHDA lesioned rats, perhaps by enhancing descending frontal-cortical inhibitory influences on subcortical structures to compensate for losses of parallel effects of DA (Pliszka et al., 1996; Schwartz and Huston, 1996; Solanto, 1998; Biederman and Spencer, 1999; Arnsten, 2000). Seemingly inconsistent with the view that enhanced central noradrenergic activity may be helpful in clinical ADHD are observations that the α2-autoreceptor agonists clonidine and guanfacine can benefit hyperactivity in ADHD patients (Popper, 2000). However, direct postsynaptic α2 agonism may be involved (Arnsten et al., 1996; Arnsten, 2000). Also, selective inhibitors of NE transport are emerging as potentially therapeutic in ADHD (Biederman and Spencer, 1999; Popper, 2000), and it remains to be clarified whether tolerance develops to their benefits in ADHD after prolonged treatment (Wender, 1998).

Conclusions

We found that selective inhibitors of NE and 5-HT transport consistently antagonized motor hyperactivity in juvenile male rats after selective neonatal lesioning of the DA system with 6-OHDA, suggesting an important role by NE or 5-HT in the effects of stimulant agents that act on neuronal release and reuptake of NE or 5-HT as well as DA in this widely employed model of clinical ADHD. Methylphenidate (d- and dl-methylphenidate) and d-amphetamine are relatively selective for NE and DA transporter, compared with 5-HT transporter (Kula et al., 1999; Fleckenstein et al., 2000; Table 1). Also, amphetamine can release DA, NE, and 5-HT in intact rats, with possibly the greatest action on NE (Rothman et al., 2001), whereas methylphenidate increases release of both DA and NE effectively, with much less effect on 5-HT (Kuczenski and Segal, 1997). These several observations provide consistent additional support for a contribution of NE to...
the antihyperactivity effects of both stimulants in 6-OHDA lesioned rats, with a parallel contribution to stimulant treatment in clinical ADHD remaining to be proved. The neuronal circuits and interactions underlying the beneficial effects of inhibitors of the uptake of both NE and 5-HT remain incompletely understood and may involve interactions between NE and 5-HT systems, and interactions of both with DA neurons (Heffner and Seiden, 1982; Jacobs and Fornal, 1997; Yehiyaian et al., 1997; Sasaki-Adams and Kelley, 2001).

Finally, despite inherent risks in generalizing from laboratory models to clinical therapeutics, the present findings encourage clinical consideration of alternatives to stimulants. Stimulants are highly effective in ADHD with low risk of tolerance, but they have significant adverse effects and a potential for abuse and illicit trafficking (Barkley, 1997; Popper, 2000). SRIs also might be expected to contribute to the treatment of ADHD with low risk of tolerance, but they have significant adverse effects and a potential for abuse and illicit trafficking (Barkley, 1997; Popper, 2000). SRIs also might be expected to contribute to the treatment of attention-deficit/hyperactivity disorder: current perspectives. J Am Acad Child Adolesc Psychiatry 35:231–272. Popper CW (2000) Pharmacologic alternatives to psychostimulants for the treatment of attention-deficit/hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 9:65–646.


To conclude, 6-OHDA and neonatal 6-hydroxydopamine treatment may serve as a useful model for the behavioral and neurochemical consequences and implications for neurotoxicity. Eur J Pharmacol 406:1–13.


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