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ORIGINAL PAPER

Hyperserotonemia in Adults with Autistic Disorder

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Abstract Hyperserotonemia is the most consistent serotonin-related finding in autism. The basis of this phenomenon, and its relationship to the central serotonergic dysfunction remains unclear. Platelet serotonin level (PSL) in 53 autistic adults and 45 healthy controls was measured. Mean PSL in autistic group (75.7 ± 37.4 ng/μL) was significantly higher than the control sample (59.2 ± 16.2 ng/μL) due to a presence of hyperserotonemic subjects which comprised 32% of the patients. PSL of autistic subjects did not correlate with the severity of symptoms, as measured by total CARS score, or the degree of mental retardation. However, significant negative relationship was observed between PSL and speech development, indicating the relationship between the peripheral 5HT concentrations and verbal abilities in autistic subjects.

Keywords Autism · Hyperserotonemia · Platelet serotonin level · Speech development · Verbal communication

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Introduction

The autism spectrum disorders (ASDs) are a group of neurodevelopmental syndromes, with onset in early childhood, that include autistic disorder, Rett's disorder, childhood disintegrative disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD NOS). The spectrum of symptoms varies among individuals, but includes some shared characteristics such as disturbances in social interactions, language, and communication, as well as the presence of stereotyped behaviors and interests (Owley, Leventhal, & Cook, 2003). The general population prevalence of ASDs is 3–6 per 1,000. However, there is a high-recurrence risk of 2–8% in siblings of the diseased, and up to 92% in monozygotic twins, pointing to the strong genetic component of this disorder (Muhle, Trentacoste, & Rapin, 2004). Recent findings indicate the involvement of multiple genes, interacting with one another and with environmental factors to lead to the alterations in early brain development (Jones & Szatmari, 2002).

Several lines of evidence suggest that alterations in serotonergic neurotransmitter system might represent one of the biological substrates of the disease. Serotonin (5-hydroxytryptamine, 5HT) has been shown to play an important role in brain development by regulating both, serotonergic outgrowth and maturation of target regions (Whitaker-Azmitia, 2001). Pharmacological manipulation of serotonergic transmission has been shown to influence some of the autistic symptoms—selective serotonin reuptake inhibitors (SSRI) reduce repetitive behaviors in adults and children with autistic disorder (McDougle et al., 1996a; Hollander et al., 2005), while depletion of serotonin precursor tryptophan increases autistic behavior in the diseased adults (McDougle et al., 1996b). Positron emission tomography studies have demonstrated an altered serotonin synthesis in the dentatothalamocortical pathway of autistic boys as well as an altered brain 5HT synthesis capacity of autistic children (Chugani et al., 1997, 1999).

Perhaps the most intriguing 5HT-related finding in autistic disorder is hyperserotonemia. For several decades, elevated blood 5HT levels have been consistently found in about one third of autistic children (Cook & Leventhal, 1996; Owley et al., 2003). Increased density of platelet 5HT transporter (Marazziti et al., 2000), increased synthesis of 5HT in the intestine (Croonenberghs, Verkerk, Scharpe, Deboutte, & Maes, 2005), altered release from the enterochromaffine cells (Janusonis, 2005), or diminished release from platelets (Cook & Leventhal, 1996) have been suggested as possible causes. However, the mechanism of the observed phenomenon, as well as its relation to the central 5HT dysfunction has remained unclear. It is still not understood why some, but not all autistic subjects display hyperserotonemia, is it connected to a certain autistic subtype or symptom, and could it represent an endophenotype of the ASDs. Furthermore, it is not clear whether hyperserotnemia is confined only to the pre-pubertal autistic children (McBride et al., 1998, Croonenberghs et al., 2000), or does it persist through the adulthood (post-pubertal subjects being far less studied, often grouped with pre-pubertal children, or analyzed separately as a relatively small subgroup).

In this work, we have studied platelet 5HT levels in a relatively large, ethnically homogenous sample of adults rather severely affected with ASDs. Our aim was to determine: (a) whether a subgroup of patients with the increased platelet 5HT concentrations could be identified among the autistic adults, and (b) whether platelet 5HT levels relate to certain aspects of behavioral expression: the severity of autistic symptoms, the level of mental retardation (MR), and the verbal abilities.

Methods

Participants

Autistic patients were recruited from the center for autism Zagreb, Croatia, after being examined by a psychiatrist and two psychologists. The group consisted of 53 subjects (38 males, 15 females, aged between 16 and 45 years, mean ± SD: 26.1 ± 6.6) diagnosed with ASDs (48 with autism, one with Asperser's syndrome, and four with PDD NOS), according to DSM-IV criteria. Severity of behavioral symptoms was measured using the Childhood Autism Rating Scale (CARS, Schopler, Reichler, DeVellis, & Daly, 1980). Degree of MR was assessed according to the standardized intelligence or developmental tests, depending on the apparent developmental level of each individual. Subjects were assigned to one of the following categories: severe MR (IQ < 30), moderate MR (IQ 30–50), and mild MR (IQ 51–70). The level of speech development was also clinically evaluated according to the following criteria: undeveloped speech (no expressive language), poorly developed speech (subject speaks a few words, or displays echolalia), and well-developed speech (subject builds sentences, although the speech is qualitatively and quantitatively less developed than that of healthy peers). Drug therapy included, SSRI (six patients), typical neuroleptics (seven patients), anticonvulsants (five patients), and a combination of neuroleptics and anticonvulsants (26 patients, 18 with typical and eight with atypical neuroleptics).

Control group consisted of 45 healthy blood donors (44 males, one female, aged between 20 and 55 years, mean ± SD: 39.2 ± 9.2). None of the control subjects had a history of mental illnesses, behavioral disorders, substance abuse, or treatment with psychotropic drugs or other 5HT-related medications.

All subjects were of Croatian (southern Slavic) origin. After an informative talk, a written consent for inclusion in the study was obtained from the control subjects and from the patients' parents. The study has been carried out in accord with the Declaration of Helsinki, and was approved by the Ethics Committee of the Medical Faculty of the University of Zagreb.

Experimental Procedure

Blood sampling was performed between 9 and 11 a.m. Venous blood (6 mL) was collected into vacutainers containing 1 mL of ACD anticoagulant. Control and patient samples were simultaneously processed in batches of 10–15 samples per group. After a thorough

mixing, blood was transferred to the 15 mL Falcon tubes and centrifuged at 1,050*g* for 2 min to obtain PRP. Separated PRP was aliquoted for an automated determination of platelet number and volume, and for measurements of 5HT concentration (in duplicates).

Platelet pellet, obtained by centrifugation of a diluted PRP sample (1 mL PRP + 3 mL saline) at 8,500*g* for 5 min, was sonicated in 1 mL of deionized water. Following deproteinisation with $ZnSO_4$/NaOH, 5HT content was measured by orthophtaldialdehyde-enhanced fluorometry at 345/485 nm, as previously reported (Jernej, Cicin-Sain, & Iskric, 1988; Jernej et al., 2000). Results were expressed as ng 5HT per μL of total platelet volume (calculated as the product of mean platelet volume and platelet count).

Statistical Analyses

Data were processed by the use of GraphPad InStat 3.01 software. Normality of distributions of the measured parameters was tested by Kolmogorov/Smirnov and Shapiro–Wilk methods, while the equality of SDs was tested by Bartlett's test. Calculations of the lognormal descriptives were done according to Limpert, Stahel, and Abbt (2001). Mean values of normally distributed parameters were compared using unpaired *t*-test or using one-way analysis of variance (ANOVA) with Tukey's post-test. Mean values of parameters that were not normally distributed, or that differed significantly in there SDs, were compared using Welch's corrected *t*-test or using non-parametric Kruskal–Wallis method with Dunn's post-test. Values were correlated using Pearson or Spearman correlation. The level of significance was set to 0.05. The values were expressed as means ± standard deviations.

Results

In the present study, the values of the measured 5HT level were expressed per total platelet volume. No significant effects of gender ($F_{(1,97)} = 0.98$, $p = 0.32$), diagnosis ($F_{(1,97)} = 0.26$, $p = 0.61$), or therapy ($F_{(3,97)} = 1.68$, $p = 0.18$) on the total platelet volume, nor its significant correlation with age ($r = 0.174$, $p = 0.09$), were observed in the integral sample of 53 autistic and 45 control subjects.

The effect of several parameters on PSL in the autistic group is shown in Table 1.

Since we have studied an adult autistic group that was in large part medicated, we had to check for the influence of medication on platelet serotonin levels (PSL) before doing any further comparisons with this parameter. There was a strong effect of drug treatment

Table 1 The effect of several parameters on platelet serotonin level (*PSL*) measured in the integral autistic sample (medication) or in the autistic sample without SSRI-treated group (demographic parameters and behavioral measures)

Category	*N*	PSL ng/μL (ng/10^9platelets)	Mean value of category	Statistics
Medication				KW = 15.66, $p < 0.01$
SSRI	6	19.0 ± 5.8 (148 ± 56)		
Neuroleptics (NL)	7	63.1 ± 30.2 (433 ± 181)		
Anticonvulsants (AC)	5	70.2 ± 24.2 (477 ± 149)		
NL + AC	26	80.4 ± 43.9 (545 ± 334)		
Non-medicated	9	74.8 ± 28.4 (534 ± 217)		
Gender				$t = 0.47$, 45 *df*
Male	33	77.4 ± 39.9 (537 ± 307)		$p = 0.64$
Female	14	71.7 ± 32.0 (477 ± 196)		
Age			25.1 ± 5.9	$r = -0.164$, $p = 0.27$
Degree of MR				$F_{(2,45)} = 1.41$, $p = 0.26$
Mild	12	61.0 ± 29.0 (427 ± 170)		
Moderate	17	75.7 ± 22.4 (491 ± 145)		
Severe	17	77.3 ± 25.8 (538 ± 177)		
Speech				$F_{(2,45)} = 5.30$, $p < 0.01$
Well-developed	8	46.3 ± 18.2 (321 ± 108)		
Poorly-developed	29	77.3 ± 25.5 (521 ± 157)		
Undeveloped	9	80.0 ± 20.9 (551 ± 170)		
CARS score				
Total			42.5 ± 8.32	$r = 0.208$, $p = 0.197$
Verbal			3.1 ± 0.87	$r = 0.326$, $p < 0.05$

PSL in ng/10^9 platelets is given in parentheses to allow comparisons with previous studies. Statistics is performed only on data expressed as ng/μL

on PSL (KW = 15.66, $p < 0.01$), apparently due to a SSRI-treated subgroup, which had a significantly lower (about four times) mean PSL value compared to the non-medicated subgroup, while the other types of medication did not significantly alter PSL values. Therefore, SSRI-treated individuals were excluded from further calculations. Platelet 5HT concentrations did not differ between males and females ($t = 0.47$, $df = 45$, $p = 0.64$), nor did they correlate significantly with the patients' age ($r = -0.164$, $p = 0.27$).

The degree of MR did not significantly influence the measured parameter ($F_{(2,45)} = 1.41$, $p = 0.26$). On the other hand, a significant difference in the mean platelet 5HT concentrations was observed among subgroups of patients varying in the level of speech development ($F_{(2,45)} = 5.30$, $p < 0.01$). Subjects with well-developed speech had significantly lower (about 40%) 5HT concentrations than the subjects with poorly developed or undeveloped speech. PSLs did not significantly correlate with the severity of autistic symptoms, as measured by the total CARS score ($r = 0.208$, $p = 0.197$). However, a significant correlation was observed with the score for verbal communication ($r = 0.326$, $p < 0.05$).

We further compared PSL values of ASDs group to those of the control sample. The difference in the mean age between autistic patients and control group was significant ($t = 8.1$, $df = 96$, $p < 0.001$). However, as in the ASDs group, 5HT levels in the control sample did not correlate with age ($r = 0.051$, $p = 0.74$).

Mean PSL values of the control and ASDs group were 59.2 ± 16.2 ng/μL (419 ± 125 ng/10^9 platelets) and 75.7 ± 37.4 ng/μL (519 ± 277 ng/10^9 platelets), respectively. While the platelet 5HT concentrations were normally distributed in the control group ($W = 0.982$, $p = 0.63$), the normality of PSL distribution in the patients' sample was rejected by the Shapiro–Wilk test ($W = 0.831$, $p < 0.001$) in favor of a log-normal distribution (KSL, $D = 0.109$, n.s.). The latter distribution is therefore more accurately described with a geometric mean of 67.91 ng/μL and a multiplicative SD of 1.62 ng/μL. Difference in the mean PSL values of the logarithmically transformed data between autistic and control samples was significant ($t = 2.142$, 76 df, $p < 0.04$, Welch's corrected t-test).

Since the normally and log-normally distributed values could be conveniently compared in terms of the confidence intervals (CI), we further determined 95.5% CIs for PSL distribution in the two groups.

In the control sample, the CI ranged from 26.72 to 91.64 ng/μL, and in the autistic sample from 25.88 to 178.22 ng/μL (Fig. 1). It could be noticed that, while the lower confidence limits of PSL for both control and autistic patients were nearly identical, the autistic patients were more likely to exhibit high values of PSL, including the extreme values not normally encountered in the control population. Indeed, when the cutoff value for hyperserotonemia (defined as values above the controls mean + 2 SD) was set at 91.6 ng/μL, we found that 15 out of 47 autistic individuals fitted into this category.

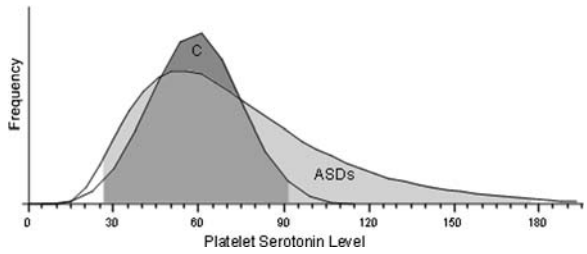

Fig. 1 Frequency distribution of platelet serotonin level in the group of patients with autism spectrum disorders (*ASDs*), $N = 47$, and in the group of healthy controls (*C*), $N = 45$. Shaded areas represent 95.5% confidence intervals

Discussion

Hyperserotonemia is a well-documented but still unexplained phenomenon found in a portion of subjects suffering from the ASDs. In an effort to contribute to the elucidation of the role of the disturbed peripheral 5HT homeostasis in etiopathogenesis of autism, we have studied platelet 5HT levels in a group of adults with ASDs.

According to the suggestion of McBride et al. (1998) that the expression of platelet 5HT in terms of total platelet volume should be more widely used, and to our own observations that individuals with higher platelet number had lower mean platelet volume and vice versa (data not shown), platelet 5HT levels in this study were expressed in units of ng 5HT per μL of total platelet volume to minimize the influence of individual and group differences in platelet count and size. Mean PSL values expressed in that manner were very similar to those reported by McBride et al. (1998) for white subjects (78 ± 23 ng/μL in autistic and 68 ± 17 ng/μL in control group).

Mean level of platelet serotonin in our ASDs sample was significantly higher than the mean PSL of the control sample. One out of three recent studies analyzing blood 5HT concentrations in post-pubertal patients, also reported significantly higher values of about 25% in autistic group (Mulder et al., 2004), while the other two reported no significant differences

between autistic and control samples (McBride et al., 1998; Croonenberghs et al., 2000). The reason for this discrepancy might lie in different compositions of the investigated autistic samples (ethnicity, level of functioning), as well as in the small sample sizes of the two latter studies. Our sample was relatively large, ethnically homogenous, and consisted of rather seriously affected individuals in terms of MR (more than 70% of subjects were moderately/severely retarded) and the severity of autistic symptoms (the average CARS score fitted to a seriously affected category). It is possible that the mentioned characteristics of the sample allowed for detection of the elevation in 5HT level—a phenomenon that might truly exist in autistic adults, but in a more discrete, harder to detect, manner than in the young children.

On the other hand, our ASDs sample contained several limitations, which might have represented a source of a false positive finding and need to be discussed. First, autistic subjects were in large part medicated. Second, their mean age and male to female ratio were significantly lower than those of the control group. Finally, the lack of the use of the gold standard diagnostic instruments (beyond DSM-IV criteria) might have affected the composition of the sample. However, regarding the first, our analysis revealed that only SSRI significantly affected platelet 5HT concentrations (subjects were excluded from further comparisons), while other medication, not directly acting on 5HT system, did not change PSL. This is in line with the observations reported by Mulder et al. (2004). Regarding the second, platelet 5HT level is considered a stable parameter after adolescence (Ritvo et al., 1971). In our previous study on a large sample of 500 healthy blood donors (Jernej et al., 2000), we showed that, post-pubertally, PSL significantly decreases only after the age of 55, which was a cutoff age for the inclusion of controls in the present study. The mentioned study also did not reveal any significant influence of gender on the PSL.

Distribution of PSL values in the patient sample mostly overlapped with the distribution in the control sample, but extended toward higher values. Similar observations were reported for a group of autistic children (Coutinho et al., 2004) and for a group of mixed pre- and post-pubertal autistic subjects (Mulder et al., 2004). Distribution in the latter study was bimodal and, compared to the log-normal distribution in our sample, enabled a more straightforward visual identification of a normoserotonemic and a hyperserotonemic subgroup. Still, the statistical definition of hyperserotnemia (Cook et al., 1993; Coutinho et al., 2004), used in this study, also revealed the existence of a group of autistic subjects with the elevated platelet 5HT levels. Proportion (32% of the patients) and magnitude (48% elevation in the mean PSL in comparison to the control mean) of hyperserotonemia were in the ranges reported for autistic children (reviewed in Anderson, 1987, Owley et al., 2003) and similar to those reported for autistic adolescents (Mulder et al., 2004).

Perhaps the most important issue regarding hyperserotonemia is whether the elevated 5HT levels in the periphery reflect in any way serotonergic abnormalities in the brain. The potential relationship between the peripheral and central serotonergic dysfunction has been indirectly studied by relating blood 5HT levels to the various aspects of behavioral expression of autism (mainly the IQ, the verbal abilities, and the severity of symptoms), with, so far, inconclusive results. Spivak et al. (2004) reported negative correlation between plasma-free 5HT concentration and the level of aggressiveness in autistic adults. Mulder et al. (2004) investigated PSL in relation to the severity of behavioral symptoms using several types of scoring, but neither displayed significant correlation. In spite of the initial finding of significantly higher blood 5HT levels in severely retarded autistic children, compared to those moderately/mildly retarded (Campbell, Friedman, DeVito, Greenspan, & Collins, 1974), correlation of platelet 5HT concentrations with the IQ (Kuperman, Beeghly, Burns, & Tsai, 1987) or cognitive performance (Cook et al., 1990) have not been found. While Mulder et al. (2004) did not find effects of platelet 5HT concentrations on several aspects of language development in autistic subjects, two earlier studies (Cook et al., 1990; Cuccaro, Wright, Abramson, Marsteller, & Valentine, 1993) reported significant negative correlation between the whole blood 5HT levels and verbal abilities in the samples consisting of autistic probands and their first-degree relatives.

In our sample, subjects with moderate or severe MR had about 25% higher PSL than the mildly retarded ones, but the observed difference was not significant. However, a significant negative relationship was observed between 5HT concentrations in platelets and language development. Patients with poorly developed or undeveloped speech displayed significantly higher PSL values than the patients with well-developed speech. Since the level of MR and the level of speech development were related, the observed (non-significant) difference in PSL among different MR categories could have been the result of different representation of well-speaking and non-speaking individuals, and might explain discrepancies in the previous MR-related findings. PSL values in our sample did not

significantly correlate with the total CARS score, but did display significant correlation with the score for verbal communication. Although the level of speech development mainly reflects the ability to speak, while the verbal communication score mainly reflects willingness to speak, they both point to the same direction: the relation between PSL values and verbal abilities in autistic subjects. Interestingly, using the positron emission tomography to measure regional changes in the brain serotonin synthesis in autistic subjects, Chugani et al. (1997) found abnormalities in dentatothalamo-cortical pathway, important for language development, which may represent aberrant innervation by serotonergic terminals.

In conclusion, in a sample of Croatian adults severely affected with the ASDs we have identified hyperserotonemic individuals, and detected a negative relationship between the platelet 5HT levels and verbal abilities of autistic subjects. Our future studies on autistic patients will focus on the function of the regulators of the peripheral 5HT homeostasis, as well as on the variability of the genes coding for 5HT elements expressed both, in the brain and in the periphery. Hopefully, our results will help to bring solutions to the two, still unanswered, questions dealing with hyperserotonemia: what causes the elevated 5HT levels in blood, and how are they involved in development of autistic symptoms.

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