Neurochemistry in the Pathophysiology of Autism

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Significant progress has been made in the search for underlying pathophysiologic mechanisms in autism over the past 50 years. The cause of the disorder, however, remains largely unknown. This article reviews neurochemical contributions to the pathophysiology of autism with a focus on monoamines, glutamate/γ-aminobutyric acid systems, and neuropeptides. As these efforts move forward, it will be important to begin to integrate genetic studies with those involving neuroimaging and postmortem research in each of these 3 areas, as well as with pharmacologic treatment approaches.

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Research into the pathophysiology and etiology of autistic disorder (autism) has been ongoing for nearly a half century. Despite these significant efforts, the cause remains unknown. This review will discuss neurochemical aspects of the pathophysiology of autism. Three primary areas will be highlighted, including monoamines (serotonin [5-hydroxytryptamine, 5-HT], dopamine [DA], norepinephrine [NE]), glutamate/γ-aminobutyric acid (GABA) systems, and neuropeptides. Where data are available, peripheral and central neurochemistry, genetics, neuroimaging, and postmortem findings will be presented.

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MONOAMINES

Serotonin

Serotonin neurons are widely distributed throughout the mammalian brain. This neuronal system is one of the earliest to develop, and the turnover rate of 5-HT is higher in the immature mammalian brain than at any other time in life. Serotonin plays a critical role as a growth factor in the immature brain, directing both proliferation and maturation.[1]

Initial studies on the pathophysiology of autism focused on the 5-HT system. A recent chapter provided a detailed review of the results from those investigations, including peripheral and central neurochemistry, behavioral/neuroendocrine challenges, genetics, and neuroimaging.[2] We will provide a brief summary of those findings.

Schain and Freedman[3] were the first to study whole blood serotonin (WBS) in autism. Their sample included 3 groups: mildly retarded children, autistic children who were severely retarded, and severely retarded children without autism. Consistent unusual elevations of WBS were found only in the autistic children, although the mean WBS level of the other severely retarded group was higher than that of the mildly retarded group. No differences were found in presenting clinical symptoms between the 6 autistic children with the highest WBS levels and those who had normal levels. These results were largely replicated by Ritvo and colleagues.[4] In 1987, Anderson and others from Yale published results from their laboratory and reviewed and summarized data on WBS levels in autism to that date.[5] WBS concentrations were significantly higher in drug-free autistic subjects than in normal controls, with 38% of the subjects determined to be "hyperserotonemic." Results from a subsequent study by McBride et al.[6] led the investigators to conclude that the prevalence of hy-

perserotonemia in autism may have been previously overestimated because of failure to control for race and pubertal status.

In a large study by Leboyer et al.,[7] WBS levels were determined in 62 subjects with autism aged 3 to 23 years, 91 healthy controls aged 2 to 16 years, and 118 healthy subjects over 16 years of age. Twenty-nine (48%) of the 60 autistic subjects for whom there was a sample met criteria for hyperserotonemia. Among controls, WBS values diminished with age, whereas WBS levels among autistic subjects appeared to be age-independent. In this same study, 51% of mothers, 45% of fathers, and 87% of siblings (older than 16 years) of autistic subjects had hyperserotonemia.

In summarizing results from studies of WBS in autism, many but not all investigations have found elevated WBS levels in younger autistic subjects that tend to remain higher than those of normal controls across the age range. In contrast, most studies of normal subjects have demonstrated an age-related decline in WBS levels with increasing age. Some investigators have suggested that these results could be explained, in part, by abnormal maturational processes of the 5-HT system in autistic subjects.[5,7] Additional factors that may affect WBS levels include race, pubertal status, and treatment with psychotropic medication. Whether WBS levels will prove to be a useful quantitative measure in the search for genetic susceptibility to autism remains to be determined.

In general, studies of urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA),[8] the primary metabolite of 5-HT, and whole blood tryptophan concentrations[5] have not found significant differences between autistic subjects and controls.

Studies of baseline levels of a measure of central 5-HT function, cerebrospinal fluid (CSF) 5-HIAA, have found no difference between children with autism and controls.[9–11] Two studies that utilized probenecid to block the transport of 5-HIAA out of the CSF found similar[12] or slightly lower[13] levels in autistic children compared with nonautistic children with psychosis.

Behavioral/neuroendocrine challenge studies have been conducted in autistic subjects. The immediate precursor of 5-HT, 5-hydroxytryptophan (5-HTP), was administered to children with autism and adult normal control subjects.[14,15] Prolactin response to 5-HTP was reduced in the children with autism, suggesting diminished central 5-HT responsivity. Blunted prolactin release was also found in response to fenfluramine 60 mg given orally, in an investigation of 7 male young adults with autism and matched healthy controls.[16] Utilizing a different strategy, the acute tryptophan depletion (ATD) paradigm was administered to 17 drug-free adults with autism by McDougle and colleagues.[17] The ATD resulted in a significant reduction in plasma free and total tryptophan, whereas administration of sham depletion (containing tryptophan) led to a significant increase in these plasma measures. Eleven of the 17 subjects showed a worsening of symptoms, including a significant increase in whirling, flapping, pacing, banging, hitting self, rocking, and toe walking, with ATD compared to sham depletion. Another set of challenge studies involved the 5-HT_{1D} receptor agonist sumatriptan, which has been shown to increase growth hormone release. Eleven adults with autism or Asperger's disorder and 9 controls were given subcutaneous sumatriptan and placebo separated by 1 week.[18] The research subjects had a significantly greater growth hormone response than controls, suggesting that a hypersensitivity of the 5-HT_{1D} receptor may exist. In a related study, Hollander et al.[19] reported that the severity of repetitive behavior at baseline in these subjects was positively correlated with the growth hormone response to sumatriptan. The same investigators recently found that the oral administration of *m*-chlorophenylpiperazine (*m*-CPP) resulted in a significant increase in repetitive behaviors and prolactin in adults with autism or Asperger's disorder in comparison with controls.[20]

A number of investigations of genes involved in the 5-HT system have been conducted in autism. The 5-HT transporter (5-HTT), the site of action of serotonin reuptake inhibitors, has been considered a candidate gene for autism. Cook et al.[21] were the first to report the presence of an association between the short variant of a functional insertion-deletion polymorphism in the promoter region of 5-HTT (HTTLPR) and autism. In contrast, Klauck et al.[22] identified preferential transmission of the long variant of HTTLPR in their sample of autistic subjects. A number of subsequent studies involving subjects from various countries have reported similar results or have been unable to replicate either finding.[2]

Results from studies involving other genes contributing to the 5-HT system, including the genes encoding the 5-HT_7 receptor[23] and the 5-HT_{2A} receptor,[24] respectively, have not identified a significant association with autism. Tryptophan 2,3 dioxygenase (TDO2) is the rate-limiting enzyme in the catabolism of tryptophan, the precursor of 5-HT. A study by Nabi et al.[25] demonstrated a significant difference in the transmission of TDO2 haplotypes to autistic subjects, suggesting the presence of a susceptibility mutation in the TDO2 or a nearby gene. Recent investigations have sought a relationship between a subset of autistic subjects with prominent rigid-compulsive behaviors and 5-HTT with some preliminary encouraging results.[26,27]

Neuroimaging studies of the 5-HT system have also been completed in autism. The first investigation utilized positron emission tomography (PET) to assess the tracer α-[^{11}C]methyl-L-tryptophan (AMT) as an indicator of 5-HT synthesis in 8 autistic children and 5 of their siblings.[28] Gross asymmetries of 5-HT synthesis in frontal cortex, thalamus, and cerebellum were found in all 7 of the autistic boys but not in the only female autistic subject. Such asymmetries were not identified in the frontal cortex

or thalamus of the siblings, although 1 sibling showed increased [^{11}C]AMT accumulation in the right dentate gyrus. This boy had a history of calendar calculation, and he ritualistically lined up his toys. The investigators concluded that the focal abnormalities in [^{11}C]AMT accumulation may represent either aberrant innervation by 5-HT terminals or altered function in anatomically normal pathways. A subsequent study by the same investigators,[29] again using PET and [^{11}C]AMT, found that for nonautistic children, 5-HT synthesis capacity was more than 200% of adult values until the age of 5 years and then declined toward adult values. In autistic children, 5-HT synthesis capacity increased gradually between the ages of 2 years and 15 years to values 1.5 times adult normal values. It was concluded that humans undergo a period of high brain 5-HT synthesis capacity during childhood, and that this developmental process is disrupted in autistic children.

Dopamine

The monoamine DA is integral to motor and cognitive functioning, as well as hormone release.[30] A role for DA in autism has been postulated, in part, based upon the beneficial effects observed with the use of DA D_2 receptor antagonists in treating this population. This class of drugs has been shown to effectively target symptoms commonly exhibited by individuals with autism, such as aggression, self-injurious behavior, and hyperactivity.[31]

To a large extent, neurochemical research in this area has centered on the measurement of the major DA metabolite, homovanillic acid (HVA), in urine and plasma, as well as CSF. When considering this research, it is important to understand that only approximately 25% of urine and plasma HVA appears to result from central DA turnover, and that peripheral measures are primarily able to identify only significant alterations in central DA metabolism.[32]

In a study of catecholamine metabolism in 22 youths with autism aged 5 to 16 years and controls matched for age and sex, no significant difference in urinary DA was found between groups.[33] Minderaa and colleagues[34] investigated plasma HVA and prolactin levels, as well as urine HVA and DA excretion, in medicated and unmedicated autistic subjects and unmedicated controls. The authors found no significant differences between the autistic and control groups, suggesting normal peripheral indices of DA functioning. Martineau et al.[35] measured urine levels of DA and its derivatives, including HVA, 3-methoxytyramine (3MT), and NE + epinephrine (EPI), in 156 children with autism, compared with age-matched mentally retarded and normal controls. The levels were found to decrease significantly with age in all 3 groups. Significantly decreased levels of DA and HVA were found in medicated versus unmedicated autistic youth. The authors hypothesized that the results may be secondary to a defect in maturation of monoaminergic systems in autism.

Several studies that measured CSF HVA levels have been published. Gillberg and Svennerholm[10] reported that group mean levels of CSF HVA were elevated by approximately 50% in autistic children, compared to an age- and sex-matched control group with neurologic disorders. However, similar to previous findings by Cohen and colleagues,[12,13] a controlled study of CSF monoamine effects with fenfluramine treatment in 9 youths with autism reported normal levels of CSF HVA.[36] In addition, Narayan and colleagues[11] also found normal levels of CSF HVA in their controlled study of 17 children with autism. The authors reported that the results were consistent with the majority of earlier studies that did not find a group difference in this metabolite in CSF.

Some genetic studies of DA involvement in autism have been completed. Comings and colleagues[37] suggested that the A1 allele of the DA D_2 receptor gene may be associated with a number of behavioral disorders in which it may act as a modifying gene. In their case-control study, the authors examined a variety of neuropsychiatric disorders, including autism, which are believed to involve defects in DA neurotransmission. The prevalence of the A1 allele was noted to be significantly increased in the group with autism.

Another study examined the DA D_1 and D_5 receptor genes in autism via restriction endonuclease fingerprinting.[38] One novel missense change (L88F) occurred in transmembrane domain II at a highly conserved amino acid in all DA receptors, as well as in α_1- and β-adrenergic receptors. The mutation was identified in a Caucasian male patient with autism.

Robinson and colleagues[39] examined the DA-β-hydroxylase (DβH) gene as a candidate locus in 37 families with 2 or more children with pervasive developmental disorders (PDDs) using the affected sib-pair method. DβH is an enzyme that catalyzes DA to NE. There was no increased concordance for DβH alleles in affected siblings, but the mothers had a higher frequency of alleles containing a 19-base pair deletion. The authors hypothesized that lowered maternal serum DβH activity may produce a suboptimal uterine environment, which, in combination with a genetic susceptibility, could result in PDDs in some families.

Dopaminergic activity has also been investigated via neuroimaging techniques in autism. Using the PET tracer [^{18}F]fluorodopa (FDOPA), Ernst and colleagues[40] studied 14 children with autism (8 males; age, 10–17 years) and 10 controls (7 males; age, 12–17 years). In the autistic group, regional FDOPA accumulation in the anterior medial prefrontal cortex was significantly reduced by 39%.

In another study employing PET, 6 children aged 3 to 5 years with autism were treated with 6R-L-erythro-5,6,7,8,-tetrahydrobiopterin (R-BH_4), a cofactor for tyrosine hydroxylase in the biosynthetic pathway of catecholamines.[41] Study subjects were included only if the investigators

noted a relatively low level of R-BH_4 in the CSF. Prior to treatment, PET revealed increased DA D_2 receptor binding in the caudate and putamen as a whole. After treatment, a 10% decrease in DA D_2 receptor binding was observed. In addition, CSF levels of R-BH_4 were found to be significantly increased.

Norepinephrine

The neurotransmitter NE is associated with arousal, memory, anxiety, and autonomic activity.[30] Produced from DA, NE is metabolized to vanillylmandelic acid (VMA) in the periphery, and to 3-methoxy-4-hydroxyphenylglycol (MHPG) in the central nervous system. Plasma and urine levels of NE and its metabolites have been considered to be generally well correlated with central functioning.[42] However, research has also shown that estimates of the proportion of MHPG in blood and urine originating in the central nervous system, relative to that from the periphery, have been uncertain, ranging from 10% to 60%.[43]

Regarding studies of blood measures of NE and its metabolites, Lake et al.[44] investigated levels of NE and DβH in autistic and normal control subjects. The authors recorded a significantly higher level of blood NE in the group with autism. In contrast to this finding, lower levels of DβH were found in the autistic group, perhaps due to the enzyme's longer half-life.

A study of plasma MHPG in youth with autism and normal controls recorded similar group means of 3.7 ng/mL and 3.2 ng/mL, respectively.[45] Similarly, Minderaa and colleagues[46] found no significant difference in mean ± SD plasma MHPG levels in unmedicated autistic (3.1 ± 0.6 ng/mL; N = 17), medicated autistic (3.3 ± 1.0 ng/mL; N = 23), and normal control (3.2 ± 1.2 ng/mL; N = 20) groups. In addition, no significant mean differences in levels of MHPG, NE, and EPI were recorded between subjects with autism and normal controls when evening and overnight urines were examined. The authors suggested that notable abnormalities in basal NE measures did not appear to be present in autism.

As a whole, studies of CSF MHPG in autism have found no significant differences compared to controls. Young and colleagues[47] reported a mean CSF MHPG concentration of 9 ng/mL in subjects with autism, a level comparable to that recorded in normal subjects. Another larger study of 25 youths with autism and age- and sex-matched controls also found similar values between groups.[10]

GLUTAMATE AND GABA

Glutamate, the primary excitatory amino acid neurotransmitter, is found in high concentrations throughout the brain. It is thought to be crucial in neuronal plasticity and higher cognitive functioning.[48] Glutamate receptors are divided into metabotropic and ionotropic types. The ionotropic receptors are further classified into the following 3 families: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate. Several researchers have postulated that glutamate dysfunction may play a role in autism.[49,50]

GABA, another amino acid neurotransmitter, is the primary inhibitory neurotransmitter in the brain. It is synthesized from glutamate by glutamic acid decarboxylase (GAD). Investigators have also hypothesized that GABA may have an important role in the pathophysiology of autism.[51]

Despite the important roles that these neurotransmitters may play, there has been a relative paucity of literature directly examining glutamatergic or GABA function in autism. This section will review neurochemical, genetic, and postmortem studies concerning the function of these neurotransmitters in autism.

Several reports have suggested that peripheral levels of glutamate are elevated in the plasma of subjects with autism and other PDDs. Aldred et al.[52] collected blood from 23 subjects aged 4 to 29 years with autism or Asperger's disorder and 55 of their family members (32 parents, 23 siblings) and measured amino acid concentrations. They found that concentrations of glutamate, phenylalanine, lysine, and asparagine were significantly higher in both subjects and family members compared to age-matched controls. Glutamine levels were significantly lower. Moreno-Fuenmayor et al.[53] also measured amino acid levels in 14 children with autism (all under 10 years) and age- and sex-matched controls. They found that aspartate was higher and glutamine and asparagine were lower in subjects than in controls. However, another analysis is in disagreement with these findings. Rolf et al.[54] measured amino acid content and GABA in platelet-rich plasma. They found that aspartate and glutamate were decreased in 18 drug-free children aged 8 to 14 years with autism compared to 14 age-matched healthy controls. GABA and glutamine levels were also significantly lower. In contrast to this, Dhossche et al.[51] reported elevated plasma GABA levels (measured by gas chromatography/mass spectrometry) in a small, heterogeneous sample of 9 subjects aged 5 to 15 years with autism compared to 9 control subjects with attention-deficit/hyperactivity disorder (ADHD). Most of the autistic subjects were taking prescribed psychotropic or anticonvulsant drugs, and all of the ADHD controls were taking psychostimulants. In summary, studies reporting on peripheral amino acid levels in autism present mixed results. Interpretation of these results is also difficult given the small sample sizes, possible medication effects, and different methodologies used.

A number of genetic studies of the glutamate and GABA systems have been conducted in autism. Jamain et al.[55] showed that the glutamate receptor ionotropic kainate 2 (GRIK2) or glutamate receptor 6 (GluR6) gene is in disequilibrium with autism, and that an excess of maternal transmission of the GRIK2 haplotype exists. Interestingly,

maternal transmission disequilibrium for GRIK2 has also been found by the same group in schizophrenia.[56] More importantly, this finding was recently replicated by Shuang et al.[57] in 174 Chinese Han parent-offspring trios. GRIK2 is located in the chromosome 6q21 region, which has been identified as a potential autism susceptibility region by at least 1 genome-wide scan study.[58]

GAD1 encodes glutamic acid decarboxylase 67kDa protein (GAD67), an enzyme important in the conversion of glutamate to GABA. As a decarboxylase, it requires vitamin B_6 as a cofactor, which some believe may have efficacy in autism.[59] It also occurs on chromosome 2q, which shows evidence for linkage in several genome-wide scans. Rabionet et al.[60] recently performed association studies on several candidate genes in this region including GAD1. They found no evidence for significant association between these genes and autism.

Ramoz et al.[61] did find linkage for 2 single nucleotide polymorphisms (SNPs) on another chromosome 2q gene, SLC25A12. This gene encodes for the mitochondrial aspartate/glutamate carrier. However, the report by Rabionet et al.[60] referenced their own unpublished data, which failed to replicate this finding in their sample.

Serajee et al.[62] found suggestive evidence for linkage disequilibrium between autism and the metabotropic glutamate receptor 8 (GRM8) gene, which occurs on chromosome 7q, another region implicated in genome-wide scans.

Several lines of evidence implicate the 15q11-q13 chromosome region as potentially harboring autism susceptibility genes. This includes numerous reports suggesting that duplications and other abnormalities in this region may occur in as many as 3% of autistic individuals.[63] This genetic region has also been implicated in Prader-Willi and Angelman syndromes, which share clinical features with autism. Finally, this region contains several GABA type A receptor subunit genes, which are candidates as autism susceptibility genes.

Cook et al.[64] tested several loci in this region for linkage disequilibrium and were the first to report an association between a marker (155CA-2) within the GABA receptor subunit β-3 gene (GABRB3) and autism in a sample of 140 trios. Linkage disequilibrium for this marker has been found in one other sample,[65] but not others.[66–69] However, Martin and colleagues[68] did report linkage disequilibrium with another nearby marker (GABRB3) in this same region.

Menold et al.[70] examined 16 SNPs located within GABRB3, GABRA5, and GABRG3 for linkage disequilibrium using the Pedigree Disequilibrium Test. Two SNPs located within the GABRG3 gene were in disequilibrium, suggesting that the GABRG3 gene or a nearby gene may contribute to genetic risk for autism. McCauley et al.[71] performed linkage disequilibrium mapping across a region containing a cluster of GABA receptor subunit genes on chromosome 15q12. Six markers individually, across GABRB3 and GABRA5, and several haplotypes inclusive of those markers, demonstrated nominally significant association.

In summary, there is emerging evidence that the GRIK2 gene may be involved in autism. There is conflicting evidence as to the role of other genes that encode GABA receptor subunits and the mitochondrial aspartate/glutamate carrier. Findings regarding GAD and other glutamate receptors await replication.

The glutamate and GABA systems have also been evaluated in postmortem studies in autism. Purcell et al.[72] used complementary DNA (cDNA) microarray technology, additional measurements of messenger RNA (mRNA) and protein levels, as well as receptor autoradiography to study the cerebellum and hippocampus in a total of 10 persons with autism and 23 matched controls. They found several genes to be up-regulated in autism, most notably the excitatory amino acid transporter 1 and the glutamate receptor AMPA 1 (GluR1) genes. In addition, higher levels of the corresponding proteins were found by Western blotting. Finally, AMPA receptor density was decreased in both the granule cell layer and molecular cell layer of the cerebellum. Other notable findings were no significant differences in GAD 1/2 protein levels (by Western blotting) or NMDA receptor density (by autoradiography) in the cerebellum.

Both GAD65 and GAD67 (2 isoforms of GAD) levels were measured in postmortem cerebellar (N = 5) and parietal cortices (N = 5) of persons with autism compared to controls.[73] In this study, GAD65 (but not GAD67) was significantly lower in cerebellar cortices and GAD67 (but not GAD65) was significantly reduced in parietal cortices. In an autoradiographic study, Blatt et al.[74] reported decreased density of GABA receptors in hippocampal sections of brain in cases with autism (N = 4) compared to controls (N = 3). The density of 6 other receptors, 5-HT_{1A}, 5-HT_2, cholinergic M_1, high affinity choline uptake site, NMDA, and kainate, did not differ significantly.

In summary, postmortem studies suggest that genes and proteins involved with glutamate and GABA functioning may be abnormal in autism. However, definitive conclusions are difficult to make given the limited number of studies and small sample sizes.

NEUROPEPTIDES

Oxytocin and Vasopressin

The 9 amino acid peptides oxytocin (OT) and vasopressin (AVP) have been implicated in the social behavior of mammals.[75] These neuropeptides are synthesized in the hypothalamus and secreted from the posterior pituitary gland, exist solely in mammals, and differ at only 2 amino acids.[76] Receptors for these peptides have been found throughout the limbic system, in the forebrain, and in brain stem autonomic centers.[77]

Animal models of OT and AVP involvement in regulating behavior have laid the groundwork for hypotheses that these peptides may be involved in the pathophysiology associated with PDDs.[78–80] OT knockout mice have been found to have impaired social memory in the presence of intact olfactory and general cognitive abilities.[81] OT has been found to promote pair bonding,[75] and OT antagonist infusion into the nucleus accumbens has inhibited the formation of partner preference in female prairie voles.[82] In rats, AVP has been shown to facilitate social memory,[76] and in male prairie voles, AVP has been found to promote pair bonding.[75]

The possible role of OT in the etiology of the social impairment of PDDs has been evaluated at epidemiologic, neurochemical, and therapeutic levels. The hypothesis that human neonatal exposure to OT (pitocin) during labor induction may lead to long-term OT receptor down regulation has been evaluated in 2 studies comparing affected children and controls.[83] A retrospective review of the birth histories of 41 children with autism compared with the records from 25 age- and IQ-matched controls found no increased incidence of pitocin exposure in the children with PDDs.[84] A comparison of the birth records of 633 preschool children with language disorders, autism, or generalized low IQ found similar rates of labor induction among all groups evaluated.[85]

Plasma, but not central, OT levels have been evaluated in a cohort of 30 autistic male children.[86,87] This investigation compared the OT levels in patients with those of age-matched but not IQ-matched control children. The first report on this cohort noted a decreased level of plasma OT in the autistic children.[86] The second report using the same cohort again reported lower OT levels in the children with autism but also increased levels of OT extended form, a precursor molecule with a C-terminal 3 amino acid extension.[87] The authors hypothesized that a deficit in an unspecified prohormone convertase may be responsible for the low OT levels found in this sample of autistic children. The finding of abnormal plasma OT levels in male autistic children is interesting in light of the animal model of OT impacting social attachment, which found a predominant female predisposition to OT susceptibility.[75]

A single study has evaluated the intravenous administration of OT in subjects with PDDs. Using a repeated infusion model with each subject as his own control, 6 adults with autism and 9 adults with Asperger's disorder were monitored for 6 different types of repetitive behavior in 60-minute intervals following infusion of OT or placebo for up to 4 hours.[88] This investigation reported no significant main effect for drug and a significant reduction in combined repetitive behaviors over time, but no significant difference for any single repetitive behavior was reported. No difference in response was noted between the subjects with autism or Asperger's disorder, and no social measures were obtained during the challenge paradigm.

While OT has been evaluated on several levels in autistic individuals, AVP has been primarily investigated at the genetic level looking for polymorphisms in the gene coding for arginine vasopressin receptor 1A (AVPR1A).[89,90] One analysis looking at 125 independent autistic probands and 65 autism-affected sibling pair families concluded that differences at the amino acid level in the AVPR1A gene are not likely to confer genetic vulnerability to autism, but that there may be some significant transmission disequilibrium outside of the gene itself in the 5` flanking region.[89] An analysis of 115 autism trios genotyped 2 polymorphisms from the 5` flanking region of the AVPR1A gene and found nonsignificant transmission patterns.[90] Interestingly, both studies found different 5` flanking region polymorphisms. This difference was hypothesized to represent varying genetic backgrounds in the 2 samples, or spurious false positive findings.[90]

Investigations of the social impairment associated with PDDs have not yet found significant epidemiologic, neurochemical, therapeutic, or genetic evidence to support a primarily OT- and/or AVP-mediated etiology. Future work may need to focus on central levels of OT and AVP, and/or possibly focus more on how the sexually dimorphic effects of OT and AVP seen in lower mammals may translate into differential effects between genders in individuals with PDDs.

Opioids

Several observations of autistic children led to the early evaluation of opioid dysregulation as a possible etiologic explanation of PDDs. These have included elevated pain threshold, little interest in social interactions, and episodes of motor hyperactivity.[91] These findings appeared to match those seen in infant animals administered opiates.[92] In one way, the human opioid system relates to the hypothalamic-pituitary-adrenal (HPA) axis and other neuropeptides because β-endorphin is excreted at the same time as corticotropin (ACTH) from the anterior pituitary. This occurs because these peptide hormones are initially part of the same preprohormone.[93]

Evaluations of β-endorphin (and β-endorphin metabolites in some cases) levels in the serum, CSF, and urine of patients with PDDs compared to controls have yielded conflicting results (see Tordjman et al.,[94] for review). Ten studies enrolling a total of 142 patients with PDDs have evaluated serum β-endorphin levels, and the results represent a relatively equal mix of increased, decreased, or similar β-endorphin levels found in the patients compared to control subjects. In 2 investigations of CSF β-endorphin levels in patients with PDDs, 1 reported increased and 1 reported decreased levels in affected patients.

Investigation of urinary opioid peptides has attempted to look at whether inadequate processing of exogenous opioids by the gastrointestinal tract may result in overabsorption and finally urinary excretion of the peptides.

Such an evaluation analyzed the urine of 10 children with autism compared to 11 adult controls and found no difference between groups.[95] This study also specifically evaluated dipeptidyl-peptidase in the serum of patients and found no difference compared with controls. This enzyme is present at the intestinal brush border, and is expected to be involved in the cleaving of exogenous dietary opioids. These findings have been challenged by those who have presented earlier data pointing toward abnormal urinary opioid levels in patients with PDDs.[96] Disagreement over the preparation of samples and the limits of detection methods emerged among the different groups reporting contradictory results with regard to urinary opioids in patients with PDDs.

The use of naltrexone, an opioid receptor antagonist, has been evaluated in several open-label and placebo-controlled trials in patients with PDDs having unknown, elevated, or normal levels of serum β-endorphin.[97] Again, as in the studies looking at serum and CSF β-endorphin levels, conflicting results on the efficacy of naltrexone exist, but most controlled studies suggest that the core symptoms of autism and associated maladaptive behavior are not significantly affected by naltrexone.

Overall, the importance of endogenous or exogenous opioid systems in patients with PDDs is subject to much debate driven by contradictory evidence. This uncertainty has led to the hypothesis that it may not be opioid levels per se that may contribute to symptoms commonly seen in PDDs, but the manner in which the endogenous opioid system interacts with other neurotransmitter systems such as the 5-HT, DA, NE, glutamate, or GABA systems.[91] To date, these potential multisystem interactions have not been extensively explored. A final manner in which opioid activity may impact theories of the pathophysiology of PDDs could be derived from the knowledge that β-endorphin has been found to inhibit oxytocin activity in rats.[98] It is clear that more research is needed to better understand how the endogenous opioid system interacts both with traditional neurotransmitters and with other neuropeptides with respect to the pathobiology and treatment of PDDs.

Cortisol/ACTH

Levels of the anterior pituitary hormone ACTH and the adrenal product cortisol have been evaluated in patients with PDDs as a means to evaluate the HPA axis in these subjects. As with opioids, conflicting results have been the rule rather than the exception.[94,99] In patients with PDDs, plasma ACTH and cortisol, and cortisol levels in response to the dexamethasone suppression test, have each been found to be similar, lower, or elevated compared to control subjects in different studies.[94,99] Recently, investigators reported elevated ACTH and low cortisol levels in 36 autistic individuals, commenting that their results were "difficult to interpret" in light of conflicting data previously reported.[99] One report on the circadian rhythm of cortisol, as measured by urinary secretion in 19 autistic patients compared to control subjects, found no significant difference in the daily rhythm of cortisol secretion.[100]

While it is clear that a simple excess or deficit of ACTH or cortisol is not present in the majority of patients with PDDs, it is less clear how abnormally elevated or decreased levels can be further interpreted at an individual patient level to ascribe any causal relationship between these peptides and common PDD symptomatology.

Melatonin

It has been hypothesized that hypersecretion of melatonin from the pineal gland may be responsible for a cascade of events impacting the HPA axis, thus possibly leading to an "autistic-like" phenotype.[101] This theory is based on evidence (primarily from animal studies) that increased melatonin can lead to decreased corticotropin-releasing hormone (CRH) secretion from the hypothalamus, leading to decreased pituitary ACTH and β-endorphin excretion while at the same time causing, by an unknown mechanism, an elevation in whole brain 5-HT.[101] One study to date[102] has systematically evaluated levels of melatonin in patients with PDDs. Ten patients with autism aged 16 to 30 years had melatonin levels drawn over a 24-hour period, and these levels were compared to findings from control subjects. The authors reported no difference in mean daily melatonin concentration, with only a trend toward a lower amplitude of melatonin peak at night in autistic patients. The exact role of melatonin in patients with PDDs remains to be fully characterized and understood. Little, if any, evidence to date exists pointing to a primary melatonin dysfunction.

Secretin

While the role of secretin as a classical hormone in the gastrointestinal system is well known, its role as a neuropeptide is continuing to be defined.[103] This potential neuroactivity has been further investigated, in part, because of preliminary reports of effective secretin treatment of social and communication impairment in patients with PDDs.[104] While controlled studies have failed to demonstrate the efficacy of secretin in treating autistic symptomatology,[104,105] recent evidence of the neuroactive properties of secretin has become clear.[103,106–108] In human and rat samples[108] and rat samples alone,[106] secretin immunoreactivity has been shown in Purkinje cells of the cerebellum,[106,108] central cerebellar nuclei,[108] pyramidal cells of the motor cortex,[108] primary sensory neurons,[106,108] and the brainstem.[106] Additionally, in separate work in rats, mRNA coding for the secretin receptor has also been found in cerebellar GABA interneurons.[107] This work postulated that in the cerebellum, secretin may be secreted by Purkinje cells, then act as a retrograde messenger modulating GABA activity. It is clear that secretin likely has neuroac-

tive effects. Its potential contributions to the pathophysiology of PDDs remains poorly understood. Based upon the results of numerous placebo-controlled studies, however, secretin has been determined to be an ineffective treatment for autism.[104,105]

Thyroid Hormone

Thyroid-stimulating hormone (TSH), released by the pituitary gland, has been evaluated in several patients with autism. In 2 separate analyses, each with 10 affected children compared with a similar number of adult controls, no difference in TSH levels was found.[102,109]

Other Anterior Pituitary Hormones

Plasma growth hormone,[99] prolactin,[99,109] luteinizing hormone,[109] and follicle-stimulating hormone[109] levels have been shown to be no different between autistic individuals and controls in small studies.

Other Neuropeptides and Neurotrophins

A novel analysis of neuropeptides and neurotrophins from frozen blood samples of neonates subsequently diagnosed with a PDD (N = 69), mental retardation without autism (N = 54), cerebral palsy (N = 63), and normal control patients (N = 54) found significantly elevated levels of several measured substances in the PDD and mental retardation groups.[110] Concentrations of neonatal vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), brain derived neurotrophic factor (BDNF), and neurotrophin 4/5 were significantly higher in both the PDD and mental retardation groups. No significant differences between the PDD and mental retardation groups were noted. Concentrations of substance P, pituitary adenylate cyclase-activating polypeptide (PACAP), nerve growth factor (NGF), and neurotrophin 3 were all similar among all groups tested. In a different study, CSF levels of the neurotrophic factor insulin-like growth factor-I were found to be similar in an analysis of 11 autistic patients and 11 age-matched "disabled" controls.[111] While the results from the neonatal samples may not be specific to PDD, their significance lies in pointing demonstrably to how neuropeptide/neurotrophin dysregulation early in development may set the brain on a course toward disordered development, including, in some cases, a course toward PDDs.[112]

CONCLUSION

This review has explored the available literature on neurochemical contributions to the pathophysiology of autism, with a focus on monoamines (5-HT, DA, NE), glutamate/GABA systems, and neuropeptides. With respect to monoamines, the majority of studies that have focused on basal measures of plasma, urine, and CSF have been negative. The 1 exception is that of elevated WBS or "hyperserotonemia," which has been replicated in multiple investigations. Its underlying mechanism, however, remains unclear. Behavioral challenges of monoaminergic systems have primarily involved 5-HT. A number of significant differences have been found between autistic subjects (primarily adults) and controls, although most results have not yet been replicated. Furthermore, results from behavioral challenges are largely based on peripheral responses, with central effects often being inferred. Preliminary PET studies involving 5-HT and DA systems have yielded potentially important findings. Concerns about radiation exposure in youth may limit further studies utilizing currently available technology, although investigations in adults may be possible. Postmortem assessment of monoaminergic involvement needs to be completed. Encouraging results from preliminary genetic studies of the glutamate and GABA systems are emerging. The use of magnetic resonance spectroscopy (MRS) will allow for an indirect assessment of these systems in the living brain. Finally, results from animal studies indicate that the OT and AVP systems are important, if not critical, for affiliative behavior. It will be important to continue to incorporate genetic studies into ongoing neuroimaging and postmortem investigations in each of the 3 areas of neurochemistry discussed in this review.

Drug names: naltrexone (Revia and others), probenecid (Probalan and others), sumatriptan (Imitrex).

Disclosure of off-label usage: The authors have determined that, to the best of their knowledge, naltrexone is not approved by the U.S. Food and Drug Administration for the treatment of aggression in autism; probenecid is not approved for use in cerebrospinal fluid studies in autism; and sumatriptan, fenfluramine, and *m*-chlorophenylpiperazine are not approved for use in behavioral challenge studies in autism.

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