Special Article

Neurobiology of Rett Syndrome

Michael V. Johnston, MD; Brendan Mullaney, BA; Mary E. Blue, PhD

ABSTRACT

Girls with Rett syndrome display signs of neuronal dysfunction including mental retardation, seizures, stereotyped movements, and abnormal breathing and autonomic control. Decelerating head growth during infancy might reflect a disorder in production or pruning of neuronal synapses or both. Recent immunocytochemical studies in rodent brain investigating development of MeCP2, the transcription factor mutated in Rett syndrome, suggest that expression is delayed until the time of synapse formation. These findings are consistent with other evidence that Rett syndrome disrupts genetic programs that establish and refine synaptic connections. (J Child Neurol 2003;18:688–692).

ABNORMALITIES IN EXCITATORY GLUTAMATE SYNAPSES

Glutamate is the major excitatory neurotransmitter in the brain, and most neurons have some receptors that respond to glutamate. Glutamate synapses mediate primary senses such as hearing and vision, activate motor acts such as speaking or walking, and play essential roles in learning and memory. The high incidence of seizures and abnormal electroencephalographic activity in girls with Rett syndrome, hyperkinetic movements, and abnormal breathing patterns appear to be one of the major developmental processes disrupted in Rett syndrome (Figure 1).5,7,9

Figure 1. Schematic diagram of rise and fall in synaptic number and density in human cerebral cortex during childhood, based on work by Huttenlocher. Data from human brain tissue suggest that deceleration of head growth during the first year and microcephaly in Rett syndrome are caused by abnormal synapse proliferation and pruning, as shown by the dotted line.
all suggest dysfunction of excitatory synapses.\textsuperscript{12,13} Electro-
physiologic studies also indicate that cerebral cortex could be more excitable in girls with Rett syndrome.\textsuperscript{13} Studies of cerebrospinal fluid and of brain using magnetic resonance spectroscopy suggest that levels of brain glutamate are elevated in Rett syndrome.\textsuperscript{14,15} Studies of postmortem brain tissue indicate that densities of receptors for N-methyl-D-aspartate (NMDA) and \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)–type glutamate receptors are above control levels in the frontal cerebral cortex and basal ganglia of young patients aged 2 to 8 years with Rett syndrome but well below the control levels in older girls.\textsuperscript{16,17} Interestingly, these changes are relatively selective in that densities of receptors for two other glutamate receptors, kainate and metabotropic receptors, are no different between girls with Rett syndrome and age-matched postmortem controls. Studies using \([^{18}\text{F}]\)fluorodeoxyglucose also showed relative increases in brain glucose metabolism in the frontal lobes of young girls with Rett syndrome compared with those of older girls, consistent with increased glutamate cycling.\textsuperscript{18,19} These findings are consistent with the multiphasic evolution of clinical signs in girls with Rett syndrome, with signs of enhanced excitatory activity are seen in younger girls with emerging encephalopathy but signs of reduced excitatory activity in older girls left with severe cognitive impairment.\textsuperscript{20,21} Reductions in NMDA and \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors would be expected to lead to severe impairment in learning and memory.\textsuperscript{11}

Dynamic changes in excitatory glutamate receptors in Rett syndrome are also likely to be closely linked to changes in the proliferation, pruning, and refinement of synapses.\textsuperscript{8} Activity at \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA receptors determines which immature synapses will be refined and maintained and which will be pruned.\textsuperscript{22,23} The shape and number of dendritic spines are also determined by activity at glutamate receptors, and prominent abnormalities in dendrites have been reported in postmortem tissue from patients with Rett syndrome.\textsuperscript{2,24,25} A study of postmortem cerebral cortex from infants who have died indicates that the density of NMDA receptors in cerebral cortex is higher than at later times.\textsuperscript{26} This suggests that the higher number of NMDA receptors in young patients with Rett syndrome could reflect a state of relative immaturity. It is unclear just how defects in the transcriptional repressor MeCP2 affect the interplay between glutamate receptors and developing synapses, but these processes seem to be one of the major targets of the disease process.

Expression Profiling for Synaptic Genes

Gene expression profiling for messenger ribonucleic acid in postmortem brain also points to abnormalities in synapses in Rett syndrome.\textsuperscript{27} Messages for the NMDA receptor R1 subunit and metabotropic (m)GluR1 receptor were up-regulated along with glial transporters for glutamate and the inhibitor transmitter \(\gamma\)-aminobutyric acid (GABA).\textsuperscript{27} In contrast, several genes associated with synapses were down-regulated, including microtubule associated protein 2, synapsin II, synaptotagmin, synaptogyrin, and syntaxin. Consistent decrements in several presynaptic markers suggest that there are deficits in the development of axonal nerve terminals in Rett syndrome. MeCP2 might normally function to suppress genes needed for prenatal development but can suppress the development of presynaptic terminals if "left on" during postnatal development. Impaired development of presynaptic terminals combined with increased expression of \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA receptors during the early stages of Rett syndrome would be expected to cause a reduction in synaptic density (Figure 2).\textsuperscript{8}

EXPRESSION OF MECP2 IN RODENT BRAIN

Immunocytochemistry with antibodies directed against MeCP2 has been used to examine the developmental expression of the protein in normal rodent brain and human postmortem tissue. Shahbazian et al reported that MeCP2 expression in mouse and human brain correlated with maturation of the central nervous system, with ontogenetically older structures such as the spinal cord becoming positive before structures such as the hippocampus or cerebral cortex.\textsuperscript{5} They suggested that the protein becomes abundant only when neurons reach a certain degree of maturity. This is consistent with the delayed onset of neurologic signs in MeCP2-null mice or mice with a truncated MeCP2 mutation.\textsuperscript{28} Cohen et al studied the expression of MeCP2 in rodent olfactory receptor neurons that undergo postnatal neurogenesis in the nasal epithelium.\textsuperscript{29} In this model, they found that MeCP2 expression correlates with the maturational state of the neurons but precedes synaptogenesis. We examined developing rats and found that MeCP2 immunoreactivity was expressed in the cerebellum in Purkinje cells at birth but that expression in the cerebellar granule cells was delayed to approximately 30 days after birth (Figure 3). This suggests that MeCP2 plays only a small role in postnatal neurogenesis of cerebellar granule cells but is important for establishment of synaptic connections with Purkinje cells.
Figure 3. Immunocytochemistry performed with an antibody directed against the C-terminal of MeCP2 (Upstate Biotechnology, Lake Placid, NY) in the adult rat cerebellum showing dense staining in neurons. Strong staining in the Purkinje cells (PCL) is present at birth, whereas staining in the granule cells (GCL), which undergo postnatal neurogenesis, is delayed to about 21 days postnatal. (Original magnification: A, ×2.5, B, ×20.)

Figure 4. Diagram of the neuronal circuitry in the cerebellum. Purkinje cells are born prenatally and are well established at birth in the rodent, with strong staining for MeCP2. Granule cells are born in the postnatal period and migrate inward from the external granule cell layer. Granule cells form excitatory synapses between their axonal parallel fibers and the candelabra-like dendrites of the Purkinje cells.

(Figure 4). Neuropathologic changes have been reported in the cerebellum of patients with Rett syndrome, and some of their neurologic signs are consistent with involvement of the cerebellum. All of these data are consistent with the hypothesis that MeCP2 becomes important for neurons to establish and refine developing synaptic connections.

MECP2 AND THE NUCLEUS BASALIS CHOLINERGIC PROJECTION

Subcortical projections to the cerebral cortex from the brain stem and basal forebrain can be impaired in Rett syndrome. The nucleus basalis projection of cholinergic neurons from the basal forebrain to the cerebral cortex has been reported to be involved in the neuropathology of Rett syndrome, and we found that immunoreactivity for MeCP2 is strongly expressed in nucleus basalis neurons in neonatal rats and remains present to adulthood. The nucleus basalis projection to cerebral cortex plays an important role in modulating cortical plasticity. Kilgard and Merzenich studied the reorganization of a cortical representational map for sounds of different frequency in adult rats and identified a receptive tonotopic map located in the auditory cortex in which low tones activated an area to the left, and each tone of higher frequency activated an area farther to the right. They could change the responsiveness of this map dramatically by exposing animals to repeated stimuli of a single-tone frequency combined with a behavioral paradigm. They could replace the behavioral paradigm with an electrical stimulus delivered to the nucleus basalis of Meynert. These experiments suggest that remodeling of cortical neuronal networks for sound is influenced by direct auditory input and by activation of the subcortical nucleus basalis, which attaches behavioral significance to the sound. The nucleus basalis is also important for enabling the plasticity of somatosensory maps in immature rodents. Early ablation of the nucleus basalis in neonatal rodents also causes shrinkage of neurons and simplification of the axonodendritic arbor similar to the changes found in Rett syndrome. This information suggests that MeCP2 can play several roles in neuronal development: a primary role in establishment and refinement of synaptic connections between many neurons and a secondary role in projection pathways such as the nucleus basalis that modulate and facilitate neuronal connections and activity-dependent plasticity.
NEUROBIOLOGIC HYPOTHESIS FOR RETT SYNDROME

Recent progress following the discovery that mutations in the gene for MeCP2 cause Rett syndrome has led to greater insight into the neurobiology of the disorder. Immunocytochemical studies indicate that MeCP2 is expressed primarily in neurons, consistent with earlier suggestions based on neuropathologic observations that neurons bear the brunt of the disease. Earlier developing parts of the brain express MeCP2 strongly at birth, whereas expression is delayed in populations that undergo postnatal neurogenesis, such as the cerebellar granule cells. Studies of nasal epithelial neurons using reagents that can accurately classify stages of development indicate that MeCP2 appears prior to synaptogenesis. These data support the hypothesis that MeCP2 allows synapses to be formed, refined and stabilized, or pruned by suppressing genes that antagonize this process. The characteristic onset of the disease during the first year of life probably reflects the importance of synapse proliferation in cerebral cortex to levels surpassing adult levels at about 2 years of age in the human. Deceleration of head growth is prominent in the human infant with Rett syndrome because of the important role that synaptogenesis plays in the postnatal growth spurt in brain size. The prominent clinical signs of Rett syndrome during the first decade, including seizures, hand wringing, abnormal breathing, and psychomotor regression, probably reflect enhanced excitability in neurons at this time, probably because excitatory receptors play such a critical role in synapse formation and pruning during this period. As the brain matures, these signs abate. Compared to the human, the onset of signs in rodents with loss of MeCP2 function is relatively delayed, possibly because overproduction of synapses is not nearly as robust or functionally important as it is in rodents.

POTENTIAL RELEVANCE TO THERAPY FOR RETT SYNDROME

Greater insight into the pathogenesis of Rett syndrome suggests potential strategies to improve outcome. These include attempts to enhance expression of MeCP2, improving the affinity of the mutated protein to DNA binding sites, or enhancing the effects of alternative transcriptional repressors that could substitute for it. Molecules that control development of synapses are also potential targets, including modulation of excitatory receptors, replacement of acetylcholine, or stimulation of other intracellular signaling pathways. Although expression of many genes appears to be controlled by MeCP2, it could be that only a few of the genes are important for controlling synaptogenesis.

CONCLUSION

Converging information from humans and rodent models indicates that neurons and especially synapses are the primary targets of disrupted development in Rett syndrome. Based on data from rodent brain, including the cerebellum and nasal epithelium, expression of MeCP2 protein is delayed for a substantial interval after completion of neurogenesis, appearing prior to formation of synapses. Presynaptic terminals appear to be preferentially disturbed in the disorder, and elevations in both extracellular glutamate concentrations and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA glutamate receptors contribute to seizures, movement disturbances, and other signs of hyperexcitability. Mutations in MeCP2 also appear to disrupt development of the cholinergic nucleus basalis, which modulates the activity-dependent plasticity of neuronal circuits in the cerebral cortex. This information is contributing to a clearer picture of the neurobiology of Rett syndrome.

References


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