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Reduced platelet vesicular monoamine transporter density in Tourette's syndrome pediatric male patients

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Abstract The vesicular monoamine transporter (VMAT2) plays a major role in the synaptic accumulation and quantal release of monoamines. In this study, we assessed high affinity [^{3}H] dihydrotetrabenazine binding to platelet VMAT2, in a group of untreated male Tourette's syndrome (TS) patients (age: 8–17.5 years, $n=9$) and in a group of age- and sex-matched healthy controls (age: 9–16 years, $n=16$). Significantly decreased platelet VMAT2 density (B_{max}) (−23%, $p=0.016$) was observed in the TS patients. The affinity (K_d) of the ligand to platelet VMAT2 was similar in both groups. If the lower platelet VMAT2 density also occurred in the brain, it may have serve as an adaptive mechanism geared to decrease dopamine storage in the presynaptic neurons and thereby to attenuate the dopaminergic overactivity and ameliorate the movement disorder.

1. Introduction

The observation that pharmacological agents which antagonize dopaminergic neurotransmission, such as haloperidol, suppress tics (Cohen et al., 1992), led to the dopaminergic theory of Tourette's syndrome (TS). This theory assumes a disruption (hyperactivity) in the dopaminergic system at the basal ganglia, as being the cause for TS symptoms (Albin and Mink, 2006).

Using the platelet plasma membrane serotonin transporter as a peripheral model for this transporter in the brain, studies have revealed alterations in the expression of platelet serotonin transporter in patients with major depression (Paul et al., 1981), obsessive–compulsive disorder (OCD) (Weizman et al., 1986; Sallee et al., 1996), and TS-related OCD (Weizman et al., 1992).

The brain vesicular monoamine transporter (VMAT2) transports intracellular monoamines into the synaptic vesicles and is expressed in all monoaminergic neurons (Liu and Edwards, 1997). VMAT2 is also expressed in platelets

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(Cesura et al., 1990; Zucker et al., 2001), and a line of evidence suggests that brain and platelet vesicular monoamine transporters have identical genetic coding sequences and similar pharmacological properties (Lesch et al., 1994; Zucker et al., 2001). Therefore, similar to the serotonin transporter, the platelet VMAT2 may serve as a possible peripheral model for brain VMAT2 (Zucker et al., 2001).

In the present study, we assessed the pharmacodynamic characteristics of platelet VMAT2, using [^{3}H]dihydrotetrabenazine ([^{3}H]TBZOH) as a ligand, in untreated TS patients and in a healthy control group.

2. Experimental procedures

2.1. Subjects

Nine untreated male patients with Tourette's syndrome, were recruited from the Schnieder Children's Hospital's TS outpatient clinic to participate in the study. All underwent a psychiatric interview adhering to the guidelines of the Hebrew version of the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime (K-SADS-PL) (Shanee et al., 1997), and the diagnosis of TS was established according to the criteria of DSM-IV-TR. The severity of TS and the severity of obsessive–compulsive symptoms were assessed by the Yale Global Tic Severity Scale (YGTSS) and the Children's Yale–Brown Obsessive–Compulsive Scale (C-YBOCS), respectively. The study participants were interviewed for history of psychiatric disorder among first relatives. Sixteen age- and sex-matched (all male) healthy subjects from the community served as controls. We used a control group almost twice the size of the patient group, in order to receive an authentic distribution of the general population.

The study was approved by the Geha Mental Health Center Review Board, and informed consent was obtained from all participants after the nature of the study was fully explained to them.

2.2. Platelet membrane preparation

Blood samples (25 ml) were collected and inserted 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), diluted 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 50 mM pH 8.0 containing 300 mM sucrose (buffer A) and centrifuged twice at 27000 ×*g* for 20 min. It was then resuspended in 4.5 ml buffer A to yield a protein concentration of about 1 mg/ml.

2.3. [^{3}H]TBZOH binding

For [^{3}H]TBZOH binding, 100 µl membranes were incubated at 25 °C with 50 µl of [^{3}H]TBZOH (eight concentrations, 0.5–8.0 nM; specific activity: 20 Ci/mmol; American Radiolabeled Chemicals Inc., St. Louis MO) and 50 µl buffer A. After 30 min of incubation, the mixture was filtered using vacuum on glass fiber filters (GF/C). The filters were rinsed four times with ice-cold buffer A, and the radioactivity was counted in scintillation liquid using a ß-counter (Packard 1600 TR). Nonspecific binding was measured in parallel samples in the presence of 1 µM tetrabenazine (Fluka, Buch, Switzerland). This ligand was shown to inhibit [^{3}H]TBZOH binding to human platelet VMAT2 in a competitive manner (IC_{50} 10 nM) (Zucker et al., 2001). The nonspecific binding at K_d value (3.2 nM) did not exceed 20%. Protein concentration was determined according to Bradford's (1976) method. The ligand affinity (K_d) to VMAT2 and the [^{3}H]TBZOH binding capacity (B_{max}) were assessed by Scatchard analysis.

2.4. Statistical analysis

Two-tailed Student's *t*-test was used for between-group comparisons. Pearson correlation test was used when appropriate. All results are expressed as mean ± SD.

3. Results

All participants were physically healthy and did not abuse drugs or alcohol.

The epidemiological, clinical and biochemical data of the patients and controls are given in Tables 1, 2 and 3. The mean age was similar in both groups (Table 3). As shown, of the nine TS patients, three suffered also from OCD (patient 9) or obsessive–compulsive symptoms (patients 7, 8).

The main finding (Table 3) was a significantly lower density (B_{max}) of the VMAT2 (−23%) in the platelets of TS patients as compared to the healthy control group (688 ± 202 vs. 890 ± 197 fmol/mg protein, $t = 7.61$, $df = 23$, $p = 0.016$). No difference was found in the affinity of the ligand to VMAT2 between the two groups (K_d: 3.70 ± 0.86 vs. 3.74 ± 0.88 nM, $t = 0.07$, $df = 23$, $p = 0.94$, NS).

No correlation was found in the TS patients between the YGTSS score and the density of VMAT2 (B_{max}) ($r = -0.34$, $n = 9$, two-tailed p = 0.37, NS) or between the YGTSS score and the dissociation constant (K_d) ($r = 0.17$, $n = 9$, two-tailed $p = 0.66$, NS).

Table 1 Clinical characteristics of the TS patients

Patient no.	Age (years)	Sex	Comorbidity	Family history	YGTSS score	C-YBOCS score	TS onset (years)	OCD/OCS onset (years)
1	9.5	M	–	Mo:D+A+MT	22	1	4.0	–
2	10.0	M	–	–	17	0	6.0	–
3	11.5	M	–	–	6	0	7.0	–
4	12.5	M	–	–	22	0	3.5	–
5	15.5	M	–	–	28	0	7.0	–
6	12.5	M	–	–	10	2	5.0	–
7	8.0	M	OCS	Mo:OCS	29	12	2.8	6.0
8	10.5	M	OCS	F:TS+Sc, 3B:TS	21	12	5.0	7.0
9	17.5	M	OCD	Mo:OCS, 2B:OCS	14	28	6.0	9.5

A=ADHD (attention deficit hyperactivity disorder), B=brother (3B=three brothers), C-YBOCS=Children's Yale–Brown Obsessive–Compulsive Scale; D=depression, F=father, M=male, Mo=mother, MT=motor tics, OCD=obsessive–compulsive disorder, OCS=obsessive compulsive symptoms, Sc=schizophrenia, TS=Tourette's syndrome; YGTSS=Yale Global Tic Severity Scale.