The serotonin system in autism
Edwin H. Cook, Jr, MD, and Bennett L. Leventhal, MD

The serotonin system has been implicated as a factor in some cases of autism since the finding in 1961 of elevated serotonin (5-hydroxytryptamine) levels in the blood of patients with autism. This has been clarified as elevation in the platelet content of serotonin. Subjects with elevated whole blood serotonin levels have been shown to have elevated platelet serotonin transport into platelets and decreased serotonin 5-HT$_2$ receptor binding. Most individuals with autism who are treated with potent serotonin transporter inhibitors have a reduction in ritualistic behavior and aggression. Reduction of central nervous system serotonin, induced by acute tryptophan depletion, causes a worsening of stereotyped behavior. Recent developments in the molecular biology of serotonin receptors are reviewed.

Although the function of serotonin (5-hydroxytryptamine [5-HT]) in the central nervous system is still being clarified, various studies have indicated an important role for serotonin in central nervous system (CNS) development, social behavior, sleep, aggression, anxiety, and affective regulation. Therefore, it is not surprising that serotonin has been the most intensively studied neurochemical in autism over the past three decades. Recent advances in pharmacology, molecular biology, and genetics have increased our knowledge of the serotonin system and hold out the promise of the development of improved pharmacologic treatment for at least some aspects of autism, ritualistic behaviors and aggression.

**Hyperserotonemia**

In 1961, Schain and Freedman [1] reported elevation of whole blood serotonin levels (hyperserotonemia) in patients with autism. A series of studies were performed to test the hypothesis that increased blood serotonin levels indicated a toxic metabolic accumulation of serotonin. A similar hypothesis was supported for the contemporary finding of phenylketonuria. However, the hypothesis that hyperserotonemia in autism was a reflection of toxic levels of serotonin was not supported. In the process of testing this hypothesis, several possible artifacts, including diet, were excluded [2,3], and the finding of hyperserotonemia in at least 25% of individuals with autism was confirmed.

An assay for platelet-poor plasma serotonin developed by Anderson et al. [4] revealed that more than 99% of whole blood serotonin is contained in platelets. Use of this assay revealed that when whole blood serotonin levels were elevated, platelet-poor plasma ultrafiltrate serotonin levels were not elevated in subjects with autism [5]. Because serotonin is both actively transported into platelets and released from platelets, either an increase in serotonin transport into platelets or a decrease in the release of serotonin from platelets would lead to an increased steady-state level of serotonin in platelets.

From a technical perspective, direct measurement of platelet serotonin levels is very difficult, requiring several steps that introduce error, including destruction of platelets, release of serotonin from platelets, or size-dependent and shape-dependent yield of platelets. Review of platelet serotonin studies suggests less consistency in findings when platelets are directly studied by centrifugal separation. Given the technical limitations and the demonstration that virtually all the serotonin in blood...
is stored in platelets [4,5] whole blood serotonin and platelet serotonin are essentially interchangeable, and the most accurate method for measuring platelet serotonin levels is to measure it in whole blood. For this review, the two will be considered as synonymous.

**Familiality of platelet serotonin**

Several studies have shown a positive correlation of platelet serotonin levels between probands with autism and their parents and siblings [6–9]. McBride et al. (Paper presented at 34th Annual Meeting of the American Academy of Neuropsychopharmacology, San Juan, 1995) recently presented results from a study that confirmed the importance of controlling for race and ethnicity in studies of platelet serotonin [10]. Black and Hispanic subjects had higher levels of platelet serotonin than did white subjects. There is no evidence for a physiologic consequence of this difference, but the finding does prompt the reassessment of many previous studies. For example, it was reported in one study that there was a correlation between whole blood serotonin levels of mothers and fathers of probands with autism [8]. In a subsequent study, this finding was not confirmed [9]. The correlation in the earlier study was accounted for by two "outlier" couples who were black. In contrast, the findings of positive correlations of serotonin between probands and their mothers were present when white subjects were considered separately.

Interestingly, subjects with autism who had a sibling with autism had higher platelet serotonin levels than subjects who did not have a sibling with autism [11]. Dominant or recessive inheritance, rather than oligogenic inheritance, is more likely in families with two autistic children than in families with only one such child. Thus, this finding suggests that hyperserotonemia may be an indicator of autism with a higher risk of sibling recurrence. It would be useful to study families without a proband with autism or attention-deficit hyperactivity disorder to see whether there is general heritability of platelet serotonin levels, independent of diagnosis. Platelet serotonin levels have been demonstrated to be stable after the age of 9 years [12], supporting the hypothesis that platelet serotonin levels are under genetic regulation. Because platelets are fragments of multinucleated megakaryocytes, genetic regulation may occur at the level of the megakaryocyte. Studies of megakaryocytes are obviously not practical. However, it is possible to study genes for regulation of the serotonin transporter and 5-HT2A receptors, because both are expressed in platelets and on pre- and postsynaptic neurons at serotonergic synapses [13,14].

**Studies of the mechanism of increased platelet serotonin**

A few studies have examined platelet serotonin function in subjects with elevated platelet serotonin levels. Platelet 3H-lysergic acid diethylamide (LSD)-labeled 5-HT2 receptor binding was decreased in adults with autistic disorder when compared with binding in normal adult control subjects [15]. There was a relatively strong but insignificant (n=9) negative correlation between 5-HT2 receptor binding sites and platelet serotonin levels. In another study, children with autistic disorder did not differ from their parents or siblings when 125I-spiroperidol-labeled platelet 5-HT2 receptor binding was studied [16]. There was no difference between subjects with normal and high platelet serotonin levels (Cook, Unpublished data). A more recent study found that 3H-LSD-labeled platelet 5-HT2 receptor binding was lower in hyperserotonemic first-degree relatives of autistic children when compared with normoserotonemic relatives [17]. The difference in outcome between these two studies may be related to several factors, including a putative point mutation in the 5-HT2A receptor altering affinity for agonists but not antagonists [18]. The two studies were performed during different seasons (February vs July–September). LSD has been shown to bind with high affinity to transfected 5-HT3A [19], 5-HT6 [20], and 5-HT2 [21] receptors. It is also possible that there was binding to platelet serotonin receptors other than the 5-HT2A receptor by LSD, but not spiroperidol, under the conditions used (Table 1).

A correlation was also found between platelet serotonin levels and rate of platelet serotonin transport [17]. The increase in transport is interesting in light of the therapeutic effect of potent serotonin transporter inhibitors on ritualistic behaviour and irritability in autism (see "Pharmacology").

**Relationship of elevated platelet serotonin levels to clinical subgroups**

No consistent pattern between symptoms of autism and elevated whole blood serotonin levels has emerged [22], but these studies have not used reliable instruments to assess autistic symptoms, such as the Autism Diagnostic Interview [23] or the Autism Diagnostic Observation Schedule [24]. In particular, future studies of serotonin levels in autism should focus on symptoms pertaining to restricted and repetitive interests or aggression, because these symptoms respond partially to treatment with potent serotonin transporter inhibitors. (See "Pharmacology").

**Pharmacology**

The most compelling evidence for the relationship between serotonin levels and autism is the efficacy of antidepressant medications that potently inhibit serotonin transport. Potent serotonin transporter inhibitors include the tricyclic antidepressant clomipramine and the selective serotonin reuptake inhibitors fluoxetine, sertraline, fluvoxamine, and paroxetine. Potent serotonin transporter inhibitors have been shown to reduce rituals
Table 1

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Signal transduction</th>
<th>Notes</th>
<th>Amino acid variants</th>
<th>Chromosome</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT_{1A}</td>
<td>↓ AC</td>
<td>8-OH-DPAT; autoreceptor; stimulates neurite branching</td>
<td>128V, G22S</td>
<td>5</td>
<td>Sikkot et al. [44], Fargin et al. [49], Goldman et al. [50], Nakshai et al. [61], Erdmann et al. [52]</td>
</tr>
<tr>
<td>5-HT_{1B}</td>
<td>↓ AC</td>
<td>= 5-HT_{1D}</td>
<td>F124C</td>
<td>6q13</td>
<td>Ramboz et al. [65], Hartig et al. [54], Lappalainen et al. [55], Saudou et al. [56], Nothen et al. [57]</td>
</tr>
<tr>
<td>5-HT_{1D}</td>
<td>↓ AC</td>
<td>= 5-HT_{1D}</td>
<td></td>
<td></td>
<td>Hartig et al. [54], Ozaki et al. [58]</td>
</tr>
<tr>
<td>5-HT_{1E}</td>
<td>↓ AC</td>
<td>= 5-HT_{1E}</td>
<td></td>
<td>1p35</td>
<td></td>
</tr>
<tr>
<td>5-HT_{1F}</td>
<td>↓ AC</td>
<td>= 5-HT_{1F}</td>
<td>6q14-15</td>
<td></td>
<td>Goldman et al. [50], McAllister et al. [59], Levy et al. [60]</td>
</tr>
<tr>
<td>5-HT_{1G}</td>
<td>↓ AC</td>
<td>= 5-HT_{1G}</td>
<td>3p</td>
<td></td>
<td>Lovenberg et al. [61], Cook (Unpublished data)</td>
</tr>
<tr>
<td>5-HT_{5A}</td>
<td>↑ PLC</td>
<td>5-HT_{5A} sequence identical brain and platelet [14]; LSD</td>
<td>T29N, A439V, H444Y</td>
<td>13q14.1</td>
<td>Cook et al. [14], Julius et al. [62], Ozaki et al. [63], Erdmann et al. [64]</td>
</tr>
<tr>
<td>5-HT_{5B}</td>
<td>↑ PLC</td>
<td>= 5-HT_{5B}</td>
<td>13q14.2</td>
<td>15q26.3-2q37.1</td>
<td>Schmuck et al. [65], Lappalainen et al. [66], Fedor et al. [67], Kaufman et al. [70]</td>
</tr>
<tr>
<td>5-HT_{5C}</td>
<td>↑ GC</td>
<td>= 5-HT_{5C}; sudden death in knockout mice; choroid plexus; alternative splice variant</td>
<td>C23S</td>
<td>Xq21</td>
<td>Julius et al. [67], Lappalainen et al. [66], Fedor et al. [67], Kaufman et al. [70]</td>
</tr>
<tr>
<td>5-HT_{6}</td>
<td>↓ AC</td>
<td>Ondansetron; splice variants</td>
<td>11q23.1-23.2</td>
<td>5q</td>
<td>Uetz et al. [71], Miyake et al. [72], Weiss et al. [73]</td>
</tr>
<tr>
<td>5-HT_{7}</td>
<td>↑ AC</td>
<td>Zopiclone; two splice variants-5-HT_{7L} and 5-HT_{7H}; human 5-HT_{7} 18 amino acids shorter than rat, autoreceptor</td>
<td>7q36.1</td>
<td></td>
<td>Adham et al. [74], Gerald et al. [75], Cook (Unpublished data)</td>
</tr>
<tr>
<td>5-HT_{1A}</td>
<td>↓ AC</td>
<td>= REC17; colocalized with mouse reeler mutant and human homologues of Eph, 3, LSD</td>
<td></td>
<td></td>
<td>Matthies et al. [19], Schanen et al. [76]</td>
</tr>
<tr>
<td>5-HT_{1D}</td>
<td>↓ AC</td>
<td>= MR22; pseudogene in humans</td>
<td></td>
<td>2q11-1q13</td>
<td>Matthies et al. [19], Grailhe et al. [77]</td>
</tr>
<tr>
<td>5-HT_{2A}</td>
<td>↑ AC</td>
<td>= 5-HT_{2A}</td>
<td>2p35-36</td>
<td>10q</td>
<td>Kohn et al. [78]</td>
</tr>
<tr>
<td>5-HT_{7}</td>
<td>↑ AC</td>
<td>Antagonists: eg. clozapine, risperidone; agonists: LSD, 5-HT_{2A}, 8-OH-DPAT; also 80%-90% homologous pseudogene</td>
<td>P279L</td>
<td>10q</td>
<td>Bard et al. [21], Gelernter et al. [79], Goldman et al. [80]</td>
</tr>
<tr>
<td>5-HT_{11}</td>
<td>Transporter</td>
<td>= SLC 6A4</td>
<td>17q11.1-1q12</td>
<td></td>
<td>Gelernter and Frimer [81]</td>
</tr>
</tbody>
</table>

AC=adenyl cyclase; GC=guananyl cyclase; LSD=lysergic acid diethylamide; 8-OH-DPAT=8-hydroxy-2-(di-n-propylamino)tetrinal; PLC=phospholipase C.

associated with anxiety and to reduce aggression in more than 50% of children and adults with autism in open [25-29] and double-blind trials [30,31]. These drugs have also been used successfully to treat self-injurious behavior and stereotypic movements in patients with mental retardation without autism [29,32]. The acute effect of administration of these drugs in healthy adults is a reduction in basolateral limbic system (amygdala and hippocampus) metabolism [33]. Chronic administration in rodents leads to an increase in serotonergic neurotransmission through downregulation of presynaptic terminal autoreceptors [34].

Preliminary evidence of the efficacy of risperidone has been reported in autism [35,36]. The effects appear to be similar to those of potent serotonin transporter inhibitors but are more rapid in onset (Cook et al., Unpublished observation). Risperidone is an antagonist at dopamine D_{2} and D_{4} receptors [37]. It is also an antagonist at 5-HT_{2A} and 5-HT_{7} receptors [38]. Development of more specific antagonists may allow study of the possibility that the rapid action of risperidone in reducing rituals in autism may be due to antagonism of presynaptic 5-HT_{7} autoreceptors, which would lead to a more rapid onset of increased serotonin neurotransmission than potent inhibition of the presynaptic serotonin transporter. In addition, absence of D_{2} receptor antagonism is likely to reduce or eliminate the risk of tardive dyskinesia.

Further evidence of serotonin involvement in autism comes from a pharmacologic study using tryptophan depletion. Tryptophan depletion leads to reduced serotonin synthesis, release, and neurotransmission. McDougle et al. [39] found exacerbation of behaviors such as whirling, flapping, pacing, banging and hitting self, rocking, toe walking, and anxiety in more than 50% of adults with autism after tryptophan depletion. This finding is consistent with the finding of a decreased ratio of the level of serum tryptophan to the level of large neutral amino acids in idiopathic infantile autism relative to control subjects [40]. This decreased ratio would lead to a lower basal level of serotonin synthesis, vulnerability to tryptophan depletion, and response to pharmacologic manipulations that increase serotonin neurotransmission (Fig. 1).
Molecular biology of serotonin receptors and the serotonin transporter

Several recent developments in the molecular biology of serotonin receptors are relevant in the study of autism. Past studies of 5-HT_2 receptors must be reconsidered, because the 5-HT_2A, 5-HT_2C, 5-HT_3, 5-HT_7 receptors have been cloned. Table 1 lists much of the information about serotonin receptors and the serotonin transporter that may be of relevance in developing a better understanding of the role of the serotonin system in autism. The expression of the 5-HT_2A receptor and the serotonin transporter in autism provides the most logical connection between platelet findings and the relevant CNS mechanism. Because pharmacologic evidence suggests that more than 50% of patients with autism have an abnormality in serotonergic neurotransmission, it is possible that one or more of these mechanisms (decreased postsynaptic 5-HT_2 receptor binding or increased serotonin transporter function) may lead to platelet findings, but other possible specific mechanisms may not be expressed in platelet changes (eg, increased 5-HT_1A autoreceptor sensitivity).

Role of serotonin in central nervous system development

Serotonin may have a role in the developmental neuropathologic abnormalities found in the hippocampus, amygdala, and cerebellum in autistic disorder [41]. As a specific example, decreased neurite branching has been observed in the hippocampus in autism [42]. Stimulation of the 5-HT_1A receptor by 8-hydroxydipropylaminotetralin (and possibly 5-HT_1A receptor [43]) decreases neurite branching during development of the nervous system [44]. In addition, the 5-HT_1A receptor has neurotrophic effects in the hippocampus [45]. The 5-HT_1A receptor is also expressed in the fetal and neonatal but not the adult human cerebellum [46]. The 5-HT_1A receptor is expressed in the hippocampus and cerebellum [47]. The 5-HT_6 receptor is expressed in the amygdala, hippocampus, and cerebellum [48].

Our laboratory has recently studied expression of serotonin receptor in an amygdala cDNA library and found a pattern of serotonin receptor mRNA of the order 5-HT_1A > 5-HT_1D > 5-HT_1B > 5-HT_2C > 5-HT_2B > 5-HT_2A > 5-HT_3 > 5-HT_7 (Cook et al., Unpublished observation). The serotonin transporter is expressed at all presynaptic serotonin terminals.

Conclusions

Convergent findings from behavioral neuroscience, platelet, pharmacologic, and genetic studies indicate the involvement of serotonin in many of the symptoms of autistic disorder. Over the next decade, as the molecular biology of serotonin-related proteins is elucidated and medications are developed using this knowledge, it will be possible to assist people with autism to more effectively control the disabling symptoms of aggression, anxiety, and inflexible rituals and routines. As the molecular biology of the role of serotonin in CNS development is elucidated, it may become possible to treat or prevent the development of the social and cognitive dysfunction associated with autism through rational pharmacologic or dietary intervention.

Acknowledgment

The authors would like to acknowledge the inspiration, encouragement, and constant push to understand the mechanism of hyperserotonemia provided by Daniel X. Freedman.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* Of special interest
** Of outstanding interest


This paper represents the first double-blind study in which both efficacy and the absence of long-term irreversible side effects (e.g., neuroleptics) have been replicated for a class of medications—potent serotonin transporter inhibitors—in the treatment of core symptoms of autistic disorder. This paper is also significant because it is the first double-blind study showing efficacy for a selective serotonin reuptake inhibitor in autistic disorder, confirming an earlier systematic open trial with fluoxetine. Although the core social and communicative impairments are not addressed by this class of medications, symptoms such as the performance of rituals, preoccupations, and routines associated with anxiety and aggression often respond.


38. Roth B, Craigo S, Choudhary S, Uluer A, Monsa Jr F, Hen Y, Melzer H, Sibley D: Binding of typical and atypical antipsychotic drugs to 5-

   This study shows worsened stereotypic behavior in subjects with autism who had undergone acute dietary depletion of tryptophan (which acutely reduces serotonin synthesis).

40. D’Eufemia P, Finocchiare R, Celli M, Viozzi L, Monteleone D, Giardini O:
   Reduction in the ratio of tryptophan levels to large neutral amino acid levels has been shown in other studies to lead to decreased tryptophan transport across the blood-brain barrier and subsequently to decreased serotonin synthesis. This is an interesting study in relationship to the findings of worsening of stereotypic symptoms after tryptophan depletion.


74. Asham N, Gerald C, Vayse P-J, Wennhark R, Brancheck T: Pharmacological characterization of two splice variants of the cloned

The serotonin system in autism

Cook and Leventhal 353


