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PLATELET SEROTONIN STUDIES IN FAMILIAL HYPERSEROTONEMIA OF AUTISM

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Summary

Platelet serotonin (5-HT) studies were conducted with 12 hyperserotonemic and 12 normoserotonemic age-, sex-, and relationship-matched relatives of autistic probands. Each group consisted of 7 mothers, 4 fathers, and 1 sister of autistic children and adolescents. The density (B_{max}) of platelet 5-HT_2 receptor binding sites, labelled with [^{3}H]-lysergic acid diethylamide (LSD), was significantly lower in 11 hyperserotonemic subjects compared to 12 normoserotonemic subjects (40.9 ± 13.5 fmol/mg protein, 59.6 ± 13.2; $p < 0.004$). The affinity (K_d) for [^{3}H]-LSD binding did not differ. Although the density (B_{max}) of [^{3}H]-paroxetine binding did not differ between groups, there was a small difference in the affinity (K_d) of [^{3}H]-paroxetine binding (hyperserotonemic 47.6 ± 9.0 pM, normoserotonemic 54.8 ± 12.1; $p < 0.05$). There were no significant differences in platelet 5-HT uptake, or in thrombin-stimulated 5-HT release. Basal, 5-HT-stimulated, and arginine-vasopressin (AVP)-stimulated inositol phosphate production, as well as basal, prostaglandin E_1 (PGE_1)-, and forskolin-stimulated cAMP production did not differ. There were signficant correlations between whole blood 5-HT levels and LSD B_{max} ($r_s = -0.63$, N=23, $p < 0.002$) and whole blood 5-HT levels and 5-HT uptake V_{max} ($r_s = 0.56$, N=18, $p < 0.02$). However, [^{3}H]-LSD labelled 5-HT_2 binding and 5-HT uptake were not correlated with each other. Hyperserotonemia of autism may be heterogeneous with one subgroup of subjects with increased 5-HT uptake and another subgroup with decreased 5-HT_2 binding.

Investigation of serotonergic measures in autism began with a report (1) that more than 25% of children with autism had hyperserotonemia, which was defined as a whole blood 5-HT level greater than 1.67 S.D. above the normal control mean. This finding has been extensively replicated and factors such as platelet count (2,3), platelet volume (4), and diet (5) have been excluded. A normal developmental course with a decrease of whole blood 5-HT to adult levels by 7 to 9 years (6) has been demonstrated in normal humans. Whole blood 5-HT levels are remarkably stable over time in normoserotonemic, adult control subjects (7).

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Since autistic disorder is most probably an etiologically heterogeneous clinical syndrome, hyperserotonemia has been of interest as a possible marker of a more homogeneous subgroup which may share either a common etiology or common pathophysiological mechanism (5). However, hyperserotonemic autistic children have symptoms which are similar to normoserotonemic autistic children (8). Recently, several studies have indicated familiality of hyperserotonemia of autism (9-12). A recent report has suggested that hyperserotonemia may be a marker of a genetic subtype of autistic disorder (13).

Since autistic disorder is a neuropsychiatric disorder without a sufficient animal model, this study used measures designed to study proteins involved in central nervous system function through use of the human platelet model. We report the results of a study of platelet 5-HT_2 receptor binding, 5-HT uptake, 5-HT release, and signal transduction, in hyperserotonemic subjects compared to normoserotonemic subjects.

Methods

Subjects

Two groups (hyperserotonemic and normoserotonemic) of 12 age- and sex-matched subjects were studied. The mean age of the hypersertonemic subjects (38.2 ± 6.7 yr.) did not differ from that of the normoserotonemic subjects (38.1 ± 6.4). Each group consisted of 7 mothers, 1 sister, and 4 fathers of autistic children. The autistic children had all been diagnosed by DSM-III (14) or DSM-III-R (15) criteria by clinical interview (B.L., E.C.). Hyperserotonemic subjects were selected from earlier studies of the familiality of hyperserotonemia (11,12) and screening of 60 parents of autistic children at the 1991 Illinois Society for Autistic Citizens annual meeting. All parents who had hyperserotonemia were contacted to participate. Several hyperserotonemic parents could not participate because they could not discontinue prescribed medication for the study. Eleven hyperserotonemic parents were able to participate and another adult hyperserotonemic sibling was included. The definition of hyperserotonemia was set at greater than 2 standard deviations above the mean. Whole blood 5-HT levels in our laboratory (16) using a liquid-liquid extraction and spectophotofluorometry (7) revealed normal women had whole blood 5-HT levels of 193 ± 50 ng/ml (mean ± S.D.) and normal men had whole blood 5-HT levels of 170 ± 56 ng/ml. For subjects previously assayed with this method, hyperserotonemia was defined for this study as whole blood 5-HT greater than 293 ng/ml for women or greater than 282 ng/ml for men. Samples assayed since 1987 have been determined by HPLC-fluorometry (17), which is highly correlated with the spectrophotofluorometric method but which yields 0.75 fold lower values in our laboratory based on 84 samples which were assayed with both methods. Therefore, hyperserotonemia using the HPLC assay in our laboratory was defined as greater than 221 ng/ml for adult females or greater than 212 ng/ml for adult males.

Subjects were excluded if they had a history of psychotropic medication use within 6 months of the study or aspirin or anti-inflammatory drugs within 2 weeks of blood drawing. Twenty patients took no medication during the month before blood sampling. One patient took 3000 mg acetaminophen 3 weeks before blood sampling, and 3000 mg. acetaminophen, 150 mg phenylpropanolamine HCl, and 132 mg phenyltoloxamine citrate 2 weeks before blood sampling, 1 patient took 975 mg of acetaminophen, 37.5 mg phenylpropanolamine HCl, 3 mg chlorpheniramine maleate and 30 mg dextromethorphan HBr 3 weeks before the blood sampling; 1 patient took 650 mg of acetaminophen 1 week before the blood sampling, and 1 patient took loperamide 24 hours before blood sampling.

One normoserotonemic mother had obsessive compulsive disorder, which had not been treated with medication and which was mild at the time of blood sampling. She had responded well to a self-directed behavioral approach. One hyperserotonemic mother had Sjogren's syndrome and a history of panic disorder, major depression, and post-traumatic stress disorder. Her psychiatric symptoms were in untreated remission at the time of the blood sampling. One normoserotonemic and one hyperserotonemic patient, respectively, had a history of major depression, but their symptoms were in remission at the time of the blood sampling.

Procedures

Blood Collection

Blood was drawn at the University of Chicago from 9:30 to 10:30 A.M. between July 30, 1992 and October 2, 1992. Subjects had a 21 gauge butterfly inserted, after resting in a supine position for 20 minutes. Blood was then withdrawn in the following order: 25.5 cc into a 30 cc syringe with 4.5 cc acid citrate dextrose (ACD)(15% v/v) for determination of platelet inositol phosphate production and platelet 5-HT release; 20 cc into a 30 cc syringe with 2.0 cc 3.8% sodium citrate for determination of platelet cAMP production; 3.75 cc into a vacutainer containing K_3EDTA for determination of whole blood 5-HT and platelet count, 10 cc into a polypropylene tube with 1.5 ml CPD (citric acid, sodium citrate, NaH_2PO_4, dextrose) for determination of platelet 5-HT uptake; and 36 cc into 60 cc syringe with 4 cc of 1% Na_2EDTA in 0.9% NaCl buffer for determination of [^{3}H]-paroxetine and [^{3}H]-LSD binding; and 10 cc into a heparin-containing Vacutainer tube for development of lymphoblastoid cell lines. The total amount of blood removed was 105.25 cc.

Platelet 5-HT Release

100 µl of whole blood was added to 1.0 ml of saline buffer in a siliconized (Sigmacoat, Sigma Co., St. Louis, MO) tube prewarmed to 37^{o}C. Fluoxetine (final concentration 10 µM) was added to block re-uptake of 5-HT. Tubes were incubated at 37^{o}C for 1 min.. Thrombin (Thrombinar®, Armour Pharmaceutical Co., Kankakee, IL.) was added at 7 final concentrations ranging from 0 to 0.5 U/ml. Incubation continued at 37^{o}C for 60 seconds. These assay parameters were sufficient to determine a dose-response curve based upon data obtained during development of this release assay (18). At the end of the release period, 3.0 ml of ice-cold saline buffer was added to each tube and the contents of each tube were immediately filtered through a 2.4 cm Whatman GF-C glass fiber filter, using a sampling manifold (Millipore, Bedford, MA). Tubes were immediately rinsed with 3.0 ml of ice-cold buffer and the rinse was filtered.

To analyze 5-HT remaining in the platelet after thrombin-stimulated release, each filter was placed in a microcentrifuge tube with 440 µl of 10 % ascorbic acid solution containing 10 ng 5-HTP (internal standard). Tubes were kept on ice until 100 µl of 3.4 M perchloric acid was added. Samples were vortex mixed for 5 seconds and subsequently placed on ice for 10-15 minutes. Each tube was centrifuged at 15,000 rpm for 5 minutes at 0-4^{o}C. The supernatant was transferred to a 1.5 ml tube and stored at -70 ^{o} C until analysis by HPLC with fluorometric detection (17).

Washed Platelet Basal and Stimulated Inositol Phosphate Production

25 cc of whole blood anticoagulated with ACD was spun at 200 x g for 20 min.. The supernatant was centrifuged at 1000 x g for 15 min.. The platelet pellet was resuspended in 0.8 ml of modified HEPES buffer (134 mM NaCl, 12 mM $NaHCO_3$, 2.9 mM KCl, 0.6 Units/ml Apyrase, 500ng/ml Prostaglandin I_2 Sodium Salt, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM glucose, pH 7.4) with 1 mM EDTA. This platelet suspension was incubated in a shaking water bath at 37^{o}C for 3 hours with 27 µCi myo-[^{3}H]-inositol (specific activity 118 Ci/mmol; Amersham, Arlington, Heights, IL). The platelet preparation was centrifuged at 1000 x g for 10 min and was washed

with 8 ml of buffer and resuspended in 1.7 ml of stimulation buffer (HEPES buffer above but without
EDTA, Apyrase, and Prostaglandin and with 10 mM LiCl, 10 mM inositol, and 1.3 mM $CaCl_2$ added).

After incubation in stimulation buffer for 10 min., 500 µl aliquots of washed platelets (2-7 x 10^8 platelets per aliquot) were incubated with 10^{-5} M 5-HT (19), 10^{-7} M AVP (20), or stimulation buffer. After 10 min., platelet stimulation was terminated by transferring the platelets into tubes containing 2 ml of chloroform:methanol:concentrated hydrochloric acid (50:100:1, v/v/v).

Inositol phosphates were extracted (21) with 1.24 ml chloroform:H_2O (1:1, v/v). The upper water-soluble fraction was analyzed for [^{3}H]-inositol phosphate content by using Dowex anion exchange columns. [^{3}H]-inositol monophosphates were determined by liquid scintillation counting using Cytoscint (ICN Biomedicals, Inc., Irvine, CA).

Platelet Basal and Stimulated cAMP Production

Intact platelets were prepared by a modification of previously reported methods (22,23). Erythrocytes and white cells were removed by centrifugation at 400 x g. The platelet-rich supernatant was then centrifuged at 5000 x g to obtain the platelet pellet. Platelets were resuspended in 200 µl of phosphate-buffered balanced salt solution and appropriate drugs (1-Isobutyl-3-methylxanthine (IBMX), PGE_1 and forskolin) were added in 20 µl volumes and incubated with platelets for 30 minutes at 37^{o}C. IBMX (2.5 mM) was used to inhibit phosphodiesterase so that cAMP production could be measured without interference of hydrolyzing enzymes. At the end of the incubation period, platelets were precipitated with trichloroacetic acid (TCA), protein precipitate was separated by centrifugation, and protein was determined by the method of Lowry (24). TCA was removed from the supernatant by ether extraction, and samples were evaporated (Speedvac, Savant, Farmingdale, NY) and stored for cAMP radioimmunoassay (RIA). The cAMP RIA was performed by the method of Brooker et al. (25). Data were analyzed using appropriate computerized RIA programs.

Whole blood 5-HT and Platelet Count

Whole blood 5-HT was chosen as the most reliable measure of platelet content of 5-HT. Greater than 99% of whole blood 5-HT is in the platelet fraction (26). Direct measurement of platelet 5-HT by centrifugation adds laboratory error due to 5-HT release during processing or variable platelet yield. Whole blood 5-HT was measured by HPLC with fluorometric detection (17). Intra-assay and inter-assay coefficients of variation (C.V.) were 1.7% and 6.2%, respectively. Platelet count was determined with a Thrombocounter C (Coulter Electronics, Hialeah, FL) to correct basal platelet IP production.

Platelet 5-HT Uptake, [^{3}H]-Paroxetine Binding, [^{3}H]-LSD Binding

Platelet 5-HT uptake, 5-HT uptake binding ([^{3}H]-paroxetine binding), and 5-HT_2 binding ([^{3}H]-LSD binding) were assayed in the laboratory of Dr. Ramesh Arora at Hines V.A. Hospital. Samples were transported on ice to his laboratory within one hour of collection. Because of difficulty in blood drawing, 1 sample did not have sufficient tissue for [^{3}H]-LSD binding, and 6 samples could not be assayed for platelet 5-HT uptake because of logistical considerations.

Platelet 5-HT uptake

Platelet-rich plasma (PRP) was obtained by centrifugation at 600 x g for 2.5 min. in a Sorvall RC-5 centrifuge at 0^{o}C. Platelets were counted electronically by means of a Coulter thrombocounter in triplicate. 5-HT uptake was studied as described earlier (27). In brief, PRP (0.3 ml) was preincubated with Kreb's phosphate buffer (0.6 ml, pH 7.4) minus $CaCl_2$ for 10

minutes at 37°C. Next 0.1 ml of [^{14}C]-5-HT (0.3-1.0 x 10^{-5} M) was added. The final concentration of 5-HT in the incubation mixtures was 0.3 μM, 0.5 μM, 0.75 μM, and 1 μM. One set of tubes was
immediately immersed in an ice bath after addition of 5-HT while the other set of tubes was incubated for an additional 4 minutes. The platelet pellet obtained by centrifuging the tubes at 10,000 x g for 15 minutes was dissolved in Tissue Solubilizer (RPI, Chicago, IL) and counted as described earlier (27). Active uptake was determined from the differences in disintegrations per minute (dpm) in the tubes incubated for 4 minutes at 37°C and the tubes immediately immersed in ice water.

Binding Studies

Isolation of Platelets. PRP was obtained by centrifugation at 600 x g for 2.5 min. in an IEC centrifuge using a swinging bucket rotor at room temperature. Blood volume was readjusted to its original volume with phosphate-glucose-sodium chloride buffer (pH 7.2) and centrifuged again at the same speed and time. PRP was taken off and mixed with the PRP collected earlier. The process was repeated 5 times. PRP was centrifuged at 16,000 x g for 10 mins. at 0-4°C. This method has yielded more than 95% of the total platelet population (28).

Preparation of Platelet Membranes. Platelet membranes were prepared following a previously described method (29). In brief, the platelet pellet was lysed by suspending the pellet in hypotonic Tris-EDTA (5 mM Tris, 0.1% EDTA, pH 7.5) buffer by homogenization using a polytron, setting 10, for 15 seconds. The membrane suspension was centrifuged at 30,000 x g for 10 min. at 0-4°C. The process was repeated twice, once with hypotonic buffer and finally with incubation buffer (Tris HCl 50 mM, pH 7.4 + NaCl 120 mM + KCl 5 mM). The platelet pellet was then resuspended into the incubation buffer to form the final membrane suspension for binding studies.

Determination of [^{3}H]-LSD Binding. Platelet 5-HT_2 binding was determined using [^{3}H]-LSD as binding ligand. An aliquot (0.2 ml) containing 100-150 μg membrane protein was incubated with [^{3}H]-LSD (sp. act. 79.9 Ci/mmole) in Tris HCl + NaCl + KCl buffer (pH 7.4) for 4 hr. at 37°C in the presence and absence of spiperone (300 nM). After incubation, the reaction was terminated by the addition of Tris HCl, pH 7.7 (50 mM) containing 0.1% bovine serum albumin and rapidly filtered through GF/F filters. The filters were washed three times with 4 ml of ice cold buffer and the radioactivity was counted after overnight digestion with Bio-Safe II (RPI, Chicago, IL).

The specific binding of [^{3}H]-LSD was defined as the difference in binding in the presence and absence of spiperone. Five to six concentrations of [^{3}H]-LSD (0.2-4 nM) were used in the incubation mixture to determine the dissociation constant (K_d) and the density of [^{3}H]-LSD binding sites (B_{max}) by Scatchard analysis.

Determination of [^{3}H]-Paroxetine Binding. Platelet membranes were incubated with [^{3}H]-paroxetine (0.01-1 nM) in Tris-HCl (pH 7.4 at 25°C, 50 mM) buffer containing 120 mM NaCl + 5 mM KCl for 2 hours at 22°C. The incubations were done in the presence and absence of fluoxetine (10 μM). After incubation, the reaction was terminated by the addition of 4 ml of ice-cold buffer and filtered immediately through GF/F filters. The filters were washed with 3 x 4 ml of the same buffer and the radioactivity was counted in Bio-Safe II after overnight digestion. The specific binding of [^{3}H]-paroxetine was defined as the difference in binding in the presence and absence of fluoxetine. The K_d and B_{max} of [^{3}H]-paroxetine binding was calculated by Scatchard analysis.

Data Analysis

Non-parametric statistics were employed because the sample size did not permit sufficient power to reject non-normal distribution of the data. The Mann-Whitney Test was utilized to compare measures between hyperserotonemic and normoserotonemic subjects. Relationships between platelet measures and whole blood 5-HT levels were determined by Spearman's rank order correlation.

Results

Whole blood 5-HT levels were higher in hyperserotonemic subjects (253 ± 51) than nor-/moserotonemic subjects (149 ± 40), as expected. There were no group differences in thrombin-stimulated platelet 5-HT release (See Table I.), nor were there significant differences in platelet basal and stimulated inositol phosphate and cAMP production (See Tables II and III.).

Platelet 5-HT uptake K_m (U=34, $p < 0.61$)and V_{max} (U=25, $p < 0.20$) did not differ significantly between hyperserotonemic and normoserotonemic subjects, although 4 hyperserotonemic subjects had 5-HT uptake V_{max} above 11.25 pmol/10^7 platelets/min, which was more than 1 S.D. above the highest normoserotonemic subject V_{max}. The V_{max} of 5-HT uptake was significantly correlated with whole blood 5-HT ($r_s = 0.56$, N=18, $p < 0.02$). Platelet [^{3}H]-paroxetine K_d was significantly decreased in hyperserotonemic subjects (U=37, $p < 0.05$) but [^{3}H]-paroxetine B_{max} did not differ (U=67, $p < 0.80$)(See Table IV.).

[^{3}H]-LSD B_{max} was significantly lower in 11 hyperserotonemic subjects compared to 12 normoserotonemic subjects (40.9 ± 13.5 fmol/mg protein, 59.6 ± 13.2; U=20, $p < 0.004$)(See Figure 1.). The age of these 11 hyperserotonemic subjects (38.5 ± 6.5 yr.) remained close to that of the 12 normoserotonemic subjects (38.1 ± 6.4). When the subjects who had received any medication during the month before blood drawing or who had a history of psychiatric illness were dropped, [^{3}H]-LSD B_{max} remained lower in the hyperserotonemic subjects (U=2.0, $p < 0.001$).

[^{3}H]-LSD B_{max} was significantly negatively correlated with whole blood 5-HT levels ($r_s = -0.63$, N=23, $p < 0.002$). [^{3}H]-LSD B_{max} and 5-HT uptake V_{max} were not significantly correlated. Post-hoc multiple regression analysis with whole blood 5-HT as the dependent variable and [^{3}H]-LSD B_{max} and 5-HT uptake V_{max} as independent variables was signficant (F 10.9, df 2,14, $p < 0.002$; $R^2 = 0.61$, [^{3}H]-LSD B_{max} Beta = -0.46, $p < 0.02$; 5-HT uptake V_{max} Beta = 0.59, $p < 0.004$). [^{3}H]-LSD Kd did not differ between normoserotonemic (0.336 ± 0.144) and hyperserotonemic (0.316 nM ± 0.099) subjects (U=63.5,$p < 0.89$).

Discussion

Most of the 5-HT related measures were similar in normoserotonemic and hyperserotonemic subjects. However, [^{3}H]-LSD binding was significantly decreased in platelets from hyperserotonemic first-degree relatives of children with autistic disorder. This finding is consistent with a previous comparison of autistic men to normal controls (30) and the negative correlation between [^{3}H]-LSD Bmax and whole blood 5-HT levels is of a similar direction and magnitude in both studies. A previous study in our laboratory found no significant difference in [^{125}I]-spiroperidol labelled platelet 5-HT_2 binding sites between normoserotonemic and hyperserotonemic subjects and no correlation between whole blood 5-HT levels and platelet [^{125}I]-spiroperidol Bmax (31).

Although these three binding studies were not performed on the same subjects, normal binding with [^{125}I]-spiroperidol, an antagonist, but decreased binding in two studies with a partial

agonist, LSD, may indicate a possible alteration in the structure of the 5-HT_2 receptor in some hyperserotonemic subjects. Further work, including a detailed study of the same subjects with several 5-HT_2 receptor binding ligands is necessary.

TABLE I

Percentage of 5-HT Remaining in Platelets after Thrombin-Stimulated Release

	N	0.005 U/ml	0.01 U/ml	0.02 U/ml	0.05 U/ml	0.1 U/ml	0.5 U/ml
Normoserotonemic	12	100 ± 10	98 ± 19	74 ± 16	49 ± 17	42 ± 21	41 ± 23
Hyperserotonemic	12	106 ± 7	104 ± 20	77 ± 14	48 ± 13	39 ± 13	38 ± 15

Data is reported as mean ± S.D..

TABLE II

Platelet Inositol Phosphate Production

	N	Basal cpm/10^8 plat.	5-HT-Stimulation % over basal	AVP-stimulation % over basal
Normoserotonemic	12	52	39 ± 67 %	59 ± 77 %
Hyperserotonemic	12	71	6 ± 42 %	67 ± 79 %

TABLE III

Platelet cAMP Production (pmol/mg protein)

	N	Basal	IBMX	PGE_1 0.1 μM	PGE_1 1.0 μM	PGE_1 10 μM	Forskolin 1.0 μM	Forskolin 10 μM	Forskolin 50 μM
Normo-serotonemic	10	34 ± 20	163 ± 132	1250 ± 500	2670 ± 830	3320 ± 1360	1260 ± 990	3780 ± 1380	7730 ± 3420
Hyper-serotonemic	10	113 ± 140	208 ± 218	2280 ± 3710	3740 ± 3960	4450 ± 3560	2050 ± 3670	3940 ± 3720	6890 ± 2450

TABLE IV

Platelet 5-HT Uptake and [^{3}H]-Paroxetine Binding

	Uptake N	Uptake Km μM	Uptake Vmax pmol/10^7 plat per min	Binding N	Paroxetine Kd pM	Paroxetine Bmax fmol/mg prot
Normo-serotonemic	9	0.39 ± 0.11	7.83 ± 1.26	12	54.8 ± 12.1	832 ± 171
Hyper-serotonemic	9	0.51 ± 0.31	10.01 ± 4.05	12	47.6 ± 9.0[a]	810 ± 114

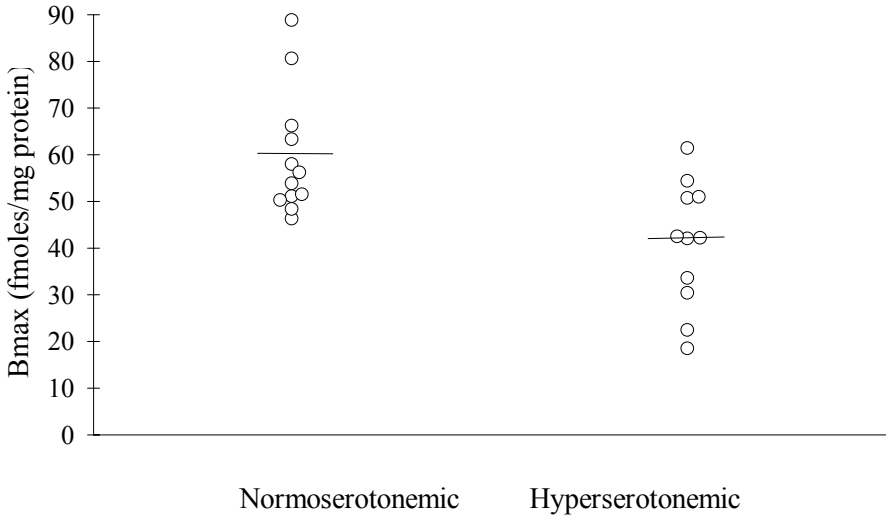

FIG. 1

B_{max} of [^{3}H]-LSD binding in the platelets of normoserotonemic and hyperserotonemic first-degree relatives of autistic probands. Line indicates mean for each group.

The finding of a positive correlation between 5-HT uptake V_{max} and whole blood 5-HT is consistent with a positive correlation between 5-HT uptake V_{max} and whole blood 5-HT levels in vervet monkeys (32). However, analysis of the specific subject data suggests that the hyperserotonemic subjects comprise 2 subgroups. Of the 8 hyperserotonemic subjects who had both 5-HT uptake and 5-HT_2 binding studies performed, 4 subjects had 5-HT uptake at least 1 S.D. above the highest normoserotonemic subject and 3 subjects had 5-HT_2 binding which was at least 0.95 S.D. below the lowest normoserotonemic subject. This suggests intergenic heterogeneity within familial hyperserotonemia of autism.

cAMP metabolism was remarkably similar between groups, although two hyperserotonemic subjects had markedly elevated rates of cAMP production. This is in contrast to a related developmental neurobiological disorder, Fragile X syndrome, in which a decrease in platelet cAMP production has been reported (33).

Thrombin-stimulated 5-HT release was not different between normoserotonemic and hyperserotonemic subjects. Subjects with increased platelet 5-HT uptake and normal 5-HT release would be expected to have higher steady state whole blood 5-HT levels. However, the current data do not explain higher steady state whole blood 5-HT levels associated with decreased 5-HT_2 binding. One possibility, which was not examined in the current study, is that signal transduction coupled to 5-HT_2 receptor binding may be related to platelet dense granule 5-HT uptake (34).

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