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Review

Autism and Parkinson's Disease: Animal Models and a Common Etiological Mechanism

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Abstract

Autism is a behaviorally-defined neurodevelopmental disorder that is difficult to diagnose, treat, and study due to its unknown etiology, widely varying symptoms, and lack of an identified neuropathology. In contrast, Parkinson's disease is a neurodegenerative disease of unknown etiology that is well defined both behaviorally and neuropathologically. While clinically these two disorders appear to have little in common, we would like to present the hypothesis that their respective etiologies may share similar features. Both autism and Parkinson's disease are thought to be the result of interactions between environmental insult, genetic polymorphisms, and age. In addition, since no particular environmental toxicant has been identified as causal for either condition, we propose that any of a number of possible environmental toxicants interact with the individual's genetic sensitivity to cause the underlying neuronal damage. We suggest that while low levels of exposure to any of these individual toxicants may be well-tolerated by most individuals during early development, various combinations of the toxicants may exert a cumulative, deleterious effect. Likewise, although several genes have been linked to autism and Parkinson's disease, respectively, no single gene has been identified that can account for a majority of cases for either. Therefore, various combinations of genetic alterations predispose individuals to their respective disorders but it remains the case that the environmental exposure(s) must occur during critical periods of development for the eventual disease manifestation. The objective of this review is to provide examples from animal models that the etiologies of autism and Parkinson's disease have common features of toxicant-induced oxidative stress together with genetic deficiencies in the ability to manage reactive oxygen species and, furthermore, that therapeutic intervention using antioxidants delivered at the time of toxicant exposure may be beneficial for both.

Key Words: animal models, autism, Parkinson's disease, reactive oxygen species, antioxidants

Introduction

The etiologies of autism and Parkinson's disease remain elusive but may be similar in that they are likely the result of gene by environment by age interaction. That is, exposure to a given environmental toxicant may result in different behavioral and neuropathological outcomes when encountered at different ages. Thus, although autism and Parkinson's disease have dissimilar phenotypic expression, when viewed from a gene by environment by age interaction, similar etiologic features become apparent. We suggest that excess reactive oxygen species, which can result from toxicant exposure or faulty oxidative stress defense genes, contribute to the etiology of both disease states. The particular genetic alterations confer susceptibility of different brain regions to the deleterious effects of toxicant-induced oxidative stress. This, together with the timing of the toxicant exposure leads to the disease state. In both autism

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and Parkinson's disease, toxicants induce oxidative stress in individuals who are compromised in their ability to manage the reactive oxygen species because of genetic alterations. In this way, oxidative stress links the toxicant exposure and genetic alterations underlying each disease state. Thus, we believe that antioxidant treatment may be of benefit if delivered during the critical period of toxicant exposure.

Reactive oxygen species contain an unpaired electron and are produced through a variety of physiologic and pathologic processes (122). The brain is particularly vulnerable to reactive oxygen species and lipid peroxidation because it has a high concentration of polyunsaturated fatty acids. Neuronal oxidative stress can result in either necrosis or apoptosis when the normal balance between oxidative events and antioxidant defenses is disrupted (10). In order to prevent oxidative damage, neurons contain three important antioxidant enzymes, glutathione peroxidase, catalase, and superoxide dismutases. In addition, the intra- and extracellular fluid contains antioxidant free radical scavengers and lipid peroxidation inhibitors (10, 75). In this review, we would like to present recent data generated through use of our animal model of autism and prior data generated through use of our Parkinson's disease model that show that the deleterious effects of the respective toxicants can be prevented by pretreatment with antioxidants. While the disease states and models do vary considerably, we believe this convergence of observations may reveal similarities in the underlying etiologies of autism and Parkinson's disease, similarities to the extent that one may speculate about possible treatment/prevention strategies common to both.

Autism

Epidemiology of Autism

Autism is a neurobiological disorder involving a wide range of symptoms including impaired communication, impaired social interaction, and abnormal motor behaviors. Autism typically appears before three years of age with many parents noticing abnormal development as early as one year. However, in cases of autistic regression, parents report children as having normal development up to the age of three, but these developmental achievements are lost thereafter (104). Fombonne's 2003 review found that surveys conducted prior to 1991 reported median prevalence rates of 4.4/10,000 and surveys conducted between the years 1992-2001 reported median prevalence rates of 12.7/10,000. The correlation between prevalence rate and year of publication was statistically significant and points to an increase in prevalence estimates over the last 15 years (34). There are several possibilities why the autism rates have increased over the last several years. One possible explanation for the increase in autism prevalence is the recent change in autism diagnosis criteria. Given the high number of autistic individuals who are co-morbidly diagnosed with other psychiatric disorders, such as mental retardation and obsessive compulsive disorder (63), it is possible that these new diagnostic tools enhance detection of autism in instances that might have been overlooked because of other conditions.

However, epidemiological studies also lend support to the hypothesis that environmental factors are relevant to autism. In particular, several autism clusters have been identified where the prevalence of autism is unusually high (14, 118). It has been suggested that these autism clusters may be the result of high levels of exposure to environmental toxicants (118). We have recently reported that these autism clusters are in regions where there is a wide variety of toxicants to which residents are exposed (64). With an obvious limitation being that families do move from one location to another, these data may be interpreted to indicate that autism may be associated with repeated low dose exposure to toxicant mixtures.

Neuropathology of Autism

The exact neuropathology accounting for autism symptoms has remained elusive. Kanner (49) described autism as an "...innate inability to form affective contact with people in the ordinary way to which the human species is biologically disposed." Kanner did note that about 50% of his cases had an unusually large head circumference. Since then, there has been a frustrating paucity of autopsied brains of autistic individuals. Bauman and Kemper (12) reported increased cell density in the hippocampus, amygdala and mamillary body of a 29-year-old, severely retarded, autistic individual who had experienced major regression at 30 months with a seizure disorder thereafter. This individual exhibited loss of cerebellar Purkinje and granule cells and engaged in stereotypic and self-injurious behavior. The major drawback for the study was that there were no controls for mental retardation and seizure activity without autism. The lack of available controls prevents the conclusion that the observed brain pathology was solely attributable to autism.

In a later summary review of nine autistic individuals, Kemper and Bauman (50) reported reduced Purkinje cell numbers in 100% of the autistic individuals. Comparing the nine brains (age ranged from 6 to 54 years) to one control brain (age 35), the

authors did note a variability in the size of the cerebellar lobes and no consistent pattern in the widening of spaces between them. Confusing this interpretation was the fact that the authors also note that the two cerebella (one from an Asperger patient and the other from an individual with autism but who had normal intelligence) both appear very similar to the control brain. If the correct interpretation of this statement is that most of the other nine autistic individuals were also mentally retarded, the neuropathological differences cannot, with confidence be attributed to the autism alone. In an MRI study, Soto-Ares *et al*. (96) observed cerebellar atrophy in 27 out of 30 individuals with mental retardation. Given these findings, one must be cautious about concluding that the cerebellar pathology observed in Bauman and Kemper's studies is specific to autism. Ritvo *et al*. (85) found a similar reduction in the number of cerebellar Purkinje cells in the brains of four autistic individuals as compared to three controls. Of interest, three of their autistic subjects were severely retarded while the fourth (subject 2) had a normal IQ. Subject 2, who was diagnosed with autism but who was not mentally retarded, had the highest Purkinje cell counts of the autistic individuals, nearly identical to the three controls. One of their subjects (subject 1) exhibited an autistic regression at 18 months of age. The Purkinje cell counts of this individual were the second lowest of all cases they had examined.

Long believed to be associated with motor movement, recent studies have shown that the cerebellum plays an important role in tasks involving attention. Thus, it has been reasoned that developmental injury to the cerebellum may account for some of the social/cognitive deficits in autism (1). For example, fMRI studies show that motor task cerebellar activation was altered in autistic subjects when compared to controls (69). In addition, contralateral and posterior cerebellar regions were activated in simple motor tasks for autistic but not healthy individuals (1). The combination of these results show that decreased cerebellar volume is associated with a reduction in cerebellar function. Therefore, regardless of whether or not Purkinje cells are significantly altered in size or number, the cerebellum appears to play an important role in the cognitive processing differences seen in autistic individuals.

Human magnetic resonance imaging (MRI) studies have demonstrated an increase in cerebral gray and white matter in autistic children compared to normally developing children (6, 22, 39, 97). These increases appear to disappear between the ages of 5 and 12 (6, 22). However, when individual regions are compared, such as the amygdala and the hippocampus, results are inconclusive. Howard *et al*. (40) showed an increase in amygdale volume whereas Aylward *et al*. (5) showed decreases. Hippocampal studies are just as confusing, with Saitoh *et al*. (87) and Piven *et al*. (79) reporting no differences in volume and Aylward *et al*. (5) showing decreases. As noted, one potential reason for these conflicting results may be that each of these studies used populations of autistic subjects differing in mental capacity, medication history, seizure activity, obsessive behavior, regression, and/or self-injurious behaviors. Any of these factors might account for variation in the size of either the hippocampus or amygdala. In fact, these studies with their varying results highlight the importance of subtyping autism in order to obtain meaningful data and a better understanding of the etiology.

Etiology of Autism

As noted, even though the exact etiology of autism remains unknown, anecdotal reports and case studies have pointed to environmental insults ranging from prenatal drug exposure (86) to severe reactions following vaccinations (49, 112). Numerous drugs have been linked to cognitive and behavioral developmental deficits in both humans and animal models (35, 55, 67, 116). In particular, clinical studies of children exposed to thalidomide or sodium valproate in utero at the critical time of neural tube closure have identified a phenotypic pathology similar to autism and including deficits in language and communication, stereotypic and hyperexcitable behavior, and global delays in behavioral development (2, 55, 60, 67, 116). Previous studies have also demonstrated impairment in cognitive, motor, attention and social development in rodents administered sodium valproate in utero or during weaning (17, 107). The valproate-induced damage is thought to be due, at least in part, to the generation of oxygen free radicals (26, 72, 100). Furthermore, it is of interest to note that not all of the individuals exposed to these agents during the critical time window developed autism-like symptoms (32, 61). It may be speculated that those who were affected may have had a genetic sensitivity to the damaging effects of oxidative stress. Likewise, although controversial, an article by Wakefield (112) demonstrated that postnatal immune challenge results in abnormal behavioral responses similar to those seen in autism disorder, and this effect has been replicated using animal models as well (36, 92,123).

Thus, while many individuals are regularly exposed to illness, toxicants, and immune challenges early in life, these events do not routinely manifest as autistic disorder. Therefore, it is likely that other

factors in addition to environmental insult are required for the manifestation of autism. Several genetic mutations have been implicated in increasing an individual's likelihood of developing autism (68, 77).

Genetic Variation in Autism

Using strict diagnostic criteria, the monozygotic twin concordance rate for autism is 65% whereas the dizygotic twin concordance rate is about 8%, similar to the rate of subsequently born siblings (9). However, when milder social and cognitive deficits are combined, monozygotic concordance rates increase to 82% and the dizygotic rates increase to 10% (33). Although fifteen to twenty different genes have been implicated in autism, when each of them is examined individually, none can account for a substantial portion of the autistic population. Thus, in order to explain the 65% concordance rate, it has been hypothesized that several gene polymorphisms must combine to result in autism. Two gene candidates linked either directly or indirectly to oxidative stress will be examined.

Serotonin Transporters

Up to one third of all individuals with autism have hyperserotonemia and this increase in serotonin levels is up to 50% higher than that found in healthy individuals (56, 89). Autistic hyperserotonemia is unusual because it occurs only in blood plasma but not in brain (56). Wittaker-Azanita (115) suggested that, during early development, the blood brain barrier is only partially formed and may allow entrance of serotonin from plasma to brain. In the developing fetal brain, the high serotonin levels may cause the loss of serotonin terminals through negative feedback via the 5-HT1A receptor. Therefore, any genes which regulate plasma levels of serotonin could indirectly have an effect on brain development and brain serotonin levels.

There are several genes linked to autism and which regulate serotonin levels. Sugie *et al*. (99) examined children with autism who possessed two of either the long or short alleles of SLC6A4 (the serotonin transporter) to see if they responded differently to treatment with selective serotonin reuptake inhibitors (SSRI). Children with the long allele responded significantly better to SSRI treatment than the children with the short allele. In addition, they found that the SLC6A4 gene variation accounted for a small percentage of overall serotonin levels in the children. The short allele for SLC6A4 has also been associated with significantly larger amounts of gray matter in all areas of the brain except for the cerebellum (113). This finding is interesting because one of the most commonly cited neuroanatomical abnormality in post-mortem autism studies is macrocephaly in all areas of the brain except for the cerebellum. In addition, SLC6A4 is highly expressed in all areas of the brain except for the cerebellum. Countinho *et al*. (23) found a two-way interaction between SLC6A4 and ITGB3. ITGB3 is a second gene that has been shown to regulate serotonin. The two-way interaction between SLC6A4 and ITGB3 accounted for the serotonin levels in autistic subjects with 67% accuracy.

In the context of our hypothesis, it is important to note that high levels of serotonin have been associated with the generation of oxidative stress and neural damage. Specifically, methamphetamine-induced release of serotonin has been shown to result in the formation of 5,7-dihydroxytryptamine (20) which, in turn, damages serotonergic terminals through oxidative stress. Thus, high plasma serotonin in an immature brain consequent to insufficient serotonin transport may result in long-lasting neuronal damage.

Glutathione-S-Transferases

There are 21 recognized glutathione -S-transpherase genes in mice (15). One polymorphism is glutathione-S-transferase M1 (GSTM1) which detoxifies xenobiotics *via* conjugating them to glutathione (7). GSTM1 also modulates stress-mediated signals *via* interaction with MAP kinases which would, in turn, alter cellular signaling (31). MAP kinases play an important role during CNS development via their regulation of apoptosis, morphogenesis, cell survival, and the response to stress.

In addition to disorders where the disease allele acts in the affected individual, there are also disease alleles that act in a mother during pregnancy which affect her offspring (47, 74). Glutathione S-transferase polymorphic alleles have been implicated in autism, with two variants altering enzyme function and positively correlated with oxidative DNA damage (24, 43, 46, 59). Williams *et al*. (117) demonstrated that the GSTP1*A haplotype was significantly more transmitted to mothers of individuals with autism in maternal trios and may be acting as a teratogenic allele. As noted, glutathione S-transferases detoxify products of oxidative stress. GST alleles that have altered function may result in their accumulation and contribute to oxidative damage. Therefore, if a mother has the GSTP1*A haplotype and is exposed to an environmental stressor which causes oxidative stress there may be a gene by environment interaction which increases the likelihood of her offspring developing autistic disorder.

Several other genes whose alteration may contribute to increases in oxidative stress have been

implicated in autism. The reduced folate carrier (RFC) and methylenetetrahydrofolate (MTHFR are both part of the methionine cycle. The G allele of the RFC has been associated with increased risk for several birth defects. James *et al*. (44) found that children with the RFC-1 GA or GG genotypes were two times more likely to be autistic. In addition, there was an interaction between the RFC-1 GG genotype and the MTHFR 677CT genotypes which showed a three-fold increase in autism susceptibility (44). Individuals with interactions between several COMT (methylates dopamine) alleles and TCN2 (required for the cellular uptake of B12) alleles have also been shown to have a seven-fold risk for developing autism (44). Collectively, these data relate autism to genetic deficits in the neuronal response to oxidative stress (10, 44, 112).

Evidence for Oxidative Stress in Autism

Zoroglu *et al*. (122) compared oxidant and antioxidant levels in autistic and healthy control children. They found increased thiobarbituric acid-reactive substances (TBARS), superoxide dismutase, and xanthine oxidase in autistic children compared to controls. In addition, they found decreases in catalase in autistic children. The TBARS levels (a measure of lipid peroxidation) in autistic patients were increased by up to three-fold in some of the autistic patients even though superoxide dismutase was also increased in these patients. Chauhan *et al*. (19) found that autistic children had significantly increased levels of lipid peroxidation, again indicating that oxidative stress is increased. In addition, they found decreased levels of several proteins that reduce oxidative damage and there was a correlation between the reduction in these proteins and the loss of previously acquired language skills (regression) in the autistic children. James *et al*. (44) observed an imbalance in methionine and homocysteine metabolism in autistic children compared to healthy controls. They state that the autistic children's lower baseline levels of S-adenosylmethionine, cystathionine, cysteine, and glutathione combined with their increased levels of S-adenosylhomocystine, adenosine, and oxidized glutathione indicate an impaired capacity for methylation, increased oxidative stress, and increased oxidative stress vulnerability. Ming *et al*. (62) compared urinary excretions of 8-isoprostane, a lipid peroxidation biomarker, in 33 children with autism and 29 aged matched controls. They found that autistic children had significantly increased levels of 8-isoprostane, with a small portion of the autistic children having between 25- to 46-fold increases above the mean. Collectively these and other observations led Kern and Jones (51) to hypothesize that autistic children are selectively vulnerable to environmental factors which cause oxidative stress and that this oxidative stress, in turn, causes the neuronal insult seen in autism.

Autism Summary

Autism disorder has several different well defined behavioral phenotypes including impaired communication and social interaction, abnormal motor behavior and selfinjurious behavior. While the behavioral phenotypes of autism disorder are well established, the neuropathology of autism is cloudy at best, and the etiology is quite elusive. We suggest that the etiology of autism likely includes environmental insult, genetic sensitivity, and age as factors, and that oxidative stress may provide a common link.

Parkinson's Disease

Epidemiology of Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder with symptoms of resting tremor, muscular rigidity, and bradykinesia. These symptoms are the result of the degeneration of dopaminergic neurons of the substantia nigra and their projecting nerve fibers to the striatum. There are two different forms of Parkinson's disease: familial and sporadic. Familial Parkinson's disease (about 15% of all cases) is characterized when an individual has a first -degree relative who also has the disease. Sporadic Parkinson's disease is characterized by a later onset of symptoms and no first-degree relatives with the disease (27). Twelves *et al*. (105) compared the incidence of Parkinson's disease from 16 different studies performed in different countries (including the United Kingdom, the United States of America and Japan). Their analysis indicates that the prevalence of Parkinson's disease is approximately 17 per 100,000. They also found up to a two-fold increase in incidence in males. Mitchell *et al*. (66) also report an increase in incidence in males as well as an increased risk for disease development with age. In addition, the same sex difference of men having increased risk for developing Parkinson's disease occurs in both the sporadic and familial forms of Parkinson's disease (8).

Neuropathology of Parkinson's Disease

Parkinson's disease is linked to the death of nigrostriatal dopaminergic neurons. There is a bleaching of the substantia nigra as the neuromelanin-containing dopaminergic cells die. This leads to a marked reduction in the concentration of striatal

dopamine, a loss of dopamine transport pumps, and a decrease in tyrosine hydroxylase activity. In addition, as the dopaminergic terminals are lost, there is a post-synaptic proliferation of dopaminergic receptors known as dennervation supersensitivity and an increase in dopamine production and release exhibited by the remaining dopaminergic terminals. Symptoms of Parkinson's disease do not manifest until 80-90% of the nigro-striatal cells have been lost. Therefore, because of the compensatory increase in post-synaptic receptors and increased dopamine turnover, a small percentage of the remaining nigro-striatal cells carry on full function, masking disease progression until a critical threshold is reached. Once Parkinsonian symptoms do manifest, the life expectancy of the patient is approximately seven to ten years. In addition to dopaminergic cell lesions, there is a prominent lesion of the central serotonergic system that manifests as a decrease in the concentration of striatal serotonin.

Etiology of Parkinson's Disease

A number of studies have linked toxicant exposure and mitochondrial dysfunction to increased risk for developing the disease. This possible link between toxicant exposure and the neurodegenerative disorder followed case reports of druginduced Parkinson's disease following self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The toxicity of MPTP is dependent upon its conversion to MPP+ by monoamine oxidase. In turn, MPP+ causes mitochondrial impairment which increases the production of free radicals and reduces ATP synthesis, both of which act synergistically to increase oxidative stress and neurodegeneration. Based upon his epidemiology studies, Barbeau (11) was the first to propose that the etiology of Parkinson's disease was the result of multiple exposures to environmental triggers, each resulting in oxidative stress in genetically susceptible individuals. Of interest, Barbeau (11) related the consequent neurodegeneration to increased release of catecholamines, particularly dopamine.

Several other toxicants have also been implicated in causing Parkinson's disease. Dieldrin is a pesticide that was banned in the United States in 1974 and was found in the post-mortem analysis of several brains of people who had Parkinson's disease. Hatcher *et al*. (38) found that low levels of dieldrin over an extended period of time caused increases in cytosolic dopamine and oxidative stress. They also report an increase in carbonyl levels and decreases in glutathione levels in the striatium, which they believe attribute to the overall increases in oxidative stress. What these studies suggest is there is no one particular toxicant or family of toxicants which result in Parkinson's disease. Any of a number of toxicants could prime an organism such that later exposures to dopaminergic toxicants would greatly increase oxidative stress and would lead to dopamine depletion significant enough to cause Parkinson's disease.

Evidence for Oxidative Stress in Parkinson's Disease

Dexter *et al*. (29) found 10-fold increases in cholesterol lipid hydroperoxides, an early marker of lipid peroxidation, in the substantia nigra of post-mortem Parkinson's brains versus healthy controls. Jenner and Olanow (45) found increased iron, decreased glutathione and oxidative stress in post-mortem brains of individuals with Parkinson's disease. Olanow and Tatton (73) suggested that oxidative stress could develop in Parkinson's disease under three different circumstances: [1] increased dopamine turnover which causes excess peroxide formation, [2] glutathione deficiency which decreases the brain's ability to get rid of free radicals, and [3] increases in reactive iron that causes hydroxyl radical formation. In addition, structural alteration of peroxiredoxin2 (Prx2), a peroxidase that work as an antioxidant enzyme, may lead to oxidative stress. When Prx2 is S-nitrosylated it forms SNOPrx2 which is nonfunctional and causes the accumulation of cellular peroxides and leads to oxidative stress *in vitro*. SNO-Prx2 increases are seen in brains of Parkinson's patients. Other proteins, such as alpha-synuclein, that partially function to decrease damage caused by oxidative stress, have been shown to have mutations which make them nonfunctional (84). Once these proteins are nonfunctional they allow for increases in oxidative stress and possibly result in neuronal death.

Genetic Variation in Parkinson's Disease

Several gene polymorphisms have been identified that have been associated with Parkinson's disease. This has led to the suggestion that environmental toxicants together with gene polymorphisms lead to the neural degeneration associated with this disease (11).

Parkin

While the exact physiological function of parkin is unknown, it appears to be similar to the ubiquitin family of proteins and may control cell growth, differentiation, and development. Parkin is also involved in protein degradation *via* its role as an ubiquitin-protein ligase (41). Mutations in the parkin gene are linked to early onset familial Parkinson's disease cases where neuronal loss in

the substantia nigra occurs without Lewy body formation (54). However, there are no clinical symptoms that distinguish a Parkinson's patient with parkin mutation from other juvenile onset Parkinson's patients (16).

LRRK2

Leucine-rich repeat kinase 2 (LRRK2) mutations have been reported to account for between 6 to 40% of familial Parkinson's disease cases depending on the ethnicity of the population. LRRK2 has a similar distribution to E3 ubiquitin ligase parkin and is located in both the cytosol and mitochondrial outer membrane (25). Two LRRK2 mutations, G2019S and R1441C, appear to enhance kinase activity which is a condition that is observed in other disease states such as cancer (114). LRRK2 is a component of Lewy bodies in both Parkinson's disease and dementia with Lewy bodies and may therefore play an important role in Lewy body formation.

Alpha-Synuclein

Alpha-synuclein protein is 140 amino acids long in both humans and rodents and these isoforms are 95.3% identical (57). Alpha-synuclein has been shown to function as a neuroprotective protein particularly with respect to oxidative stress. Two alpha-synuclein mutations, A53T and A30P have been implicated in causing familial Parkinson's disease and in vitro studies indicate that these mutations allow alpha-ssynuclein to form amyloid fibrils. Alpha-synuclein is a major component of Lewy bodies and accumulation of alpha-synuclein may play a major role in the development of Parkinson's disease (73). Even though only a small percentage of people with familial Parkinson's disease have alpha-synuclein mutations, it appears alterations in alpha-synuclein levels or expression may play a role in many if not all forms of Parkinson's disease. Purisai *et al*. (80) stated that, in addition to genetic variants, alpha-synuclein could be toxic in and of itself. If alpha-synuclein's structure is altered *via* oxidative modification, which may occur through toxicant and alpha-synuclein interactions, it may switch its role from a neuron survival protein to a protein which leads to cell death. Purisai *et al*.'s study provided evidence for this theory because they found that one single injection of MPTP is sufficient to produce sustained up-regulation of alpha-synuclein in non-human primate brains.

The alpha-synuclein protein appears to be normally neuroprotective but its two mutant forms, A30P and A53T, decrease alpha-synuclein's chaperone activity and cause alpha-synuclein overexpression (83). One function of molecular chaperones is that they interact with destabilized proteins to prevent them from aggregating and encourage them to correctly fold (18). Oxidative stress is one of several cell stressors that can cause proteins to partially unfold and aggregate, and these altered proteins may not be able to be removed sufficiently or at all from the organism. Therefore, if one or more of an organism's chaperone molecules is genetically altered and can no longer counteract the effects of oxidative stress on protein folding, that organism is more vulnerable to oxidative stress and has a higher likelihood of sustaining damage from the environmental insult.

Animal Models of Autism and Parkinson's Disease: Antioxidant Protection

Autism

As noted, autism is a neurodevelopmental disorder characterized by impaired social interaction, limited or absent communication skills, and restricted or repetitive motor patterns. These functional deficits (core symptoms) of autism, especially the impaired social interactions and deficiencies in language development, have proven difficult to model in other species. In addition, about 75% of those diagnosed with autism are also mentally retarded making symptom identification and attempts to develop animal models of autism still more complicated. We have initiated work on a novel strategy to model autism in mice wherein the normal development of key behaviors are first carefully monitored from birth through adolescence. Once the maturation of these key behaviors is understood (*i.e*. in terms of the postnatal day(s) of life in which subjects are able to successfully perform the task or engage in the behavior), the performance of mice with genetic alterations and/or early toxicant exposure can be assessed for comparison. The model does not claim to produce "autistic" mice; rather, developmental deficits can be identified and attributed to genetic alterations, toxicant exposure, or the interaction of the two. The use of mice with either a genetic mutation or toxicant exposure clinically associated with autism makes our model highly relevant (111).

The model strategy begins by classifying behavioral manifestations of genetic- or toxicant-induced developmental disorders along a developmental time line into broad categories of retardations (defined as when a behavior fails to develop in the affected mice during a critical period), regressions (defined as when a behavior develops at about the right time but then is lost with later development as, for example, following toxicant exposure), or

intrusions (defined as the appearance of other behaviors aberrant in form or frequency which mask or overshadow the normal development). Most developmental disorders include some combination of these conditions. In this framework, the hypothesis that genetic and/or environmental triggers are involved in a developmental disorder such as autism can be readily tested. That is, acute or repeated exposure to a toxicant in the presence or absence of a gene alteration should disrupt neurobehavioral development causing behavioral retardation, regression, or intrusions. Although using retardations, regressions and intrusions as a classification scheme has clinical validity, most animal models have not examined these three scenarios but, instead, focus on single aspects of neurobehavioral development following either toxicant exposure or genetic manipulation.

As noted, our model strategy examines mice for toxicant-induced retardations, regressions or intrusions in their development of a variety of behavioral tasks that have been selected because they are associated with human autism. The selected tasks may be classified as assessing the maturation of social, emotional, cognitive and motor skills and include (among others) assessment of play, aggression, elevated plus maze, water maze, active and passive avoidance, stereotypic and self-injurious behavior, and surface and mid-air righting reflexes. Furthermore, many of the particular tasks were selected because they have been linked, at least in part, to maturation of the cerebellum, hippocampus, amygdala, and striatum. In turn, these brain areas are known to be involved in autism, are targets of selected toxicants relevant to autism, and have been linked to the function of autism-related genes. In brief, we have developed a comprehensive mouse model of autism capable of identifying genetic or toxicant-induced deficits in the normal maturation of social, emotional, cognitive and motor behaviors; in turn, these deficits can be linked to morphological and/or neurochemical changes in discrete brain regions known to be altered in human autism (111). Toward this end, we have used this model to attempt to identify both underlying mechanisms of toxicant-induced damage as well as strategies to protect the developing brain against these toxicants. Two toxicants used in our model that link autism to oxidative stress are valproic acid (VPA) and methylmercury (MeHg).

Valproic Acid and Oxidative Stress

VPA elicits an oxidative stress response as it undergoes a cytochrome P450-dependent hydroxylation and desaturation as well as mitochondrial and peroxisomal oxidation. Covalent binding of VPA to tissue macromolecules and formation of glutathione conjugates are indicative of bioactivation of VPA to reactive intermediates (48, 102). VPA decreases cellular glutathione (93) rendering the cells more susceptible to oxidative damage (119). VPA also suppresses the activities of hepatic glutathione reductase, glutathione peroxidase, and glucose-6 phosphate dehydrogenase (21). VPA, alone (103) or in the presence of allyl alcohol (52), induces lipid peroxidation in hepatocytes (52, 103) and plasma (103). These reactive oxygen species may play a role in VPA induced cytotoxicity (101). The metabolism-dependent VPA-induced *in vitro* cytotoxicity is the result of generation of hydrogen peroxide and subsequently interacting intracellularly with iron to produce the highly reactive hydroxyl free radicals. To prevent cell damage a series of antioxidant enzymes and iron chelators protect against metabolism-dependent VPA-induced *in vitro* cytotoxicity (100). Vitamin C and vitamin E treatment exert a cytoprotective effect against 4-ene VPA-induced injury in GSH-depleted hepatocytes in rats (48).

VPA therapy for seizure disorders has been demonstrated to increase serum lipid peroxides and to decrease glutathione peroxidase (GPX) activity secondary to a VPA-induced decrease in selenium concentrations. Patients with low GPX activity are at risk for the development of an idiosyncratic adverse reaction to VPA (78). In other species, VPA is associated with abnormal hindbrain neurogenesis in rats that is consistent with some of the pathological features of human autism and thalidomide teratogenesis (86). Furthermore, VPA can cause spina bifida in the fetus during early gestation. Children with spina bifida were documented to have lower erythrocyte GPX activity as compared with their age-matched healthy controls. Parents of these children also demonstrated lower GPX activity (37). These findings suggest that VPA causes oxidative stress resulting in toxicity and teratogenesis, especially in a genetically-susceptible population. It is therefore anticipated that mice over-expressing GPX1 may be resistant to VPA toxicity.

VPA is used widely as an antiepileptic drug because of its relatively few side effects in adult humans. However, as noted above, pre-natal exposure to valproate appears to produce a pattern of behavioral and neuroanatomical abnormalities in children similar to those found in autism. Clinical studies of children exposed to valproate in utero have characterized a fetal valproate syndrome with symptoms similar to autism including deficits in language and communication, stereotypic behavior, hyperexcitability, and global delays in behavioral development

(2, 55, 60, 67, 116). In addition, clinical case studies of infants with fetal valproate syndrome report physical abnormalities including low myelomeningocele lesion, minor abnormalities of the face and ear, and microcephaly (2, 67). In rodents, VPA exposure at the time of neural tube closure causes changes in Purkinje cell number and cerebellar cell volume similar to what is observed in patients with autism (42, 86, 94). Schneider and Przewlocki (90) found that rats pre-natally injected with VPA exhibit lowered sensitivity to pain, repetitive/stereotypic-like behavior combined with lower exploratory activity, and decreased numbers of social behaviors and increased latency to engage in social interactions.

Valproic Acid as an Animal Model of Autism

Our previous studies demonstrated that injections of sodium valproate at crucial developmental time points can produce a functioning animal model of autism (111). By using a battery of behavioral tests, we have demonstrated that animals injected with VPA, both pre- and post-natally, display a retardation and/or regression of certain critical behaviors such as surface righting, mid-air righting and negative geotaxis.

More recently we demonstrated that P14 exposure caused a significant decrease in social behaviors in comparison to control animals (121). In addition, early post-natal administration of valproate to mouse pups also causes a 30-fold increase in the number of cells staining for apoptosis in the cerebellum and a 10-fold increase in cells staining for apoptosis in the hippocampus (121).

Finally in the context of our present hypothesis, we previously noted that valproate is thought to exert its toxic actions through the generation of reactive oxygen species. Accordingly in an effort to determine if antioxidants could protect against the behavioral deficits caused by VPA, male BALB/c mice were treated with vitamin E or its vehicle on day P14. Both groups then received a second injection of either saline or 400 mg/kg of VPA, making a total of 4 groups. The ability to right in mid-air was evaluated on P13-19. Pups were held upside-down, 45 cm above a padded surface and dropped for 3 trials each test day. Ability to right in mid-air was determined if the pup landed with all four paws on the surface (65).

The mid-air righting response of developing mouse pups first appears on about post-natal day 14 of life. VPA treatment causes a pronounced regression in this response but pretreatment with the antioxidant, vitamin E, completely protects the pup (65). As noted, VPA has been associated with human autism and, therefore, these data suggest that antioxidants administered at the time of toxicant exposure might afford some protection against an autistic-like regression.

Methylmercury and Oxidative Stress

An organic mercury, MeHg, was selected as a second toxicant for testing because it is an important, widely distributed environmental toxicant. Though some reports have associated early exposure to organic mercury with increased risk for autism (see 13 for review), this issue remains far from resolved. Organic mercury is converted by sediment bacteria in fresh and salt water to MeHg which then bioaccumulates in the food chain with the largest fish and sea mammals having the highest body burden. Industry also contributes organic mercury to the waste stream leading to human exposure, sometimes with immediate and devastating consequences. The toxic actions of MeHg depend on dose and age, with the developing brain being most sensitive to low dose exposure. MeHg does cross the placental barrier and, in humans exposed in utero to acute high doses, was shown to cause a retardation in cognitive and locomotor development along with numerous other neurological symptoms including seizures and cerebral palsy (71).

The consequences of low dose, chronic exposure to mercury through fish consumption are somewhat more controversial, with some studies showing deleterious effects while others show no adverse consequences (13, 71). Early exposure to mercury has been shown to disrupt the neurobehavioral development of other species including rodents and primates (81, 82). A proposed mechanism through which MeHg exerts its toxicity is thought to be, in part, mediated by disruption of neural cell adhesion molecules (30), known to be important for proper synapse formation during development; however, oxidative stress may contribute to the neurotoxicity.

Oxidative stress is also involved in MeHg-induced neurotoxicity as demonstrated by the significant increase in reactive oxygen species (ROS) and thiobarbituric acid reactive substances and a reduction in glutathione levels when cultured rat oligodendrocytes, astrocytes and neurons of embryonic and neonatal rat were exposed to methylmercuric chloride (120). The neurotoxicity of MeHg in cultured neurons was blocked by the pretreatment with antioxidants (76). Trolox, a water soluble derivative of vitamin E, protects against MeHg-induced neurotoxicity in rats (106). Likewise, antioxidants (glutathione, vitamin E and selenium) produced protective effects against MeHg toxicity in cultured human neurons and astrocytes (88). The major mechanisms involved in MeHg neurotoxicity are thought to include oxidative stress, impairment of intracellular calcium homeostasis and modulation

of neurotransmitter systems, especially glutamate-mediated excitotoxicity (3). MeHg binds to sulfhydryl groups on proteins and glutathione leading to the modification of structural and functional properties of enzymes involved in detoxification of ROS (98). Indeed, ROS are known to mediate MeHg-induced neurotoxicity in multiple experimental models. For example, MeHg induces ROS formation both *in vivo* and *in vitro* in neurons and glia (95). A significant increase in ROS generation in mitochondria from MeHg-exposed rat brains has also been observed (120). *In vitro* studies suggest that the effect of MeHg on ROS generation is calcium and age-dependent, with younger neuronal cultures exhibiting greater propensity to ROS formation (70). Finally, antioxidants/oxygen radical scavengers, such as vitamin E, glutathione, and selenium have been shown to provide a certain degree of protection against MeHg neurotoxicity in cell culture as well as in experimental animals (88).

MeHg Expsoure as an Animal Model of Autism

Similar to the above VPA study, male pups were treated with phosphate buffered saline (PBS) or with 4.0 mg/kg MeHg on alternate days between P3 and P15. Additional groups received the same MeHg treatment but with Trolox 1.0 or 2.5 mg/kg before each injection of MeHg. As in the VPA study, pups acquired the mid-air righting response on about post-natal day 15. However, pups treated with MeHg alone or MeHg with 1.0 mg/kg of Trolox showed a regression, failing to acquire this behavior. Pups treated with MeHg and 2.5 mg/kg of Trolox did not differ on their performance of the mid-air righting task from the controls. Therefore, as with the VPA, antioxidant administration protected mice against MeHg toxicity in a dose-dependent fashion.

Collectively, these data indicate that two toxicants that have been associated with human autism will induce an autistic-like regression in the motor development of developing mice. Furthermore, the neurodevelopmental toxicity of both toxicants was significantly reduced by pretreatment with antioxidants.

Parkinson's Disease

Amphetamine and methamphetamine (METH) are potent stimulants that have been used clinically for treatment of obesity, minimal brain dysfunction, narcolepsy, and to counter fatigue. They are also subject to widespread abuse. Both act as indirect agonists, causing release of catecholamines, blocking their reuptake, and inhibiting their degradation by monoamine oxidase. Collectively, these effects lead to an increase in the concentration of catecholamines in the synapse as well as an overall increase in catecholaminergic activity in the brain.

Over the past 30 years, we and others have demonstrated that the repeated administration of high doses of the amphetamines leads to long-lasting depletions of central dopamine levels. These dopamine depletions are accompanied by a loss of dopamine transporters, a decrease in tyrosine hydroxylase activity, and nerve terminal degeneration. The dopaminergic toxicity occurs across a wide range of species and is sex- and age-dependent, being far more prominent in males and older subjects (see 53, 110). Collectively, these observations make the amphetamines a useful model for Parkinson's disease.

The mechanism through which METH causes the dopaminergic damage remains unknown. It has been suggested that METH-induced DA release encourages formation of 6-OHDA by auto-oxidation of DA in the elevated extracellular DA concentrations (4, 20, 91). The formation of DA-related reactive oxygen species (ROS) such as superoxide and hydroxy radicals appear to play an important role in METH-induced neurotoxicity as well. In the context of our present hypothesis, we demonstrated that administration of antioxidants, such as ascorbic acid or vitamin E, caused attenuation of METH-induced neurotoxicity (28, 58, 108, 109), whereas, inhibition of superoxide dismutase (SOD) by diethyldithiocarbamate increased its neurotoxicity (28).

Finally, as noted above, MPTP serves as an excellent model of Parkinson's disease. The demonstration that oxidative stress is involved, at least in part, as a mechanism through which it exerts its toxicity was achieved by antioxidant pretreatment studies. Specifically, for both MPTP and MPP+, we demonstrated that antioxidant pretreatment nearly completely protected the subjects against the toxicant-induced dopamine lesion (108,109). As with the animal model of autism, a variety of antioxidants have previously been shown to protect subjects against two dopaminergic toxicants.

Conclusions

Autism and Parkinson's disease are both thought to be the result of toxicant exposure acting upon genetically sensitive individuals. The toxicants thought to be involved in each condition may exert their deleterious effects through the generation of reactive oxygen species. Likewise, the genetic polymorphisms associated with each disease are thought to confer enhanced sensitivity to these same oxidative stress mechanisms. Using our animal models, we have shown that various toxicants that have been associated with autism or Parkinson's

disease induce neurobehavioral deficits that resemble the respective disease states. We have previously shown that a number of different antioxidant pretreatments completely protect our subjects against the dopaminergic toxicity exerted by methamphetamine and MPTP, two widely used Parkinson's disease models. We now add to this our recent observations that various antioxidant pretreatments completely protect our subjects against autistic-like regression induced by toxicants associated with human autism. We believe these observations indicate that the two conditions share similar etiologies and that these data may suggest prevention strategies that might be effective for each. The etiologies differ with respect to the genetic alterations. They likely differ with respect to the timing and duration of toxicant exposure. To date, most studies have used acute high-dose toxicant exposures to create autism or Parkinson's like conditions in other animals. Future studies will be directed at determining the critical periods during which low dose toxicant exposure leads to these disease states. We believe that antioxidant therapy delivered at the time of toxicant exposure (as opposed to after the damage has occurred) may protect the genetically-sensitive individual against the neural damage induced by these toxicant administered at low doses during these critical periods of developmental risk.

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