Genetics of Childhood Disorders: XXIV. ADHD, Part 8: Hyperdopaminergic Mice as an Animal Model of ADHD

RAUL R. GAINETDINOV, M.D., PH.D., AND MARC G. CARON, PH.D.

In 1937, Charles Bradley first observed that Benzedrine (amphetamine) induces a calming effect when used in hyperactive children. It is still not known how these drugs work to increase alertness in normal individuals as well as distractible and overactive children. Numerous studies have shown that psychostimulants exert their pharmacological actions through interaction with plasma membrane monoamine transporters, namely, the dopamine, serotonin, and norepinephrine transporters. The normal function of these transporters is to provide rapid removal of neurotransmitters from the synaptic cleft to permit repetitive synaptic firing. Inhibition of the transporter would prevent the normal reuptake and would lead to markedly elevated extracellular monoamine levels.

Because dopamine has been strongly implicated in the control of locomotion, particular attention has been given to the interaction of these drugs with the dopamine transporter (DAT). The elevation of extracellular dopamine levels is believed to be the primary mechanism by which psychostimulants are able to regulate locomotion. This concept, as well as the proven therapeutic efficacy of psychostimulants in attention-deficit/hyperactivity disorder (ADHD) patients, has provided the basis for the hypodopaminergic hypothesis of the disorder and suggested a possible connection between DAT and ADHD.

Many attempts to clarify the status of the dopamine system in patients with ADHD have been largely unsuccessful. This is generally thought to be due to the fact that many of the current approaches in clinical research, such as the analysis of plasma, urine, cerebrospinal fluid, or computer imaging techniques, provide equivocal estimates of the extracellular levels of dopamine in the major motor areas of the human brain. The hypodopaminergic hypothesis of ADHD, therefore, remains a theoretical concept inferred from an oversimplified understanding of the mechanism of action of psychostimulants in normal subjects.

The hypothesized connection between DAT function and ADHD has continued to spark considerable interest. There has been a reported association between a polymorphism in the DAT gene and ADHD. Specifically, a significant association was found by Cook and associates between ADHD and the 480-bp DAT1 allele (48-bp repeat sequence). Other groups of researchers later confirmed these observations in additional cohorts of patients and concluded that the 480-bp allele of DAT1 is preferentially transmitted to ADHD probands. More recently, four different analytical strategies were used to examine the association and linkage of the DAT gene and ADHD in children. Waldman and associates replicated

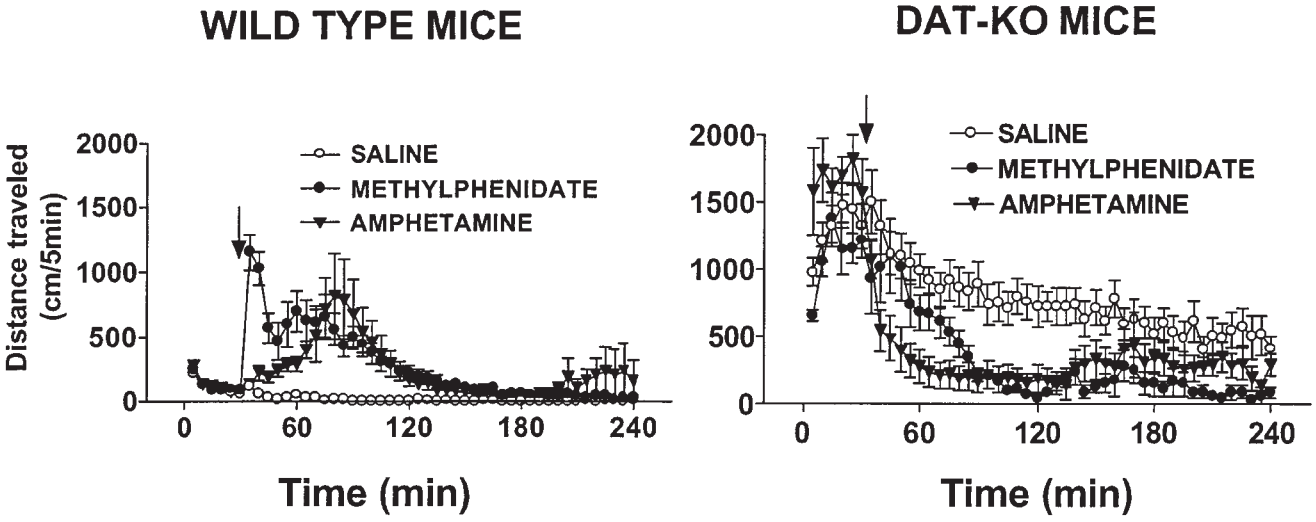

Fig. 1 Calming effect of amphetamine (2 mg/kg) and methylphenidate (30 mg/kg) on the hyperactivity of dopamine transporter knockout (DAT-KO) mice (right panel). Note that wild type mice were activated by these drugs (left panel). Adapted with permission from Gainetdinov et al., *Science* 283:397–401. Copyright 1999, American Association for the Advancement of Science.

previous findings demonstrating the 480-bp allele as the "high-risk" allele. Moreover, the relationship of the DAT1 allele to ADHD increased as symptom severity increased within the hyperactive-impulsive spectrum. Of considerable interest are the recent studies that have suggested that homozygosity for the 10-repeat allele of the dopamine transporter gene may be associated with poor response to methylphenidate. Taken together, these molecular genetic studies provide provocative evidence that alterations in DAT-mediated processes could significantly contribute to the pathogenesis of this disorder.

However, the functional consequences of this association remain unclear. It is well established that enhanced dopaminergic transmission in the basal ganglia translates into elevated energy, hyperactivity, and even euphoria. Numerous pharmacological studies with drugs that enhance or reduce dopaminergic transmission have shown that hyperactivity is a behavior of increased, not decreased, dopaminergic tone.

Additional evidence for the positive role of dopamine in hyperactivity comes from the development of mice with genetic inactivation of the dopamine transporter. Genetically engineered "knockout" mice are becoming increasing important in neuroscience as a tool to study the possible functions of a gene under study. The typical procedure is to produce a mutation in the gene in the laboratory. The mutation is one that typically renders the gene product inactive. A series of steps is then performed to introduce the inactivated gene back into the mouse.

The reintroduction of the inactivated gene is typically performed by microinjecting the mutated gene into embryonic stem (ES) cells. These are cells that can be easily grown and manipulated in culture dishes in the laboratory. Homologous recombination is a normal event that occurs in gametes when both copies of chromosomes line up next to each other and exchange genetic material between the paternal and the maternal chromosome. The mutated gene that has been injected into the ES cells will now be able to align itself with the chromosomal region that contains the normal copy and can be exchanged with it after the homologous recombination event.

ES cells that have had this rare chromosomal rearrangement can be selected to grow preferentially in the culture dish. This is possible because a second gene was included with the injected DNA. The second gene encodes a protein that conveys resistance to specific antibiotics. Normally, ES are killed by the antibiotic neomycin. Thus one grows the injected ES cells in the presence of neomycin. Only those ES cells that have had the rare recombination event will be able to grow. DNA is extracted from some of these cells to confirm the presence of the mutated gene. These cells are then introduced into the uteri of mice. The offspring, the so-called F1 generation, should now have one normal and one inactive copy of the gene. Crosses are then made between the F1 mice, and approximately one quarter of this next generation will have two copies of the inactivated gene.

Genetically engineered "knockout" mice that lack the gene encoding the dopamine transporter (DAT-KO mice) have become available. These mice demonstrate remarkable hyperactivity. This is thought to be due to the greater than 5-fold elevation of extracellular dopamine levels within the striatum, the major motor area of the brain. It is worth mentioning that the knockout mice behave essentially like their normal littermates when placed in a familiar environment. However, when place in a novel environment, the mice become much more active. Importantly, no corresponding rise in dopamine accompanies exposure to the novel environment, suggesting that these behavioral changes are regulated through more than just the dopamine system. These mice also show significant cognitive impairment in an eight-arm radial maze test, a standard approach to evaluate spatial cognitive function in rodents. Specifically, the mutant animals make significantly more perseverative errors, suggesting that these mice might suffer from poor behavioral inhibition.

An obvious question is whether the drugs commonly used in the treatment of attentional difficulties have any effect on these mice. Administration of amphetamine (Adderall®, Dexedrine®), methylphenidate (Ritalin®), or cocaine to the hyperactive knockout mice calms them; thus they show a response to psychostimulants that is similar to the response seen in humans with ADHD. Conversely, normal mice become hyperactive when given these psychostimulants (Fig. 1).

Normal mice show an expected increase in extracellular dopamine brain levels in response to an injection of methylphenidate, which blocks the dopamine transporter. Of interest, the knockout mice do not show any changes. These findings strongly suggest that psychostimulants do not affect the dopamine system in these mice and most likely exert their calming effects through modulation of other neurotransmitters targeted by these drugs.

To test this hypothesis, a selective inhibitor of the norepinephrine transporter, nisoxetine, was used. That it had no effect on the hyperactivity of the knockout mice suggests that norepinephrine is not likely to be involved in the mechanism by which the psychostimulants act. In contrast, an inhibitor of serotonin reuptake, fluoxetine (Prozac®), causes a dramatic reduction in hyperactivity, as do other drugs that either directly activate serotonin receptors (serotonin receptor agonist quipazine) or increase brain serotonin levels, such as precursors of serotonin (tryptophan and 5-hydroxytryptophan). These results suggest that hyperactivity induced by high levels of dopamine can be dampened by enhancing serotonergic tone. Accordingly, the psychostimulants are likely to modulate behavior in the mutant mice by enhancing the calming effects of serotonin rather than by acting directly on dopamine reuptake, because there are no functional DAT in these mice.

While this is not the first study to implicate serotonin in impulse regulation and inhibitory control on external stimuli-

induced behavioral activation, most researchers have long assumed that the primary action of psychostimulants like Ritalin was through the dopamine system. However, attempts to understand the calming effect of psychostimulants exclusively through the dopaminergic theory have been largely unsuccessful. We hypothesize that, at least in these mice, interaction of psychostimulants with the serotonin transporter may provide enhanced serotonergic tone, sufficient to exert inhibitory influence on behavior.

In the clinical arena, however, there are very few reports that address whether serotonergic drugs are beneficial in patients with ADHD. It is generally acknowledged that conventional serotonin reuptake inhibitors are of limited use in the management of these patients, and in fact one of the major side effects of these medications is stimulation. At the same time, some recent reports indicate some therapeutic benefit from the preferential serotonergic drugs venlafaxine and buspirone. Moreover, addition of selective serotonin reuptake inhibitors to psychostimulant treatment was reported in one small open-label trial to be beneficial in certain individuals with ADHD. Future controlled clinical studies are warranted to test the hypothesis developed from the mouse work. It should be mentioned also that serotonin can have an extremely complex set of actions on locomotor behaviors. It has been determined that there are at least 14 subtypes of serotonin receptors, and some of them mediate opposite actions on many functions, locomotion in particular. A major challenge for future research is to determine which subtype(s) of serotonin receptors are primarily involved in the calming effects of psychostimulant.

The dopamine transporter knockout mice display several key characteristics of ADHD. These include hyperactivity and cognitive impairments as well as response to psychostimulants. A similar, although less pronounced phenotype is characteristic of mice with reduced (more than 80%) DAT expression (Drs. X. Zhuang and R. Hen, unpublished observations). In contrast, mice that have higher than normal DAT expression show hypoactivity that is particularly evident in a new environment. Taken together, these genetic animal studies suggest that ADHD most likely represents a hyperdopaminergic condition and that the calming effect of psychostimulants is mediated through targets other than DAT.

In conclusion, these findings suggest that genetic defects in the dopamine transporter gene might contribute to some forms of ADHD in humans. It should be emphasized that ADHD is likely to be a heterogeneous disorder with several biochemical/genetic defects leading to similar clinical symptoms. Dopaminergic dysregulation, like that observed with the DAT knockout mice, might be produced by mutations in other components of the dopaminergic system. Moreover, dysregulation of other neurotransmitter pathways is likely to be an additional mechanism responsible for the development of ADHD-like conditions.

WEB SITES OF INTEREST

For modems at 56k: http://media.med.yale.edu:8080/ramgen/mouse/mouse-lw.rm

For faster connections: http://media.med.yale.edu:8080/ramgen/mouse/mouse-hi2.rm

ADDITIONAL READINGS

Anderson GM, Cook EH (2000), Pharmacogenetics: promise and potential in child and adolescent psychiatry. *Child Adolesc Psychiatr Clin N Am* 9:23–42

Carlsson A (1993), Thirty years of dopamine research. *Adv Neurol* 60:1–10

Cook EH Jr, Stein MA, Krasowski MD et al. (1995), Association of attention-deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 56:993–998

Donovan DM, Miner LL, Perry MP et al. (1999), Cocaine reward and MPTP toxicity: alteration by regional variant dopamine transporter overexpression. *Brain Res Mol Brain Res* 73:37–49

Gainetdinov RR, Caron MG (2000), An animal model of attention deficit hyperactivity disorder. *Mol Med Today* 6:43–44

Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG (1999), Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283:397–401

Lucki I (1998), The spectrum of behaviors influenced by serotonin. *Biol Psychiatry* 44:151–162

Popper CW (1997), Antidepressants in the treatment of attention-deficit/hyperactivity disorder. *J Clin Psychiatry* 58(suppl 14):14–29

Solanto MV (1998), Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. *Behav Brain Res* 94:127–152

Accepted September 19, 2000.

Dr. Gainetdinov is Assistant Research Professor, Cell Biology, and Dr. Caron is James B. Duke Professor of Cell Biology and an Investigator, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC.

Correspondence to Dr. Lombroso, Child Study Center, Yale University School of Medicine, 230 South Frontage Road, New Haven, CT 06520; e-mail: Paul.Lombroso@Yale.edu.

To read all the articles in this series, visit the Web site at http://info.med.yale.edu/chldstdy/plomdevelop/

Erratum

In the installment of this column that appeared in the January 2001 issue, the references in paragraphs 6 through 10 are incorrect (Barr C [2001], Genetics of Childhood Disorders: XXII. ADHD, Part 6: The Dopamine D4 Receptor Gene. *J Am Acad Child Adolesc Psychiatry* 40:118–121). The Assistant Editor regrets the error.