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Review

Developmental Neuroscience and Developmental Disorders

Hormesis, Cellular Stress Response, and Redox Homeostasis in Autism Spectrum Disorders

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In the United States, 1.1–1.5% of children have been diagnosed with autism spectrum disorders (ASD), corresponding to a 30% increase in incidence and prevalence. Social and communication impairments are the main signs and symptoms of ASD, and currently available medications have been ineffective in reducing these core deficits. Observational studies have indicated that children with ASD tend to show improved cognition and behavior after febrile illness, which is associated with alteration of metabolic pathways, leading to cellular stress responses and increased expression of heat shock proteins (Hsps). Sulforaphane and hydroxytyrosol, phytochemicals derived from cruciferous vegetables and extra virgin olive oil, respectively, can induce metabolic effects in cellular stress responses that are similar to those produced by fever. Thus, modulation of endogenous cellular defense mechanisms may be an innovative approach for therapeutic intervention in ASD and other disorders associated with neuroinflammation and neurodegeneration. This Review introduces the hormetic dose–response concept and presents possible mechanisms and applications for neuroprotection. We address the emerging role of Hsps in the neuroprotective network of redox stress-responsive mechanisms and propose the potential therapeutic utility of the nutritional antioxidants sulforaphane and hydroxytyrosol against particular signs and symptoms of ASD. We argue that such research findings must be approached with pragmatism and prudence. It is vital to capitalize on recent and ongoing investments in brain science research and to refine neuroscientific knowledge and capability for more accurate diagnosis and safe, effective, and ethically sound treatment of ASD and other neuropsychiatric spectrum disorders.

Key words: heat shock proteins; heme oxygenase; hormesis; vitagenes; autism spectrum disorder

Autism is a developmental spectrum disorder characterized by repetitive and stereotypic patterns of behavior characteristically occurring by 3 years of age that are

SIGNIFICANCE

Autism spectrum disorder (ASD) presents as several neurodevelopmental abnormalities, including impairment in the ability to communicate and interact socially, with restricted and repetitive stereotypies in behavior, activities and interests. This Review introduces the hormetic dose–response concept and putative mechanisms by which hormetic dose responses may be applicable to the induction of endogenous neuroprotective processes that may be of value in the treatment of ASD. The central neuroprotective role of heat shock protein in redox stress-responsive mechanisms and the therapeutic potential of the nutritional antioxidants sulforaphane and hydroxytyrosol are highlighted. Possible biomarkers of stress-response pathways are addressed, and novel redox active therapeutic strategies relevant to ASD are proposed. Key considerations for current and future research are also discussed.

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associated with impaired language, communication, and social skills. As a neurodevelopmental spectrum disorder (i.e., autism spectrum disorder; ASD), it includes several conditions (e.g., Asperger's syndrome, pervasive autistic disorder, not otherwise specified; Sierro et al., 2016). Autism is more prevalent in males (male:female ratio of 4.5:1), presently affects one of 68 children in the United States, and has complex genetic and epigenetic features, but it lacks a single genetic marker that is of value for diagnosis or laboratory testing (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2014 Principle Investigators and Centers for Disease Control and Prevention [CDC], 2014). ASD diagnosis is mainly phenotypically based, and therapeutic approaches are limited. Currently, a U.S. Food and Drug Administration-approved medical therapy directed to the ASD symptom core or pathophysiological processes associated with ASD is lacking (American Psychiatric Association, 2013).

PUTATIVE PATHOPHYSIOLOGIC MECHANISMS AND SUBSTRATES

Although the exact causes of ASD have yet to be elucidated, several contributory factors have been postulated and/or identified. These include dysfunctional redox regulation and oxidative stress effects in the prenatal and/or developing brain, mitochondrial dysfunction, immune dysregulation, neural (and/or peripheral) inflammation, microbiome alterations, and a host of environmental variables that may interact with and/or engage the aforementioned pathophysiological processes (Liu et al., 2016).

In particular, glutamatergic and dopaminergic neurotransmission and metabolic pathways associated with oxidative stress are increasingly recognized as potential pathologic mechanisms and are also seen as viable targets for drug development (Lim et al., 2016). Serotonin (5-HT) is a modulatory neurotransmitter, and 5-HT dysfunction has been implicated in neuropsychiatric disorders, including ASD (Gangi et al., 2016). Specifically, the kynurenine pathway of tryptophan metabolism is emerging as directly associated with serotonergically mediated neuroinflammation, subserved in part by indoleamine 2,3-dioxygenase, an enzyme induced by various proinflammatory cytokines (Lim et al., 2016). In view of the evidence that interleukin (IL)-21 and IL21 receptor antagonist are elevated in ASD, a putative link between inflammation and kynurenine pathway-induced depletion of serotonin is both justifiable and useful in explaining the disrupted affective control observed in ASD (Lim et al., 2016). Additionally, there is evidence suggestinng involvement of the dopamine (DA) system in ASD (Lim et al., 2016), although it is not yet understood whether dysfunctional 5-HT and DA metabolism are related, reciprocal, coincident, or distinct in contributing to the pathophysiology of ASD (vide infra).

In addition, in vitro studies of neural cultures exposed to various environmental or food chemicals have shown that rotenone, a pesticide associated with the etiology of Parkinson's disease, produces transcriptional changes similar to those found in brains of children with ASD (Pearson, 2016). Similar types of shared transcriptomic signatures have also been found after exposing neural cell cultures to fungicides such as famoxadone, fenamidone, pyraclostrobin, and trifloxystrobin (Pearson et al., 2016). In these experiments, oxidative stress-induced disruption of microtubules was reduced by sulforaphane; this supports a central role of redox mechanisms and oxidative stress in neural pathophysiology that may be contributory, at least in part, to ASD (Pearson et al., 2016).

Mitochondrial dysfunction and impaired brain energy metabolism have also been demonstrated in the brains of both animal models of ASD and children presenting with these disorders (Kaur et al., 2014). Kaur and colleagues (2014) and Chauhan et al. (2011) have posited that mitochondrial dysfunction in ASD may be acquired rather than inherited. Given that mitochondrial function is essential to neural bioenergetics, anecdotal reports that ASD core signs and symptoms are often ameliorated during febrile episodes (Singh et al., 2014) are of interest. It may be that fever-induced functional improvement in ASD involves cellular stress responses leading to upregulation of heat shock proteins (Hsps), which may mediate positive changes in synaptic function and long-range connectivity. Cellular stress proteome involves interaction of protein networks operating in response to stress conditions, such as fever, radiation, or hypoxia, to restore homeostasis.

In this light, pharmacological modulation of cellular stress pathways may be a viable approach for the treatment of neuropsychiatric disorders, including ASD (Singh and Zimmerman, 2016). The potential of small redox-active molecules, such as the phytochemicals sulforaphane and hydroxytyrosol, to be used as clinical tools to affect basic physiological pathways associated with pathogenesis of ASD is of interest. In this regard, molecules that function in 1) cellular stress response and vitagene network modulation, 2) restoration of redox imbalance/oxidative stress, 3) improvement in mitochondrial function, 4) immune response and modulation of neuroinflammation, 5) fever and heat shock response control, and 6) amelioration of synaptic dysfunction (Talalay and Zimmerman, 2015) are noteworthy. Identification of biomarkers to assess such functions could be useful to direct novel therapeutic strategies to mitigate these pathophysiologic processes and thus improve signs and symptoms of ASD.

Abbreviations

ARE	antioxidant-responsive element
HO-1	heme oxygenase-1
HSE	cis-acting heat shock elements
HSF1	heat shock transcription factor 1
Keap1	Kelch-like ECH-associated protein 1
Nrf2	nuclear factor erythroid 2-related factor 2

HORMESIS AND AUTISM

Hormesis is defined as a dose/concentration–response relationship characterized by stimulation effects elicited at

low doses/concentrations and inhibition produced at high doses/concentrations of ligand (Calabrese and Baldwin, 2002; see Fig. 1). However, the hormetic dose–response concept also represents an adaptive process in which a low dose/concentration of a physical or chemical agent/stressor that is usually toxic/harmful/inhibitory at higher doses/concentrations can induce responses that frequently appear to exert beneficial effects within the biological system and/or organism. In this way, hormetic processes may be considered as fundamentally adaptive responses (Mattson, 2008). Hormetic responses have specific quantitative features that characterize the magnitude/amplitude of the low-dose stimulatory response (Calabrese and Blain 2005, 2011). These responses occur in all types of biological systems (e.g., plants, microorganisms, animals) as well as across multiple levels of biological organization from cells (and in most cell types) to organs to the organism (Calabrese, 2008a). Hormetic response is also important for neurobiologic function because "the adaptive process by which neurons (and thereby nervous systems and organisms) respond to moderate levels of stress by enhancing their ability to resist a more severe stress that might otherwise be lethal or cause dysfunction or disease" (Mattson and Cheng, 2006). The roles of hormesis in neural systems and in neuropsychiatric research and its translation have been strengthened to a considerable extent by studies on neuronal and astrocytic chemoprotection (Calabrese, 2008b–e), neurite outgrowth (Calabrese, 2008f), p-glycoprotein effector induction (Calabrese, 2008g), astrocyte survival (Calabrese, 2008e), anxiolytic and antiepileptic drugs (Calabrese, 2008h,i), stroke/traumatic brain injury (Calabrese, 2008j), and pain (Calabrese, 2008k) and behavioral pharmacological applications, including treatment of addictive disorders (Calabrese, 2008l), learning disorders (Calabrese, 2008m), and neurodegenerative diseases such as Alzheimer's disease (Calabrese, 2008m), Parkinson's disease, and cerebral degeneration and cognitive decline consequential to aging (Calabrese, 2008d).

The hormetic dose response has also been studied in preconditioning experimental settings, with application to a number of biological systems and clinical conditions (E.J. Calabrese 2013a; E.J. Calabrese 2016a,b,c). Detailed assessments of preconditioning have revealed that, when such experiments involve adequate numbers and an appropriate range of doses, the preconditioning response typically conforms to quantitative features of hormesis. Additional assessment of the preconditioning phenomenon has indicated that the protective response after the challenge dose is directly related to the induction of adaptive mechanisms during the conditioning period. Specifically, optimal induction of adaptive mechanisms corresponds to the optimal protection concentration. Blocking the conditioning dose-induced adaptive pathways abolishes protection from subsequent damaging responses to the challenging dose. Thus, the preconditioning phenomenon may be seen as a specialized form of the broader hormetic dose response.

These findings may have important implications for a host of applications designed to employ mechanical, exercise, dietary, and pharmacological means to upregulate

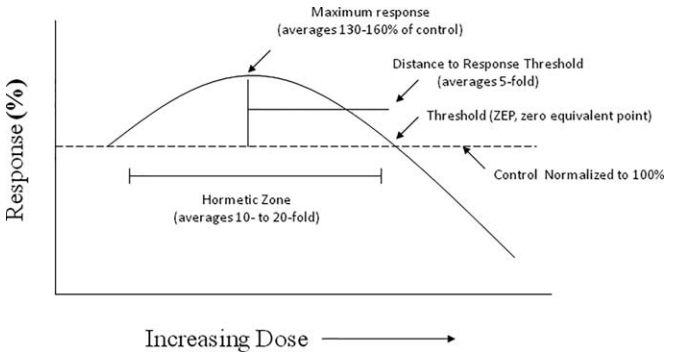

Fig. 1. Dose–response curve showing the quantitative features of hormesis.

adaptive mechanisms to protect biological systems (on a variety of scales from the cellular to the organismal) from damage from challenging or harmful events or exposures. Recently, E.J. Calabrese (2013b) documented specific receptors and cell signaling pathways for 400 different hormetic dose responses, many involving the central and peripheral nervous systems. Examination of hormetic mechanisms, especially those reported within a preconditioning framework, indicates that they appear to be a manifestation of the highly conserved cell danger response (CDR) that protects cells from endogenous and exogenous insult.

As noted by Naviaux (2014), the hormetic response is induced when physical, chemical, or biological threats exceed the cellular capacity for homeostasis. This resulting disruption in homeostasis incurs a cascade of molecular changes in cellular electron flow, oxygen consumption, and redox potential to produce alterations of cellular structures and processes, including membrane fluidity, bioenergetics, and protein folding and aggregation. The initial components of this cascade evoke the release of ATP, ADP, Krebs cycle intermediates, oxygen, and reactive oxygen species, and this is sustained by purinergic signaling (Naviaux, 2014). These initial adaptive processes eventually establish an anti-inflammatory and resilient phenotype that mediates protection from a range of potential insults (for a specific duration of time). This response, which appears to be species, gender, and organ specific, may be modulated by multiple factors, including the timing, sequence, and magnitude of applied stresses during the conditioning period. The anti-inflammatory phenotype is the result of a redox regulatory signaling pathway that may be blocked/abolished by the application of powerful antioxidants, thereby eliminating the adaptive response (E.J. Calabrese and V. Calabrese, 2013; Frey et al., 2015; Large et al., 2015; Wunderlich et al., 2015).

Naviaux (2014) has posited that understanding the CDR may be important to "reframe old concepts of pathogenesis for a broad array of chronic, developmental, autoimmune, and degenerative disorders such as autism spectrum disorders, attention deficit hyperactivity." We speculate that a better understanding of

hormetic mechanisms may provide insights for developing and evaluating extant and novel approaches in both research and clinical applications of prevention and treatment of ASD and other neuropsychiatric spectrum disorders.

CELLULAR STRESS RESPONSE AND THE VITAGENE SYSTEM: A ROLE IN ASD?

Accumulation of misfolded proteins within the endoplasmic reticulum (ER) appears to be a representative if not a causative feature of a number of neurological diseases

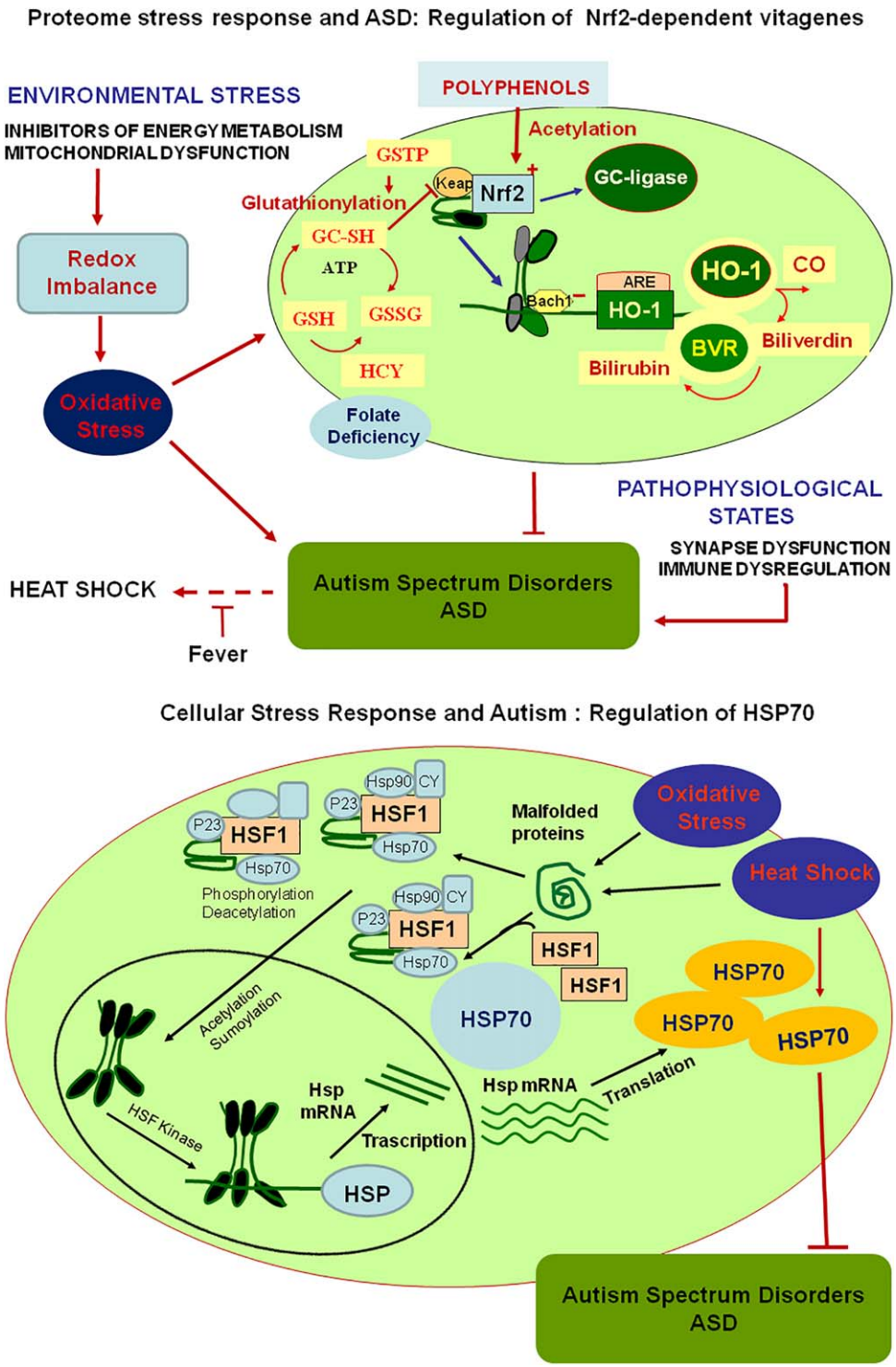

Fig. 2.

(Hetz et al., 2015). Under such conditions, perturbation of ER homoeostasis triggers an unfolded protein response that leads to adaptive cellular signaling pathways that function to restore proper functioning of ER through modulation of gene expression (Calabrese et al., 2010a; V. Calabrese et al., 2012). Children with ASD show mutations in genes coding for synaptic proteins, which suggests that abnormal synaptic function may be a risk factor for the neurodevelopmental changes that are characteristic of or contributory to ASD (Ulbrich et al., 2016). Current research is focused on ASD-associated mutations in CNTNAP2 and synaptic adhesion protein CADM1 (Fujita et al., 2010), and evidence suggests that synaptic function is one of the molecular pathways underlying the pathogenesis of ASD (see Fig. 2A).

Cellular stress response or heat shock response, represents an ancient and highly conserved cytoprotective mechanism related to the ability of a cell to counteract stressful conditions (Calabrese et al., 2010b). Production of Hsps, including protein chaperones, is essential for folding and repair of damaged proteins and serves to promote cell survival conditions that would otherwise result in apoptosis (Calabrese et al., 2007b; Morimoto, 2011). When conformationally challenged proteins are expressed the resulting aggregation-prone unfolded or misfolded proteins are rapidly degraded via the ubiquitin–proteasome pathway. Under physiological conditions, long-term cellular health is maintained by proteome homeostasis, regulated through a complex protein network modulating the equilibrium among protein biosynthesis, folding, translocation, assembly/disassembly, and clearance (Morimoto, 2011; Giffard et al., 2013). Due to centrality of proteome homeostasis for survival, emerging interest now focuses on the discovery and development of small molecules capable of modulating heat shock responses and related stress response pathways for therapeutic purposes (Calabrese et al., 2007a,b; Calamini et al., 2011; Kansanen et al., 2011).

At transcriptional, translational, and posttranslational levels, cellular stress response is regulated through shock transcription factor 1 (HSF1), which is suppressed under normal conditions by an inhibitory complex of stress proteins containing Hsp90 and other proteins under a monomeric inactive state, without the ability to bind cis-acting heat shock elements in the promoters of Hsp genes. HSF1 can be activated in response to environmental stress and several pathophysiological conditions, including cancer, ischemia–reperfusion injury, diabetes, and aging (Morimoto, 2014; see Fig. 2A). Mammalian cells possess at least three HSF family members, HSF1, HSF2, and HSF4 (Westerheide et al., 2012; Leak, 2014), and, although neurons have the ability to express HSF proteins, they appear not to exhibit the heat shock response (Van Oosten-Hawle and Morimoto, 2014). The major activator of HSF1 is proteotoxic insult, such as in the case of a prototypic stimulus, heat shock. Under these conditions, misfolded protein displaces HSF1 from the inhibitory chaperone complex, allowing HSF1 to trimerize, become phosphorylated, and translocate to the nucleus, where it binds to the heat shock element of Hsp genes (Calderwood, 2012). In the activation of HSF1, multistep processes involving multiple inducible phosphorylation, dephosphorylation, acetylation and deacetylation steps, and sumoylation operate ultimately to activate *hsp* gene transcription (Calderwood, 2012; Westerheide et al., 2012). As an extracellular signal component, the heat shock response involves tyrosine phosphorylation upstream of HSF1, the receptor tyrosine kinase HER2, and signaling cascades acting downstream via intracellular kinase Akt (Calderwood et al., 2009).

There is now strong interest in discovering and developing pharmacological agents capable of inducing the heat shock response (Calabrese et al., 2007a). Among the cellular pathways involved in cell survival and conferring protection against oxidative stress, vitagenes appear to play a central role (V. Calabrese et al., 2008a,b,c; Calabrese et al., 2009a,b,c). Vitagene products include members of the Hsp family such as heme oxygenase-1 (HO-1), Hsp72, sirtuins, and thioredoxin/thioredoxin reductase (V. Calabrese et al., 2009a,b; V. Calabrese et al.,

Fig. 2. **A**: Proteome stress responses. Expression of heat shock genes, including chaperones and components of the clearance machinery, is induced in response to physiological and environmental stress conditions, including longevity stimuli such as fasting, caloric restriction, or polyphenol antioxidants and protein conformational diseases. HSF1 can also be directly stimulated by longevity stimuli such as the histone deacetylase silent information regulator two homolog 1 that directly activates HSF1 by deacetylation, thus fostering longevity. The increased flux of damaged or misfolded proteins in response to proteotoxic environmental conditions (stress) is the trigger for induction of the cellular stress response. Damaged or misfolded proteins titrate away Hsps that are bound to HSF1 and maintain them in a repressed state before stress, resulting in their activation. Multistep activation of HSF1 involves posttranslational modifications such as hyperphosphorylation and deacetylation that allow HSF1 to trimerize and translocate into the nucleus, where inducible acetylation, phosphorylation, and sumoylation occur before binding of nuclear-localized trimers to DNA, and heat shock genes are transcribed. In particular, after it is activated, HSF1 is transiently sumoylated on lysine 298, which requires the phosphorylation of serine 303 adjacent to the consensus site. Thus, small ubiquitin-related modifier (SUMO) modification is elaborately regulated, and the SUMO substrate specificity can be determined by regulatory elements outside the consensus site. **B**: The Keap1/Nrf2/ARE pathway. Under basal conditions, transcription factor Nrf2 is bound to a cytoplasmic repressor Keap1, which targets Nrf2 for ubiquitination and proteasomal degradation via association with the cullin 3-based E3 ubiquitin ligase complex. Small-molecule inducers modify highly reactive (sensor) cysteine residues of Keap1, which loses its ability to target Nrf2 for degradation. This results in stabilization of Nrf2, binding to the ARE (in heterodimeric combinations with a small Maf transcription factor), and activation of the transcription of cytoprotective vitagenes. Legend: A) Biliverdin reductase (BVR); carbon monoxide (CO); γ-glutamyl-cysteine ligase (GC-ligase); reduced glutathione (GSH); oxidized glutathione (GSSG); Glutathione S-transferase P (GSTP); Homocysteine (HCY). B) p23-chaperone (P23); Cyclophilin 40 (CY).

2010a,c; V. Calabrese et al., 2012b). HO-1, also known as *Hsp32*, metabolizes heme into free iron, carbon monoxide, and biliverdin, the latter being the precursor of bilirubin, a linear tetrapyrrole that has been shown to neutralize oxidative and nitrosative stress effectively because of its property of interacting with nitric oxide and reactive nitrogen species (Mancuso et al., 2013). Mammalian sirtuins, a group of proteins that has been associated with metabolism, stress tolerance, and aging effects in several organisms, deacetylate a range of substrates, including histones and transcription factors, in a reaction requiring the cofactor NAD^+ (D. Liu et al., 2009; G. Liu et al., 2016). Through this activity, sirtuins regulate cell differentiation, energy transduction, glucose homeostasis, and apoptosis (D. Liu et al., 2009).

The 70-kDa Hsps (Hsp70s) function as molecular chaperones under physiological conditions, folding newly synthesized proteins and refolding damaged or misfolded proteins as well as assembling and disassembling protein complexes. Hsp70s have highly conserved domain structures (Kästle et al., 2012) consisting of an N-terminal ATPase, a middle region, and an N-terminal peptide binding domain. Thus, there are Hsp70s that localize specifically in the ER (BiP) and in the mitochondria (mortalin), although they are localized mainly to the cytosol and nucleus, where Hsp70s coexist with the heat shock cognate isoform (Hsc70).

Hsc70 is constitutively expressed, and its action as an important housekeeping protein involves folding of newly synthesized polypeptides to nascent proteins, protein translocation, and disassembly of clathrin-coated vesicles. However, in contrast to Hsc70, Hsp70 expression is induced by various stressors. Consistent with this, protein unfolding is generally associated with an increase in protein hydrophobicity, formation of toxic protein aggregates (Grune et al., 2011), and consequent initiation of events leading to fast Hsp upregulation. This is additionally accelerated by the fact that heat shock genes do not contain introns (Bozaykut et al., 2013). Generally, protein unfolding is a process accompanied by binding to Hsp70, followed either by refolding into a native functional state and release or by permanent binding to Hsp70 to protect other, nondamaged proteins.

Because of their covalent nature, most oxidative protein modifications are not reparable, so oxidized proteins are processed by the cytoplasmic, nuclear, and ER proteosomal systems. It has been documented that oxidized proteins are a natural substrate for the 20S proteosome, which, in contrast to the 26S proteasome, is able to degrade unfolded proteins in an ATP- and ubiquitin-independent manner (Grune et al., 2011). Specifically, the (ATP- and ubiquitin-dependent) 26S proteasome catalyzes degradation of regulatory proteins that are no longer required in cellular metabolism and partially misfolded proteins resulting from errors in protein synthesis. The (ATP- and ubiquitin-independent) 20S proteasome captures substrates through surface recognition of hydrophobic patches resulting from oxidation-induced charge changes that cause random and partial unfolding, a process accompanied by exposure of hydrophobic residues that were previously shielded in the conformational interior milieu of proteins (Grune et al., 2011; Labadia and Morimoto, 2014). Increasing evidence indicates that the Hsc70/Hsp70 system interacts with the proteasome to maintain the integrity and stability of the 26S proteasome, whereas Hsp90 contributes to the resistance of the 20S proteasome to oxidative damage (Conconi et al., 1998; Kikis et al., 2010).

In addition to playing a major role in protein folding, Hsp70s also regulate protein degradation processes, as occurs in chaperone-mediated autophagy of protein aggregates, a process called *aggrephagy* (Gamerdinger et al., 2011). Perhaps most importantly, stress-inducible molecular chaperone Hsp70s play a central role in the 20S proteosomal degradation of oxidized proteins. Oxidized proteins bound to Hsp70 can then migrate to 20S proteasomes, where they are degraded. Thus, two mechanisms are operative to catalyze the efficient degradation of oxidized proteins, direct recognition of oxidized protein substrates by the 20S proteasome and Hsp70-mediated pathways.

Elucidation of Hsp70s' capability to promote the degradation of oxidatively damaged proteins may be especially important for understanding mechanisms of neuroinflammation and neurodegeneration because oxidized proteins and protein aggregates accumulate in aging, various age-related diseases, and certain neurodevelopmental disorders as a consequence of proteosomal dysfunction (Grune et al., 2011). Dysregulation of the ubiquitin proteasome system may underlie Angelman syndrome, Rett syndrome, and ASD, in which dysfunction in the ubiquitin ligase (also known as *UBE3A*) has been demonstrated (Lehman, 2009).

The importance of this system in neurocognitive dysfunction is further supported by recent findings that these proteins, along with neuronal adhesion proteins, exhibit copy number variation in a cohort of over 800 patients suffering from ASD (Glessner et al., 2009). Furthermore, UBE3A dysfunction appears to produce a spectrum of pediatric cognitive dysfunction that is dependent on the mechanism and severity of UBE3A loss (Lehman, 2009).

THE KELCH-LIKE ECH-ASSOCIATED PROTEIN 1/NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2/ANTIOXIDANT-RESPONSIVE ELEMENT PATHWAY

Induction of the Kelch-like ECH-associated protein 1/nuclear factor erythroid 2-related factor 2/antioxidant-responsive element (Keap1/Nrf2/ARE) pathway, the forefront of the cellular defense (Fig. 2A), has been shown to be protective against various conditions of stress. Conversely, under conditions of Nrf2 deficiency, increased sensitization and accelerated disease pathogenesis occur (Kansanen et al., 2011). Under physiological conditions, transcription factor Nrf2 is continuously targeted for ubiquitination and proteosomal degradation by the repressor protein Keap1. Many inducers of the Keap1/

Nrf2/ARE pathway chemically target specific cysteine residues within Keap1, thereby inducing loss of Keap1's ability to target Nrf2 for ubiquitination and proteasomal degradation (V. Calabrese et al., 2010a; Zhang et al., 2011). As a consequence, accumulating Nrf2 enters the nucleus, binding as a heterodimer with a small Maf transcription factor to specific sequences located upstream in the heat shock gene promoter, called *antioxidant response elements*, thus activating transcription processes (Hayes et al., 2015).

Under basal conditions, these protective systems do not operate at maximum capacity but can be induced to higher activity levels by redox active compounds such as antioxidants, in particular sulforaphane or hydroxytyrosol, thus reducing the risks of developing malignancies and other chronic diseases (Knatko et al., 2016). Nrf2-dependent genes encode a large network of cytoprotective proteins, called *vitagenes*, that include genes involved in the metabolism and transport of an array of endo- and xenobiotics; proteins with antioxidant functions; and genes that participate in the synthesis, utilization, and regeneration of glutathione and reduced nicotinamide adenine dinucleotide phosphate. The number of genes under the transcriptional control of Nrf2 is very large, as indicated in studies integrating chromatin immunoprecipitation with parallel sequencing and global transcription profiling; 645 basal and 654 inducible genes are direct targets of Nrf2, with 244 genes at the intersection (Dinkova-Kostova et al., 2015; Tebay et al., 2015). Examples of NRF2-dependent proteins include: (1) enzymes involved in the synthesis and regeneration of glutathione (e.g., χ-CT, the core subunit of the cystine/glutamate membrane transporter, γ-glutamate cysteine ligase catalytic (GCLC) and modulatory (GCLM) subunits, glutathione reductase); (2) enzymes responsible for the synthesis of reducing equivalents (e.g., glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme); (3) anti-inflammatory enzymes, such as leukotriene B4 dehydrogenase; (4) conjugating enzymes such as glutathione S-transferases (GSTs) and uridine 5′-diphospho (UDP)-glucuronosyltransferases; (5) proteins facilitating the export of xenobiotics and/or their metabolites (e.g., solute carriers and adenosine triphosphate (ATP)-binding cassette transporters); (6) proteins active against metal overload (e.g., ferritin and metallothioneins); (7) proteins involved in removal or repair of damaged proteins (e.g., subunits of the 26S proteasome); and (8) antioxidant enzymes such as heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and thioredoxin reductase).

NEUROPROTECTION BY PHARMACOLOGICAL ACTIVATORS OF THE KEAP1/NRF2/ARE PATHWAY: POTENTIAL FOR TREATING ASD?

Sulforaphane

Sulforaphane (see Fig. 3), an isothiocyanate active component of cruciferous vegetables obtained in high concentrations from broccoli seeds and sprouts, is the product of glucoraphanin hydrolysis by myrosinase, an enzyme segregated in plant cells and released when plant cells are masticated and ingested. Sulforaphane has been shown to function in cellular defense against a broad spectrum of cellular stresses (Zhang et al., 2011). Bioavailability of sulforaphane to the brain via various routes of administration has been the subject of considerable investigation (Singh and Zimmerman, 2016).

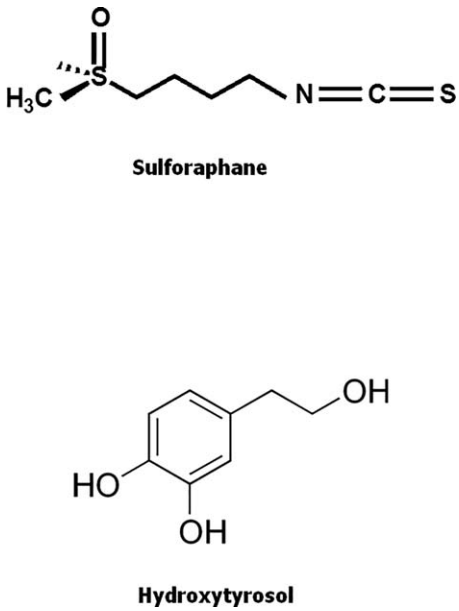

Fig. 3. Chemical structures of inducers of the Keap1/Nrf2/ARE pathway for which neuroprotective activities have been demonstrated.

Sulforaphane upregulates Hsps and related mechanisms central to multiple cellular processes in the CNS, including synaptic transmission, and may improve cortical connectivity (Zhang et al., 2011). It is of note that these aspects of brain function and structure have been shown to be reduced in ASD (H. Liu et al., 2016). As a principal inducer of the Nrf2-dependent genes, sulforaphane has been shown to exert protective effects against certain neurodegenerative diseases. Induction of HSF1-dependent genes by sulforaphane is a more recent discovery (Gan et al., 2010; Zhang et al., 2011; Sharma et al., 2012; Dinkova-Kostova et al., 2015).

Sulforaphane is an efficient stress proteome and Hsp activator, and it appears to function in a manner similar to fever. Reports that, in some instances, febrile illness has been shown to reduce certain cognitive and behavioral dimensions of ASD (Zhang et al., 2011; Singh et al., 2014) are of interest. It is also known that fever upregulates Hsps whose function is central in various cellular processes of CNS connected to synaptic transmission, and thus capable of improving long-range cerebral cortical connectivity which is depressed in ASD (Liu et al., 2016). Based on these findings, a promising study by Singh et al. (2014) of pediatric ASD patients receiving sulforaphane derived from broccoli sprout extracts at daily oral doses of 50–150 μmol for 18 weeks

followed by 1 month without treatment showed significant improvement in social interaction and verbal communication in sulforaphane-treated patients compared with untreated patients. After discontinuation of sulforaphane, improvements in social behaviors and communication returned to pretreatment levels.

Hydroxytyrosol

Hydroxytyrosol (HT; see Fig. 3), a product of the hydrolysis of oleuropein, is formed during the maturation of olives, storage of olive oil, and preparation of table olives. As a consequence of their polar character, phenolic compounds, including HT, are found in significant quantities in the remains of oil processing, such as pomace olive oil, olive mill waste water, and rinse waters. HT has received increasing attention because of its multiple pharmacological activities, such as antioxidant activity and apoptosis induction (Zrelli et al., 2015).

HT efficiently scavenges free radicals and protects biomolecules from free radical-induced oxidative damage. HT activates the Nrf2–ARE signaling pathway, leading to the upregulation of phase II enzymes and protection of neural cells exposed to hydrogen peroxide or 6-hydroxydopamine (Bernini et al., 2015; De la Cruz et al., 2015; Peng et al., 2016). As a potent antioxidant and novel small molecule that can induce the Nrf2–ARE pathway, HT is currently being viewed with interest with regard to pharmacological mitigation of neurodegenerative processes. In this light, there have been attempts either to synthesize HT or to recover it from olive oil production wastes.

Two known sources of HT are the ingestion of natural products that contain HT or its precursors and derivation from DA oxidative metabolism (De la Torre et al., 2006). Indeed, HT can be endogenously produced as a product of DA oxidation as a component of 3,4-dihydroxyphenylethanol (DOPET; Schröder et al., 2009). In the metabolism of DA by monoamine oxidase, oxidative-deamination of DA generates 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is further oxidized to the carboxylic acid 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase.

Although DOPAC is the major metabolite of DA in the brain, a small portion of DOPAL is reduced to DOPET by aldehyde or aldose reductase (Rodríguez-Morató et al., 2015). DOPAL is a highly reactive metabolite that is toxic to dopaminergic cells, suggesting that DOPAL might be a toxic DA metabolite in vivo (Goldstein et al., 2015, 2016). In ASD, disruption of DA metabolism is associated with increasing oxidative stress conditions, in which highly reactive aldehydes are formed, such as 4-hydroxynonenal and malondialdehyde, that can inhibit aldehyde dehydrogenase 2. As a consequence, the oxidation of DOPAL to DOPAC is prevented, and (neurotoxic) DOPAL accumulates (Rodríguez-Morató et al., 2015). Given these findings, DOPET formation may be viable and of value to reduce levels of DOPAL in children with ASD. We posit that this putative link between oxidative stress and the generation of endogenous neurotoxic substances will be important for further research focusing on mechanisms involved in ASD and, possibly, neuropsychiatric spectrum disorders in general.

TOWARD AN INVESTIGATIONAL AND TRANSLATIONAL TRAJECTORY

We opine that the mechanisms described here may contribute to the relative function and/or dysfunction of neural systems involved in ASD. These systems are selectively sensitive to initial conditions and very low-level fluctuations in the microenvironment (i.e.- unstable homoclinic effects), and adapt by alteration of output effects (i.e.., synaptic weighting, etc.) to modulate performance and/or prevent insult (Ermentrout, 1998). Activity and response parameters of components of the system (i.e., "bottom-up" effects) are likely responsive to, and affected by network properties and/or activity of the system as a whole (i.e., "top-down" effects). The expression and extent of these system effects reflect response(s) of particular units and/or component networks, and can vary in different individuals and at different times during development and/or throughout the life span (Kampis, 1991). Thus, we believe that a vital next step will be to further and sustain research toward both deepening understanding of these mechanisms, and engaging these findings in the development of clinical diagnostics and therapeutics. Perhaps most ideal in this pursuit will be an inter-disciplinary approach, which to date has proven useful and of value in the neurosciences, and which is now seen as important (if not essential) to the *Brain Research through Advancing Innovative Neurotechnologies* – BRAIN- initiative (see: www.whitehouse.gov/BRAIN) and for the execution of precision (viz.- personalized) medicine (see: www.whitehouse.gove/precision medicine). The iterative use of extant methods, coupled to ever newer, and increasingly more effective techniques and tools of assessment (e.g.- conjoined types of neuroimaging; neurogenetics; biomarker identification and evaluation) and intervention (e.g.- more precise ligands and effective pharmaceutical preparations, and/or neuromodulatory technologies) afford avenues of may offer considerable potential in both research and practice (Giordano 2010). But this also speaks to the need to continually re-assess techniques of assessment, analysis and translational engagement. It is our hope that reciprocal advances in methods will lead to increased knowledge and capabilities to more accurately define, diagnose, treat and perhaps prevent ASD and other neurological disorders.

The epidemiology of ASD suggests that acquiring additional information and clinical capability remains an essential task and a goal to meet the challenges and opportunities of large-scale investments in the brain sciences. To be sure, there are extant informational gaps with regard to the ways in which putative mechanisms subserve and affect neural functions and how current and future neuroscientific and neurotechnological advances may be employed. Such gaps serve as the impetus for ongoing research. Research findings must be approached

from a self-critical and self-revising stance toward neuroscience and its applications if we are to capitalize on investments in brain science and refine the knowledge and biomedical capability that it can afford, in ways that are both technically apt and ethically sound.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to the study and take responsibility for the integrity and the accuracy of the study concept and design. Drafting of the Review: VC, JG, MR, DB, AT, MLO, RB, EJC. Critical revision of the Review for important intellectual content: VC, JG, EJC. Study supervision: VC, JG, EJC.

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