Reduced Midbrain Dopamine Transporter Binding in Male Adolescents with Attention-Deficit/Hyperactivity Disorder: Association Between Striatal Dopamine Markers and Motor Hyperactivity

Aurelija Jucaite, Elisabeth Fernell, Christer Halldin, Hans Forssberg, and Lars Farde

Background: *The hypothesis that altered dopamine transmission underlies hyperactive-inattentive behavior in children with attention-deficit/hyperactivity disorder (ADHD) is based on genetic studies and the efficacy of psychostimulants. Most of previous positron emission tomography (PET) and single photon emission tomography (SPET) studies have shown altered binding of dopamine markers in the basal ganglia. Yet, the functional role of the neurochemical disturbances are poorly understood. The purpose of our study was to examine dopamine transporter (DAT) and dopamine D2 receptor (D2R) binding in adolescents with ADHD and to search for its relationship with cognitive functions as well as locomotor hyperactivity.*

Methods: *Twelve adolescents with ADHD and 10 young adults were examined with PET using the selective radioligands [^{11}C]PE2I and [^{11}C]raclopride, indexing DAT and D2R density. The simplified reference tissue model was used to calculate binding potential (BP) values. Attention and motor behavior were investigated with a continuous performance task (CPT) and motion measurements.*

Results: *The BP value for [^{11}C]PE2I and [^{11}C]raclopride in the striatum of children with ADHD did not differ from that of the young adult control subjects. In the midbrain, however, the BP values for DAT were significantly lower (16%; $p = .03$) in children with ADHD. Dopamine D2 receptor binding in the right caudate nucleus correlated significantly with increased motor activity ($r = .70$, $p = .01$).*

Conclusions: *The lower BP values for DAT in the midbrain suggest that dopamine signaling in subjects with ADHD is altered. Altered dopamine signaling might have a causal relationship to motor hyperactivity and might be considered as a potential endophenotype of ADHD.*

Key Words: Attention-deficit/hyperactivity disorder, positron emission tomography, dopamine D2 receptors, dopamine transporter, [^{11}C]PE2I, continuous performance task

Attention-deficit/hyperactivity disorder (ADHD) is an operationally defined diagnostic concept describing children with inattention, hyperactivity, and impulsivity. Attempts to identify a common pathophysiology have been unsuccessful, which might reflect a condition that includes several endophenotypes caused by different etiologic factors (Castellanos and Tannock 2002). The current dopamine hypothesis of ADHD is receiving increased scientific support (Swanson et al 1998). The hypothesis is based on the clinical effects of the psychostimulants methylphenidate and amphetamine, both of which increase the endogenous synaptic dopamine concentration through inhibition of the dopamine transporter (DAT), as shown in healthy adults and subjects with ADHD (Krause et al 2000; Solanto 1998; Volkow et al 2002). Furthermore, allelic variability of the DAT, dopamine D4 receptor, and D2 receptor genes have all been associated with ADHD symptoms (Comings et al 1996; Cook et al 1995; Waldman et al 1998), as well as response to treatment with methylphenidate (Rohde et al 2003). Additional support has been provided by structural magnetic resonance imaging (MRI) studies, showing reduced regional brain volumes in children with ADHD, including the caudate nucleus and the right prefrontal cortex, regions receiving dopaminergic projections from the substantia nigra and the ventral tegmentum in the midbrain (Castellanos et al 2002, 2003).

Motor hyperactivity is a prominent symptom of ADHD. Clinical and experimental results have long suggested a relationship between dopamine transmission and motor hyperactivity. In a pioneering clinical study, Teicher et al (1996) used a movement analysis system to obtain an objective measure of motor activity during cognitive testing in boys with ADHD. They found that the degree of motor activity could be related to blood flow in the basal ganglia and cerebellum (Teicher et al 2000). Interestingly, the data could be used to predict the optimal dose of methylphenidate in individual patients. Locomotor hyperactivity is usually the main measure in experimental animal models of ADHD. It can be induced by different genetic or pharmacologic manipulations of the dopamine system (Gainetdinov and Caron 2001; Giros et al 1996; Heijtz et al 2002; Russell 2002); however, the true nature and pathophysiologic mechanisms of hyperactivity in human are still unknown.

In recent research on the dopamine system in ADHD, several single photon emission tomography (SPET) and positron emission tomography (PET) studies have been performed in children, adolescents, and adults. Interestingly, the variable number of tandem repeat polymorphisms in the DAT gene has been related to the expression of DAT in the striatum as measured by PET (Heinz et al 2000). Using SPET and different radioligands, three independent groups have reported increased DAT density in the putamen and the caudate nucleus in adults and children (Cheon et al 2003; Dougherty et al 1999; Dresel et al 2000); however, these findings have not been replicated by another independent group reporting unaltered DAT binding (van Dyck et al 2002b). Presynaptic synthesis of dopamine has been studied with [^{18}F]fluorodopa ([^{18}F]DOPA) (Ernst et al 1998, 1999). In a first study of

From the Department of Woman and Child Health (AJ, EF, HF), and the Section of Psychiatry, Department of Clinical Neuroscience (CH, FL), Karolinska Institutet, Stockholm, Sweden.

Address reprint requests to Aurelija Jucaite, Astrid Lindgrens's Barnsjukhus, Neuropediatrics Q2:7, Stockholm S-171 76, Sweden; E-mail: aurelija.jucaite@kbh.ki.se.

Received May 24, 2004; revised October 11, 2004; accepted November 1, 2004.

0006-3223/05/$30.00
doi:10.1016/j.biopsych.2004.11.009

17 adults with ADHD, a reduced ratio of [^{18}F]DOPA in the prefrontal cortex was shown. In a subsequent study on 10 children and adolescents (12–17 years of age) with ADHD, the investigators reported an increased accumulation of [^{18}F]DOPA in the right midbrain and no other regional differences. Taken together, several SPET and PET studies indicate an abnormal regulation of presynaptic biological markers of dopaminergic transmission in subjects with ADHD.

To evaluate the effects of treatment with psychostimulants, dopamine D2 receptor (D2R) density in the striatum has been investigated with PET in two studies in children with ADHD (Ilgin et al 2001; Rosa Neto et al 2002). These investigations have shown that methylphenidate decreases D2R availability in the striatum after administration of a single dose (Rosa Neto et al 2002), as well as after dosing for 3 months (Ilgin et al 2001). The effect correlated with the rate of reduction in impulsivity and hyperactivity and was dependent on the initial levels of [^{11}C]raclopride binding. The studies inferred that there are lower levels of endogenous dopamine in the striatum of children with ADHD. Comparison groups, however, were not included.

The discrepancy between early studies might be explained by several experimental and biological conditions. The use of different imaging techniques (PET and SPET) and of different radioligands might account for inconsistencies in estimates of regional radioligand binding. In addition, the density of marker proteins might be changed by the medication used (Brandon and Steiner 2003) or affected by age (van Dyck et al 2002a). Moreover, various endophenotypes of ADHD might have diverse neurochemical substrates (Castellanos and Tannock 2002; Pliszka et al 1996). The differences in reported results might also be related to the perinatal history of subjects. It has been shown that the D2R density in the striatum in infants might be upregulated after hypoxia (Tranquart et al 2001) and that there is a relationship between neonatal cerebral blood flow and increased D2R binding in ADHD children (Lou et al 2004).

A basic and yet unanswered question is whether the relation between alterations in the dopamine system can be related to the degree and type of the functional deficits of ADHD. In the present PET study, the nigrostriatal dopamine system was examined in adolescents with ADHD and young adult control subjects. The study included quantification of dopamine markers in the midbrain, the site of dopaminergic cell bodies and their projection region, the neostriatum. The PET protocol was designed for examination of dopaminergic markers of pre- and postsynaptic neurons with the new radioligand [^{11}C]PE2I and [^{11}C]raclopride, respectively. In addition, we searched for the correlations between dopamine markers and behavior, as well as cognitive performance of adolescents with ADHD.

Methods and Materials

Subjects

The study was approved by the Research Ethics and Radiation Safety Committees of the Karolinska Hospital. Participation in the study was voluntary, and the subjects agreed to participate after receiving written and oral information. Written informed consent was obtained from the subjects and/or their parents in accordance with the Declaration of Helsinki. The study was carried out at the PET Centre, Department of Clinical Neuroscience, Karolinska Institutet (Stockholm, Sweden).

The comparison group consisted of 10 healthy men aged 19–38 years (mean age 29.5 ± 5.8 years), recruited among employees and students at Karolinska Institutet. They were healthy according to medical history, physical examination, and routine blood and urine tests, and they did not use nicotine.

The study group included 12 boys with ADHD 12–15 years of age (mean 13.8 ± 1.2 years). They were recruited in cooperation with child neurologists/psychiatrists at university hospitals in the county of Stockholm. Inclusion criteria were a clinical diagnosis of ADHD combined type (DSM-IV; American Psychiatric Association 1994), no neurologic comorbidity, and no mental retardation. Before inclusion, a pediatric neurologist (EF) interviewed the patients and the parents, confirmed the diagnosis and rated the severity of symptoms according to DSM-IV criteria (scores 0–4). Subjects with comorbid psychiatric conditions (e.g., autistic spectrum disorders, obsessive compulsive disorders, conduct disorders, and anxiety) were excluded. Nine of the 12 boys were drug naïve (i.e., they had not previously been treated with any psychostimulants). Three boys had used methylphenidate; two of them had not taken medication for 2 years and, for one boy, medication was withdrawn 1 week before the PET measurements. No other medications were used at the time of the study. No anatomic brain abnormality was detected on MRI in any subject from either group, as evaluated by a neuroradiologist at the Karolinska Hospital.

PET Experimental Procedure

Each subject participated in two PET measurements that were performed on the same day, at least 2 hours apart. In the first measurement, [^{11}C]PE2I was used to determine DAT binding; in the second, [^{11}C]raclopride was used to determine D2R binding.

MRI and the Head Fixation System. The anatomic brain images were obtained with the MRI system GE Signa (Milwaukee, Wisconsin), 1.5 T. A standard spin-echo sequence with a 512 × 256 matrix was used with a repetition time of 400 msec and echo time of 9 msec for T1-weighted images, acquired in all subjects. A head fixation system with an individual plaster helmet was used both for MRI and PET measurements, immobilizing the head and allowing identical repositioning to be used for the co-registration between two imaging modalities (with accuracy of 2 mm) (Bergström et al 1981).

Radiochemistry. [^{11}C]PE2I and [^{11}C]raclopride were prepared by methylation of the desmethyl precursors with [^{11}C]methyl triflate, as described in detail in earlier publications (Halldin et al 2003; Langer et al 1999).

PET Measurement Procedure. A sterile phosphate buffer (pH = 7.4) containing radioligand was injected as a bolus over a period of 2 sec into the right cubital vein. The cannula was then immediately flushed with 10 mL saline solution. The mean specific radioactivity of [^{11}C]PE2I at the time of injection was .05 ± .03 MBq/mmol. In the group of adolescents with ADHD, the radioactivity injected varied between 90 MBq and 120 MBq (100 ± 8 MBq, mean ± SD); in the adult group it varied between 120 MBq and 282 MBq (205 ± 5 MBq). The mean specific radioactivity of [^{11}C]raclopride at the time of injection was .04 ± .02 MBq/mmol. In the ADHD group, the radioactivity injected was between 97 MBq and 128 MBq (mean 110 ± 7 MBq). In the adult control subjects the radioactivity of the [^{11}C]raclopride ranged from 164 MBq to 344 MBq (mean 234 ± 56 MBq); it was injected intravenously.

The PET system used was ECAT Exact HR 47 (Siemens/CTI, Knoxville, Tennessee), with an in-plane and an axial resolution of 3.6 mm and 4.0 mm, respectively, full width at half maximal (Wienhard et al 1994). The radioactivity in brain was measured continuously for 63 min ([^{11}C]PE2I) or 56 min ([^{11}C]raclopride) immediately after the injection with a preprogrammed sequence