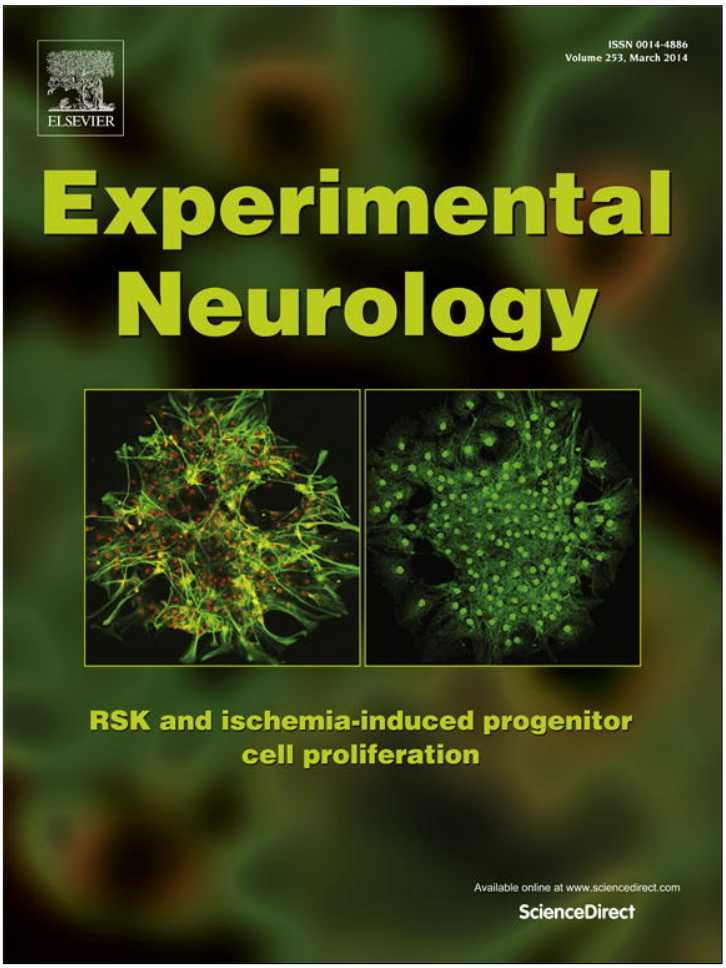


Experimental Neurology 253 (2014) 192–196

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review

Non-protein amino acids and neurodegeneration: The enemy within

Kenneth J. Rodgers *

School of Medical and Molecular Biosciences (04.06.35A), University of Technology, Harris Street, Sydney, NSW 2007, Australia

ARTICLE INFO

Article history:
Received 14 October 2013
Revised 9 December 2013
Accepted 17 December 2013
Available online 24 December 2013

Keywords:
Protein misfolding
Alzheimer's disease
Motor neurone disease
Parkinson's disease
Multiple sclerosis
Non-protein amino acid
BMAA

ABSTRACT

Animals, in common with plants and microorganisms, synthesise proteins from a pool of 20 protein amino acids (plus selenocysteine and pyrolysine) (Hendrickson et al., 2004). This represents a small proportion (~2%) of the total number of amino acids known to exist in nature (Bell, 2003). Many 'non-protein' amino acids are synthesised by plants, and in some cases constitute part of their chemical armoury against pathogens, predators or other species competing for the same resources (Fowden et al., 1967). Microorganisms can also use selectively toxic amino acids to gain advantage over competing organisms (Nunn et al., 2010). Since non-protein amino acids (and imino acids) are present in legumes, fruits, seeds and nuts, they are ubiquitous in the diets of human populations around the world. Toxicity to humans is unlikely to have been the selective force for their evolution, but they have the clear potential to adversely affect human health. In this review we explore the links between exposure to non-protein amino acids and neurodegenerative disorders in humans. Environmental factors play a major role in these complex disorders which are predominantly sporadic (Coppede et al., 2006). The discovery of new genes associated with neurodegenerative diseases, many of which code for aggregation-prone proteins, continues at a spectacular pace but little progress is being made in identifying the environmental factors that impact on these disorders. We make the case that insidious entry of non-protein amino acids into the human food chain and their incorporation into protein might be contributing significantly to neurodegenerative damage.

Contents

Toxicity of non-protein amino acids . . . 192
Use of non-protein amino acids in translation of mRNA to protein . . . 193
Specific effects of proteomimetic amino acids on human health . . . 193
Proteomimetic amino acids and neurological disease . . . 193
Cellular effects of incorporation of proteomimetic amino acids . . . 194
Animal studies using proteomimetic amino acids . . . 194
Concluding remarks . . . 194
Acknowledgments . . . 195
References . . . 195

Toxicity of non-protein amino acids

Some non-protein amino acids are able to compete in metabolic pathways involving protein amino acids: in other words, they function as antimetabolites (Rubenstein, 2000). They can also be mistakenly utilised in protein synthesis ('proteomimetics') (Fowden et al., 1967; Rodgers and Shiozawa, 2008; Rubenstein, 2000). Incorporation of non-protein amino acids (amino acid analogues) into proteins was systematically studied in the 1960s but this phenomenon and its possible implications have been largely overlooked (reviewed in (Hendrickson et al., 2004)). An exception is the arginine-mimetic canavanine, which occurs in Papilionoideae such as the jack bean, and kills the larvae of predators by replacing arginine in the peptide chain of newly-synthesised proteins (Thomas and Rosenthal, 1987). Canavanine, if supplied in a high enough concentration, is lethal to rats (Thomas and Rosenthal, 1987) and is currently being investigated as a human anti-cancer agent (Vynnytska et al., 2010).

In other examples, fescue grasses out-compete other plants by releasing a phytotoxic root exudate of which *meta*-tyrosine, a proteomimetic amino acid, is the primary component (Bertin et al., 2007). *Meta*-tyrosine interferes with root development in competing

* Fax: +61 2 9550 3302.
E-mail address: kenneth.rodgers@uts.edu.au.

0014-4886/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.12.010

plants by being charged to $tRNA^{phe}$ and replaces phenylalanine in the protein sequence (Bertin et al., 2007). *Meta*-tyrosine is also a product of phenylalanine oxidation and is utilised by mammalian cells *in vitro*, resulting in the synthesis of aberrant proteins which are toxic or lethal (Rodgers et al., 2002). Similarly, the proline mimetic azetidine-2-carboxylic acid, which is synthesised by *Convallaria majalis*, is lethal to other plant species that do not synthesise this imino acid and competes with proline for insertion into proteins (Peterson and Fowden, 1965).

Use of non-protein amino acids in translation of mRNA to protein

Proteomimetic amino acids, being close structural analogues of the 20 protein amino acids, enter mammalian cells and cross the blood–brain barrier on amino acid transporters and once inside the cells compete with protein amino acids for charging onto tRNA (Hendrickson et al., 2004). A high level of fidelity in protein synthesis is maintained because of a very low error rate on the part of tRNA synthetases when selecting the correct protein amino acid for esterification to the cognate tRNA and from the abortive termination of protein synthesis following detectable errors in this process (Zaher and Green, 2009). tRNA synthetases have evolved to discriminate efficiently between the 20 protein amino acids, and in some cases require an additional proof-reading step (Hendrickson et al., 2004) but are less efficient at discriminating against non-protein amino acids with a similar, size, shape and charge to a protein amino acid (Fowden et al., 1967; Hendrickson et al., 2004). For example, L-3,4 dihydroxyphenylalanine (L-DOPA), a very close structural analogue of L-tyrosine, can be charged to $tRNA^{tyr}$ in mammalian cells resulting in the synthesis of full-length proteins containing biosynthetically incorporated L-DOPA (Rodgers et al., 2002).

The most effective defence against proteomimetic amino acids is to evolve a more selective tRNA synthetase. The bruchid beetle, a canavanine-resistant predator, has adopted this strategy, possessing an advanced arginyl tRNA synthetase which has a low affinity for canavanine relative to arginine (Malinow et al., 1982; Rosenthal et al., 1976). Similarly, the jack bean has been shown to possess an advanced arginyl tRNA synthetase that prevents auto-toxicity from canavanine insertion into its own proteins (Igloi and Schiefermayr, 2009).

Specific effects of proteomimetic amino acids on human health

Proteomimetic amino acids impact on human health only when high enough quantities are ingested to compete effectively with the 'parent' protein amino acid for insertion into polypeptide chains. In humans, exposure to canavanine from ingestion of alfalfa seeds or tablets can cause systemic lupus erythematosus (SLE)-like symptoms, an effect also seen experimentally in cynomolgus macaques (Malinow et al., 1982). Similarly, ingestion of 1,1′-ethylidene-bis[L-tryptophan] (EBT), a contaminant in a synthetic tryptophan preparation, resulted in over 1500 cases of Eosinophilia–Myalgia Syndrome (EMS) and 38 deaths (Rubenstein, 2000). Administration of EBT to rats resulted in the development of EMS and confirmed that the amino acid was incorporated into proteins (Silver et al., 1994). The L-tyrosine mimetic L-DOPA, which is present in seeds from the highly insect-resistant Central American plants of the genus *Macuna* (at ~6–9%), is incorporated into proteins by mammalian cells in place of L-tyrosine (Rodgers et al., 2002, 2004) and generates protease-resistant, aggregate-prone proteins in human cells *in vitro* (Dunlop et al., 2008; Rodgers et al., 2004).

Incorporation of a proteomimetic amino acid into proteins is a random process and, although no specific proteins are targeted, aggregation-prone or 'intrinsically disordered proteins' (IDP) such as α-synuclein and tau protein could be more sensitive to an amino acid substitution (Uversky et al., 2008). The extent to which an amino acid substitution destabilises a protein depends, amongst other factors, on which amino acid is substituted and its location in the folded protein; internal or solvent-exposed (Ozawa et al., 2005). Post-mitotic cells such as neurons and retinal pigment epithelial (RPE) cells are less well-equipped to handle terminally aggregated proteins than rapidly dividing cells, since they are unable to reduce the burden of protein aggregates by distributing them amongst daughter cells. In these ways global errors in protein synthesis can cause tissue-specific dysfunction (Drummond and Wilke, 2009), as was evident in a study in which neurodegeneration was the primary pathology in a mouse with a minor translational proof-reading defect (Lee et al., 2006). Proteins which have been implicated in neurological disorders are generally aggregation-prone or contain domains with a high aggregation propensity (King et al., 2012): mutations in genes encoding these proteins, however, are not always 'causative' for the disorder to which they are linked, so are considered to be 'susceptibility genes' that by themselves do not give rise to a phenotype (Rocchi et al., 2003). Incorporation of proteomimetic amino acids into proteins could therefore be an environmental trigger which accelerates the rate of protein aggregation in individuals carrying susceptibility genes.

Proteomimetic amino acids and neurological disease

Sufferers from Parkinson's disease are exposed to the L-tyrosine mimetic L-DOPA (levodopa) over many years as a therapeutic agent for their medical condition. L-DOPA is efficiently incorporated into proteins by mammalian cells *in vitro* (Rodgers et al., 2002) and proteins containing incorporated DOPA are present in blood and brain extracts from L-DOPA-treated individuals (Chan et al., 2012; Rodgers et al., 2006). Despite being in use for over 40 years there is still much debate about whether L-DOPA might accelerate the progression of Parkinson's disease (Zesiewicz, 2011) this has been hard to resolve since almost all Parkinson's patients are eventually treated with L-DOPA. It is likely that oxidative stress from L-DOPA is an *in vitro* artefact (Clement et al., 2002), however, proteins containing incorporated L-DOPA are cytotoxic *in vitro* (Chan et al., 2012) and would be capable of producing a slowly progressive chronic toxicity *in vivo*. When present in proteins or other polymers, L-DOPA is one of nature's most effective adhesives and cross-linking agents (Messersmith, 2010; Miserez et al., 2008) and is capable of generating protein aggregates in cells preventing their removal by proteolysis (Dunlop et al., 2008).

The non-protein amino acid β-methylamino-L-alanine (BMAA) has been linked to neurological diseases. BMAA has been implicated in a complex neurological disorder on the South Pacific island of Guam in which individuals (from different cultural and genetic backgrounds) slowly developed features of amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and dementia (known collectively as ALS-PDC) (Bradley and Mash, 2009). This disorder occurred at a 100-fold greater incidence than that of ALS in the USA and other developed countries (Kurland, 1988). Steele and Guzman reported a higher incidence of ALS–PDC in villages in the south of Guam where cycad flour was eaten more frequently and made the observation that Chamorro people on the nearby island of Saipan, who did not consume cycad flour, had no increased incidence of ALS–PDC (Steele and Guzman, 1987). This supported Whiting's hypothesis that seeds of *Cycas circinalis* contained a neurotoxin (Whiting, 1963). Although cycad flour contained acute toxins that caused diarrhoea, vomiting and in some cases death (Whiting, 1963), these were generally removed prior to use by an extensive (but variable) washing procedure, leaving lipid soluble substances as well as insoluble or protein-bound material (Whiting, 1963). Washed cycad flour has a complex toxicity profile in mice that includes glutamate release, gait disturbances, loss of muscle strength and balance, as well as apoptotic cell death in the spinal cord and brain (Shaw and Wilson, 2003). Acute toxins such as sterol glucosides remain in the flour after washing and appear to contribute significantly to the excitotoxic effects of the washed cycad flour (Khabazian et al., 2002). The amino acid BMAA was identified in cycad seeds by Bell et al. (Vega et al., 1968) and in a landmark study, Spencer demonstrated that administration of BMAA to cynomolgus monkeys resulted in the development of a degenerative motor-system disease (Spencer et al.,

1987a, 1987b). BMAA, which commonly occurs in a 'protein-associated' form, is synthesised by cyanobacteria (genus *Nostoc*), which are root symbionts of cycad palms on Guam and is biomagnified in cycad seeds and flying foxes (1000-fold) (Cox et al., 2003). It has been recently shown that BMAA is mistakenly incorporated into proteins by human cells in place of L-serine and this results in the development of protein aggregates in vitro (Dunlop et al., 2013). It is feasible that the incorporation of BMAA into proteins promoted misfolding of a number of aggregation-prone proteins in the brains of Chamorro natives on Guam, thus triggering three distinct disorders. Some individuals experienced symptoms of ALS, others had symptoms more consistent with PD and dementia, while some individuals displayed symptoms of all three disorders (Plato et al., 2003). These disorders, presumably involving different proteins and tissues, were manifest in a distinctive sequence: with the highest incidence of ALS, occurring around 10 years prior to that of Parkinson's disease and dementia (Plato et al., 2003). Seeding of protein aggregates in neurons could also account for the late development of ALS–PDC in Guamanian migrants after periods of absence from Guam of up to three decades (Garruto et al., 1980).

Neurolathyrism, an upper motor neurone disease that is seen clinically as a spastic paralysis of the lower limbs, and which occurs in periodic outbreaks in Ethiopia and the Indian sub-continent following exposure to a range of non-protein amino acids consumed from certain *Lathyrus* species in times of famine (Getahun et al., 1999) could originate by a similar mechanism. The non-protein amino acid β-*N*-Oxalylamino-L-alanine (BOAA), present in chickling pea (*Lathyrus sativus*) (Rao et al., 1964), has been implicated in neurolathyrism (Spencer and Schaumburg, 1983). When BOAA was given to primates by gavage, neurological symptoms developed after 2 to 4 weeks but the onset of the symptoms was delayed (3 to 10 months) if BOAA was present in a diet fortified with the same amount of BOAA (Spencer et al., 1986). In humans, the onset of symptoms usually occurs 3 to 6 months after ingestion of a diet rich in BOAA but has been reported to develop more rapidly in individuals with a high level of physical activity (Woldeamanuel et al., 2012). The delayed onset of symptoms when BOAA was administered to primates with food is consistent with a reduction in the rate of incorporation of a proteomimetic amino acid in the presence of the parent amino acid in the diet.

Edward Rubenstein provided evidence of a tight link between the worldwide prevalence of multiple sclerosis (MS) and the geography of beet agriculture (*Beta vulgaris*) (Rubenstein, 2008). Beets contain the non-protein amino acid azetidine-2-carboxylic acid (AZE) which can replace proline in proteins. Exposure to AZE can lead to both neurodegeneration and autoimmune disorders (Rubenstein, 2000) and is proposed to alter the structure and function of myelin basic protein by replacing certain proline residues (Rubenstein et al., 2006).

Cellular effects of incorporation of proteomimetic amino acids

There is evidence of two primary pathological outcomes from incorporation of proteomimetic amino acids into proteins. If proteins containing proteomimetic amino acids are efficiently degraded, or generate truncated proteins, they can give rise to peptides which can produce autoimmune symptoms due to recognition of the new, proteomimetic-containing epitopes as non-self antigens; alternatively, if the proteins generated resist degradation and accumulate (an effect that might also be related to the level of incorporation), they can increase the intracellular burden of misfolded proteins and initiate or promote aggregate formation (Dunlop et al., 2008). Post-mitotic cells, such as RPE cells, would be more sensitive to the accumulation of protein aggregates than rapidly-dividing cells. It is of interest therefore that an unusual retinopathy, known as linear retinal pigment epitheliopathy (LRPE), was reported on Guam (Cox et al., 1989). LRPE was shown to be uniquely associated with ALS–PDC and is a predictor of the later development of symptomatic ALS–PDC (Hanlon and Steele, 1993).

Animal studies using proteomimetic amino acids

It could be argued that if incorporation of proteomimetic amino acids into proteins can trigger neurological changes it would have been observed more often in animal studies. Generally however, these studies are designed to examine acute effects, and animals are not exposed to the proteomimetic amino acid long enough to produce pathological changes resulting from the slow accumulation of protein aggregates. In addition, proteomimetic amino acids are most often supplied with food so that the protein amino acid competing with the mimetic for charging to the tRNA synthetase is also present at high levels. Spencer et al. supplied BMAA to primates by gavage, separating it from dietary amino acids, and reported motor neuron dysfunction in BMAA-treated animals (Spencer et al., 1987a, 1987b). In contrast to those studies in which BMAA is administered with food (reviewed in (Karamyan and Speth, 2008)), the delivery of BMAA by intraperitoneal injection has consistently produced neurological changes in chicks, mice and rats (Polsky et al., 1972; Seawright et al., 1990). An important finding in the primate study of Spencer was that, while early signs of motor-neuron dysfunction were observed at high doses of BMAA, signs of extrapyramidal compromise developed slowly in animals fed lower doses of BMAA, this led the authors to suggest that chronic toxicity might be mechanistically distinct from acute toxicity (Spencer et al., 1987b). Acute effects of BMAA have been reported in a wide range of species including zebrafish (*Danio rerio*) (Purdie et al., 2009b), brine shrimp (*Artemia salina*) (Purdie et al., 2009a) and fruit fly (*Drosophila melangaster*) (Goto et al., 2012) and were attributable to the excitotoxic effects of BMAA. Other studies have reported what appeared to be both acute and chronic effects. For example, brains of 6 month old rats that had received BMAA by subcutaneous injection on postnatal days 9 and 10, exhibited changes that could be attributed to excitotoxicity as well as disturbances in protein turnover with the deposition of polyubiquitinated proteins in the hippocampus (Karlsson et al., 2012). Excitotoxic effects and those related to protein misfolding and ER stress were also reported in rats that received BMAA for 5 days intraperitoneally (de Munck et al., 2013). Evidence of an interaction between neuroproteins and BMAA was provided in a study in which radiolabelled BMAA was shown to be retained in proteins in specific regions of the brain following intravenous injection (Xie et al., 2013).

Other things being equal, a low-protein diet will increase susceptibility to the adverse effects of proteomimetic amino acids, and conversely a high protein (and proteomimetic amino acid-free) diet will be protective. It was notable therefore that in the study of Morzova et al. that examined dietary risk factors for ALS, the most significant result was that chicken was significantly protective (Morozova et al., 2008). Interestingly, other high protein foods such as beef and pork showed an obvious trend towards protection against ALS (Morozova et al., 2008). A significantly lower incidence of ALS has been reported in Brazil (Matos et al., 2012), and a low incidence in Uruguay, (Chio et al., 2013) countries with a high consumption of red meat.

Inhalation is a route of exposure that could potentially separate proteomimetic amino acids from dietary proteins. Intriguing hypotheses have been proposed by Cox linking inhalation of BMAA present in desert dust to the higher incidence of ALS observed in deployed Gulf war veterans (Cox et al., 2009) and by Stommel linking ALS clusters in New Hampshire, USA to inhalation of aerosolised toxins from lakes with regular cyanobacterial blooms (Caller et al., 2009).

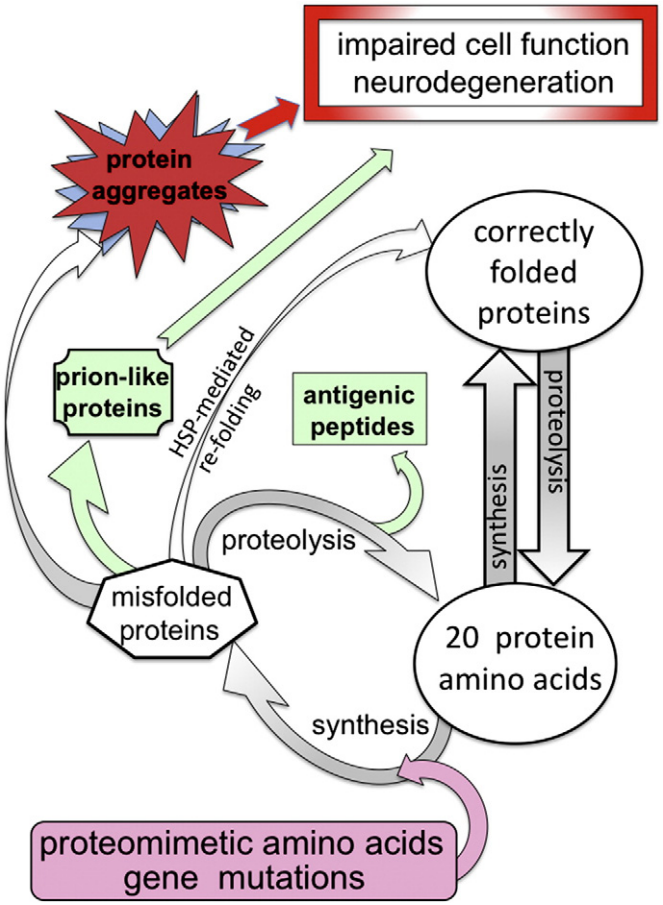
Concluding remarks

Proteomimetic amino acids are effective weapons of survival in the silent wars of plants and, in what could be considered collateral damage, can invade the pool of protein amino acids in humans and become mistakenly inserted into proteins. If this substitution leads to an increase in protein misfolding and aggregation, the burden of protein aggregates will slowly increase in neurons and other post-mitotic cells over the

lifetime of the individual. The rate of accumulation of protein aggregates might be further increased in individuals carrying mutations in certain proteins, and their effects might become more pronounced in the ageing nervous system. This would provide a link between environmental factors and genetic susceptibility (Scheme. 1), a currently held paradigm for the aetiology of many neurodegenerative diseases.

BMAA was detected in brain proteins from ALS, Parkinson's and Alzheimer's patients but not in brains from non-neurological controls (Bradley and Mash, 2009; Pablo et al., 2009). Other studies which utilised different analytical approaches however failed to detect BMAA in Chamorro brains or brains of patients diagnosed with Alzheimer's disease (Snyder et al., 2009, 2010). Interestingly, in the study of Pablo, no BMAA was detected in patients with Huntington's disease, which is a familial disorder (Pablo et al., 2009). The difficulties associated with accurate detection and quantification of BMAA in complex biological samples are comprehensively discussed in an excellent critical review by Cohen in which the author concludes 'the absence of standardised or validated methods makes comparison of the disparate findings difficult' (Cohen, 2012). Clearly additional independent analyses are required to determine if BMAA is present in the brain of individuals diagnosed with sporadic neurological disorders. Elevated levels of DOPA-containing proteins are present in the brains of L-DOPA-treated Parkinson's disease patients (Rodgers et al., 2006). Many proteomimetic amino acids might be lodged in aggregates in the human brain, and careful proteomic analysis is required to reveal their identity. Indirect (and silent) exposure to proteomimetic acids is currently the most alarming aspect of this phenomenon, since recent evidence has shown bioconcentration of BMAA in aquatic species occurs in South Florida (Brand et al., 2010) and the Baltic sea (Jonasson et al., 2010), and levels of BMAA detected in pink shrimp are comparable to those in flying foxes consumed in Guam (Brand et al., 2010).

In summary, proteomimetic amino acids may have evolved as weapons in the silent war between plants, but their insidious entry into the human food chain may be causing significant collateral neurodegenerative damage.



Scheme 1. Schematic representation of the possible outcomes of proteomimetic amino acid insertion into proteins by mammalian cells. Proteomimetic amino acids can be mistakenly incorporated into proteins resulting in the synthesis of proteins that are unable to fold correctly. The rate of generation of misfolded proteins can be accelerated in the presence of gene mutations in specific proteins (a gene–environment interaction). In some cases the heat shock protein (HSP) network can refold abberrant proteins or deliver them to the proteolytic machinery of the cell for degradation. In some instances this can result in the generation of peptides containing non-protein amino acids which are antigenic. If not detected or degraded, misfolded proteins can accumulate seeding further protein aggregation in the cell resulting in impaired neuronal function or apoptosis. Incomplete proteolysis can generate prion like proteins that can promote protein aggregation in adjacent cells.

Acknowledgments

The author expresses his gratitude to Dr Christopher L. Jackson from the University of Bristol, U.K. and Dr Peter B. Nunn from the University of Portsmouth, U.K. for preliminary review of the manuscript and for their insightful comments.

References

Bell, E.A., 2003. Nonprotein amino acids of plants: significance in medicine, nutrition, and agriculture. J. Agric. Food Chem. 51 (10), 2854–2865.
Bertin, C., Weston, L.A., Huang, T., Jander, G., Owens, T., Meinwald, J., Schroeder, F.C., 2007. Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. Proc. Natl. Acad. Sci. U. S. A. 104, 16964–16969.
Bradley, W.G., Mash, D.C., 2009. Beyond Guam: the cyanobacteria/BMAA hypothesis of the cause of ALS and other neurodegenerative diseases. Amyotroph. Lateral Scler. 10 (Suppl. 2), 7–20.
Brand, L.E., Pablo, J., Compton, A., Hammerschlag, N., Mash, D.C., 2010. Cyanobacterial blooms and the occurrence of the neurotoxin beta-N-methylamino-L-alanine (BMAA) in South Florida aquatic food webs. Harmful Algae 9, 620–635.
Caller, T.A., Doolin, J.W., Haney, J.F., Murby, A.J., West, K.G., Farrar, H.E., Ball, A., Harris, B.T., Stommel, E.W., 2009. A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. Amyotroph. Lateral Scler. 10 (Suppl. 2), 101–108.
Chan, S.W., Dunlop, R.A., Rowe, A., Double, K.L., Rodgers, K.J., 2012. L-DOPA is incorporated into brain proteins of patients treated for Parkinson's disease, inducing toxicity in human neuroblastoma cells in vitro. Exp. Neurol. 238, 9.
Chio, A., Logroscino, G., Traynor, B.J., Collins, J., Simeone, J.C., Goldstein, L.A., White, L.A., 2013. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 41, 118–130.
Clement, M.V., Long, L.H., Ramalingam, J., Halliwell, B., 2002. The cytotoxicity of dopamine may be an artefact of cell culture. J. Neurochem. 81, 414–421.
Cohen, S.A., 2012. Analytical techniques for the detection of alpha-amino-beta-methylaminopropionic acid. Analyst 137, 1991–2005.
Coppede, F., Mancuso, M., Siciliano, G., Migliore, L., Murri, L., 2006. Genes and the environment in neurodegeneration. Biosci. Rep. 26, 341–367.
Cox, T.A., McDarby, J.V., Lavine, L., Steele, J.C., Calne, D.B., 1989. A retinopathy on Guam with high prevalence in Lytico-Bodig. Ophthalmology 96, 1731–1735.
Cox, P.A., Banack, S.A., Murch, S.J., 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc. Natl. Acad. Sci. U. S. A. 100, 13380–13383.
Cox, P.A., Richer, R., Metcalf, J.S., Banack, S.A., Codd, G.A., Bradley, W.G., 2009. Cyanobacteria and BMAA exposure from desert dust: a possible link to sporadic ALS among Gulf War veterans. Amyotroph. Lateral Scler. 10 (Suppl. 2), 109–117.
de Munck, E., Munoz-Saez, E., Miguel, B.G., Solas, M.T., Ojeda, I., Martinez, A., Gil, C., Arahuetes, R.M., 2013. beta-N-methylamino-l-alanine causes neurological and pathological phenotypes mimicking Amyotrophic Lateral Sclerosis (ALS): the first step towards an experimental model for sporadic ALS. Environ. Toxicol. Pharmacol. 36, 243–255.
Drummond, D.A., Wilke, C.O., 2009. The evolutionary consequences of erroneous protein synthesis. Nat. Rev. Genet. 10, 715–724.
Dunlop, R.A., Dean, R.T., Rodgers, K.J., 2008. The impact of specific oxidized amino acids on protein turnover in J774 cells. Biochem. J. 410, 131–140.
Dunlop, R.A., Cox, P.A., Banack, S.A., Rodgers, K.J., 2013. The non-protein amino acid BMAA is misincorporated into human proteins in place of l-serine causing protein misfolding and aggregation. PLoS One 8, e75376.
Fowden, L., Lewis, D., Tristram, H., 1967. Toxic amino acids: their action as antimetabolites. Adv. Enzymol. Relat. Areas Mol. Biol. 29, 89–163.
Garruto, R.M., Gajdusek, C., Chen, K.M., 1980. Amyotrophic lateral sclerosis among Chamorro migrants from Guam. Ann. Neurol. 8, 612–619.
Getahun, H., Mekonnen, A., TekleHaimanot, R., Lambein, F., 1999. Epidemic of neurolathyrism in Ethiopia. Lancet 354, 306–307.

Goto, J.J., Koenig, J.H., Ikeda, K., 2012. The physiological effect of ingested beta-N-methylamino-L-alanine on a glutamatergic synapse in an in vivo preparation. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 156, 171–177.
Hanlon, S.D., Steele, J.C., 1993. An unusual retinal pigment epitheliopathy endemic to the island of Guam. Optom. Vis. Sci. 70, 854–859.
Hendrickson, T.L., de Crecy-Lagard, V., Schimmel, P., 2004. Incorporation of nonnatural amino acids into proteins. Annu. Rev. Biochem. 73, 147–176.
Igloi, G.L., Schiefermayr, E., 2009. Amino acid discrimination by arginyl-tRNA synthetases as revealed by an examination of natural specificity variants. FEBS J. 276, 1307–1318.
Jonasson, S., Eriksson, J., Berntzon, L., Spacil, Z., Ilag, L.L., Ronnevi, L.O., Rasmussen, U., Bergman, B., 2010. Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. Proc. Natl. Acad. Sci. U. S. A. 107, 9252–9257.
Karamyan, V.T., Speth, R.C., 2008. Animal models of BMAA neurotoxicity: a critical review. Life Sci. 82, 233–246.
Karlsson, O., Berg, A.L., Lindstrom, A.K., Hanrieder, J., Arnerup, G., Roman, E., Bergquist, J., Lindquist, N.G., Brittebo, E.B., Andersson, M., 2012. Neonatal exposure to the cyanobacterial toxin BMAA induces changes in protein expression and neurodegeneration in adult hippocampus. Toxicol. Sci. 130, 391–404.
Khabazian, I., Bains, J.S., Williams, D.E., Cheung, J., Wilson, J.M., Pasqualotto, B.A., Pelech, S.L., Andersen, R.J., Wang, Y.T., Liu, L., Nagai, A., Kim, S.U., Craig, U.K., Shaw, C.A., 2002. Isolation of various forms of sterol beta-D-glucoside from the seed of Cycas circinalis: neurotoxicity and implications for ALS-parkinsonism dementia complex. J. Neurochem. 82, 516–528.
King, A., Al-Sarraj, S., Troakes, C., Smith, B.N., Maekawa, S., Iovino, M., Spillantini, M.G., Shaw, C.E., 2012. Mixed tau, TDP-43 and p62 pathology in FTLD associated with a C9ORF72 repeat expansion and p.Ala239Thr MAPT (tau) variant. Acta Neuropathol. 125 (2), 303–310. http://dx.doi.org/10.1007/s00401-012-1050-0.
Kurland, L.T., 1988. Amyotrophic lateral sclerosis and Parkinson's disease complex on Guam linked to an environmental neurotoxin. Trends Neurosci. 11, 51–54.
Lee, J.W., Beebe, K., Nangle, L.A., Jang, J., Longo-Guess, C.M., Cook, S.A., Davisson, M.T., Sundberg, J.P., Schimmel, P., Ackerman, S.L., 2006. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. Nature 443, 50–55.
Malinow, M.R., Bardana Jr., E.J., Pirofsky, B., Craig, S., McLaughlin, P., 1982. Systemic lupus erythematosus-like syndrome in monkeys fed alfalfa sprouts: role of a nonprotein amino acid. Science 216, 415–417.
Matos, S.E., Conde, M.T., Favero, F.M., Taniguchi, M., Quadros, A.A., Fontes, S.V., Oliveira, A.S., 2012. Mortality rates due to amyotrophic lateral sclerosis in Sao Paulo City from 2002 to 2006. Arq. Neuropsiquiatr. 69, 861–866.
Messersmith, P.B., 2010. Materials science. Holding on by a hard-shell thread. Science 328 (5975), 180–181. http://dx.doi.org/10.1126/science.1187598.
Miserez, A., Schneberk, T., Sun, C., Zok, F.W., Waite, J.H., 2008. The transition from stiff to compliant materials in squid beaks. Science 319, 1816–1819.
Morozova, N., Weisskopf, M.G., McCullough, M.L., Munger, K.L., Calle, E.E., Thun, M.J., Ascherio, A., 2008. Diet and amyotrophic lateral sclerosis. Epidemiology 19, 324–337.
Nunn, P.B., Bell, E.A., Watson, A.A., Nash, R.J., 2010. Toxicity of non-protein amino acids to humans and domestic animals. Nat. Prod. Commun. 5, 485–504.
Ozawa, K., Headlam, M.J., Mouradov, D., Watt, S.J., Beck, J.L., Rodgers, K.J., Dean, R.T., Huber, T., Otting, G., Dixon, N.E., 2005. Translational incorporation of L-3,4-dihydroxyphenylalanine into proteins. FEBS J. 272, 3162–3171.
Pablo, J., Banack, S.A., Cox, P.A., Johnson, T.E., Papapetropoulos, S., Bradley, W.G., Buck, A., Mash, D.C., 2009. Cyanobacterial neurotoxin BMAA in ALS and Alzheimer's disease. Acta Neurol. Scand. 120, 216–225.
Peterson, P.J., Fowden, L., 1965. Purification, properties and comparative specificities of the enzyme prolyl-transfer ribonucleic acid synthetase from Phaseolus aureus and Polygonatum multiflorum. Biochem. J. 97, 112–124.
Plato, C.C., Garruto, R.M., Galasko, D., Craig, U.K., Plato, M., Gamst, A., Torres, J.M., Wiederholt, W., 2003. Amyotrophic lateral sclerosis and parkinsonism–dementia complex of Guam: changing incidence rates during the past 60 years. Am. J. Epidemiol. 157, 149–157.
Polsky, F.I., Nunn, P.B., Bell, E.A., 1972. Distribution and toxicity of alpha-amino-beta-methylaminopropionic acid. Fed. Proc. 31, 1473–1475.
Purdie, E.L., Metcalf, J.S., Kashmiri, S., Codd, G.A., 2009a. Toxicity of the cyanobacterial neurotoxin beta-N-methylamino-L-alanine to three aquatic animal species. Amyotroph. Lateral Scler. 10 (Suppl. 2), 67–70.
Purdie, E.L., Samsudin, S., Eddy, F.B., Codd, G.A., 2009b. Effects of the cyanobacterial neurotoxin beta-N-methylamino-L-alanine on the early-life stage development of zebrafish (Danio rerio). Aquat. Toxicol. 95, 279–284.
Rao, S.L., Adiga, P.R., Sarma, P.S., 1964. The isolation and characterization of beta-N-oxalyl-L-alpha, beta-diaminopropionic acid: a neurotoxin from the seeds of Lathyrus Sativus. Biochemistry 3, 432–436.
Rocchi, A., Pellegrini, S., Siciliano, G., Murri, L., 2003. Causative and susceptibility genes for Alzheimer's disease: a review. Brain Res. Bull. 61, 1–24.
Rodgers, K.J., Shiozawa, N., 2008. Misincorporation of amino acid analogues into proteins by biosynthesis. Int. J. Biochem. Cell Biol. 40, 1452–1466.
Rodgers, K.J., Wang, H., Fu, S., Dean, R.T., 2002. Biosynthetic incorporation of oxidized amino acids into proteins and their cellular proteolysis. Free Radic. Biol. Med. 32, 766–775.
Rodgers, K.J., Hume, P.M., Dunlop, R.A., Dean, R.T., 2004. Biosynthesis and turnover of DOPA-containing proteins by human cells. Free Radic. Biol. Med. 37, 1756–1764.
Rodgers, K.J., Hume, P.M., Morris, J.G., Dean, R.T., 2006. Evidence for L-dopa incorporation into cell proteins in patients treated with levodopa. J. Neurochem. 98, 1061–1067.
Rosenthal, G.A., Dahlman, D.L., Janzen, D.H., 1976. A novel means for dealing with L-canavanine, a toxic metabolite. Science 192, 256–258.
Rubenstein, E., 2000. Biologic effects of and clinical disorders caused by nonprotein amino acids. Medicine (Baltimore) 79, 80–89.
Rubenstein, E., 2008. Misincorporation of the proline analog azetidine-2-carboxylic acid in the pathogenesis of multiple sclerosis: a hypothesis. J. Neuropathol. Exp. Neurol. 67, 1035–1040.
Rubenstein, E., Zhou, H., Krasinska, K.M., Chien, A., Becker, C.H., 2006. Azetidine-2-carboxylic acid in garden beets (Beta vulgaris). Phytochemistry 67, 898–903.
Seawright, A.A., Brown, A.W., Nolan, C.C., Cavanagh, J.B., 1990. Selective degeneration of cerebellar cortical neurons caused by cycad neurotoxin, L-beta-methylaminoalanine (L-BMAA), in rats. Neuropathol. Appl. Neurobiol. 16, 153–169.
Shaw, C.A., Wilson, J.M., 2003. Analysis of neurological disease in four dimensions: insight from ALS–PDC epidemiology and animal models. Neurosci. Biobehav. Rev. 27, 493–505.
Silver, R.M., Ludwicka, A., Hampton, M., Ohba, T., Bingel, S.A., Smith, T., Harley, R.A., Maize, J., Heyes, M.P., 1994. A murine model of the eosinophilia-myalgia syndrome induced by 1,1′-ethylidenebis (L-tryptophan). J. Clin. Invest. 93, 1473–1480.
Snyder, L.R., Cruz-Aguado, R., Sadilek, M., Galasko, D., Shaw, C.A., Montine, T.J., 2009. Lack of cerebral bmaa in human cerebral cortex. Neurology 72, 1360–1361.
Snyder, L.R., Hoggard, J.C., Montine, T.J., Synovec, R.E., 2010. Development and application of a comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry method for the analysis of L-beta-methylamino-alanine in human tissue. J. Chromatogr. A 1217, 4639–4647.
Spencer, P.S., Schaumburg, H.H., 1983. Lathyrism: a neurotoxic disease. Neurobehav. Toxicol. Teratol. 5, 625–629.
Spencer, P.S., Roy, D.N., Ludolph, A., Hugon, J., Dwivedi, M.P., Schaumburg, H.H., 1986. Lathyrism: evidence for role of the neuroexcitatory aminoacid BOAA. Lancet 2, 1066–1067.
Spencer, P.S., Hugon, J., Ludolph, A., Nunn, P.B., Ross, S.M., Roy, D.N., Schaumburg, H.H., 1987a. Discovery and partial characterization of primate motor-system toxins. Ciba Found. Symp. 126, 221–238.
Spencer, P.S., Nunn, P.B., Hugon, J., Ludolph, A.C., Ross, S.M., Roy, D.N., Robertson, R.C., 1987b. Guam amyotrophic lateral sclerosis–parkinsonism–dementia linked to a plant excitant neurotoxin. Science 237, 517–522.
Steele, J.C., Guzman, T., 1987. Observations about amyotrophic lateral sclerosis and the parkinsonism–dementia complex of Guam with regard to epidemiology and etiology. Can. J. Neurol. Sci. 14, 358–362.
Thomas, D.A., Rosenthal, G.A., 1987. Toxicity and pharmacokinetics of the nonprotein amino acid L-canavanine in the rat. Toxicol. Appl. Pharmacol. 91, 395–405.
Uversky, V.N., Oldfield, C.J., Dunker, A.K., 2008. Intrinsically disordered proteins in human diseases: introducing the D2 concept. Annu. Rev. Biophys. 37, 215–246.
Vega, A., Bell, E.A., Nunn, P.B., 1968. The preparation of L- and D-alpha-amino- beta-methylaminopropionic acids and the identification of the compound isolated from Cycas circinalis as the L-isomer. Phytochemistry 7, 1885–1887.
Vynnytska, B.O., Mayevska, O.M., Kurlishchuk, Y.V., Bobak, Y.P., Stasyk, O.V., 2010. Canavanine augments proapoptotic effects of arginine deprivation in cultured human cancer cells. Anticancer Drugs 22, 148–157.
Whiting, M.G., 1963. Toxicity of cycads. Econ. Bot. 14, 270–302.
Woldeamanuel, Y.W., Hassan, A., Zenebe, G., 2012. Neurolathyrism: two Ethiopian case reports and review of the literature. J. Neurol. 259, 1263–1268.
Xie, X., Basile, M., Mash, D.C., 2013. Cerebral uptake and protein incorporation of cyanobacterial toxin beta-N-methylamino-L-alanine. Neuroreport 24, 779–784.
Zaher, H.S., Green, R., 2009. Quality control by the ribosome following peptide bond formation. Nature 457, 161–166.
Zesiewicz, T.A., 2011. Parkinson disease: the controversy of levodopa toxicity in Parkinson disease. Nat. Rev. Neurol. 8, 8–10.