Neuroscience Research 69 (2011) 41–50

Contents lists available at ScienceDirect

Neuroscience Research

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

Sub-optimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and D-amphetamine, but unaffected by the COMT inhibitor tolcapone

Neil E. Paterson*, Jennifer Ricciardi, Caitlin Wetzler, Taleen Hanania

PsychoGenics, Inc., 765 Old Saw Mill River Rd., Tarrytown, NY 10591, United States

ARTICLE INFO

Article history:
Received 3 February 2010
Received in revised form 1 October 2010
Accepted 1 October 2010
Available online 8 October 2010

Keywords:
Tolcapone
5-CSRTT
Variable inter-trial interval
Impulsivity
Attention
Atomoxetine
Methylphenidate
D-Amphetamine
Rat

ABSTRACT

Prefrontal cortical dopamine (DA) and norepinephrine (NE) are implicated in multiple aspects of cognitive function assessed via the 5-choice serial reaction time task (5-CSRTT) in rodents. The present studies assessed the effects of the NE reuptake inhibitor atomoxetine (0.5–2.0 mg/kg), the mixed DA/NE reuptake inhibitor methylphenidate (0.1–2.0 mg/kg), the catecholamine releaser D-amphetamine (0.1–1.0 mg/kg) and the catecholamine-o-methyl-transferase (COMT) inhibitor tolcapone (3.0–30.0 mg/kg) in rats that exhibited sub-optimal performance (reduced accuracy: <70% correct) in the 5-CSRTT. Increased ITI durations were associated with increased premature responding. Decreased ITI durations resulted in increased percent omissions, increased perseverative responses and increased response latencies, but had no effects on magazine latencies or percent correct. Atomoxetine decreased premature responding at prolonged ITI durations and methylphenidate decreased percent omissions at low doses (0.1 and 0.5 mg/kg). By contrast, D-amphetamine increased premature and perseverative responding in a dose-dependent manner (0.3–1.0 mg/kg). Finally, tolcapone had no effects on sub-optimal performance in the variable ITI 5-CSRTT. These results suggest minimal potential of tolcapone as a therapeutic agent for ADHD and implicate cortical NE, not DA, in impulsive action.

1. Introduction

Impaired attention and increased impulsivity are characteristic of several disease states. The continuous performance test (CPT), used in humans, is capable of detecting attention and impulsivity deficits in a number of disorders, including attention deficit hyperactivity disorder (ADHD; Riccio et al., 2001), schizophrenia (Lee and Park, 2006) and mild cognitive impairment (Levinoff et al., 2006). The pre-clinical analogue of the CPT is the 5-choice serial reaction time task (5-CSRTT), which has been described in detail elsewhere (e.g., Bari et al., 2008). Alteration of certain test parameters [specifically the introduction of variable inter-trial intervals (vITI)] results in increased premature responding in the prolonged ITI trials (e.g., Bizarro et al., 2004; Day et al., 2007). This premature responding is hypothesized to provide a measure of impulsive action, i.e., a failure to withhold an inappropriate response, and has been shown to be sensitive to atomoxetine (Navarra et al., 2008) and the histamine H3 receptor antagonist ciproxifan (Day et al., 2007). The present studies utilized a vITI version of the 5-CSRTT to assess the effects of clinically efficacious ADHD medications and the catecholamine-O-methyl-transferase (COMT) inhibitor tolcapone.

The 5-CSRTT has been useful in the elucidation of the neurobiological substrates of attention and executive function (for review, see Robbins, 2002). The majority of studies utilize animals that perform at a relatively high level of accuracy (typically 75% correct responding). Nonetheless, many neuropsychiatric disorders are associated with decrements in attentional and executive function. Therefore, in the search for effective therapeutics, subjects that exhibit sub-optimal performance in the 5-CSRTT may be useful due to increased dynamic range of the test and the use of a sub-optimal performing population. Previously, the cognitive-enhancing properties of nicotine were observed in rats that exhibited sub-optimal, but not optimal, 5-CSRTT performance (Grottick et al., 2003; Semenova et al., 2007). Interestingly, dopamine D1 receptor activation in the PFC was shown to enhance accuracy in rats that performed poorly in the 5-CSRTT (Granon et al., 2000). Therefore, the present studies compared the effects of atomoxetine, methylphenidate and D-amphetamine in rats that exhibited sub-optimal performance levels in the vITI 5-CSRTT.

Atomoxetine, a NET inhibitor (Wong et al., 1982), attenuates impulsivity and enhances attention in clinical (Michelson et al., 2003; Chamberlain et al., 2007) and preclinical (Blondeau and

* Corresponding author at: Behavioral Pharmacology, PsychoGenics, Inc., 765 Old Saw Mill River Rd., Tarrytown, NY 10591, United States. Tel.: +1 914 406 8058; fax: +1 914 406 8090.
E-mail address: Neil.Paterson@PsychoGenics.com (N.E. Paterson).

0168-0102/$ – see front matter © 2010 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2010.10.001

Dellu-Hagedorn, 2007; Navarra et al., 2008; Robinson et al., 2008) studies. The pro-cognitive effects of atomoxetine may be due to enhanced cortical noradrenergic neurotransmission (Bymaster et al., 2002; Swanson et al., 2006). Nonetheless, atomoxetine also stimulates DA overflow in the prefrontal cortex (Bymaster et al., 2002; Swanson et al., 2006), due to the role of the NE transporter in regulating DA overflow in the PFC (Carboni et al., 1990; Gresch et al., 1995; Yamamoto and Novotney, 1998). Thus, it is currently unclear whether the effects of atomoxetine on impulsive action are mediated via enhanced cortical DA, NE or both. The mixed DA/NE reuptake inhibitor methylphenidate (Han and Gu, 2006) increased NE and DA overflow in the PFC (Bymaster et al., 2002) and improved performance in multiple components of cognitive function including spatial and reference memory, and attention (Kantak et al., 2008; Dow-Edwards et al., 2008). Finally, D-amphetamine induces the pre-synaptic release of monoamines (Holmes and Rutledge, 1976); although D-amphetamine can inhibit multiple forms of impulsivity in human testing (de Wit et al., 2002), preclinical studies indicated that D-amphetamine has a dis-inhibitory effect on operant responding, with increased premature and perseverative responses in the 5-CSRTT (Cole and Robbins, 1987; Harrison et al., 1997; van Gaalen et al., 2006; Higgins et al., 2007). Current ADHD medications include Adderall®, a mixture of amphetamine salts. Due to the multiple mechanisms of action of all of these compounds, it is currently unclear whether PFC DA and NE play separable roles in attention and impulsive action in the 5-CSRTT, respectively (for review, see Arnsten, 2009).

Tolcapone inhibits catecholamine-O-methyl-transferase (COMT), the enzyme responsible for catecholamine metabolism in the central nervous system (Zürcher et al., 1990) and implicated in regulation of DA, but not NE, overflow in the PFC (Gogos et al., 1998; Tunbridge et al., 2004). Administration of tolcapone had no effects on basal DA efflux, but significantly enhanced increased dopamine overflow after pharmacological challenge (Acquas et al., 1992; Napolitano et al., 2003; Tunbridge et al., 2004), physiological challenge (Tunbridge et al., 2004) or food reward (Lapish et al., 2009). Further, tolcapone enhanced performance in PFC dopamine-dependent behavioral tasks (Lapish et al., 2009; Tunbridge et al., 2004). Based on the selective effect of tolcapone on cortical dopamine versus norepinephrine, and the suggested involvement of cortical DA versus NE in attention and impulsive action, it was hypothesized that tolcapone would enhance accuracy but would not attenuate premature responding in the vITI 5-CSRTT.

In summary, the present studies aimed to compare the effects of the clinically effective ADHD treatments atomoxetine, methylphenidate and D-amphetamine against the COMT inhibitor tolcapone on impulsive action and attentional performance in sub-optimal rats in the variable ITI 5-CSRTT procedure.

2. Methods and materials

2.1. Subjects

Male Long-Evans rats (275–300 g) were obtained from Harlan Laboratories (Indianapolis, IN). Upon arrival, the rats were assigned unique identification numbers (tail marked). Rats were single-housed in OptiRAT cages and acclimated for 7 days prior to commencing a food-restriction regimen: rats were held at 85% of age-matched free-feeding control body-weights, receiving approximately 10–20 g of rat chow daily. Water was provided ad libitum, except during testing. Animals were maintained in a 12/12 h light/dark cycle (lights on at 07:00 EST) with room temperature maintained at $22 \pm 2\,^{\circ}C$ and the relative humidity maintained at approximately 50%. All animals were examined, handled and weighed prior to initiation of the study to assure adequate health and suitability and to minimize non-specific stress associated with testing. All efforts were made to minimize discomfort of any sort at all times during the conduct of the studies. The 5-CSRTT sessions were performed during the animal's light cycle phase. All experiments were approved by the Institutional Animal Care and Use Committee of PsychoGenics, Inc. in AALAC-accredited facilities, and in accordance with the Guide to the Care and Use of Laboratory Animals (NIH, 1996).

2.2. Apparatus

The apparatus consisted of 10 aluminum and Plexiglas chambers with grid floors (width 31.5 cm, depth 25.0 cm, and height 33.0 cm), housed in sound-attenuating cabinets (Med Associates, St. Albans, VT). Each cabinet is fitted with a low-level noise extractor fan. The left wall of each chamber was concavely curved with 5 apertures evenly spaced, located approximately 2.5 cm from the floor. Each aperture contained a standard 3W LED to serve as stimulus lights. The opposite wall contained a food magazine, located approximately 3.0 cm from the floor. Each chamber was illuminated with a 3 W house-light located in the center of the ceiling panel. After each test session the apparatus was cleaned with 70% ethanol. The apparatus was controlled, and all data were captured, by a computer using K-Limbic software (Conclusive Software Ltd., distributed by Med Associates, St. Albans, VT).

2.3. Experimental procedure

2.3.1. Training

Animals were trained to monitor the five apertures for stimulus light illumination. Each session is initiated by the illumination of the house light, and the delivery of a food reward into the magazine. The first trial begins when the rat opens the magazine to obtain the food pellet. After the inter-trial interval (ITI) one of the stimulus lights is illuminated for 500 ms. The rat must nose-poke in the illuminated aperture either during or within 5 s of stimulus light illumination. Such a response is defined as a correct response, and is rewarded with delivery of a food pellet. Collection of the pellet initiates the next trial. A nose-poke response in a non-illuminated aperture (incorrect response) or a nose-poke after the 5 s limited hold (missed trial) results in termination of the trial with extinction of the house-light and imposition of a time-out period. Nose-pokes made during the inter-trial interval (premature responses) and nose-pokes made after the first response to the stimulus presentation (perseverative response) were not punished by imposition of a time-out, but were counted. Rats took 40–60 sessions to acquire the task.

2.3.2. Testing

After acquisition of the 5-CSRTT (less than 75% correct and at least 50 trials completed per session for 5 consecutive sessions), drug testing began. The average percent accuracy across all studies was $64.0 \pm 0.9\%$. From the total pool of trained rats, approximately 25% of subjects fit the criteria for sub-optimal performance. During variable ITI test sessions, the ITI was 10, 7, 5 or 4 s in duration (presented in banks of 4 trials; each ITI duration was presented once in a randomized order within each bank) for 60 min. All rats received all drug treatments, according to a randomized-order, counterbalanced and within-subjects design. Drug tests were performed on Wednesdays and Fridays of each week, only when performance was less than 75% correct for a minimum of 50 trials completed in the previous test session.