REVIEW ARTICLE

A POSSIBLE CENTRAL MECHANISM IN AUTISM SPECTRUM DISORDERS, PART 2: IMMUNOEXCITOTOXICITY

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In this section, I explore the effects of mercury and inflammation on transsulfuration reactions, which can lead to elevations in androgens, and how this might relate to the male preponderance of autism spectrum disorders (ASD). It is known that mercury interferes with these biochemical reactions and that chronically elevated androgen levels also enhance the neurodevelopmental effects of excitotoxins. Both androgens and glutamate alter neuronal and glial calcium oscillations, which are known to regulate cell migration, maturation, and final brain cytoarchitectural structure. Studies have also shown high levels of DHEA and low levels of DHEA-S in ASD, which can result from both mercury toxicity and chronic inflammation.

Chronic microglial activation appears to be a hallmark of ASD. Peripheral immune stimulation, mercury, and elevated levels of androgens can all stimulate microglial activation. Linked to both transsulfuration problems and chronic mercury toxicity are elevations in homocysteine levels in ASD patients. Homocysteine and especially its metabolic products are powerful excitotoxins.

Intimately linked to elevations in DHEA, excitotoxicity and mercury toxicity are abnormalities in mitochondrial function. A number of studies have shown that reduced energy production by mitochondria greatly enhances excitotoxicity. Finally, I discuss the effects of chronic inflammation and elevated mercury levels on glutathione and metallothionein. (*Altern Ther Health Med.* 2009;15(1):60-67.)

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EXCESSIVE ANDROGENS AND AUTISM

There is strong evidence that mercury exposure in humans increases androgen levels. For example, Barregård et al reported that there was a significant correlation between increasing concentration of mercury in chloralkali workers and testosterone levels.[1] Animal studies also show a link between sex steroid production and mercury dosing.[2] Studies have also shown a link between elevated prenatal testosterone,[3] postnatal serum testosterone,[4] and autism spectrum disorders.

As to the mechanism of testosterone elevation by mercury exposure, it has been suggested that Hg^{2+} directly causes a defect in adrenal steroid biosynthesis by inhibiting the activity of 21 alpha-hydroxylase,[5] while others have suggested inactivation of hydroxysteroid steroid sulfotransferase either directly[6] or by way of inflammation.[7] It has also been shown that DHEA-S, the proposed storage form of active DHEA, is also significantly lowered in autistic disorders.[8]

Kim et al have shown that even very small doses of LPS (1 nmoL) can dramatically decrease the levels of mRNA for SULT2A1 and PAPSS2, which are responsible for sulfonation of a number of endogenous hydroxysteroids, bile acid, and xenobiotics as well as sulfonation of DHEA to DHEA-S.[7] Normally, DHEA-S plasma levels are 300- to 500-fold higher than DHEA levels. Kim et al found that TNF-α and IL-1ß were responsible for the decrease. Unlike in autistic patients, DHEA levels were not increased in LPS-exposed animals, which can occur with mercury toxicity. Reductions in DHEA-S are common with other chronic inflammatory disorders, such as rheumatoid arthritis.[9]

In keeping with the finding of a defect in transsulfuration, one frequently sees associated elevations in androgens and elevations in homocysteine. For instance, several workers have found elevated levels of homocysteine in cases of polycystic ovary syndrome.[10,11] Normally, men have higher homocysteine levels than women, thought to be secondary to higher androgen levels.[12]

Androgen excess interferes with the conversion of homocysteine to cysthathionine, which by conversion to cysteine becomes a major source of glutathione.[13] Thus androgen excess can not only raise homocysteine levels, it can lower glutathione, a major antioxidant in brain. Other pathways in the methionine cycle are also affected, which may partially explain the significant reduction in methionine seen in autistic children, as well as s-adenosyl methionine levels.[4,14]

James et al found not only low total glutathione levels in autistic subjects but also oxidized glutathione levels that were 2-fold higher, which strongly indicate oxidative stress.[14] Several of

the enzymes utilized in the methionine cycle, such as methionine synthase, betaine-homocysteine methyltransferase, and methionine adenosyltransferases, are known to be redox-sensitive enzymes.[15,16] With the chronic elevation of ROS, RNS, and lipid peroxidation products in the autistic brain, one would not be surprised by suppression of these enzymes.

Vitamin B_{12} and folate interplay in generating methyl groups during the methionine cycle. A recent study found an increased frequency in mutations of the C677T allele of methylenetetrahydrofolate reductase enzyme in autistic children.[17] The same genetic mutation causes elevations in homocysteine.[18] In addition, studies have shown abnormal absorption of vitamin B_{12} from the ileum of autistic children.[19]

It is accepted that there is a dimorphic influence of sex steroids on both external male/female morphology as well as brain structure and behavior.[20] In addition, it has been suggested that autism represents a form of "extreme male brain," with normal male behaviors, such as a reduced ability to read nonverbal skills, different language skills, and low theory of mind function, being accentuated.[21,22]

Support for this theory arises from studies of children with congenital adrenal hyperplasia (CAH), which is characterized by high levels of circulating androgens in both afflicted males and females. For example, in one such study, Knickmeyer et al found that females affected with high androgen levels scored higher on the Autism Spectrum Quotient test than normal females.[23]

While this is suggestive of a link, despite high levels of testosterone in children with CAH, few are fully autistic, even though they may share some behavioral symptoms. In addition, many have other metabolic disorders that could contribute to symptomatology, such as electrolyte disorders.

This is not to say that these studies on CAH didn't show behavior effects; it's just that the serious defects in social cognitive function seen with autism are not observed. This indicates that more is involved with autism than elevated androgen levels early in development. For example, elevated androgen levels do not explain the chronic extensive immune activation seen in the autistic brain or the prolonged, widespread activation of microglia and astrocytes. It also doesn't completely explain the extensive neuropathological findings and abnormal pathway development found in the autistic brain.

A number of studies have shown abnormalities in both morphology and function in the amygdala and prefrontal cortex of autistic children, something not accounted for with androgen excess alone.[24-26] Estrada el al have shown that supraphysiologic levels of testosterone (micromolar ranges) can initiate apoptosis of neuronal cells in culture, which should affect neural development.[27] Likewise, Geier and Geier found rather dramatic and rapid improvement in 11 consecutively treated autistic children using both mercury chelation and leuprolide acetate, a drug that lowers androgen levels.[28] The children experience a 2-fold drop in serum testosterone levels over 3 months. Improvements were seen in sociability, cognitive awareness, and aggressive behavior, due mostly to lowered androgen levels, as the effects of mercury chelation usually take longer to manifest.

It should be noted that children fasting for blood test have been noted to show similar rapid improvements in behavior. The combination of elevated androgens, reduced glutathione protection against oxidative stress, and elevated levels of homocysteine would be of considerable concern during brain development.

The Role of Androgens and Estrogens on Microglial Activation and Excitotoxicity

The question to be answered is by what mechanism does androgen excess affect neurodevelopment and neurologic function? There are several possibilities, yet they may be interrelated.

It is known that both testosterone and estrogen, at basal levels, are neuroprotective and play a significant role in neuronal development, migration, dendritic outgrowth, and synaptogenesis.[29,30] Central to the effect of androgen excess appears to be generation of calcium oscillations by androgens, which have been shown to regulate not only neurite outgrowth but also neuron migration.[31] These oscillations of calcium are not caused by stimulation of nuclear gene androgen receptors but rather by rapidly acting cell membrane G-protein-regulated receptors that activate endoplasmic reticulum calcium release by inositol 1,4,5-triphosphate and diacylglycerol signal transduction.[32] It was also shown that the calcium oscillations were not secondary to conversion of testosterone to estrogens by brain aromatase. These oscillations of intracellular calcium also code for cell differentiation in the CNS.[33]

The recent finding by Balthazart et al that the glutamatergic system, primarily acting through the AMPA/kainate receptors, rapidly inhibits brain aromatase activity demonstrates another mechanism by which brain testosterone levels remain elevated in the autistic child.[34] Brain aromatase, as an inducible enzyme, converts testosterone into 17ß-estradiol as an inducible enzyme.[35]

Studies have shown that both NMDA receptors and androgen receptors play a role in neuronal differentiation, migration, and dendritic outgrowth by regulating calcium oscillations.[36,37] Calcium waves have also been shown to regulate growth cone function.[38] Of particular interest was the finding by Estrada et al that low concentrations of testosterone induced calcium oscillations, but high concentrations produced sustained dose/dependent elevations in intraneuronal calcium levels, something that would be expected to produce abnormal neuronal migration and neurotoxicity.[27] In their study, they indeed found that higher doses of testosterone triggered apoptosis human neuroblastoma cells. The effect was dose-dependent, with 1 µmol measured in inducing significant cell death and 10 µmol being significantly more lethal. It is also of note that the recent finding of region specific 5α-reductase, which converts testosterone to the more potent dihydrotestosterone, can result in specific regions of the CNS having testosterone levels higher than plasma levels.[39]

Others have noticed that there is a sex difference in terms of the outcome of neurological injury, with females making better neurological recoveries than males.[40,41] Experimentally, Hawk et al found that chronic testosterone replacement increased stroke damage, and 17ß-estradiol treatment decreased damage

in castrated male rats.[42] This is in keeping with the demonstrated protective effects of estrogens on brain, at least when in physiological ranges.

While androgen receptors have been demonstrated in the hypothalamus, hippocampus, preoptic area, amygdala, and medial hypothalamus, they have also been demonstrated throughout frontal lobe areas as well and influence frontal lobe $GABA_A$ receptor regulation.[43-48] This finding demonstrates a more expanded behavioral effect of androgens than merely reproductive behavioral effects.

In another study, Yang et al using both a murine hippocampal culture and an in vivo study using Sprague-Dawley rats found that 10 µmol of testosterone in vitro significantly increased glutamate toxicity.[49] Likewise, 10 µmol of estradiol significantly ameliorated glutamate toxicity. In the in vivo study, they used an implanted testosterone pellet for slow release of the hormone to minimize the stress of repeated injections. Using a middle cerebral artery stroke model, they found that the testosterone-implanted animals had a significantly larger volume of stroke damage than did controls.

Androgens, like excitotoxins, have been shown to enhance the inflammatory mediator NF-kB and thereby increase COX-2 and iNOS activation, leading to free radical generation, lipid peroxidation, and increased secretion of glutamate from microglia.[50,51] Using both an excitotoxic and stab wound injury to hippocampus, García-Ovejero et al demonstrated that both lesions could induce androgen and estrogen receptors on glia.[51] Estrogen receptor alpha (ERalpha) was expressed on astrocytes, and androgen receptors (AR) were expressed on microglial membranes.

Both receptors were observed to appear 3 days after the injury, with the maximum of ERalpha and AR immunoreacting glia appearing at day 7 and returning to baseline at 28 days. Taken together, these studies indicate that chronic elevation of testosterone activates microglia, triggering the release of a number of neurotoxic elements including the excitotoxins glutamate and quinolinic acid. Indeed, DonCarlos et al have shown that of the glial cells only activated microglia express androgen receptors, whereas activated astrocytes express estrogen receptors.[52] They also found that AR immunostaining was heavier in frontal cortex than the hypothalamic-limbic structures. In addition, the demonstration that microglia direct neuronal precursor cell migration and differentiation and that activated microglia can increase neuronal numbers significantly may explain the hypercellularity seen in certain areas of the autistic brain, particularly the amygdala.[53]

When androgen levels are chronically elevated, microglial activation would not only be enhanced, but toxicity of secreted glutamate and inflammatory cytokines would be exaggerated. Unlike the adult brain, this combination of inflammatory cytokines, androgens, and excitotory neurotransmitters would not only precipitate chronic neurodegeneration but also alter progenitor cell differentiation and maturation, dendrite outgrowth and arborization, synaptic development and stabilization, and neuronal migration.

HOMEOCYSTEINE, EXCITOTOXICITY, AND THE DEVELOPING CENTRAL NERVOUS SYSTEM

Homocysteine, which is elevated in many autistic children, is involved in various transsulfuration reactions, such as cysteine synthesis, remethylation for methionine synthesis and transmethylation of DNA, proteins, and lipids, and the biosynthesis of neurotransmitters and some hormones. While cysteine itself is known to be a powerful excitotoxin,[54] especially in an alkaline environment, in the autistic low cysteine levels are seen.[55]

Elevated homocysteine, even to moderate levels, is associated with Alzheimer's disease,[56] age-related memory loss,[57] schizophrenia,[58] neural tube defects,[59] seizures,[60] and neurobehavioral toxicity of chemotherapeutic agents.[61] Homocysteine oxidizes to a number of L-glutamate analogues (L-homocysteine sulfinic acid [L-HCSA] and L-homocysteic acid [L-HCA]) and L-aspartate analogues (L-cysteine sulfinic acid [L-CSA] and L-cysteic acid [L-CA]) with significantly greater excitotoxic effects than homocysteine itself.[62]

Recent studies have shown that oxidized homocysteine metabolites activate NMDA receptors as well as metabotropic receptors and that in cerebellar granule cells, neurotoxicity involves a co-stimulation of NMDA receptors and Group I metabotropic receptors.[63] Others have confirmed potent stimulation of excitatory metabotropic glutamate receptors by homocysteine metabolites.[64,65]

Lockhart et al found that hippocampal neurons were especially sensitive to excitotoxicity induced by the homocysteine oxidative product, L-homocysteic acid.[66] There is growing evidence that L-homocysteic acid may be a glial transmitter, acting through astrocytic NMDA receptors.[67] One sees a powerful amplification of the excitotoxic cascade with the metabotropic receptors of group I, as well as NMDA receptors, being activated by homocysteic acid and homocysteine sulfinic acid, especially when in combination with high levels of extraneuronal glutamate.

There is also evidence that Purkinje cells have unique receptor properties in that they have few NMDA receptors and greater expression of non-NMDA receptors.[68] Homocysteic acid has been shown, as an excitotoxin, to act through NMDA receptors in hippocampal neurons and via non-NMDA receptors in Purkinje cells. With proinflammatory cytokines, ROS/RNS, lipid peroxidation products, and mitochondrial depression-caused amplification of excitotoxicity, one can better understand the widespread loss of Purkinje cells seen in the cerebella of autistic cases. In essence, this is less of a direct autoimmune injury and more characteristic of bystander injury described by McGeer and McGeer as autotoxicity.[69]

Because both inotropic and metabotropic glutamate receptors, as well as androgens, act through excess intracellular calcium accumulation, one can readily understand the critical role played by each in the process, as explained in the next section. Homocysteine oxidation products, such as homocysteic acid, homocysteine sulfinic acid, and cysteic acid, along with glutamate, inflammatory cytokines, chemokines, and inflammatory prostaglandins, trigger the autotoxic injury to a widespread area surrounding the immune reaction, thus explaining the autopsy picture seen in the autistic brain.

THE ROLE OF MERCURY IN AUTISM

Both mercury and aluminum are considered neurotoxic metals, with mercury being significantly more toxic. Autistic children are exposed to a number of sources of mercury and aluminum. Mercury exposure can be from atmospheric sources, dental amalgam, fish consumption, certain pesticides and herbicides, and vaccines. In most cases, children are exposed a number of such sources. Of particular concern to the child's developing brain is *in utero* exposure to mercury from the mother's dental amalgam, seafood consumption, or vaccinations during pregnancy or immediately before conception. Because of the human brain's extensive postnatal development, mercury exposure after birth is also of major concern. Mercury has been shown to pass through the placental barriers rather easily, thus entering the fetus's circulatory system, and hence, brain.[70,71] The leading sources of aluminum are food and vaccines.

A number of studies have shown architectonic abnormalities in the fetus following maternal exposure to mercury.[72-75] This can result in abnormalities in neuronal and glial proliferation, neuronal migration, and the final cytoarchitecture of the brain, especially the cerebellum.

There is also evidence that ionic mercury is the most toxic form of mercury within the CNS and that organic mercury is slowly demethylated in the brain to form ionic mercury, which can then be redistributed over time. Vahter et al, for example, studied demethylation of methylmercury in *Macaca fascicularis* monkeys after oral dosing with 50µg/kg of methylmercury for 6, 12, or 18 months and found that the concentration of inorganic mercury slowly increased in all brain sites but especially in the thalamus and pituitary.[76]

Recent studies have shown that there are toxicological and pharmacokinetic differences between methylmercury from seafood and ethylmercury from the vaccine preservative thimerosal. For example, Burbacher et al, using monkeys exposed either to methylmercury (MeHg) or vaccines with thimerosal at birth and then at 1, 2, and 3 weeks of age, found a significant difference in the blood half-life, with thimerosal's initial and terminal half-life being 2.1 and 8.6 days respectively and MeHg being 21.5 days.[77] They also found that ethylmercury's brain concentration was 3-fold lower than MeHg. Yet, of significant importance was the finding that 34% of ethylmercury was converted to ionic mercury in the monkeys' brains vs 7% for MeHg. Ionic mercury, besides being more toxic, is much more difficult to remove from the CNS, even with chelation.

Two studies measured the mercury burden of children receiving the recommended childhood vaccines. Redwood et al found that at birth an infant received 12.5 µg of mercury, 62.5 µg at 2 months, 50 µg at 4 months, 62.5 µg at 6 months, and 50 µg at 18 months, for a total mercury burden of 237.5 µg of ethylmercury during the first 18 months of life, which exceed the environmental protection agency safety guidelines for an adult.[78] In the second study, similar infant mercury exposures were seen.[79]

Effect of Mercury on Neurons, Microglia, and Astrocytes

One of the most obvious toxic effects of mercury is the generation of abundant free radicals and lipid peroxidation products, with antioxidants providing considerable protection against mercury-induced neurotoxicity.[80] Yet a more complicated process appears to be involved in the generation of these free radicals since blocking the NMDA glutamate receptor also significantly attenuates MeHg toxicity and reduces ROS generation as well.[81,82] It has also been shown that free radicals dramatically increase the toxic sensitivity of immature neurons to MeHg, so that previously nontoxic concentrations of MeHg became fully toxic,[83] just as in the case of excitotoxins.[84]

One of the most involved free radicals in both mercury neurotoxicity and excitotoxicity is peroxynitrite, formed by an interaction between nitric oxide (NO) and superoxide.[83-85] Peroxynitrite is known to especially target the mitochondria, which reduces energy production and enhances ROS formation.[86] In addition, peroxynitrite, as a reactive nitrogen species, reacts with cellular proteins, particularly L-tyrosine residues, producing nitrotyrosine accumulation.

New evidence points to a strong connection between inflammation in the brain, mitochondrial failure, and excitotoxicity through calcium-activated inducible nitric oxide synthetase (iNOS) and the formation of peroxynitrite.[87] Activated microglia are known to upregulate iNOS and generate large amounts of peroxynitrite, which in turn not only triggers excitotoxicity but reduces cellular energy levels.[88,89] Reduction in cellular energy enhances excitotoxicity to the degree that even physiological concentrations of extracellular glutamate can be excitotoxic.[90] Recent studies have shown that mitochondrial dysfunction is commonly found in neurodegenerative diseases.[91,92] Also of note, studies have shown the mitochondria to have the highest intracellular levels of mercury on exposure to ionic mercury.[93]

One of the major functions of mitochondria, besides energy production, is calcium buffering. During excitotoxicity, much of the cytosolic calcium is removed by either the smooth endoplasmic reticulum (SER) or mitochondria, and dysfunction of either can result in exacerbation of intracellular signaling, with resulting free radical generation, lipid peroxidation, and activation of cellular death signals. Mercury, by disrupting cellular calcium channels and activating SER calcium signaling, further exacerbates the problem, leading to abnormal neurogenesis and neurodegeneration as well as microglial activation as described previously.[94]

Systemic stimulation of immunity utilizing LPS increases brain oxidative stress, thus increasing sensitivity to excitotoxins and mercury.[95] In addition, as we have seen, systemic inoculation with LPS also increases brain microglial activation, inflammatory cytokine activation, and enhancement of excitotoxicity. Likewise, these events are characterized by disruptions of calcium homeostasis, mitochondrial dysfunction, and cellular energy loss—again, all events that have been shown to disrupt neurogenesis and induce neurodegeneration. The effect of overstimulation of glutamate receptors, particularly NMDA and AMPA receptors, is further enhanced by ROS, lipid peroxidation products, and inflammatory cytokines, especially TNF-α.[96,97] Aluminum, like mercury, is a powerful inducer of brain ROS and LPO production.[98,99] Measures of oxidative stress and lipid peroxidation have

shown significant elevations in children with autism.[100,101]

It should also be noted that high levels of DHEA interfere with mitochondrial energy production, and as we have seen, DHEA levels are increased as much as 2-fold in some studies of children with autism spectrum disorders.[102] In this study, it was found that high levels of DHEA suppressed complex I (NADH quinone oxidoreductase) in primary cultures of cerebellar granule cells without affecting other mitochondrial electron transport enzymes. In the in vivo part of the study, adult male mice were fed a diet containing 0.6% DHEA for 10 weeks followed by a normal diet to exclude acute effects of DHEA. They found that the neuron density was significantly lower in the primary motor cortex and hippocampus. They also noted that under hypoglycemic conditions, the toxic effect of DHEA was significantly more pronounced. Because of the effects of complex I inhibition on neurogenesis, one would expect a different histological picture in immature or fetal mice. With DHEA levels being significantly elevated in autism spectrum disorders, it is reasonable to assume depression of mitochondrial function would occur, especially in the presence of other mitochondrial depressing factors such as elevated levels of peroxynitrite and mercury toxicity.[8]

Charleston et al[103] in their study of long-term exposure of monkeys to methylmercury described extensive microglial, as well as astrocytic activation throughout the brain as described in the brains of autistics by Vargas et al.[104] Of special importance, they found continued microglial activation in the group of monkeys in which MeHg exposure was stopped for 6 months, demonstrating that microglial activation persists long after exposure. It should also be noted that with priming by mercury-induced activation of microglia, further immune activation from any cause, vaccinations, systemic infections, food allergies, etc, would be expected to exaggerate brain excitotoxicity and inflammation.

While astrocytes are the major source of glutamate as well as critical inflammatory cytokines, microglia act as the primary mechanism of astrocyte activation, and they can also secrete excitotoxic levels of glutamate upon stimulation.[105,106] This is especially so under conditions of mitochondrial dysfunction, magnesium deficiency, and hypoxia/ischemia.

With astrocytes acting as the sink for mercury, concentrations reach significantly higher, neurotoxic levels in this cell type. Astrocytes also act as the primary site for glutamate uptake. A large number of studies have shown that glutamate uptake can be significantly altered by extracellular toxins, including TNF-α, ROS, RNS, and lipid peroxidation products and that uptake is sensitive to even small concentrations of mercury.[107-111] In fact, Brookes demonstrated that concentrations of mercuric chloride as low as 0.5 µg inhibited glutamate transport into astrocytes by 50% and that no other metal tested—Al^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Sr^{2+}, Cd^{2+}, or Zn^{2+}—inhibited glutamate transport.[112] At this concentration, mercury is considered not to be directly cytotoxic.

Glutamate uptake is not the only neurotransmitter affected. Dave et al found that methylmercury not only inhibited glutamate uptake in primary astrocyte cultures but that it also inhibited Na+-dependent and fluoxetine-sensitive [^{3}H] 5-HT uptake as well.[113] This could in part explain the elevated serotonin levels seen in autism.[114] Of concern with chronically elevated levels of serotonin is the fact that one of its metabolic products, quinolinic acid, is also an excitotoxin secreted from activated microglia.[115]

Effect of Mercury on Glutamate Transporters

Glutamate regulation occurs through 4 primary mechanisms: the X_{AC}^- transporters (excitatory amino acid transporters—EAAT1-5), the X_c^- cystine/glutamate antiporter, conversion of glutamate into glutamine by glutamine synthetase, and metabolic diversion into Kreb's cycle. Inhibition of the EAAT glutamate transporters may be primarily through oxidation, since antioxidants can reverse the inhibition.[116,117] The transporters contain sulphydryl groups, which would make them vulnerable to mercury as well as oxidation.[118] It is also known that the transporters are dependent on protein kinase C and that mercury inhibits its function.[119,120] One of the mechanisms for estrogen protection against excitotoxicity is its ability to enhance glutamate transport into the astrocyte.[121]

Not only do the glutamate transporters play a vital role in preventing excitotoxicity, they also play a major role in brain development, as there is a programmed rise and fall in the different transporters during brain development.[122] In one study, Kugler and Schleyer found that the glutamate transporter GLAST (EAAT1) was expressed in higher levels earlier in development than GLT-1 (EAAT2) in the rat hippocampus and that both the glutamate transporters and glutamate dehydrogenase were increased at birth and rose to adult levels between P20 and P30, indicating an important control system over glutamate levels during postnatal development.[123] Mercury has also been shown to suppress glutamate dehydrogenase activity as well.[124]

It has also been shown that Purkinje cells are very dependent on GLAST and EAAT4 for resistance against excitotoxicity induced by hypoxia/ischemia.[125] GLAST is expressed in Bergmann glia and EAAT4 in the perisynaptic region of Purkinje cell spines.[126] This could also explain the dramatic loss of Purkinje cells in autism, since mercury toxicity alone usually spares the Purkinje cells and targets cerebellar granule cells.[127] A combination of inflammatory bystander injury, ROS-RNS/LPO accumulation, androgen excess, and excitotoxicity dramatically increase the damage, mainly because of hyperexcitability of NMDA and AMPA receptors and chronic microglial activation with release of neurotoxic elements.

Juárez et al demonstrated a dramatic increase in extracellular glutamate following methylmercury instillation in the frontal cortex of 15 freely moving awake rats using a microdialysis probe.[128] They found a 9.8-fold rise in extracellular glutamate following a MeHg dose of 10 µmol and 2.4-fold rise using a 100 µmol dose. It is known that a dose of 10 µmol of MeHg produces a 50% inhibition of glutamate uptake into astrocytes.[129] Brain trauma in rats has been shown to produce a 2.8-fold rise in extracellular glutamate.[130]

Mercury is also known to be a potent inhibitor of glutamine synthetase activity, which when inhibited, causes a buildup of

extracellular glutamate.[131] This can lead to excitotoxicity and an alteration in neuronal migration and progenitor cell differentiation.

Mercury's Effect on Glutathione, Metallothionein, Excitotoxicity, and Autism

Another frequent finding in autism is lower glutathione levels, which is also common with mercury toxicity and excitotoxicity.[132-134] As one of the principal intracellular antioxidants, glutathione scavenges a number of reactive oxygen and nitrogen species, including peroxynitrite. It has also been shown to have a neurotransmitter function, binding to its own synaptic receptors, and in addition has been shown to modulate glutamatergic excitatory neurotransmission by displacing glutamate from ionic receptors.[135,136] At high extracellular concentrations glutathione enhances NMDA receptor activity, increasing the risk of excitotoxicity.[135]

Astrocytes are the sole source of glutathione for neurons, making glutathione particularly susceptible to mercury inactivation, since astrocytes are also the principle site of mercury accumulation in the CNS.[137] Mercury has been shown to lower glutathione levels in embryonic neuronal cells as well as adult neurons.[138,139] Low glutathione levels have been associated with a number of neurodegenerative conditions, especially Parkinson's disease, as an early event.[140-142]

Glutathione production by astrocytes is dependent on the sodium-independent X_c^- cystine/glutamate antiporter, which exchanges intracellular glutamate for extracellular cystine utilized by the astrocyte to produce glutathione.[143] High levels of glutamate inhibit cystine entry into astrocytes, resulting in low glutathione levels, as we would expect with the elevated glutamate levels seen in autistics and those exposed to mercury.[144]

Another protective system impacted by mercury is metallothionein. Rising et al have shown that exposure of rat neonatal primary astrocytes to methylmercury constitutively increase the production of metallothionein-1 (MT-1) and MT-2.[145] Aschner et al demonstrated a 14-fold increase in MT-1 mRNA upregulation in full-term fetal rats exposed *in utero* to elemental mercury vapor.[146]

Beside their role in heavy metal detoxification, metallothioneins function to control inflammation and oxidative stress and to promote brain repair.[147] They have also been found to play a significant role in protection against excitotoxicity.[148] MT-1 and MT-2 play the most significant role in protection against neuroinflammation and have been shown to reduce the number of activated microglia during injury.[149] With a significant number of metallothionein molecules bound with mercury, they would be less able to carry out their antiinflammatory and antioxidant functions.

There is abundant evidence that mercury, particularly in its ionic form, is toxic to neurons and less so glial cells and that organic forms of mercury are demethylated slowly to form ionic mercury, with accompanying redistribution in the CNS. Because of mercury's effects on a number of enzymes, mitochondrial function, gene function, microglial activation, inflammatory cytokine release, antioxidant systems, and glutamate metabolism, it becomes a major player in abnormal brain development as well as neurodegenerative-associated excitotoxicity. Most of these effects occur at very low micromolar or submicromolar concentrations.

Because few studies have looked at total accumulated concentrations from multiple sources, such as atmospheric mercury, seafood sources, thimerosal-containing vaccines, and dental amalgam, the impact of mercury has been grossly underestimated by many experts in autism spectrum disorders.

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