Neuropharmacology 64 (2013) 321–328

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

A selective dopamine reuptake inhibitor improves prefrontal cortex-dependent cognitive function: Potential relevance to attention deficit hyperactivity disorder

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ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form
28 June 2012
Accepted 3 July 2012

Keywords:
ADHD
Prefrontal cortex
Cognition
Psychostimulants
Norepinephrine
Dopamine

ABSTRACT

Drugs used to treat attention deficit hyperactivity disorder (ADHD) improve prefrontal cortex (PFC)-dependent cognitive function. The majority of ADHD-related treatments act either as dual norepinephrine (NE) and dopamine (DA) reuptake inhibitors (psychostimulants) or selective NE reuptake inhibitors (SNRIs). Certain benztropine analogs act as highly selective DA reuptake inhibitors while lacking the reinforcing actions, and thus abuse potential, of psychostimulants. To assess the potential use of these compounds in the treatment of ADHD, we examined the effects of a well-characterized benztropine analog, AHN 2-005, on performance of rats in a PFC-dependent delayed-alternation task of spatial working memory. Similar to that seen with all drugs currently approved for ADHD, AHN 2-005 dose-dependently improved performance in this task. Clinically-relevant doses of psychostimulants and SNRIs elevate NE and DA preferentially in the PFC. Despite the selectivity of this compound for the DA transporter, additional microdialysis studies demonstrated that a cognition-enhancing dose of AHN 2-005 that lacked locomotor activating effects increased extracellular levels of both DA and NE in the PFC. AHN 2-005 produced a larger increase in extracellular DA in the nucleus accumbens, although the magnitude of this was well below that seen with motor activating doses of psychostimulants. Collectively, these observations suggest that benztropine analogs may be efficacious in the treatment of ADHD or other disorders associated with PFC dysfunction. These studies provide a strong rationale for future research focused on the neural mechanisms contributing to the cognition-enhancing actions and the potential clinical utility of AHN 2-005 and related compounds.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is conservatively estimated to affect 3%–5% of children and adults (Solanto, 2001; Wilens et al., 2004). Psychostimulants are currently the most effective treatment for ADHD (Greenhill, 2001). However, the abuse potential of these drugs raises significant concerns about their widespread use. Thus, there is a need for new drug treatments for ADHD that display comparable efficacy while lacking the abuse potential of psychostimulants.

Extensive studies demonstrate that ADHD-approved medications improve cognitive processes dependent on the prefrontal cortex (PFC), including working memory, planning, response inhibition and the regulation of impulsivity (Chamberlain et al., 2007; Diamond, 2005; Mehta et al., 2001; Turner et al., 2005). These observations are consistent with imaging data demonstrating ADHD is associated with PFC dysfunction (Castellanos and Tannock, 2002). Importantly, the cognition-enhancing actions of ADHD-related drugs are not limited to ADHD, with similar effects observed in both normal human and animal subjects (Arnsten and Dudley, 2005; Berridge et al., 2006; Devilbiss and Berridge, 2008; Elliott et al., 1997; Gamo et al., 2010; Mehta et al., 2001; Rapoport and Inoff-Germain, 2002). Collectively, these observations suggest that the clinical efficacy of drugs used in the treatment of ADHD involves, at least in part, an ability to improve PFC-dependent function.

Psychostimulants used in the treatment of ADHD (i.e. methylphenidate, amphetamine) act as non-selective catecholamine reuptake inhibitors (Berridge and Devilbiss, 2011). Additionally, selective norepinephrine reuptake inhibitors (SNRIs) are effective in the treatment of ADHD, though these drugs are typically viewed as less efficacious than psychostimulants (Berridge and Devilbiss, 2011). To date, selective DA reuptake inhibitors (SDRIs) have not been utilized in ADHD, largely due to a limited number of compounds that display selectivity for the DA transporter (DAT)

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http://dx.doi.org/10.1016/j.neuropharm.2012.07.005

while lacking the abuse potential of psychostimulants. However, a series of benztropine analogs has been described that display high selectivity and affinity for the DAT while lacking reinforcing effects in rodents and monkeys (Hiranita et al., 2009; Li et al., 2005; Woolverton et al., 2001, 2000). The behavioral and pharmacological profiles of these compounds suggest they may be efficacious in the treatment of ADHD while lacking significant abuse potential.

The behavioral and neurochemical actions of the benztropine analog, N-allyl-3α[bis(4fluorophenyl)methoxy]tropane (AHN 2-005), have been well-characterized. Prior work demonstrates that this compound displays high selectivity for the DAT relative to other transporters and receptors and lacks reinforcing effects as measured in conditioned place preference and self-administration paradigms at doses that produce robust increases in extracellular DA concentrations (Hiranita et al., 2009; Katz et al., 1999, 2004; Raje et al., 2005). To assess the potential use of AHN 2-005 in ADHD, we first examined the degree to which this compound improves PFC-dependent function of rats as measured in a delayed-response task of working memory. Importantly, the pharmacology of performance in this task aligns closely with the pharmacology of ADHD: all major classes of drugs used to treat ADHD (psychostimulants, SNRIs, α_2-agonists) improve performance in this task (Arnsten, 2009; Berridge and Devilbiss, 2011). Thus this task is a useful preclinical screen for ADHD-related compounds. In the current studies, AHN 2-005 dose-dependently improved performance in this task, comparable to that seen with ADHD-related drugs.

Available evidence indicates that clinically-relevant, cognition-enhancing doses of psychostimulants and SNRIs simultaneously and preferentially elevate extracellular NE and DA within the PFC (Berridge et al., 2006; Bymaster et al., 2002). This has been posited to reflect, in part, a prominent role of the NET in the clearance of DA within the PFC (Berridge and Devilbiss, 2011; Carboni et al., 2006; Yamamoto and Novotney, 1998). These and other observations indicate a pivotal role of PFC catecholamines in the cognition-enhancing/therapeutic actions of ADHD-related drugs (Arnsten, 2009; Arnsten and Dudley, 2005; Devilbiss and Berridge, 2008; Spencer et al., 2012). If NE and DA binding at the NE transporter (NET) in the PFC is competitive, elevations in extracellular DA are expected to elevate extracellular NE levels. To test whether this occurs with AHN 2-005, additional microdialysis studies examined the degree to which a cognition-enhancing dose of AHN 2-005 (10 mg/kg) simultaneously impacts extracellular DA and NE within the PFC, the nucleus accumbens and the medial septal area. Similar to that seen with clinically-relevant doses of psychostimulants and SNRIs, AHN 2-005 elicited moderate increases in extracellular levels of *both* DA and NE within the PFC and medial septal area, and modestly higher increases in extracellular levels of DA in the nucleus accumbens. These neurochemical effects were observed in the absence of locomotor activating effects, consistent with results of earlier studies (Li et al., 2005).

Collectively, these preclinical observations suggest that AHN 2-005 and other benztropine analogs may have utility in the treatment of ADHD and other conditions associated with PFC dysfunction.

2. Methods and material

2.1. Animals and surgery

Male Sprague–Dawley rats (260–280 g, Charles River, Wilmington, MA) were housed in pairs with *ad lib* access to food and water on an 11:13 h light:dark cycle (lights on 7:00 AM). For microdialysis studies, probes were surgically implanted under isoflurane anesthesia, as previously described (Berridge et al., 2006). All procedures were in accordance with NIH guidelines and were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

2.2. Spatial delayed alternation/working memory testing

Training and testing were similar to that used previously (Berridge et al., 2006; Devilbiss and Berridge, 2008). Briefly, animals were pair housed and placed on a restricted feeding schedule in which they were allowed to eat 15–25 g of standard chow immediately after each training/testing session. The quantity of food/chow was titrated for each animal to maintain motivation for food rewards (chocolate chips) while avoiding weight loss. The testing apparatus was a T-maze consisting of a runway (91 cm), and two arms (66 cm) perpendicular to the runway and placed at the end of the runway farthest away from the experimenter. The runway and arms were 10 cm in height and width. 20 cm from the end of the runway closest to the experimenter was an 18 cm tall removable gate that, when in position, created a start-box from which the animal could not enter the rest of the maze.

For this task, animals were rewarded (chocolate chip) when they entered the arm of the maze not chosen on the previous trial (10 trials per session, 1 session per day). Following each trial, the animal was placed in the start box for a delay period. Inter-trial delays were titrated for each animal to elicit performance accuracy in the range of 60–80%. If an animal exceeded this range, the delay was lengthened on the following testing day and baseline testing resumed. Stable performance was defined as two consecutive days in which performance did not differ by more than 10%. Accuracy of performance increases over time, necessitating periodic increases in delays to maintain performance level in the target range. Given the need for demonstrating stable baseline and the fact delays are periodically adjusted, animals received a treatment on average of once every two weeks. To ensure prominent PFC-dependency, delays were limited to 120-s. We have previously observed that with delays up to 80–120-s in length, temporary inactivation of the medial PFC of rats reduces performance to chance levels (unpublished observations, Spencer et al., 2012). Thus, even at 120-s delays this task is highly PFC-dependent. This range of delays is identical that that used in our previous studies documenting cognition-enhancing effects of methylphenidate in this task (Berridge et al., 2006). Given the 120-s cut-off for delays, not every animal received all treatments prior to reaching the 120-s cut-off.

Spatial cues were minimized by black plastic draping that surrounded the maze. All training and testing were conducted by a single individual. The maze was cleaned with 5% ethanol between animals. For a given animal, fecal boli and urine were removed/absorbed by a dry tissue prior to the start of the next trial. Intraperitoneal treatments were counter-balanced within and across animals and were administered 20-min prior to testing.

2.3. Microdialysis studies

On the day prior to testing, a microdialysis probe was inserted into one or two of the following regions: PFC (A+3.2; L0.8; V-5.2), the nucleus accumbens (A+1.7; L1.4; V-7.85), or the medial septal area (A+0.25; L1.05; V-6.5 at 6° from vertical) as described previously (Berridge et al., 2006; Berridge and Stalnaker, 2002). The last 0.5–1.0 mm of a dialysis probe contained an epoxy plug (corresponding to the ventral-most portion of the probe when implanted). The length of functional membrane was 4 mm for PFC, 3 mm for the medial septal area and 2 mm for the nucleus accumbens. This active membrane began immediately above the epoxy plug. Animals were housed in a Plexiglas testing chamber (32 × 32 × 40 cm) contained within a ventilated, sound-attenuating outer chamber for 1–2 days (see below). Artificial extracellular fluid (AECF: 147 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 2.5 mM KCl; pH 7.4) was perfused through the dialysis probe.

DA and NE were measured in dialysate samples using HPLC with electrochemical detection as previously described (Berridge and Stalnaker, 2002). Briefly, AECF was delivered at a rate of 1.5 μl/min through dialysis membrane (MW cut-off 13,000, o.d. 250 μm; Spectrum Labs, Rancho Dominguez, CA). 30-min samples were collected prior to and following vehicle or AHN 2-005 treatment. For the PFC and medial septal area, samples were split and analyzed for both DA and NE. 20 μl aliquots were injected onto an HPLC-EC system consisting of an ESA Model 582 pump set at 0.6 ml/min and an ESA 5100A Coulochem II detector with 2 electrodes in series: −.025V, +.220V (ESA Inc. Boston, MA). For DA, samples were injected onto a Velosep C18 100 × 3.2 mm column with a mobile phase consisting of: 200 mM sodium phosphate (pH 3.0–4.5), 0.1 mM EDTA, 0.3 mM sodium octyl-sulfate, and 5% v/v methanol. For NE, samples were injected onto an ion exchange column (ESA, MD-16, #70–7277) and the mobile phase consisted of 150 mM ammonium acetate (pH 6.0), 0.14 mM EDTA, 15% v/v methanol, and 5% acetonitrile. The quantitation limit for NE and DA (using a criterion of 3 times background noise) was approximately 0.3 pg. NE levels display robust elevations during quiet waking relative to sleep (Berridge and Stalnaker, 2002). To avoid potential arousal-state related increases in NE release, baseline samples were collected during periods when the animal was awake a majority of the time (this occasionally required gentle tapping on the chamber and/or leaving the outer chamber door ajar). An average baseline value was calculated from three 30-min baseline samples displaying no greater than 10% variation from the average value. The mean baseline concentration of NE per sample was 1.50 ± 0.12 pg within the PFC (n = 14) and 1.15 ± 0.15 pg within the medial septal area (n = 8). The mean baseline concentration of DA was