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The α_{2C}-adrenergic receptor mediates hyperactivity of *coloboma* mice, a model of attention deficit hyperactivity disorder

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Drugs that modify noradrenergic transmission such as atomoxetine and clonidine are increasingly prescribed for the treatment of attention deficit hyperactivity disorder (ADHD). However, the therapeutic targets of these compounds are unknown. Norepinephrine is also implicated in the hyperactivity exhibited by *coloboma* mice. To identify the receptor subtypes that regulate the hyperactivity, *coloboma* mice were systematically challenged with adrenergic drugs. The β-adrenergic receptor antagonist propranolol and the α_1-adrenergic receptor antagonist prazosin each had little effect on the hyperactivity. Conversely, the α_2-adrenergic receptor antagonist yohimbine reduced the activity of *coloboma* mice but not control mice. Subtype-selective blockade of α_{2C}-, but not α_{2A}- or α_{2B}-adrenergic receptors, ameliorated hyperactivity of *coloboma* mice without affecting activity of control mice, suggesting that α_{2C}-adrenergic receptors mediate the hyperactivity. Localized in the basal ganglia, α_{2C}-adrenergic receptors are in a prime position to impact locomotor activity and are, therefore, potential targets of pharmacotherapy for ADHD.

Keywords: Locomotor activity; *coloboma* mouse; Attention deficit hyperactivity disorder; Norepinephrine; α_{2C}-adrenergic receptor; β-adrenergic receptor; Dose–response; MK 912; Yohimbine; Clonidine

Introduction

Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, inattention, and impulsivity and is a common pediatric neuropsychiatric disorder (Olfson, 1992; Faraone et al., 2003; CDC, 2005). The stimulants amphetamine and methylphenidate, which are indirect agonists that increase extracellular monoamine concentrations by inhibiting reuptake and/or promoting release, are the primary treatments for ADHD (Robison et al., 1999). Amphetamine benefits some patients, methylphenidate benefits others, and still others respond to both or neither medication (Winsberg et al., 1974; Arnold et al., 1978). It is not understood how stimulants ameliorate symptoms of some patients or why subpopulations of patients respond differently to stimulants. A better understanding of the mechanisms underlying ADHD would resolve these questions.

The efficacy of stimulants suggests that dopaminergic and/or noradrenergic dysregulation contribute to the expression of ADHD. In support of this assertion, genetic association studies implicate molecules that regulate both dopaminergic and noradrenergic neurotransmission, including receptors and transporters (Cook et al., 1995; Comings et al., 1996, 1999, 2000; Daly et al., 1999; Faraone et al., 2001; Holmes et al., 2002; Maher et al., 2002; Grady et al., 2003; Roman et al., 2003; Kustanovich et al., 2004). Further, in several different studies assessing catecholamine utilization, both the dopamine metabolite homovanillic acid (HVA) and the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) are correlated with the behavioral signs of ADHD in children. For example, ADHD patients display a positive correlation between severity of hyperactivity and HVA concentration in cerebrospinal fluid (Castellanos et al., 1994, 1996), and between scores on the Test of Variables of Attention (a standardized reaction time test that measures sustained attention and impulsivity) and norepinephrine metabolites in urine (Llorente et al., 2006). Diversity in psychostimulant response, genetic polymorphisms, and neurochemical abnormalities suggests that ADHD is not attributable to any single pathophysiologic mechanism. Consequently, it is unlikely that a universal treatment will ameliorate symptoms of all ADHD patients. In the absence of specific therapeutic targets, the treatment strategy progresses from stimulants to nonstimulant drugs that increase synaptic norepinephrine (reuptake inhibitors), to drugs that are thought to decrease noradrenergic transmission (clonidine, guanfacine, or propranolol—alone or in combination with stimulants) (Silver, 1999; Pliszka, 2003).

Coloboma mice may be a useful tool for developing rational therapeutic strategies for ADHD. These mice exhibit spontaneous hyperactivity caused by a semidominant deletion mutation that includes the *Snap25* gene. This mutation results in a 50% reduction in the expression of the protein SNAP-25 (Hess et al., 1992). SNAP-25 concentrates in presynaptic terminals and is expressed in

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neurons throughout the brain, with the highest levels of expression found in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra, and cerebellar granule cells (Oyler et al., 1989). SNAP-25 is associated with the plasma membrane of axon terminals and has been identified as a component of the machinery essential for docking synaptic vesicles at the presynaptic membrane in readiness for Ca^{2+}-triggered neurotransmitter exocytosis (Sollner et al., 1993a,b). Important for this work, several independent research groups identify an association between the *SNAP25* gene and ADHD in humans (Barr et al., 2000; Brophy et al., 2002; Mill et al., 2002; Kustanovich et al., 2003). Also similar to ADHD patients, amphetamine ameliorates hyperactivity of *coloboma* mice (Hess et al., 1996), suggesting a relationship between abnormal catecholamine regulation and hyperactivity in these mice. Indeed, brain norepinephrine concentrations are increased in *coloboma* mice compared to control littermates, and depletion of norepinephrine ameliorates hyperactivity of *coloboma* mice (Jones et al., 2001; Jones and Hess, 2003), suggesting that dysregulation of norepinephrine contributes to the *coloboma* mouse phenotype. To determine the adrenergic receptor subtypes that regulate locomotor hyperactivity in *coloboma* mice, we systematically tested the effects of noradrenergic compounds on locomotor activity of *coloboma* mice, beginning with nonselective drugs and progressing to increasingly selective compounds.

Materials and methods

Mice

Coloboma (*Cm*/+) mice and control (+/+) C3H/HeSnJ mice (Jackson Laboratories, Bar Harbor, Maine) were bred and housed in group cages (2–4 mice/cage) with corncob bedding at Johns Hopkins University vivarium (lights on at 7 a.m. and off at 9 p.m.). Mutants and controls were age- and sex-matched male and female mice; most mutant and control pairs were littermates. Water and standard laboratory rodent food (2018SX; Harlan Teklad, Madison, WI) were available ad libitum throughout all experiments. Experiments were performed in compliance with The Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University.

Hyperactivity of coloboma mice is obvious when they are 2 weeks of age and continues into adulthood (Hess et al., 1992; Heyser et al., 1995); the degree of hyperactivity exhibited by each coloboma mouse is stable throughout life. Therefore, mice were included in this study when they reached 2 months of age (adulthood) and participated in the study for up to 10 months.

Locomotor activity

Automated photocell activity cages (29.2 × 50.5 cm) with 12 2-cm-high infrared beam detectors arranged in a 4 × 8 grid were used to measure locomotor activity (San Diego Instruments, San Diego, CA, USA). Changes in beam status were assessed 18 times per second, and beam breaks were recorded and compiled every 10 min. Mice were habituated to locomotor activity chambers containing corncob bedding, food and water for ≥4 h prior to testing.

Drug challenge

There is considerable variability in the locomotor activity of *coloboma* mice. This arises from inter-animal variability, likely due to the variability in the penetrance of this semidominant mutation. In contrast, the locomotor activity of individual mice is quite stable and consistent across test sessions. Because intra-animal variability is very low, mice are tested in paired or repeated measures designs. Mice were tested in a dose–response paradigm with doses, including vehicle, administered in a pseudorandom order and a ≥2-day drug holiday between doses. Saline or vehicle was administered as part of the dose–response paradigm for each drug. Mice were injected with 10 mL/kg drug or vehicle approximately 45–60 min after the start of the dark cycle. The effect of drug on activity is expressed as a percentage of baseline (vehicle) beam breaks over 1 h, beginning immediately after injection. In some cases, data are also expressed as fine and ambulatory movements, a detection feature of the San Diego Instruments software. Fine movements are defined as repetitive interruptions of a single beam, whereas ambulatory movements represent larger movements that require the sequential interruption of 2 adjacent beams.

Drugs and doses

Doses and routes of administration for all drugs (Table 1) were determined prior to the start of the test based on the response of normal mice. Drug doses that caused any unusual behavioral effects–such as seizures, abnormal posturing, or obvious sedation–were excluded. Dose ranges for many drugs were already defined and determined to be behaviorally relevant based on our previous work in mice (Fureman and Hess, 2005).

The following drugs were purchased from Sigma (St. Louis, MO, USA): Clonidine HCl, Guanabenz acetate, Guanfacine HCl, Phentolamine HCl, Prazosin HCl, BRL 44408 maleate, MK 912 HCl, Propranolol HCl, and Desipramine HCl. The following drugs were purchased from Tocris (Ellisville, MO, USA): Yohimbine HCl, ARC 239 HCl_2, and Atomoxetine HCl. Saline served as

Table 1
Noradrenergic agents used in this study

Drug	Dose	Pharmacology	Site of action
Phentolamine	0.5–10 mg/kg, s.c.	Antagonist (nonselective)	α-adrenergic receptors
Propranolol	1–10 mg/kg, s.c.	Antagonist (nonselective)	β-adrenergic receptors
Prazosin	0.5–8 mg/kg, i.p.	Antagonist	α_1-adrenergic receptors
Yohimbine	0.5–6 mg/kg, s.c.	Antagonist	α_2-adrenergic receptors
BRL 44408	0.25–5 mg/kg, i.p.	Antagonist	α_{2A}-adrenergic receptors
ARC 239	1–20 mg/kg, i.p.	Antagonist	α_{2B}-adrenergic receptors
MK 912	0.1–3 mg/kg, i.p.	Antagonist	α_{2C}-adrenergic receptors
Clonidine	12.5–200 μg/kg, s.c.	Agonist (nonselective)	$\alpha_{1/2}$-adrenergic receptors
Guanfacine	50–1000 μg/kg, s.c.	Agonist	$\alpha_{2A/B/C}$-adrenergic receptors
Guanabenz	5–100 μg/kg, s.c.	Agonist	α_{2A}-adrenergic receptors
Desipramine	1–30 mg/kg, i.p.	Antagonist	Norepinephrine transporter
Atomoxetine	1–20 mg/kg, i.p.	Antagonist	Norepinephrine transporter