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ORIGINAL INVESTIGATION

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Stereoselective effects of methylphenidate on motor hyperactivity in juvenile rats induced by neonatal 6-hydroxydopamine lesioning

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Abstract *Rationale:* The psychostimulant *dl-threo*-methylphenidate is commonly used to treat attention deficit-hyperactivity disorder (ADHD). Consistent with its effects in ADHD patients, racemic methylphenidate antagonizes behavioral hyperactivity in several animal models of ADHD, including juvenile rats with neonatal 6-hydroxydopamine (6-OHDA) lesions of forebrain dopamine projections. The enantiomers of methylphenidate differ markedly in stimulant potency but have not been compared in the 6-OHDA lesion model. *Objective:* Locomotor-inhibiting effects of methylphenidate enantiomers were compared in 6-OHDA-lesioned rats to test the hypothesis that *d*-methylphenidate is more potent than *dl*- and *l*-methylphenidate. *Methods:* Selective dopamine lesions were made using 6-OHDA (100 µg, intracisternal, IC) on postnatal day (PD) 5 after desipramine (25 mg/kg, SC) pretreatment to protect noradrenergic neurons. Effects of *d*-, *l*- and *dl-threo*-methylphenidate on locomotor activity of lesioned and sham control rats were quantified at PD 23–27. *Results:* Lesioning yielded robust motor hyperactivity at PD 23–27. Both *d*- and *dl*-methylphenidate stimulated locomotor activity in intact rats, and inhibited activity in lesioned rats. *l*-Methylphenidate did not affect locomotor activity in either lesioned rats or controls. *d*-Methylphenidate (ED_{50}=1.66 mg/kg) was 3.3 times more potent than *dl*-methylphenidate (ED_{50}=5.45 mg/kg) in reducing locomotor hyperactivity in lesioned rats. In addition, pretreatment of lesioned rats with *l*-methylphenidate significantly reduced the motor inhibiting effects of *d*-methylphenidate. *Conclusions:* The more active enantiomer, as predicted, was *d*-methylphenidate, but the *l*-enantiomer interfered with its effects, suggesting that clinical potency of *d*-methylphenidate may be more than twice that of the racemate.

Keywords Attention deficit-hyperactivity disorder (ADHD) · Enantiomers · Hyperactivity · 6-Hydroxydopamine · Methylphenidate · Neonatally lesioned rat · Potency

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Introduction

Attention deficit-hyperactivity disorder (ADHD) is a prevalent neuropsychiatric disorder characterized by inattention, hyperactivity and impulsivity, most often diagnosed in boys (Barkley 1997). A very commonly prescribed medication for ADHD is *dl-threo*-methylphenidate (MPD; Ritalin) (Swanson et al. 1995; Findling and Dogin 1998; Wender 1998). Despite extensive research on the molecular action of this drug, the precise mechanisms by which it alleviates symptoms of ADHD remain uncertain. It is widely assumed that its ability to facilitate the release and activity of dopamine (DA) in forebrain is involved, since a primary molecular target of methylphenidate in the mammalian central nervous system is the neuronal membrane DA transporter (DAT) protein (Ritz et al. 1987; Gatley et al. 1996). A recent brain imaging study by Volkow et al. (2001) demonstrated that methylphenidate increased extracellular DA in human brain at doses commonly used to treat ADHD patients.

Currently clinically employed methylphenidate is racemic *R,S-(±)-threo*-methyl-2-phenyl-2-[2′-piperidyl]acetate. Dissimilar biological activities of separate enantiomers have been reported for numerous drugs (Sheldon 1993). For chiral agents, potential advantages of single-enantiomer preparations include smaller doses and potentially reduced risk of adverse effects (Thall 1996). Several studies in laboratory animals have indicated that the *d*-enantiomer of methylphenidate, *R,R-(+)-threo*-methylphenidate, is more potent than the racemate and

far more active than the *l*-methylphenidate *S,S-(–)-threo*-enantiomer (Patrick et al. 1987; Aoyama et al. 1996; Kula et al. 1999). Moreover, a preliminary double-blind, randomized crossover study of nine boys with ADHD compared acute doses of *d*- and *l-threo*-methylphenidate with the racemate and placebo, using computerized behavioral tests of attention (Srinivas et al. 1992). At 5 mg *d*-methylphenidate produced similar acute effects to 10 mg of *dl*-methylphenidate, and the *l*-enantiomer was indistinguishable from placebo. This finding suggests that the potency ratio of *d*- versus *dl*-methylphenidate may be close to 2.0, as expected if the *l*-isomer is psychopharmacologically inert.

However, the separated enantiomers of other dopaminergic agents, including aporphines, exert dissimilar or even contradictory actions to the primary, psychopharmacologically active enantiomer (Froimowitz et al. 1986; Neumeyer et al. 1988; Campbell et al. 1990, 1991). In keeping with this possibility, recent findings from this laboratory in intact adult rats indicate that *l*-methylphenidate is not merely inactive, but rather interferes with the stimulant effect of the *d*-enantiomer (R.J. Baldessarini, A. Campbell, N.S. Kula, in preparation).

Accordingly, we further tested the hypothesis that the *d-threo*-enantiomer of methylphenidate is the more active enantiomer, quantified the potency ratio of *d*- versus racemic drug, and tested for a possible interaction between the enantiomers. We employed a widely used laboratory model of ADHD based on motor hyperactivity in juvenile male rats [maximal at postnatal days (PD) 20–30] following neonatal lesioning of the cerebral DA system with 6-hydroxydopamine (6-OHDA; Shaywitz et al. 1976, 1978, 1984; Erinoff et al. 1979; Concannon et al. 1983; Kostrzewa et al. 1994; Zhang et al. 2001). Such animals show motor hyperactivity with deficient adaptation to novel environmental stimuli, as well as learning deficits (Shaywitz et al. 1976, 1978, 1984; Erinoff et al. 1979; Archer et al. 1988; Takasuna and Iwasaki 1996; Luthman et al. 1997). In this model, motor hyperactivity and some learning deficits are dose-dependently reversed by psychostimulants including *d*-amphetamine and *dl*-methylphenidate (Shaywitz et al. 1978; Heffner and Seiden 1982; Wool et al. 1987; Luthman et al. 1989; Zhang et al. 2001).

Materials and methods

Neonatal 6-hydroxydopamine lesioning

Neonatal 6-OHDA lesioning followed methods previously detailed by this laboratory (Teicher et al. 1986, 1998; Zhang et al. 2001). Sprague-Dawley rats (Charles River Labs, Wilmington, Mass., USA) were maintained under a 12/12-h artificial-daylight/dark schedule (on, 0700–1900 hours), with free access to tapwater and standard commercial rat chow. On PD 1, male pups were randomly assigned to lactating dams (ten per dam). On PD 5, pups were given subcutaneous (SC) injections of desipramine hydrochloride (25 mg/kg body weight; Sigma Chemicals, St Louis, Mo., USA). At 45 min later, under hypothermal anesthesia, subjects were randomly given an intracisternal (IC) injection of 20 µl vehicle [0.9% (w/v) sodium chloride containing 0.1% (w/v) ascorbic acid], or 6-OHDA hydrobromide (100 µg free base; Sigma-RBI, Natick, Mass., USA). Pups were returned to nursing dams in home cages after regaining consciousness. All procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee and conducted in a facility that fully complies with the NIH Guide for the Care and Use of Laboratory Animals of 1996 and other federal and local requirements.

Behavioral testing

Racemic *dl*-methylphenidate (*dl-threo*-methylphenidate hydrochloride, chemical purity >99% by thin layer chromatography) was obtained from Sigma-RBI, and the pure *d-threo*- and *l-threo*-methylphenidate hydrochloride enantiomers (enantiomeric purity for both >99% by liquid chromatography) were generously donated by Celgene Corporation (Warren, N.J., USA). The reported plasma half-life of *dl*-methylphenidate in adult rat is about 1–2 h (Wargin et al. 1983). Accordingly, to avoid carryover effects, each subject was tested in three sessions separated by 48 h, at PD 23, 25 and 27 in experiments 1–3. A total of 14 sham, and 55 lesioned rats was tested.

Experiment 1

For each sham-lesioned animal, one testing session involved vehicle [0.9% (w/v) saline] as a within-subject control, and others two involved randomly assigned *d*-, *l*-, or *dl*-methylphenidate at 3 mg/kg. Thus, the experiment involved a total of 14 vehicle, and 28 drug treatments.

Experiment 2

Each 6-OHDA-lesioned animal was tested on three occasions, in random order, with vehicle [0.9% (w/v) saline] as a within-subject control, and twice with *d*-, *l*-, or *dl*-methylphenidate at one of four selected doses (0.3, 1, 3, or 10 mg/kg). That is, each 6-OHDA-lesioned rat was treated with two of 12 possible active treatments on 2 drug treatment days. This experiment included a total of 44 lesioned rats given 44 vehicle and 88 drug treatments.

Experiment 3

Separate subjects were tested for a possible influence of *l*-methylphenidate on the actions of the *d*-enantiomer by pretreating 6-OHDA-lesioned rats with saline vehicle or *l*-methylphenidate (10 mg/kg), followed by vehicle or *d*-methylphenidate (10 mg/kg) in random order 15 min later. This experiment included 11 lesioned animals, given 11 treatments with vehicle (vehicle plus vehicle) and 22 drug treatments (vehicle plus *d*-methylphenidate or *l*-methylphenidate plus *d*-methylphenidate).

To avoid order effects, treatments were randomized with stratification, so that every treatment group was represented similarly at testing days PD 23, 25 or 27. All test treatments were given intraperitoneally (IP) in a volume of 5.0 ml/kg just before electronic recording of locomotor activity for 90 min. Rats were immediately returned to lactating dams after testing.

Rats were tested individually in a novel testing environment in 43.2×20.3×20.3 cm transparent plastic cages exposed to 4×8 horizontal infrared beams in a photobeam activity monitoring system (San Diego Instruments; San Diego, Calif., USA), between 1000 and 1600 hours in the absence of food and water (Zhang et al. 2001). Breaking of consecutive photobeams was scored as locomotor activity counts, accumulated at 5-min intervals by a microcomputer.

Lesion verification

Lesioning with 6-OHDA was verified by autoradiographic analysis of the density of binding of [^{3}H]2-β-carbomethoxy-3-β-[4′-iodophenyl]tropane (β-CIT; Kula et al. 1999) to DA transporter proteins, as an index of the concentration of DA terminals in rat forebrain. For this purpose, rats were killed at PD 29 by decapitation at 48 h after final behavioral testing, and their brains were quickly removed and frozen. Coronal brain sections (10 µm) were prepared in a cryostat at –17°C, thaw-mounted on gelatin-coated microscopic slides, and stored at –80°C. 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 $MgCl_2$. Sections were then incubated for another 60 min in fresh buffer containing 2 nM [^{3}H]β-CIT (64.7 Ci/mmol; Tocris Cookson Ltd, Bristol, UK). Specific binding was defined with excess GBR-12909 (1 µM; Sigma-RBI). 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 [^{3}H]-sensitive Hyperfilm radiographic film (Kodak, Rochester, N.Y., USA) at 4°C for 14 days with [^{3}H]standards, and developed for standard autoradiographic processing. Radioligand binding was quantified in lateral and medial caudate-putamen (CPu) and nucleus accumbens septi (NAc) with a computerized image analyzer (MCID-M4; Image Research Inc., St Catherines, Ontario, Canada), and converted to nCi/mg tissue using [^{3}H]reference standards to express results as mean±SEM fmol/mg tissue. These methods are detailed in previously published reports (Tarazi et al. 1997, 2000).

Data analysis

Lesion effects on DAT density were analyzed by two-way analysis of variance (ANOVA; for overall changes across treatments and brain regions), followed by post-hoc Dunnett's *t*-tests for planned comparisons. Behavioral data were analyzed similarly using ANOVA with Statview-5 programs (SAS Corp.; Cary, N.C., USA), but with locomotor activity as a repeated measure. All data are presented as means±SEM. Differences between treatment groups were considered statistically significant at $P \leq 0.05$ in two-tailed tests. Median-effective inhibitory doses (ED_{50}) and their 95% confidence intervals (CI) were computed with the Allfit program adapted to the Macintosh microcomputer (Munson and Rodbard 1980; Baldessarini et al. 1992; Kula et al. 1999). Relationships between lesion-induced motor hyperactivity and DAT levels were evaluated by nonparametric Spearman rank correlation (r_s).

Results

Effects of lesioning on dopamine terminals

Neonatal 6-OHDA lesioning at PD 5 produced profound reductions in DAT binding in both CPu and NAc following death on PD 29 after completion of behavioral and pharmacological testing. Losses of DAT binding were consistent among 6-OHDA-lesioned rats (range, in lateral CPu: 28.7–47.1, medial CPu: 17.4–34.1, NAc: 18.7–42.6 fmol/mg, all groups n=14) and in sham controls (lateral CPu: 100.5–159.3, medial CPu: 74.1–128.1, NAc: 47.1–95.6, all groups n=10). Average losses compared to sham-lesioned controls were 71% in lateral CPu, 75% in medial CPu, and 57% in NAc (Table 1).

Table 1 Effects of neonatal 6-OHDA lesioning on binding of [^{3}H]β-CIT to dopamine transporters quantified autoradiographically in juvenile rats. Data are specific binding (mean fmol/mg tissue±SEM) for n=10–14 rats/group

	Sham controls	6-OHDA lesioned	% of control
Lateral caudate-putamen	131±7.7	37.9±1.4[a]	28.9
Medial caudate-putamen	102±7.9	24.9±1.5[a]	24.4
Nucleus accumbens septi	71.6±7.4	30.8±1.9[a]	43.0

[a] P<0.001 versus corresponding sham-lesioned controls

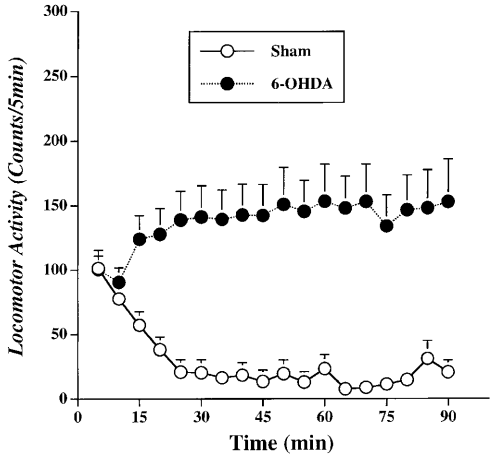

Fig. 1 Effects of neonatal 6-OHDA lesioning on motor activity. Data are mean±SEM locomotor activity scores collected at 5-min interval for 90 min in a novel testing environment between PD 23–27. Responses differ highly significantly between the sham controls and lesioned rats treated with vehicle and pooled from all experiments (P<0.00l)

Lesion-induced motor hyperactivity

Neonatal 6-OHDA lesioning resulted in markedly increased and sustained spontaneous locomotor activity in juvenile lesioned rats placed in a novel environment, pooling from all subjects tested between PD 23–27 (in n=55 lesioned versus n=14 sham-controls: P<0.001; Fig. 1). Motor activity of lesioned rats was similar to that of sham controls for the first 5–10 min of testing, 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 throughout the 90-min session. Motor activity levels in 6-OHDA-lesioned rats on separate days (PD 23, 25, 27) did not differ significantly despite repeated testing in novel environments across testing days [$F(2,52)$=1.23, P=0.30]. Activity levels in identically treated 6-OHDA-lesioned rats from behavioral experiments 2 (n=44) and 3 (n=11) also did not differ from each other [$F(1,53)$=0.22, P=0.64].

In association with minor individual variation in DAT labeling, no correlation was found between motor hyperactivity in 6-OHDA-lesioned rats and the loss of DAT binding in individual animal (r_s for all three brain regions averaged 0.139 (all P averaged 0.64; n=14).

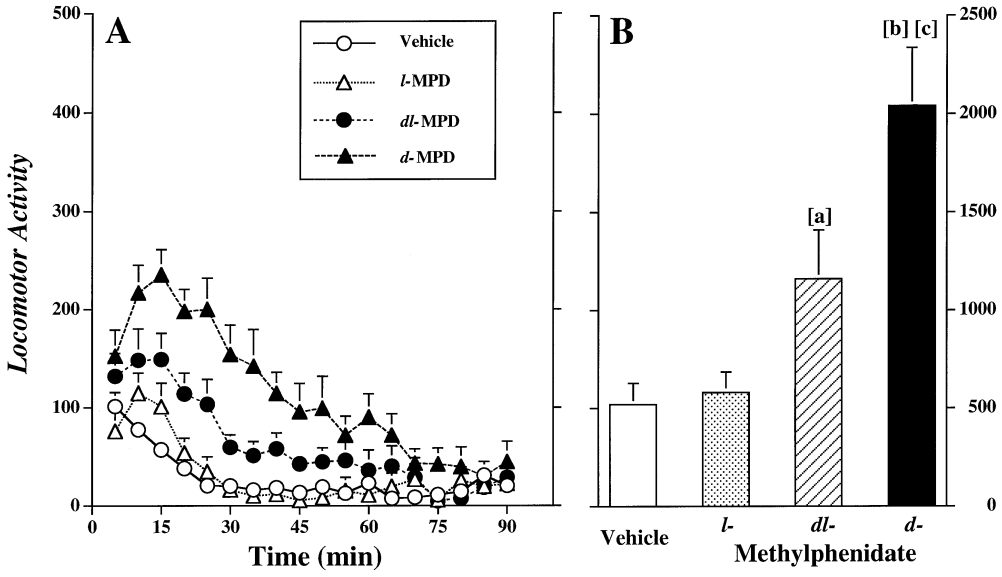

Fig. 2A, B Effects of *d*-, *dl*-, *l*-methylphenidate (*MPD*; all at 3 mg/kg, IP) and vehicle on locomotor activity in sham-lesioned controls. **A** Locomotor activity scores collected at 5-min intervals; **B** activity scores for the entire session. *[a]* $P<0.05$, and *[b]* $P<0.00$l versus vehicle controls, *[c]* $P<0.01$ versus *dl*-methylphenidate

Behavioral effects of methylphenidate enantiomers in sham-lesioned rats (experiment 1)

There was no evidence of a group-order artifact since motor activity with vehicle treatment did not differ among rats given different drugs [$F(2,25)=0.15$, $P=0.86$]. Accordingly, we pooled the vehicle data ($n=14$) to comprise the controls for the drug-treated condition with *d*- ($n=9$), *dl*- ($n=10$), and *l*-methylphenidate ($n=9$) in sham rats.

When tested at 3 mg/kg, *dl*-methylphenidate and *d*-methylphenidate (both $P<0.001$ versus vehicle control) produced strong stimulatory responses in unlesioned rats with marked increases of locomotor activity that returned to vehicle-treated control levels within 90 min. In contrast, *l*-methylphenidate had no significant effect at the dose tested (Fig. 2A). Total activity scores for the entire 90-min sessions also show the stimulating effects of *dl*-methylphenidate ($P<0.05$ versus vehicle control) and *d*-methylphenidate ($P<0.001$ versus vehicle control) and lack of effect with *l*-methylphenidate (Fig. 2B). *d*-Methylphenidate was more active than *dl*-methylphenidate in increasing locomotor activity in sham rats ($P<0.001$, Fig. 2A).

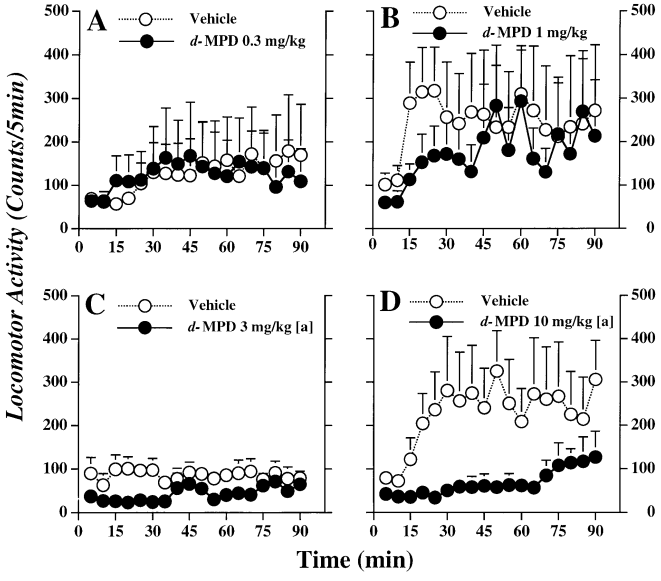

Fig. 3 Effects of *d*-methylphenidate (*MPD*) on motor hyperactivity in 6-OHDA-lesioned juvenile male rats, at doses of: **A** 0.3, **B** 1.0, **C** 3.0, or **D** 10.0 mg/kg, IP. *[a]* $P<0.001$ versus vehicle controls

Behavioral effects of methylphenidate enantiomers in 6-OHDA-lesioned rats (experiment 2)

Pure *d*-methylphenidate dose-dependently and potently antagonized 6-OHDA lesioning-induced hyperactivity. By intra-individual comparison of vehicle treatment to doses of *d*-methylphenidate: 0.3 mg/kg ($n=8$), $P=0.98$; 1 mg/kg ($n=6$), $P=0.07$; 3 mg/kg ($n=9$), $P<0.001$; 10 mg/kg ($n=6$), $P<0.001$ (Fig. 3).

The racemate also antagonized 6-OHDA lesioning-induced hyperactivity, comparing intra-individual vehicle treatment to doses of *dl*-methylphenidate: 0.3 mg/kg ($n=7$), $P=0.95$; 1 mg/kg ($n=9$), $P=0.98$; 3 mg/kg ($n=9$), $P<0.05$; 10 mg/kg ($n=8$), $P<0.001$ (Fig. 4).

In contrast, *l*-methylphenidate did not appreciably alter locomotor activity in lesioned rats at any dose, comparing intra-individual vehicle treatment to doses of *l*-methylphenidate: 0.3 mg/kg ($n=5$), $P=0.32$; 1 mg/kg ($n=8$), $P=0.84$; 3 mg/kg ($n=5$), $P=0.94$; 10 mg/kg ($n=7$), $P=0.29$.

Comparison of drug effects of *d*- and *dl*-methylphenidate on locomotor activity scores in a range of doses (0 and 0.3–10 mg/kg, IP) are compiled from data of Figs 3 and 4 (Fig. 5). Pure *d*-methylphenidate inhibited lesion-induced hyperactivity with an ED_{50} of 1.66 (95%

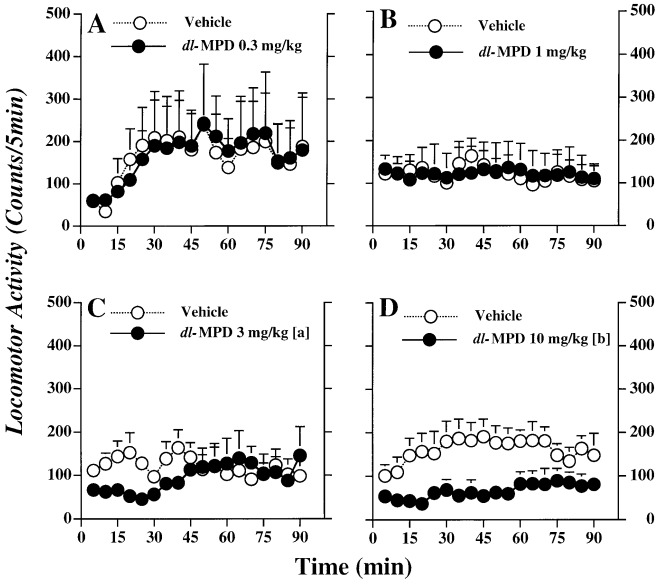

Fig. 4 Effects of *dl*-methylphenidate (*MPD*) on motor hyperactivity in 6-OHDA-lesioned juvenile male rats, at doses of: **A** 0.3, **B** 1.0, **C** 3.0, or **D** 10.0 mg/kg, IP. *[a]* $P<0.05$; *[b]* $P<0.001$ versus vehicle controls

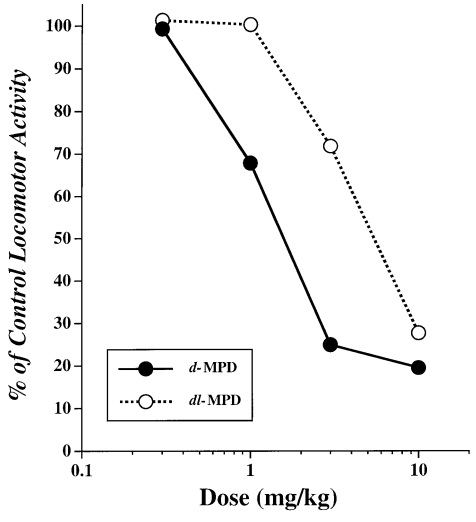

Fig. 5 Potency of *d*- versus *dl*-methylphenidate (*MPD*) in inhibiting lesion-induced motor hyperactivity, based on doses of 0, and 0.3–10 mg/kg, and total activity scores for 90-min sessions. Computed ED_{50} as 1.66 mg/kg for *d*-methylphenidate, 5.45 mg/kg for *dl*-methylphenidate (potency ratio=3.3)

CI=1.08–2.24) mg/kg, compared to *dl*-methylphenidate ED_{50} of 5.45 (CI=4.57–6.33) mg/kg, indicating a 3.3-fold potency difference. If *l*-methylphenidate were completely inactive, a theoretically predicted ED_{50} value for the *d*-enantiomer would be one-half that of the racemate, or 2.72 (CI=2.16–3.28) mg/kg, compared to the nearly significantly different observed value of 1.66 (CI=1.08–2.24).

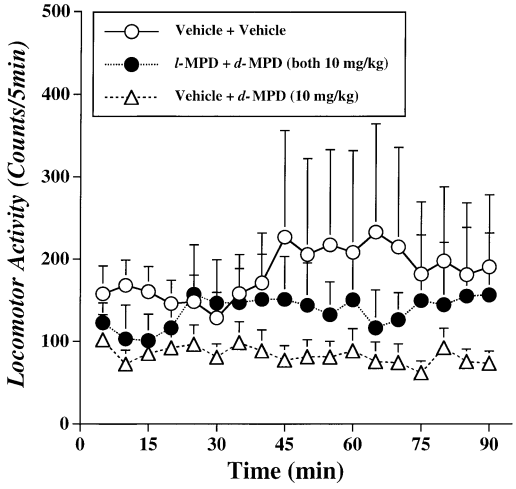

Fig. 6 Effects on locomotor responses (mean activity counts/5 min±SEM) in 6-OHDA-lesioned rats, treated with vehicle injections (*open circles*), *d*-methylphenidate (*MPD*; 10 mg/kg; *open triangles*; $P<0.001$ versus vehicle controls), or *l*- plus *d*-methylphenidate (both 10 mg/kg, IP; *filled circles*; $P<0.001$ versus *d*-methylphenidate)

Interaction study (experiment 3)

Interaction of *l*- with *d*-methylphenidate was tested in rats by pretreating with *l*-methylphenidate (10 mg/kg, IP) at 15 min before challenging with the *d*-enantiomer (10 mg/kg, IP). Pretreatment with the *l*-enantiomer significantly attenuated the effects of *d*-methylphenidate ($P<0.001$, $n=11$; Fig. 6). In these experiments *d*-methylphenidate also strongly reduced hyperactivity compared to vehicle ($P<0.001$; Fig. 6).

Discussion

Consistent with previous studies (Shaywitz et al. 1976, 1978, 1984; Erinoff et al. 1979; Zhang et al. 2001), 6-OHDA lesioning of developing DA projections in rat forebrain of neonatal male rat pups resulted in robust locomotor hyperactivity and lack of adaptation to a novel environment when subjects were tested at a later juvenile stage (Fig. 1).

We also verified by autoradiographic analysis of DAT binding with an improved radioligand (Kula et al. 1999; Tarazi et al. 2000), that neonatal 6-OHDA lesioning led to major losses of DA innervation in forebrain tissue of juvenile rats with limited variance and without significant correlation to individual motor activity levels (Table 1). Motor hyperactivity induced by neonatal lesioning with 6-OHDA is particularly associated with destruction of mesolimbic DA pathways (Heffner et al. 1983; Shaywitz et al. 1984). However, individual behavioral responses have not been found to be closely related to DA concentrations in brain regions in individual animals (Schwarting and Huston 1996).

At a screening dose of 3 mg/kg in sham-lesioned control rats, *d*-methylphenidate was more effective than *dl*-

methylphenidate in stimulating locomotor activity, and the *l*-enantiomer had little effect (Fig. 2). These results accord with previous observations that the *d*-enantiomer was the active enantiomer in stimulating motor activity (Patrick et al. 1987; Aoyama et al. 1996) and reducing milk consumption in intact adult rats (Eckerman et al. 1991). Similar enantiomeric differences were verified in a preliminary experiment in the present study with intact juvenile rats (Fig. 2). Our previous in vitro studies indicated that the *d*-enantiomer was 10- to 20-fold more potent than the racemate in competing for binding of several chemically dissimilar radioligands at DAT in homogenates of rat CPu tissue, and in inhibiting the transport of [^{3}H]DA into isolated nerve terminals prepared from fresh rat CPu (Kula et al. 1999).

Other differences between the enantiomers of methylphenidate may include their stereoselective tissue absorption or metabolic disposition (Kimko et al. 1999; Challman and Lipsky 2000). Notably, plasma concentrations of *d*-methylphenidate were higher than those of the *l*-enantiomer in human subjects given identical oral doses (Hubbard et al. 1989; Srinivas et al. 1992). On the other hand, plasma levels and clearance in human subjects given the enantiomers intravenously were indistinguishable, suggesting that lower concentrations of *l*-methylphenidate after oral administration might be due to a stereoselective first-pass metabolic effect (Srinivas et al. 1993). A recent study using positron-emission tomographic (PET) brain imaging found that competition of [^{11}C]*d*-methylphenidate to DA-rich CPu tissue in human brain was much greater than that of [^{11}C]*l*–methylphenidate (tracer doses for both 6–7 mCi), with much greater enantiomeric selectivity than was found in cerebral cortex or cerebellum (Ding et al. 1997). However, these differences may reflect a pharmacodynamic preference for the *d*-enantiomer at active target sites including the DAT in forebrain tissue.

We now report for the first time, that *d-threo*-methylphenidate was also more potent than the racemate in reducing locomotor hyperactivity in the 6-OHDA-lesioning model of ADHD in juvenile male rats, whereas the *l*-enantiomer was virtually inactive by itself at the doses tested (Fig. 3, Fig. 4). The ED_{50} ratio for *d*- versus *dl*-methylphenidate was substantially greater than the theoretical value of 2.0 that would be expected if the *l*-enantiomer were pharmacologically inert. Instead, the observed *d*- versus *dl*-methylphenidate potency ratio of 3.3 (Fig. 5) is consistent with the idea that the *l*-enantiomer, including that in the racemate, may interfere with the actions of *d*-methyphenidate. This hypothesis is consistent with our recent finding of a dose-dependent inhibitory effect of *l*-methylphenidate against *d*-methylphenidate in intact adult rats (R.J. Baldessarini, A. Campbell and N.S. Kula, in preparation). In a direct test of such an interaction using the lesioned juvenile rat model, pretreatment with *l*-methylphenidate significantly attenuated locomotor responses of 6–OHDA lesioned rats to a subsequent challenge with an equal dose of *d*-methylphenidate (Fig. 6).

Mechanisms underlying an evident antagonistic action of *l*-methylphenidate against *d*-methylphenidate are not clear. It may be that the less potent *l*-enantiomer competes for DAT sites in vivo to reduce the net effectiveness of *d*-methylphenidate. Such a mechanism would be analogous to the competition by a weak partial-agonist against a potent or full agonist of DA receptors to yield a net functional antagonistic action, as we have reported previously in the antagonism by *S(+)*-enantiomers of various aporphines against their more DA agonistic *R(–)*-enantiomers, or against DA itself (Campbell et al. 1990, 1991). Such a competitive interaction may also account for paradoxical, apparently antidopaminergic effects of other relatively weak DA partial agonists in intact nervous system, including ergolines that act as more potent agonists at up-regulated or sensitized DA receptors, such as in 6-OHDA lesioned rat brain and perhaps also in Parkinson's disease (Campbell et al. 1989; Coward et al. 1990).

In conclusion, the present experiments demonstrated that the locomotor inhibiting effects of *dl*-methylphenidate in juvenile 6-OHDA-lesioned rat model of clinical ADHD can be attributed solely to the *d*-enantiomer, and that the *l*-enantiomer interacts with the *d*-enantiomer to limit this effect. Clinical evaluation of *d*-methylphenidate for the treatment of ADHD should consider this interaction and the possibility that the potency of *d*- versus *dl*-methylphenidate may be greater than the 2:1 ratio expected if *l*-methylphenidate were pharmacologically inert.

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References

Aoyama T, Kotaki H, Sawada Y, Iga T (1996) Pharmacokinetics and pharmacodynamics of methylphenidate enantiomers in rats. Psychopharmacology 127:117–122

Archer T, Danysz W, Fredriksson A, Jonsson G, Luthman J, Sundstrom E, Teiling A (1988) Neonatal 6-hydroxydopamine-induced dopamine depletions: motor activity and performance in maze learning. Pharmacol Biochem Behav 31:357–364

Baldessarini RJ, Kula NS, Campbell A, Bakthavachalam V, Yuan J, Neumeyer JL (1992) Prolonged D_2 antidopaminergic activity of alkylating and nonalkylating derivatives of spiperone in rat brain. Mol Pharmacol 42:856–863

Barkley RA (1997) Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull 121:65–94

Campbell A, Baldessarini RJ, Cremens C, Teicher MH, Marsh E, Kula NS (1989) Bromocriptine antagonizes behavioral effects of cocaine in the rat. Neuropsychopharmacology 2:209–224

Campbell A, Baldessarini RJ, Gao Y, Zong R, Neumeyer JL (1990) *R(–)* and *S(+)* stereoisomers of 11-hydroxy- and 11-methoxy-*N*-*n*-propylnoraporphine: central dopaminergic behavioral activity in the rat. Neuropharmacology 29:527–536

Campbell A, Yeghiayan S, Baldessarini RJ, Neumeyer JL (1991) Selective antidopaminergic effects of *S*(+)-N-*n*-propylnoraporphines in limbic versus extrapyramidal sites in rat brain:

comparisons with typical and atypical antipsychotic agents. Psychopharmacology 103:323–329
Challman TD, Lipsky JJ (2000) Methylphenidate: its pharmacology and uses. Mayo Clin Proc 75:711–721
Concannon JT, Braughler JM, Schechter MD (1983) The development of brain biogenic amines, cyclic nucleotides and hyperactivity in 6-OHDA-treated rat pups. Pharmacol Biochem Behav 18:477–482
Coward DM, Dixon AK, Urwyler S, White TG, Enz A, Karobath M, Shearman G (1990) Partial dopamine-agonistic and atypical neuroleptic properties of the amino ergolines SDZ 208-911 and SDZ 208-912. J Pharmacol Exp Ther 252:279–285
Ding YS, Fowler JS, Volkow ND, Dewey SL, Wang GJ, Logan J, Gatley SJ, Pappas N (1997) Chiral drugs: comparison of the pharmacokinetics of [^{11}C]*d-threo* and *l-threo*-methylphenidate in the human and baboon brain. Psychopharmacology 131:71–78
Eckerman DA, Moy SS, Perkins AN, Patrick KS, Breese GR (1991) Enantioselective behavioral effects of *threo*-methylphenidate in rats. Pharmacol Biochem Behav 40:875–880
Erinoff L, MacPhail RC, Heller A, Seiden LS (1979) Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. Brain Res 164:195–205
Findling RL, Dogin JW (1998) Psychopharmacology of ADHD: children and adolescents. J Clin Psychiatry 59:42–49
Froimowitz M, Neumeyer JL, Baldessarini RJ (1986) A stereochemical explanation of the dopamine agonist and antagonist activity of stereochemical pairs. J Med Chem 29:1570–1573
Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS (1996) Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 58:231–239
Heffner TG, Seiden LS (1982) Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. Brain Res 224:81–90
Heffner TG, Heller A, Miller FE, Kotake C, Seiden LS (1983) Locomotor hyperactivity in neonatal rats following electrolytic lesions of mesocortical dopamine neurons. Brain Res 285:29–37
Hubbard JW, Srinivas NR, Quinn D, Midha KK (1989) Enantioselective aspects of the disposition of *dl-threo*-methylphenidate after the administration of a sustained-release formulation to children with attention deficit-hyperactivity disorder. J Pharmacol Sci 78:944–947
Kimko HC, Cross JT, Abernethy DR (1999) Pharmacokinetics and clinical effectiveness of methylphenidate. Clin Pharmacokinet 37:457–470
Kostrzewa RM, Brus R, Kalbfleisch JH, Perry KW, Fuller RW (1994) Proposed animal model of attention deficit hyperactivity disorder. Brain Res Bull 34:161–167
Kula NS, Baldessarini RJ, Tarazi FI, Fisser R, Wang S, Trometer J, Neumeyer JL (1999) [^{3}H]β-CIT: a radioligand for dopamine transporters in rat brain tissue. Eur J Pharmacol 385:291–294
Luthman J, Fredriksson A, Lewander T, Jonsson G, Archer T (1989) Effects of *d*-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment. Psychopharmacology 99:550–557
Luthman J, Bassen M, Fredriksson A, Archer T (1997) Functional changes induced by neonatal cerebral 6-hydroxydopamine treatment: effects of dose levels on behavioral parameters. Behav Brain Res 82:213–221
Munson PJ, Rodbard D (1980) Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 107:220–239
Neumeyer JL, Froimowitz M, Baldessarini RJ, Campbell A, Gao Y (1988) Neuropharmacology and stereochemistry of dopamine receptor agonist and antagonist enantiomeric pairs. J Recept Res 8:83–96
Patrick KS, Caldwell RW, Ferris RM, Breese GR (1987) Pharmacology of the enantiomers of *threo*-methylphenidate. J Pharmacol Exp Ther 241:152–158
Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223
Schwarting RKW, Huston JP (1996) The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 50:275–331
Shaywitz BA, Yager RD, Klopper JH (1976) Selective brain dopamine depletion in developing rats: an experimental model of minimal brain dysfunction. Science 191:305–308
Shaywitz BA, Klopper JH, Gordon JW (1978) Methylphenidate in 6-hydroxydopamine-treated developing rat pups. Effects on activity and maze performance. Arch Neurol 35:463–469
Shaywitz BA, Teicher MH, Cohen DJ, Anderson GM, Young JG, Levitt P (1984) Dopaminergic but not noradrenergic mediation of hyperactivity and performance deficits in the developing rat pup. Psychopharmacology 82:73–77
Sheldon RA (1993) Chirotechnology: industrial synthesis of optically active compounds. Marcel Dekker, New York
Srinivas NR, Hubbard JW, Quinn D, Midha KK (1992) Enantioselective pharmacokinetics and pharmacodynamics of *dl-threo*-methylphenidate in children with attention deficit hyperactivity disorder. Clin Pharmacol Ther 52:561–568
Srinivas NR, Hubbard JW, Korchinski ED, Midha KK (1993) Enantioselective pharmacokinetics of *dl-threo*-methylphenidate in humans. Pharmacol Res 10:14–21
Swanson JM, McBurnett K, Christian DL, Wigal T (1995) Stimulant medications and the treatment of children with ADHD. In: Ollendick TH, Prinz RJ (eds) Advances in clinical child psychology. Plenum Press, New York, pp 265–322
Takasuna M, Iwasaki T (1996) Active and passive avoidance learning in rats neonatally treated with intraventricular 6-hydroxydopamine. Behav Brain Res 74:119–126
Tarazi FI, Yeghiayan SK, Baldessarini RJ, Kula NS, Neumeyer JL (1997) Long-term effects of *S*(+)N-*n*-propylnorapomorphine compared with typical and atypical antipsychotics: differential increases of cerebrocortical D_2-like and striatolimbic D_4-like dopamine receptors. Neuropsychopharmacology 17:186–196
Tarazi FI, Kula NS, Zhang K, Baldessarini RJ (2000) Alkylation of rat dopamine transporters and blockade of dopamine uptake by EEDQ. Neuropharmacology 39:2133–2138
Teicher MH, Barber NI, Reichheld JH, Baldessarini RJ, Finklestein SP (1986) Selective depletion of cerebral norepinephrine with 6-hydroxydopamine and GBR-12909 in neonatal rat. Brain Res 395:124–128
Teicher MH, Andersen SL, Campbell A, Gelbard HA, Baldessarini RJ (1998) Progressive accumbens degeneration after neonatal striatal 6-hydroxydopamine in rats. Neurosci Lett 247:99–102
Thall E (1996) When drug molecules look in the mirror. J Chem Educ 73:481–484
Volkow ND, Wang GJ, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding YS, Gatley SJ, Gifford A, Franceschi D (2001) Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 21:1–5
Wargin W, Patrick K, Kilts C, Gualtieri CT, Ellington K, Mueller RA, Kraemer G, Breese GR (1983) Pharmacokinetics of methylphenidate in man, rat and monkey. J Pharmacol Exp Ther 226:383–386
Wender PH (1998) Pharmacotherapy of attention-deficit/hyperactivity disorder in adults. J Clin Psychiatry 59:76–79
Wool RS, Weldon DA, Shaywitz BA, Anderson GM, Cohen DJ, Teicher MH (1987) Amphetamine reverses learning deficits in 6-hydroxydopamine-treated rat pups. Dev Psychobiol 20:219–232
Zhang K, Tarazi FI, Baldessarini RJ (2001) Role of dopamine D_4 receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. Neuropsychopharmacology 25:624–632