Methylphenidate Preferentially Increases Catecholamine Neurotransmission within the Prefrontal Cortex at Low Doses that Enhance Cognitive Function

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Background: *Low doses of psychostimulants, such as methylphenidate (MPH), are widely used in the treatment of attention-deficit/hyperactivity disorder (ADHD). Surprisingly little is known about the neural mechanisms that underlie the behavioral/cognitive actions of these drugs. The prefrontal cortex (PFC) is implicated in ADHD. Moreover, dopamine (DA) and norepinephrine (NE) are important modulators of PFC-dependent cognition. To date, the actions of low-dose psychostimulants on PFC DA and NE neurotransmission are unknown.*

Methods: *In vivo microdialysis was used to compare the effects of low-dose MPH on NE and DA efflux within the PFC and select subcortical fields in male rats. Doses used (oral, 2.0 mg/kg; intraperitoneal, .25–1.0 mg/kg) were first determined to produce clinically relevant plasma concentrations and to facilitate both PFC-dependent attention and working memory.*

Results: *At low doses that improve PFC-dependent cognitive function and that are devoid of locomotor-activating effects, MPH substantially increases NE and DA efflux within the PFC. In contrast, outside the PFC these doses of MPH have minimal impact on NE and DA efflux.*

Conclusions: *The current observations suggest that the therapeutic actions of low-dose psychostimulants involve the preferential activation of catecholamine neurotransmission within the PFC.*

Key Words: Psychostimulants, ADHD, dopamine, norepinephrine, attention, working memory

Attention-deficit/hyperactivity disorder (ADHD) is characterized by attentional dysfunction, impulsivity, and excessive motor activity levels. Conservative estimates indicate prevalence rates of 3%–5% in both children and adults (Solanto 2001; Solanto 1998; Wilens et al 2004). Low-dose psychostimulants, including methylphenidate (MPH; Ritalin), are the most effective and widely used form of therapy for ADHD (for review, Greenhill 2001). Given the widespread use of psychostimulants, it is surprising that little is known about the neural mechanisms underlying the therapeutic actions of these drugs. Importantly, the behavioral and cognitive actions of low doses of these drugs are not unique to ADHD, and occur in both normal human and animal subjects (Arnsten and Dudley 2005; Kuczenski and Segal 2002; Mehta et al 2001; Rapoport et al 1980; Vaidya et al 1998). Moreover, these actions are in distinct contrast to the behavioral activating and cognition-impairing actions of higher doses of these drugs.

At higher doses, psychostimulants increase norepinephrine (NE) and dopamine (DA) efflux widely throughout the brain. In contrast, recent studies demonstrate that low doses of MPH, estimated to yield clinically relevant plasma levels, exert a minimal influence on DA efflux within the nucleus accumbens (ACC; Kuczenski and Segal 2001; Kuczenski and Segal 2002). The prefrontal cortex (PFC) is posited to play a prominent role in ADHD and to be involved in the therapeutic actions of low-dose stimulants (for review, Arnsten 2001; Castellanos and Tannock 2002, Vaidya et al 1998). Importantly, DA afferents within the PFC display a greater sensitivity to a variety of environmental and pharmacologic challenges relative to subcortical DA systems (Berridge et al 1999a; Roth et al 1988). Surprisingly, the degree to which low-dose stimulants influence DA and NE neurotransmission within the PFC is, to date, not known.

The current studies used in vivo microdialysis in unanesthetized rats to examine the degree to which low and clinically relevant doses of MPH influence NE and DA neurotransmission within the PFC and select subcortical regions (ACC and the medial septal area [MSA]). Importantly, similar to that observed in humans, these low doses of MPH improved working memory and sustained attention while having minimal effects on locomotion and electroencephalographic–electromyographic (EEG–EMG) indices of arousal. When administered in this dose range, MPH increased NE and DA efflux within the PFC while having minimal effects on NE and DA levels outside the PFC. Combined, these observations indicate a prominent role of PFC catecholamines in the cognition-enhancing and behavioral-calming actions of low-dose psychostimulants.

Methods and Materials

Animals and Surgery

Male Sprague-Dawley rats (290–370 g; Charles River, Wilmington, Massachusetts) had ad libitum access to food and water. For microdialysis testing, a microdialysis probe was lowered into the PFC (A+3.2; L1.0; V-5.0 at an angle of 4° lateral), the core subregion of the ACC (A+1.7; L1.4; V-7.8), or the MSA (A-.7; L.5; V-7.8) on the day before testing, as described previously (Berridge and Stalnaker 2002). Electroencephalographic and electromyographic electrodes were implanted, as described below. Animals were housed in the testing chamber overnight (see

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below) and artificial extracellular fluid (AECF) perfused through the dialysis probe.

Plasma Measurement of MPH

Limited plasma measurements of MPH were conducted after both oral gavage (2.0 mg/kg) and intraperitoneally (IP; .25 and .5 mg/kg) administered MPH. Blood samples were collected via cardiac puncture in isoflurane-anesthetized animals at 5-min and 15-min after IP injection and oral gavage, respectively. Blood (3–5 mL) was collected into sealed tubes containing NaF and Na-oxalate (VWR International, West Chester, Pennsylvania) and centrifuged, and the plasma decanted. Plasma was stored at −80°C and analyzed by high-pressure liquid chromatography (HPLC) with mass spectrometry (National Medical Services, Willow Grove, Pennsylvania). External standards (MPH 2–200 ng/mL) were run with each batch. The lower limit of detection in this assay was 2 ng/mL, and the interassay precision for MPH was 8.9 % and 7.6 % at 12 and 40 ng/mL, respectively.

Microdialysis and HPLC Analysis of DA and NE Concentrations

DA and NE were measured in dialysate samples by using HPLC with electrochemical detection (Berridge and Stalnaker 2002). Briefly, AECF (147 mmol/L NaCl, 1.3 mmol/L $CaCl_2$, .9 mmol/L $MgCl_2$, 2.5 mmol/L KCl [pH 7.4]) was delivered at a rate of 1.5 μL/min through a length of dialysis membrane (molecular weight cutoff: 13000; OD: 250 μm). The length of functional dialysis membrane was 4 mm for PFC and MSA, and 2 mm for ACC. Fused silica provided outflow to a sample collection vial outside the testing chamber.

Sixteen-minute samples were collected before and after vehicle or MPH injections. Baseline values were determined from three to four samples characterized by low levels of waking (i.e., sleeping). The quantitation limit for both NE and DA (three times background noise) was approximately .3 pg (20 μL samples). The mean baseline concentration of NE per sample was 1.25 ± .08 pg within PFC (n = 21) and 1.08 ± .10 pg within the MSA (n = 14). The mean baseline concentration of DA was .90 ± .06 pg (n = 41) within the PFC and 5.58 ± .40 pg (n = 41) within the ACC.

Microdialysis Experimental Procedures

A Plexiglas testing chamber (32 × 32 × 40 cm) was housed in a wooden, sound-attenuating chamber (Berridge and Foote 1996). On the day of testing, at least four 16-min dialysis samples were collected before an IP injection or oral gavage administration of either normal saline or varying doses of MPH dissolved in saline (IP: .25, .5 or 1.0 mg/kg; oral: 2.0 mg/kg). Generally, IP injections were performed without picking up the animal. One goal of the current study was to compare the neurochemical and behavioral actions of low-dose stimulants with those of higher doses of these drugs. Given that virtually all previous work on the actions of higher doses of psychostimulants has typically been conducted during the light phase of the circadian cycle, we tested the animals between the hours of 9:00 AM and 5:00 PM.

EEG and EMG Recording and Analyses

In the subset of animals receiving IP injections, bipolar EEG and EMG electrodes were placed into the PFC (+3.0 A; ± 1.5L) and dorsal neck muscle, respectively, as described previously (Berridge and Foote 1996). Using previously described criteria (Berridge and Foote 1996), we scored EEG and EMG for the following behavioral state categories 1) slow-wave sleep, 2) rapid eye movement sleep, 3) quiet-waking, and 4) active-waking. Time spent in each state was measured for each 16-min dialysis sample collection epoch by observers unaware of experimental conditions.

Locomotor Activity

Behavior was scored from videotapes starting 30-min before MPH administration. The frequency of rears (both free and wall) and quadrant entries (defined by hind legs crossing into a new quadrant) was measured.

Visual Signal Detection Testing (Sustained Attention)

Testing involved a modified signal detection task of sustained attention (Bushnell 1998; McGaughy and Sarter 1995). Briefly, animals (n = 8; 375–400 g) were reduced to 85% of their ad libitum weight. Sessions used a discrete trial format starting after a variable delay (mean = 14 sec). In one half of the trials ("signal trials"), a light-emitting diode positioned above the feeder trough was illuminated for 4 sec, after which two levers, one on either side of the feeder trough, were projected into the chamber. In the other half of the trials ("no-signal trials"), the chamber remained dark for 4 sec, after which both levers were inserted into the chamber. On signal trials, pressing the right-side lever was rewarded with two sucrose pellets (a "hit") followed by a 5-sec blackout. Pressing the left lever resulted in retraction of the levers followed by a 5-sec blackout ("miss"). On no-signal trials, a right lever press resulted in retraction of the levers and a 5-sec blackout ("false alarm"), whereas a left lever press resulted in reward delivery (two pellets; "correct rejection"), lever retraction, and a 5-sec blackout. If no response occurred within 5 sec of the levers being inserted, the levers were retracted, the 5-sec blackout was presented, and the trial scored "no response." Trials with no response rarely occurred and were excluded from the analyses. A variable intertrial interval of 14 sec, on average, with a minimum of 6 sec, elapsed before the start of a new trial. A session lasted for 100 trials over approximately 30 min. Signal length varied randomly (.25, .5, .75, 1.0, 1.5, 2.0, and 2.5 sec; with replacement).

Rats were injected with MPH (.5 mg/kg IP) or saline 30 min before testing. The order of injections was randomized across rats. Testing was conducted on consecutive days. Dependent measures included the proportion of trials with a correct response (proportion of hits + proportion of correct rejection), the probability of a hit (correct responses/number of signal trials), probability of a false alarm (correct responses/number of no signal trials), and d', a relative measure of stimulus detectability (d' = Z(N) − Z(SN). Z(N) = Z score of the Noise Distribution = Z score of (1-probability of false alarms). Z(SN) = Z score of the Signal + Noise distribution = Z score of 1-probability of a hit).

Delayed Alternation Testing (Working Memory)

Training and testing were similar to that described previously (Zahrt et al 1997). Briefly, animals were housed singly and placed on a restricted feeding schedule in which they were allowed to eat 16–25 g of standard chow immediately after testing. The quantity of food was titrated for each animal to maintain motivation for food rewards (chocolate chips). For this task, animals were rewarded when they entered the maze arm not chosen on the previous trial (10 per session, 1 session per day). Maze arm entries and response times (time between start-box opening and reward obtainment) were recorded for each trial. For delays longer than 5 sec, animals were removed from the maze and retained in a holding cage for the delay period. Animals were placed back into the start box 2 sec before the end of the delay. The intertrial delay was adjusted until performance was stable and within the range of 60% to 80%. Stable performance was