ELSEVIER

European Neuropsychopharmacology 15 (2005) 159–162

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

www.elsevier.com/locate/euroneuro

Decreased platelet vesicular monoamine transporter density in children and adolescents with attention deficit/hyperactivity disorder

Paz Toren[a,*], Moshe Rehavi[b], Anat Luski[a], Netta Roz[b], Nathaniel Laor[a,c], Michal Lask[a], Abraham Weizman[d]

[a]*Tel-Aviv Community Mental Health Center and the Sackler Faculty of Medicine, Tel-Aviv University, Israel*
[b]*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Israel*
[c]*Yale Child Study Center, New Haven, CT, USA*
[d]*Research Unit, Geha Mental Health Center, and The Laboratory of Biological Psychiatry, Felsenstein Medical Research Center, Beilinson Campus, Petah-Tikva, Israel, and the Sackler Faculty of Medicine, Tel-Aviv University, Israel*

Received 12 February 2004; received in revised form 22 July 2004; accepted 22 July 2004

Abstract

The aim of the present study was to assess vesicular monoamine transporter (VMAT2) density in attention deficit/hyperactivity disorder (ADHD), a disorder involving monoaminergic dysregulation. It was hypothesized that the hypoactivity of monoaminergic neurotransmission related to ADHD could be associated with an under-expression of VMAT2. We assessed high affinity [^{3}H]dihydrotetrabenazine [TBZOH] binding to platelet VMAT2 in untreated male ADHD children and adolescents (n=11) as compared to age-matched controls (n=14), as well as the correlation between VMAT2 density and the severity of ADHD symptoms as measured by the clinician-administered DSM-IV ADHD Scale (DAS) and the parent-administered Abbreviated Conners' Rating Scale (ACPRS). The [^{3}H]TBZOH binding capacity (Bmax) was significantly lower (17%) in the ADHD group as compared to the controls. There was no difference between the two groups in the affinity (K_d value) of [^{3}H]TBZOH to its binding site. An inverse correlation was found between the ADHD symptom scales and the Bmax values. It remains unclear whether the under-expression of platelet VMAT2 in ADHD children is reflective of a parallel change in the brain, and whether it is primary or an epiphenomenon of ADHD.

Keywords: Platelet vesicular monoamine transporter density; Attention deficit/hyperactivity disorder; Monoaminergic dysregulation

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) contains a persistent pattern of inattention and/or hyperactivity–impulsivity that is more frequent and severe than is typically observed in individuals at a comparative level of development (American Psychiatric Association, 2000). The disorder involves 6–9% of school-age children (Bird et al., 1988). Dysregulation of norepinephrine and dopamine and also, to some extent, of serotonin has been documented in this disorder (Weizman et al., 1990). Children with ADHD may benefit from drugs with a noradrenergic–dopaminergic agonistic activity including psychostimulants and antidepressants (Weizman et al., 1990; Weiss and Weiss, 2002).

Numerous secretory cells produce and store in vesicles the biogenic monoamines serotonin, dopamine and norepinephrine. Monoamine vesicles have been observed in central and peripheral neurons, and in the adrenal medulla (Henry and Scherman, 1989). The vesicular protein responsible for the accumulation of the monoamines is the vesicular monoamine transporter (VMAT). VMAT operates as an H+-amine exchanger, using the inwardly acidic pH gradient generated by vacuolar type H+ ATPase to drive

* Corresponding author. 7 Geiger Street, Basement Floor, Ramat Aviv, Tel-Aviv 69341, Israel. Tel.: +972 3 6401802; fax: +972 3 6429136.

0924-977X/$ - see front matter © 2004 Elsevier B.V. and ECNP. All rights reserved.
doi:10.1016/j.euroneuro.2004.07.002

monoamine uptake (Schuldiner, 1994). Two distinct types of VMAT have been identified: VMAT1, which is found in adrenal chromaffin granules, and VMAT2, which is expressed primarily in the brain (Erickson et al., 1992; Liu et al., 1994). Dihydrotetrabenazine (TBZOH) is a very potent inhibitor of VMAT2 and binds with high affinity to this transporter. Scherman et al. (1988) have used [^{3}H]TBZOH as ligand to label VMAT2 in human brain homogenates. The highest densities of binding sites were observed in caudate nucleus, putamen, and nucleus accumbens nucleus. VMAT2 expression does not appear to be regulated by most drugs affecting monoaminergic transmission, such as typical antipsychotics (Vander Borght et al., 1995) and monoamine oxidase inhibitors (Zucker et al., 2001a), but is sensitive to the atypical antipsychotic clozapine (Rehavi et al., 2002). VMAT2 binding sites may serve as an index of monoaminergic innervation in human psychopathological disorders (Zubieta et al., 2001). Indeed, VMAT2 concentrations in the thalamus of treated patients with bipolar disorder type I were higher than in control subjects and in treated patients with schizophrenia. Increased VMAT2 concentrations were shown in the ventral brainstem of treated patients with schizophrenia as well as in patients with bipolar disorder (Zubieta et al., 2001). Meyer et al. (1999) did not demonstrate significant differences in brain VMAT2 density or binding between subjects with Tourette's disorder (a disorder with dopaminergic dysregulation) and control subjects.

Blood platelets have been extensively used as a peripheral model for serotonin transporters in the brain (e.g., Weizman et al., 1986). In a similar pattern, it was demonstrated that platelets could be used as an accessible peripheral model for VMAT2 (Zucker et al., 2001b). VMAT non-selectively accumulates cytoplasmic biogenic monoamine neurotransmitters into the storage vesicles of presynaptic neurons and blood platelets (Lesch et al., 1994). Sequence analysis revealed that the coding sequences of the brain- and platelet-derived complementary DNAs are identical. (Lesch et al., 1994). Studies on the expression of platelet VMAT2 in monoamine-related mental and neurological disorders have been conducted in depressed (Zucker et al., 2002a) and schizophrenia patients (Zucker et al., 2002b).

Since ADHD is related to monoaminergic dysregulation, it is of interest to assess VMAT2 density in this disorder. It was hypothesized that the hypoactivity of monoaminergic neurotransmission related to ADHD could be associated with an under-expression of VMAT2. In order to evaluate the relationship between ADHD and VMAT2, we assessed VMAT2 pharmacodynamic characteristics in blood platelets of untreated male ADHD children and adolescents, as compared to age-matched controls, as well as the correlation between VMAT2 density and the severity of ADHD symptomatology.

2. Experimental procedures

2.1. Subjects

Eleven boys aged 6–16 (12.1±3.5) years diagnosed with ADHD participated in the study group. The diagnosis of ADHD was established according to the DSM-IV-TR criteria (American Psychiatric Association, 2000) by a senior child and adolescent psychiatrist (P.T.) following a psychiatric interview according to the guidelines of the K-SADS-PL (Shanee et al., 1997). All participants were drug-free for at least 2 months. Exclusion criteria included the presence of co-morbid psychiatric disorders (e.g., anxiety and mood disorders, substance abuse, psychotic disorders, conduct and oppositional-defiant disorders) as well as any chronic physical disease.

Fourteen healthy boys from the community, aged 6–16 (11.5±2.1) years, served as controls. The controls underwent a comprehensive interview (A.L.) to exclude the presence of any psychiatric or physical disorder.

The study was approved by the Institutional Review Board and an informed consent was obtained from the parents and the participants.

2.2. Psychometric Instruments

Rating scales for ADHD were completed for all participants (study and control groups), concomitantly with the blood samples collection:

(a) DSM-IV ADHD Scale (DAS) (Spivak et al., 1999), a clinician-administered rating scale including the 18 items (score range 0 to 3) of the DSM-IV criteria for ADHD (9 for inattention and 9 for hyperactivity/impulsivity).
(b) The Abbreviated Conners' Parent Rating Scale (ACPRS) (Conners and Barkley, 1985), a parent self-administered rating scale with 10 items (score range 0–3).

2.3. Platelet membrane preparation

Blood samples (25 ml) were collected between 8:00 and 10:00 h into tubes containing an anticoagulant solution of 16 mM citrate buffer and 1 mM EDTA. Platelet-rich plasma was separated from blood cells by low-speed centrifugation (350×g for 10 min), resuspended in 20 ml Hepes buffer 50 mM pH 8.0, and centrifuged at 1700×g for 20 min. The pellet was disrupted with Brinkman polytron in 20 ml Hepes buffer