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Effect of methylphenidate treatment during adolescence on norepinephrine transporter function in orbitofrontal cortex in a rat model of attention deficit hyperactivity disorder

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HIGHLIGHTS

- Methylphenidate (MPH) reduces ADHD by inhibiting norepinephrine transporter (NET).
- Long-term effects of MPH on NET function in orbitofrontal cortex (OFC) are unknown.
- NET function was greater in OFC of a rat model of ADHD compared to non-ADHD control.
- MPH during adolescence normalized NET function in ADHD model during adulthood.
- Thus, some of the therapeutic action of MPH persists long after treatment cessation.

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is associated with hypofunctional medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC). Methylphenidate (MPH) remediates ADHD, in part, by inhibiting the norepinephrine transporter (NET). MPH also reduces ADHD-like symptoms in spontaneously hypertensive rats (SHRs), a model of ADHD. However, effects of chronic MPH treatment on NET function in mPFC and OFC in SHR have not been reported. In the current study, long-term effects of repeated treatment with a therapeutically relevant oral dose of MPH during adolescence on NET function in subregions of mPFC (cingulate gyrus, prelimbic cortex and infralimbic cortex) and in the OFC of adult SHR, Wistar-Kyoto (WKY, inbred control) and Wistar (WIS, outbred control) rats were determined using in vivo voltammetry. Following local ejection of norepinephrine (NE), uptake rate was determined as peak amplitude (A_{max}) × first-order rate constant (k_{-1}). In mPFC subregions, no strain or treatment effects were found in NE uptake rate. In OFC, NE uptake rate in vehicle-treated adult SHR was greater than in adult WKY and WIS administered vehicle. MPH treatment during adolescence normalized NE uptake rate in OFC in SHR. Thus, the current study implicates increased NET function in OFC as an underlying mechanism for reduced noradrenergic transmission in OFC, and consequently, the behavioral deficits associated with ADHD. MPH treatment during adolescence normalized NET function in OFC in adulthood, suggesting that the therapeutic action of MPH persists long after treatment cessation and may contribute to lasting reductions in deficits associated with ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent neurobehavioral disorders, affecting ~12–15% of children worldwide and persisting into adulthood in ~4–5% of individuals (Froehlich et al., 2007; Polanczyk et al., 2007). ADHD etiology, although multigenetic (Faraone et al., 2005; Gizer et al., 2009; Kuntsi and Klein, 2012), has been associated with polymorphisms of the norepinephrine transporter (NET) gene (Barr et al., 2002; Kollins et al., 2008; McEvoy et al., 2002). However, altered noradrenergic function in ADHD has not been verified in clinical studies (Zimmer, 2009), in part, due to the paucity of suitable radioligands probing NET for use in positron emission tomography studies (Ding et al., 2006). Importantly, compelling evidence supporting the involvement of the noradrenergic system in ADHD pathology comes from the common mechanism of action

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of all of the currently available FDA-approved therapeutics for ADHD. ADHD medications increase noradrenergic neurotransmission either via inhibition of NET function (e.g., methylphenidate; MPH, amphetamine and atomoxetine) or by activation of postsynaptic α2A adrenergic receptors (e.g., guanfacine) (Berridge et al., 2006; Bymaster et al., 2002; Fernando et al., 2012; Ma et al., 2005). Thus, ADHD pathology is strongly connected to deficits in noradrenergic neurotransmission and the efficacy of ADHD therapeutics is associated with increased noradrenergic transmission (Levy, 2009).

The prefrontal cortex has been associated with the hallmark symptoms of ADHD and has been linked to the beneficial effects of ADHD therapeutics in preclinical models (Arnsten, 2009; Berridge and Devilbiss, 2011). For example, administration of a therapeutically relevant low dose of MPH (2.0 mg/kg, i.p.) in rats produced positive blood oxygen dependent signals in the orbitofrontal cortex (OFC), a subregion of prefrontal cortex (Easton et al., 2009; Kuczenski and Segal, 2002). Inhibition of α2 adrenergic receptors by local administration of yohimbine into the dorsolateral prefrontal cortex of primates resulted in impaired spatial working memory, increased impulsivity and increased locomotor hyperactivity, thus resulting in behaviors consistent with an ADHD profile (Li and Mei, 1994; Ma et al., 2003, 2005) and suggesting a role for noradrenergic neurotransmission in prefrontal cortex in ADHD pathology.

Effects of NET inhibition by ADHD therapeutics on prefrontal cortex function have been elaborated also using preclinical models. MPH is an equipotent inhibitor of NET and dopamine transporter (DAT), with Ki values for inhibition of [^{3}H]dopamine and [^{3}H]norepinephrine (NE) uptake of 160 nM and 40 nM, respectively (Easton et al., 2007b; Richelson and Pfenning, 1984). At therapeutically relevant doses (0.5–2 mg/kg, i.p.), MPH inhibits NET function and preferentially increases extracellular NE in prefrontal cortex compared to subcortical areas, as well as compared to dopamine in prefrontal cortex (Berridge et al., 2006). Increasing extracellular NE activates α2A receptors and decreases the concentrations of the intracellular second messenger, cyclic adenosine monophosphate (cAMP), thereby enhancing the strength and duration of firing of pyramidal neurons in prefrontal cortex (Wang et al., 2007). Enhanced pyramidal neuronal firing is hypothesized to be the mechanism by which ADHD medications improve attention and working memory and reduce impulsivity (Sagvolden, 2006; Wang et al., 2007). MPH indirectly increases the activation of cortical α2A adrenergic receptors in non-human primates and rats (Arnsten, 2009; Gamo et al., 2010). Thus, enhancing noradrenergic neurotransmission in the prefrontal cortex via inhibition of NET function is critical for the therapeutic action of MPH. However, few studies have investigated the long-term consequences of MPH treatment during adolescence on NET function during adulthood.

The spontaneously hypertensive rat (SHR) is a well-established model of ADHD that exhibits several of the behavioral and neurochemical features of ADHD (de Villiers et al., 1995; Oades et al., 2005; Russell et al., 1995; Sagvolden, 2011; Sagvolden et al., 2005). In SHR, noradrenergic systems in the locus coeruleus and hypothalamus are inadequately regulated by α2A autoreceptors and heterologous glutamatergic receptors, resulting in enhanced NE release (de Villiers et al., 1995; Howells and Russell, 2008; Russell et al., 2005). Importantly, α2A auto-receptor mediated regulation of NE release is decreased in prefrontal cortical slices from SHR (Russell et al., 2000). Notably, alterations in NET function in the prefrontal cortex of SHR have not been reported.

Previous studies in SHR found that MPH treatment during adolescence produces lasting changes in DAT function in prefrontal cortex as well as in its subregion, the medial prefrontal cortex (mPFC; Harvey et al., 2011; Somkuwar et al., 2013). Specifically, DAT function was increased in mPFC of MPH-treated SHR compared to vehicle-treated SHR as well as compared to MPH-treated Wistar Kyoto rats (WKY, inbred control) and Wistar rats (WIS, outbred control). The aim of the current study was to evaluate in vivo NET function in subregions of prefrontal cortex, including the cingulate gyrus (CG), prelimbic cortex (PrL), infralimbic cortex (IL) and lateral orbitofrontal cortex (LO). We chose to separately target the CG, PrL and the IL cortex subregions of mPFC, because these regions have distinct intracortical and subcortical connections (Heidbreder and Groenewegen, 2003; Hoover and Vertes, 2007; Vertes, 2006). Specifically, the CG and the dorsal PrL are thought to project to motor and sensory cortices, while the ventral PrL and IL project to limbic and associative areas. As such, these individual subregions may differentially influence ADHD pathology (Easton et al., 2007a). Also, the current study describes a novel method for isolating NET function in prefrontal cortex using an in vivo voltammetry approach. Differences were revealed between SHR and the WKY and WIS controls following administration of MPH or vehicle throughout adolescence on NET function during adulthood.

2. Materials and methods

2.1. Drugs and reagents

(±)-MPH was purchased from Sigma-Aldrich Corporation (St. Louis, MO). MPH was dissolved in water to obtain a concentration of 1.5 mg/ml (prepared daily) and injected into oyster crackers (Kantak et al., 2008) to attain a dose of 1.5 mg/kg for oral administration. (±)-Norepinephrine (+)-bitartarate (NE), GBR12909 (1-2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride), ascorbic acid, perchloric acid (70%), sodium chloride and Nafion perfluorinated ion-exchange resin (5% solution) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Sodium phosphate dibasic and monobasic were purchased from Fischer Scientific (Fair Lawn, NJ). Urethane and sticky wax were purchased from Sigma Life-Sciences (St. Louis, MO) and FDJ/On time LLC (Winter Park, FL), respectively.

2.2. Animals

For optimization of the NE clearance assay, male and female adult Sprague-Dawley rats were obtained from Harlan Laboratories Inc. (Indianapolis, IN). Some of the rats used for optimization experiments were drug and experimentally naïve and others experienced operant or Pavlovian conditioning, including acute treatment with morphine (10 mg/kg or 45 mg/kg, i.p.). Consequently, rats that underwent conditioning experienced greater handling and enrichment compared to the experimentally naïve rats. Although optimization experiments to identify the effect of DAT inhibition on NE clearance in PFC were conducted 1–2 weeks after treatment, large variability in the data may have been a consequence of the variation in treatment history.

For experiments investigating the effect of MPH treatment during adolescence, naive male SHR, WKY and WIS rats were obtained at postnatal day (P) 25 from Charles River Laboratories/US facilities (Kingston, NY or Raleigh, NC). Vendor location is a critical to ensure homogeneity with respect to ADHD (SHR) and non-ADHD (WKY and WIS) phenotypes. WKY from Charles River/GER have been characterized as a model for the predominantly inattentive subtype of ADHD (Sagvolden et al., 2009), and have a large number of discordant SNP genotypes (35%) relative to WKY from Charles River/US and Harlan/UK, which have only 2.5% discordant SNP genotypes (Zhang-James et al., 2013). Further, WKY from Harlan/UK is a well-recognized control for the SHR ADHD phenotype (Sagvolden et al., 2009), supporting the use of WKY from Charles River/US as the inbred control for the current studies.

All rats were individually housed with free access to food and water (unless specified otherwise) in a colony room maintained on