NEUROBIOLOGY OF AUTISM: AN UPDATE

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SUMMARY

Consideration of available studies suggests that many cases of autism are caused by a neurodevelopmental disorder. In other cases, a known disease entity is found, either during life or at autopsy. A common factor linking primary idiopathic autism with double syndrome cases having autistic behavior may be malfunction in specific neuroanatomic systems, defects in which give rise to the clinically defined autistic symptomatology. The available evidence suggests that the limbic system is abnormal in most cases of autism, and that the hippocampus, basal forebrain, cingulate and orbitofrontal cortices are specifically involved. Evidence for neocortical involvement is less strong; additional investigations will be necessary to define the role of neocortical pathology observed in some, but not all, individuals with autism. Similarly, the role of the thalamus and hypothalamus and their subdivisions needs to be better defined. The role of the cerebellum in the causation of autistic symptoms is controversial. However, the consistency of the findings of the Boston group suggest that additional study, especially studies focused on the connections of the cerebellum to the diencephalic and telencephalic structures, is warranted.

The evidence, then, suggests that autism is a disorder of connectivity, often but not exclusively arising during the gestational period and ongoing degeneration of involved neural systems may occur in some individuals. Since different investigators, who study different populations of autistic individuals, have found involvement of multiple neuroanatomic sites, neural network(s) may be involved in pathogenesis of this complex behavior. A defect at any point in the network could produce autistic behavior, and differences in the specific network defect between individuals might account for observed differences in clinical phenotype. The recent identification of abnormalities in serotonin synthesis in autistic individuals suggests that serotonergic systems are likely involved (23). However, the complexity of the brain's circuitry, especially in the limbic system (56), and the presence of multiple neurotransmitters in any given anatomic site in the brain, suggests that investigations of additional neurotransmitter systems might be useful as well.

While autism is now accepted to be an intrinsic disorder of the brain, much additional work needs to be done to elucidate the precise biochemical and physiologic defects that lead to the observed pathologic changes. Application of basic neuroscience methods to clinical material will hopefully elucidate the pathogenesis of this disorder and lead to effective therapy.

Key words: Autism, Asperger's syndrome, Rett disorder.

RESUMEN

Los conocimientos aportados por estudios recientes llevan a considerar que muchos casos de autismo se deben a trastornos del neurodesarrollo. En otros casos puede encontrarse alguna entidad nosológica bien conocida, sea en vida del paciente o en la autopsia. La disfunción de sistemas neuroanatómicos específicos que generan la sintomatología autística, clínicamente definida, constituye el denominador común entre los casos de autismo idiopático primario con los casos de síndrome doble con conducta autística. La evidencia obtenida indica que, en la mayoría de los casos de autismo, coexiste un sistema límbico anormal, y que el hipocampo, el prosencéfalo basal - corteza órbitofrontal - y la corteza del cíngulo se hallan involucradas. Sin embargo, todavía se requiere de investigaciones adicionales para definir con precisión el papel que desempeña la patología neocortical que existe en algunos, que no en todos, los casos de autismo. Lo mismo se debe decir de los núcleos talámicos e hipotalámicos afectados. La participación del cerebelo en la sintomatología autística sigue siendo tema de controversia. Al respecto, la solidez de los hallazgos del grupo de Boston garantiza la continuación de estudios enfocados a dilucidar las conexiones del cerebelo con las estructuras diencefálicas y telencefálicas.

Por lo anterior es dable considerar que el autismo es un trastorno de conectividad que frecuentemente, aunque no exclusivamente, ocurre durante la gestación y que, en algunos casos, los sistemas neurales afectados muestran degeneración progresiva. Otros investigadores, que estudian casos de autismo en otros grupos de población, han descrito afección de múltiples regiones neuroanatómicas y redes neuronales que pueden participar en la patogénesis del complejo conductual del autismo. Cualquier lesión en un punto determinado de la red neural sería capaz de producir la conducta autística, y las diferencias en cuanto al punto lesionado en una misma red, entre los individuos afectados, podría explicar las diferencias que se observan en el fenotipo clínico.

Las anomalías en la síntesis de serotonina en casos de autismo sugiere que los sistemas serotoninérgicos probablemente se hallen afectados (23). Sin embargo, la complejidad de los circuitos cerebrales, especialmente en el sistema límbico (56) y la presencia simultánea de múltiples neurotransmisores en un sitio anatómico dado del cerebro, igualmente sugiere que se lleven a cabo investigaciones adicionales sobre los sistemas de neurotransmisores.

Aunque ya se acepta que el autismo es un trastorno cerebral intrínseco, todavía se requiere precisar las anomalías bioquímicas y fisiológicas que determinan el substrato neuropatológico. Es posible que con la aplicación de métodos de neurociencia básica al material

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clínico se pueda contribuir al conocimiento de la patogenia del autismo y, eventualmente, prevenir y diseñar una terapéutica efectiva.

Palabras clave: Autismo, síndrome de Asperger, trastorno de Rett.

INTRODUCTION

In 1943, Leo Kanner (48) described an unusual group of children who were preoccupied with themselves and who did not relate well to persons around them. Although such children had previously been classified as having childhood schizophrenia, Kanner applied the term "infantile autism" to them. Simultaneously, Hans Asperger, working in Vienna and unaware of Kanner's work, described a similar group of children (5).

"Autism" is derived from the Greek "autos," meaning to be centered on the self or to live in one's own world. Autism is a subtype of the pervasive developmental disorders, which also include Asperger's syndrome, Rett's disorder and childhood disintegrative disorder. Currently, both the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (2) and the International Statistical Classification of Diseases and Related Health Problems (ICD-10) describe autism as a developmental behaviorally defined disorder of childhood. Individuals with Asperger's syndrome do not have a clinically significant delay in language, but they display the remainder of the qualitative impairments listed above and most authorities consider autism and Asperger's syndrome as a single neurobehavioral entity (36). Girls with Rett's disorder manifest autistic behavior patterns, but also show characteristic physical signs including acquired microcephaly and peculiar hand wringing gestures. Childhood disintegrative disorder is diagnosed in children who develop autistic behavior after apparently normal development for at least the first 24 months of life (2).

CLINICAL CONSIDERATIONS

Autistic behavior is characterized by qualitative impairments in social interaction and language and communication, imaginative play, and restricted and stereotyped patterns of behavior and activities, and specific diagnostic criteria are provided in the Diagnostic and Statistical Manual IV-R of the American Psychiatric Association (2), and the ICD-10 of the World Health Organization (1992) (35,59,60,70). Diagnosis of classical childhood autism requires evidence of impairment in all three of the above areas, and deficits in at least one of the three areas must have been evident before the age of three years. Furthermore, there must be no other explanation for the deficits, such as Rett's disorder (in girls) or childhood disintegrative disorder. In addition to the behavioral and communication deficits mentioned above, autistic children may also manifest sudden mood changes, insensitivity to pain, under responsiveness to spoken language, temper tantrums, unprovoked aggression, self-injurious behavior and short attention spans. They may have a preference for repetitive movements that stimulate the vestibular system, such as spinning or swinging.

Individuals with Asperger's syndrome manifest lesser degrees of these behavioral deficits, and they acquire spoken language by age three years. However, they use their spoken language in abnormal ways, and their use of language is consistent with their other behavioral deficits. Social behavior and restricted interests have been shown to be similar in Asperger's disorder and autism (34). Recent comparisons of motor, visuospatial and executive functioning in individuals with Asperger's syndrome and high functioning autism have shown no significant differences between the two groups (52).

Rett's disorder is caused by a defect in the methyl-CpG-binding protein 2 (MECP2) locus on the X chromosome (3); a defect in this gene locus is present in approximately 80% of cases. While changes in NMDA receptors have been observed in the superior frontal gyrus of Rett brains (20), no unifying hypothesis about the pathogenesis of the autistic behavior seen in Rett's disorder has yet emerged.

Childhood Disintegrative Disorder (disintegrative psychosis) is similar to infantile autism with regard to parental psychopathology, neuroradiologic findings and genetic abnormalities (55).

Since all the autistic spectrum disorders share similar neurobehavioral features, albeit with variable clinical details, the underlying brain dysfunction(s) may involve similar systems in all these clinically defined entities.

EPIDEMIOLOGY

The estimated prevalence of autistic disorders varies from report to report, probably because criteria for the diagnosis have been variable (36). When behavioral criteria (DSM-IV or ICD-10) are used to survey large groups of children, the prevalence of autism is 1-2/1000 across both Western and Asian cultural groups (21, 69). One study suggests that there are an additional 3 children with Asperger's syndrome/1000 (36). Studies performed in the United States have shown lower prevalence levels than studies performed elsewhere, and it has been suggested that the lack of comprehensive community health care and a mobile population has made U.S. case ascertainment difficult (36). The

incidence of Rett's disorder was 1:10,000 in a Swedish survey (39). The incidence of childhood disintegrative disorder is extremely low and has been estimated at 1:100,000. More boys than girls manifest autistic behavior, and the ratio is 2-3 boys per 1 girl (60). It has been suggested that genes that promote social behavior are present on the X-chromosome, and that therefore females, with two X-chromosomes, are protected in some fashion against developing the social isolation characteristic of autism (67).

While the term "autism" has been narrowly used as a label for individuals manifesting these symptoms without a known etiology ("idiopathic childhood autism"), these same autistic behaviors have also been described in association with a variety of other conditions. When an autistic spectrum disorder occurs in a patient with another known medical condition, the term "double syndrome" has been used (36). Common associated conditions include fragile X syndrome and tuberous sclerosis. Rare associated conditions include degenerative diseases such as neuroaxonal dystrophy and others (32,59,60,65,70,71), infectious processes including congenital rubella infection and herpesvirus encephalitis, toxins such as valproic acid (72) and thalidomide (68), and metabolic conditions such as phenylketonuria. By definition, autistic behavior is characteristic of Rett's disorder. The association of many different neurological conditions, some of which produce obvious pathologic changes upon neuropathologic study, with autistic behavior suggests that clues to the pathophysiology of this clinical syndrome might, therefore, be acquired from study of the brain specimens from individuals who fulfill behavioral criteria for autism, regardless of whether or not a specific etiology has been assigned to them. Study of neuropathologic characteristics common to these persons might elucidate the pathophysiologic mechanisms of autistic behavior, which are generally believed to be neurodevelopmental in origin (16,37,49,70). The recently reported linkage of the HOXA1 gene on Chromosome 7, which is involved in brainstem development, with autistic behavior, provides a useful example (47). Studies of MEPC2 have revealed that this gene product is necessary for both neurodevelopment and maintenance of mature neurons, provide another specific line of investigation that may elucidate the etiology of autistic behavior (22,38).

NEUROPATHOLOGY

Only a few descriptions of the neuropathology of childhood autism exist. Review of the literature reveals that 19 brains have been studied in Boston by Bauman and Kemper. Nine of these specimens were studied using celloidin embedding and classic neuropathologic methodology including whole brain sections and Golgi impregnations (10-12,14-18,49,61); midsagittal photographs were made from 11 cases, and brain weight was available for 19 cases. Occasional cases have been studied by other groups (7,24,37,62,63,71,73), and these total 16 specimens. Four brains embedded in both paraffin and celloidin and (apparently) stained with routine staining methods as well as with Golgi techniques have been reported as part of a collaborative effort between workers in Boston, Chicago and New York (73). Paraffin sections of four brains stained with the Nissl technique have been studied by a group in Los Angeles (62). This group made a concerted effort to obtain autistic brain specimens, but they received only four brains over a period of 11 years. One brain has been studied using paraffin sections and Nissl stains in Rochester, NY (24) and a reconstruction of the ponto-medullary junction derived from serial sections of the remaining material was recently reported (63). A single specimen was reported from France (37). Neuroaxonal dystrophy was observed in two cases reported in 1999 (71). The gross weights of four brains have been reported in 1993 by a group in London (7), who reported an additional six brains in 1998 together with brief comments on a seventh specimen (8). Brain weights, but no gross or microscopic descriptive data, were reported from an additional five specimens in 1999 (25). Therefore, assuming no overlap between these small collections of autistic specimens, at most, 51 brain specimens from patients with well-documented autistic behaviors have been reported in the literature. It is probable that other autistic brains have been studied as part of at least one series of brains from patients with "childhood psychosis," but it is difficult to ascertain whether or not a particular subject was autistic or not from the information provided in this paper (29). Most of these specimens were studied before the routine use of immunohistochemical methods employing antibodies against various neuronal, synaptic, cytoskeletal and glial epitopes became widely available.

In contrast to the paucity of studies of brains from persons with classical autism or Asperger's syndrome, Rett disorder brains are small and show neocortical pathology, especially in the dendrites (4).

Study of the 51 childhood autism specimens as well as neuroimaging data, has indicated that several different neural systems are likely to be involved in the pathogenesis of autistic behavior. Additional data derived from cerebrospinal fluid analyses, together with findings from the brains of Rett disorder patients, suggest that

investigations of connectivity in these neural circuits may be of value for elucidating the cause of the puzzling behavioral syndrome that characterizes autism.

Gross findings

The Boston collection includes 11 brains from individuals less than 12 years of age, and 8 from individuals older than 18 years. Kemper and Bauman (49) have stated that 8 of their specimens derived from persons less than 12 years old showed a significant increase in brain weight, but that no statistically significant differences were found in their specimens from persons more than 18 years of age, even though five of eight brains from autistic individuals older than 18 years weighed less than expected weight by 100-300 grams as reported in abstract form (13). The British group reported that three of four (7) and four of seven of their later specimens were megalencephalic (8). The French brain reportedly was small. Only one of five specimens reported from San Diego (25) showed megalencephaly; these workers pointed out that standards for normal brain weight vary widely. The presence of increased brain weight in some younger persons with autism, when considered together with other evidence of incomplete or delayed neuronal maturation, suggests that investigations related to neuronal migration and apoptosis might be of value. In addition, the decreased weights reported in some older individuals suggest that cell death may be occurring during life in individuals with autism.

Neocortex and cerebral white matter

Neocortical pathology has been found in five of the seven brains studied by Bailey et al (8), who noted disturbances of the cortical lamina in five cases and increased numbers of neurons, singly or in heterotopias, in four cases. In one of the 1998 cases, there were hyperconvoluted temporal gyri and upwardly rotated hippocampi, but the gyral pattern was unremarkable in the other five cases (8). The remainder of the 51 autistic brains cataloged above have not shown evidence of neocortical pathology, though the limbic cortex (cingulate and orbitofrontal) may be abnormal (*vide infra*). An extensive study of four autistic brains, which employed the Golgi methodology to demonstrate dendritic arborization and spines in multiple regions of the brain, including the neocortex, limbic system and cerebellum, revealed no conclusive abnormalities in these structures, nor any other neuropathology that could be related to the pathogenesis of the patients' autistic behavior (73). Bauman and Kemper found no evidence of neocortical changes in their cases (10,16,17,49). Only rare spheroids were noted in the neocortex of the two cases of neuroaxonal dystrophy with autistic symptomatology (71). However, investigations using MRI methods to visualize the neocortex and corpus callosum suggest that atrophy may be present in the parietal lobe and the posterior portions of the corpus callosum that carries crossing fibers derived from this region (19,26,30). In addition, a clinical study of reflex and volitional saccades (54) suggested that there may be dysfunction of the prefrontal cortex and its connections to the parietal lobe.

Limbic system

Bauman and Kemper found neuronal abnormalities in the limbic system of individuals with autism. They described an increase in cell density in the pyramidal cell layer of the hippocampus, subiculum, entorhinal and anterior cingulate cortex (16,18). While they noted an increased packing density in the amygdala, mamillary body and the medial septal nucleus, they observed a decreased number of neurons without change in cell-packing density in the diagonal band of Broca. Neuronal size was reduced in these areas as well. Golgi preparations demonstrated decreased complexity of the dendritic arbors in the pyramidal cells of CA1 and CA4 of the hippocampus (16,61). Bauman and Kemper interpreted these abnormalities in packing density, neuronal size and dendritic trees as indices of delayed maturation of these brain regions, i.e. a developmental defect (18,49). Consideration of the extensive connections of these limbic structures with the remainder of the brain suggests that these abnormalities may underlie some of the defects in social behavior, language and cognitive functioning found in autistic persons (6,16,18,28,51). In contrast, Bailey et al (8) found that the hippocampus was unremarkable in four of their seven cases, apparently unavailable for study in one, and had increased neuronal density in two. The amygdala of the seventh, briefly described case contained clusters of large neurons.

Study of two double syndrome cases having both autistic behavior and neuroaxonal dystrophy, showed pathology in the hippocampus, limbic cortices and in hypothalamic, thalamic and brainstem nuclei having limbic connections (71).

Brainstem and cerebellum

Several different groups have noted abnormalities of cerebellar architecture (11,12,15,16,62,73). The most interesting findings are those described by the Boston group, where older (more than 20 years of age) cases showed decreased numbers of neurons that were small

and pale, in the fastigial, globose and emboliform nuclei (16).

Interestingly, younger (less than 12 years of age) autistic subjects showed normal numbers of neurons, which Bauman and Kemper considered enlarged, in these areas and in the dentate nucleus. They also noted abnormal shape of the dentate nucleus (10), but they thought that the inferior olive contained normal numbers of neurons, which, however, were unusually small and pale in older cases. Again, younger subjects showed enlarged neurons which were normal in number in the dentate nucleus (16). There was also a tendency of the neurons to cluster at the periphery of the inferior olivary nucleus (16). Bauman and Kemper suggested that these abnormalities, which were not accompanied by astrogliosis, arose before 30 weeks of intrauterine life and represented persistence of fetal circuitry in this region, which could not be sustained as the patient aged, and therefore produced the small pale neurons observed in older cases (16). The extensive connections of the cerebellum with the limbic system and the neocortex may indicate that the cerebellum modulates those higher cortical functions that are disturbed in autism (16,43,44,66).

Both the British group (8) and the Boston group (11,12,15,16,49) and occasionaly other workers (62,73) have found variable loss of Purkinje cells and granular cells, with or without gliosis, in the cerebellar cortex. Also, a single specimen had heterotopic neurons in the molecular layer (62). Recent work from the British group revealed a decrease in Purkinje cells, with proliferation of Bergmann's astrocytes and/or an increase in glial fibrillary acidic protein staining, in 6/7 cases (8). The authors interpreted this pathology as acquired during life. In the seventh case, Purkinje cells were found in the molecular layer. The dentate nucleus was noted to be discontinuous, with or without patches of subcortical ectopic grey matter, in two cases. In 4/6 cases in which sections were available, the inferior olivary nuclei were dysplastic, and in 4/7, ectopic neurons were present close to the olive. Additional subtle abnormalities interpreted as of developmental origin were present in six cases (8).

Independent MRI examinations have confirmed that cerebellar abnormalities are present in some persons with autism (27) but not in others (31,58).

Recent neuroimaging studies suggest that the brainstems of some autistic patients are decreased in size (33,40,41,45,58). While this could be a nonspecific finding, as it has been noted in patients with other types of mental retardation (40), examination of autistic brainstems using modern neuropathologic methods has never been performed. The study of Rodier et al (63) employed the Kluver-Barrera method to demonstrate neurons and myelin at the ponto-medullary junction in an individual with autism and lack of facial expression. After finding severe neuronal loss in the facial nerve nucleus and superior olive in the brainstem of this woman with autism, these workers suggested, using rodent models, that disruption of neuroblasts in the basal plate of the rhombencephalon necessary to cause such a defect occurs during neural tube closure. They speculated that, since the insult to the brainstem must have occurred at this particular developmental stage, the insult(s) responsible for the remainder of the patient's autistic symptomatology might have occurred at this same developmental stage. Additional studies by this group revealed that ablation of the HOXA1 gene, which is in part responsible for the formation of the fifth rhombomere including the facial nucleus and the superior olive, produces similar defects in rodents, although autistic behavior was not documented in these rodents (47). When a population of individuals with autism and their families was surveyed for HOXA1 genotype, the relative frequencies of the various genotypes was significantly altered in the autistic population as compared to controls, suggesting that HOXA1 may be involved in the pathogenesis of autistic behavior in at least some cases (47).

Cerebrospinal fluid

Measurement of ganglioside levels in cerebrospinal fluid (CSF) specimens of autistic persons disclosed increases, suggesting that alterations in synaptic integrity might be present (50).

Further study showing alterations in high-energy phosphate and membrane phospholipid metabolism in the prefrontal cortex of autistic persons suggest that neuronal and/or synaptic membranes may be altered in this syndrome (53). Increases in glial fibrillary acidic protein in autistic CSF may be nonspecific or may correlate with lesions such as dysfunctional synapses (1) or axons.

Functional neuroimaging studies

Functional neuroimaging studies have compared brain functions among individuals with autistic spectrum disorders and normal individuals.

By necessity, each of these investigations has focused on a specific function and the relatively restricted neuroanatomic regions that are related to that function (9,42,64,74). Nonetheless, these studies support the idea that malfunction in several different regions may underly the complex behavioral peculiarities observed in autism.

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