EXCITOTOXINS: THEIR ROLE IN HEALTH AND DISEASE

*Tadvi NA¹, Qureshi SA², Naveen Kumar T³, Shareef SM⁴, Naidu CDM⁵, Venkata Rao Y⁶

¹Associate Professor, ²Assistant Professor, ³Professor, ⁴Professor and Head of Department, Pharmacology, Kamineni Institute of Medical Sciences, Marketpally.
²Post Graduate Student, Physiology, Kamineni Institute of Medical Sciences, Marketpally.
³Associate Professor, Pharmacology, Apollo Institute of Medical Sciences, Hyderabad.

*Corresponding author email: nasertadvi@yahoo.co.uk

ABSTRACT

Background: Excitotoxins are a class of substances usually amino acids or their derivatives that normally act as neurotransmitters in brain but in excessive amounts lead to over excitation of neurons leading to a state of exhaustion & death. Over 70 types of excitotoxins have been identified so far and many have a free access to our body in form of taste enhancing food additives like monosodium glutamate, aspartame, sodium casienate etc. They have been implicated for the development of a wide variety of neurological disorders like Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, and even for early ageing. Objective: The purpose of this review is to sort out truth about extent of involvement of excitotoxins in neurodegeneration from the massive propaganda against them wherein they have been implicated in almost all disorders of unknown etiology. Method: A comprehensive search strategy was developed incorporating both the peer reviewed, non peer reviewed literature and electronic databases like Medline. These were scrutinized and relevant research papers were examined. Conclusion: There is considerable evidence based research pertaining to the neurodegenerative effect of excitotoxins to the human brain. Yet the autonomous food regulating bodies like FDA refuse to recognize the immediate and long term danger to the public caused by the use of such excitotoxic food additives. Thus only means of protecting oneself from such type of neurological damage is to consume only unprocessed, fresh, whole, organic foodstuffs.

Keywords: Excitotoxins, Health Disease

INTRODUCTION & OVERVIEW

Excitotoxins refer to those substances which are capable of inducing excitotoxicity. The term excitotoxicity was coined by Dr. John Olney in the year 1969 to describe the neuronal injury that results from presence of excess glutamate in brain.¹ The histological appearance of excitotoxicity includes massive swelling of neuronal bodies & dendrites consistent with somatodendritic location of glutamate receptors and excitatory synapses². They have been implicated in wide variety of neurological disorders like Alzheimer’s disease³, Parkinson’s
Many of these substances are abundantly found in the body as well as in the environment both in animal and plant kingdom. For example in the body within the normal limits excitatory amino acids like glutamate have a crucial role in development of learning, memory, perception of pain, immune function and functioning of various special senses. But if their levels exceed it leads to excitatory damage. Over 70 types of excitotoxins have been identified so far & many have a free access to our body in the form of taste enhancing additives like monosodium glutamate, aspartame, sodium casienate etc. They also exist as variety of toxins in nature. In presence of associated risk factors long term exposure to excitotoxins has been proved in etiopathogenesis of neurodegenerative disorders. In fact, glutamate & aspartate toxicity are also thought to be responsible for memory loss, confusion, mild intellectual disorientation that frequently late middle age or old age. There is substantial evidence that shows that excitotoxic damage is related to neuronal death associated after cerebral ischemia, stroke, status epileptics and head trauma.

It is the purpose of this review to consolidate available information about excitotoxins and to segregate fact from fiction as regards to their role in etio-pathogenesis in wide variety of disorders especially neurodegenerative diseases.

MECHANISM OF ACTION OF EXCITOTOXINS

Excitotoxins along with other factors especially ageing alter the metabolic capacities of neurons. Ageing is an associated strong risk factor as with ageing there is progressive accumulation of mutations in mitochondrial genome decreasing the capacity of neurons to handle oxidative metabolism. Moreover, altering the oxidative metabolism of neurons leads to increased production of reactive oxygen metabolites. Natural antioxidant defenses fail to tackle this excessive free radicals and as a result the membrane potential is altered in such a way that the magnesium block of NMDA glutamate receptors is lifted up. This makes the receptor channel highly sensitive to even low levels of glutamate present around and leads to massive calcium influx. Increased calcium influx causes, increased water influx & osmotic damage to mitochondria, Stimulation of phospholipase A2, stimulation of nitric acid synthase, production of platelet activating factor (PAF), generation of free radicals, Activation of other enzymes like endonucleases, proteases esp calpain, phosphatases, Stimulation of phospholipase A2 causing production of arachidonic acid metabolites leading to potentiation of NMDA evoked currents leading to sustained activation of glutaminergic receptors and inhibition of absorption of glutamate into astrocytes & neuron via EAAT 2 (Essential Amino Acid Transporter 2). Stimulation of nitric oxide synthase (esp NO synthase II of oligodendrocytes) generates more nitric oxide(NO) further increasing free radical production. Constant sustained activation of glutaminergic receptors inhibits cysteine transport decreasing the intracellular levels of beneficial reducing agents in form of sulphydryl groups reduction of sulphydryl groups further potentiates free radical production.

Free radicals along with endonucleases cause DNA fragmentation and induce apoptosis. If the damage is severe enough there is rupture of lysosomes and extracellular release of intact and enzymatically modified cellular organelles causing inflammation and necrosis.

Neurons become more susceptible to glutamate toxicity in presence of astrocytes. In fact, concentrations of glutamate required to produce neurotoxicity in absence of astrocytes was 100 times more than that required in presence of astrocytes. These results confirmed that there was a soluble factor released by neurons which
signaled astrocytes of their presence and astrocytes increase the sensitivity of neurons to glutamate.

**DISTRIBUTION OF EXCITOTOXINS**

**Endogenous excitotoxins:** They are naturally present in body and in appropriate amounts are actively involved in physiological process related to survival of the organism. Important among this group are excitatory neurotransmitters glutamate and aspartate which are present throughout central nervous system.

**Glutamate:** The human body contains about 10g of free glutamate brain 2.3g muscle 6g, liver 0.7g, kidneys 0.7g & blood 0.04g. The most abundant molecular component of glutamate system is N-acetyl aspartyl glutamate (NAAG). Glutamate is produced from α-ketoglutarate an intermediate in the Krebs cycle. Once released it is taken up from synaptic cleft by both neurons & glia. Astrocytes convert it to glutamine by enzyme glutamine synthase. Glutamine then diffuses back into neurons which hydrolyze it back to glutamate by enzyme glutaminase. It has 2 subtypes of receptors called as ionotropic (iGluR) and metabotropic (mGluR) receptors. Ionotopic are ligand gated ion channels that open in response to a various agonists like kainate (ka), NMDA (N methyl D aspartic acid), AMPA (α amino 3 hydroxy 5 methyl 4 isoxalopropionic acid). Kainate receptors are simple ion channels when open permit Na influx and K efflux. AMPA has two population one leads to only Na influx & the other both Na & Ca influx. NMDA receptors are facilitated by binding of glycine which on opening allows large amount of calcium in. It also has a Mg+ ion block at resting membrane potential & it gets removed only when the neuron containing the receptor is partially depolarized by some other receptors like AMPA etc.

Metabotropic glutamate receptors operate via G protein mediated second messenger system. Based on sequence homology they have been divided in 3 groups:

- Group I: mGluR1, mGluR5
- Group II: mGluR2, mGluR3
- Group III: mGluR4, mGluR6, mGluR7

Group I stimulate Phospholipase C & eventually increase intracellular calcium and Group II, Group III are coupled to inhibition of adenyl cyclase. Activation of mGluR causes the production of inositol triphosphate which in turn activates receptors on endoplasmic reticulum that open calcium permeable channels. Glutamate is excitatory at ionotropic receptors & modulatory at metabotropic receptors. Another important function of mGluR is to modulate the function of other receptors by changing the synapse excitability. Glutaminergic neurons form an extensive network throughout the cortex, hippocampus, striatum, thalamus, hypothalamus, cerebellum, visual & auditory system & are essential for cognition, memory, movement & sensations like taste, sight & hearing. NMDA receptors bring about long term potentiation. They are pivotal in developing fetal brain for differentiation & migration of neurons mainly via calcium influx. Blockade of NMDA receptors during prenatal period with ethanol can induce apoptosis in vulnerable neurons leading to fetal alcohol syndrome.

**Aspartate:** It is also an excitatory as glutamate usually seen at synapses of pyramidal projection neurons of cortex in layers 3, 4 & 6 along with glutamate. Excitatory stellate interneurons in layer 4 of cortex also use it as neurotransmitter.

**Exogenous excitotoxins:** Although glutamate & other endogenous excitotoxins are present in the body. They are highly prevalent in the environment especially plants & animals either in free form or bound to peptides. In fact glutaminergic system is present in nearly all species of organisms including plants.

1. **Dietary glutamate:** It has been estimated that an average adult man has a daily glutamate intake of 28g and daily turnover of 48g from diet and breakdown of gut
proteins\textsuperscript{26}. Out of that only 2.5% of dietary protein escapes digestion & absorption, rest all is taken in\textsuperscript{29}. The process of digestion breaks protein into aminoacids & smaller peptides which are taken in very efficiently by active transport. At least 7 different amino acid transporters have been identified so far five of which require Na\textsuperscript{+} and two require Cl\textsuperscript{−} ions and rest are uniport mechanisms\textsuperscript{29}. Using these mechanism dietary L glutamate thus enters circulation & reaches blood brain barrier. Although tight junctions of cerebral capillaries prevent proteins from entering brain tissue , there are amino acid cotransporters present at those sites which allow acidic amino acids to enter inside\textsuperscript{29}.

2. Monosodium Glutamate (MSG): It is the sodium salt of glutamate responsible for fifth taste sensation i.e. the umami taste. In 1909 Professor Ikeda and Saburosuke Suzuki discovered taste enhancer called MSG. It is very commonly used as a food additive or a taste enhancing agent in almost all processed foodstuffs like readymade soups , curries , sauces , salad dressings , potato chips , all types of Chinese cuisine in form of ajinomoto & even baby foods. Many of the times it is present disguised under the labels of natural flavouring , malt extract , whey protein concentrate etc. Since, 1948 the rate of addition of MSG to readymade food products is doubling per decade\textsuperscript{39}. The food additive called as hydrolysed protein is more dangerous as it contains aspartate along with glutamate in very large amounts.

Extensive research has been done to study the effects of MSG on body in experimental animal models & have shown definitive effects like, neuronal cell death\textsuperscript{40}, substantial deficit in hippocampal long term potentiation\textsuperscript{41}, learning disabilities & behavioral deficits\textsuperscript{42,43}, delayed co ordination in newborns\textsuperscript{44}, retinal damage\textsuperscript{45}, hepatic and renal toxicity.\textsuperscript{46} As the consumption of MSG is increased because of increased uptake of processed food products , the extrapolation of these results to human beings is very much possible.

The general mechanism of action of MSG is very similar to excess of endogenous glutamate in form of NMDA receptor induced cation influx ultimately resulting in cellular injury in form of apoptosis or necrosis with associated inflammation\textsuperscript{17}. The determinant as to whether glutamate stress will lead to apoptosis or necrosis depends upon degree of damage to mitochondria where minor damage causes apoptosis & severe causes necrosis\textsuperscript{17,18}.

3. Aspartame : Aspartame is an artificial sweetener used extensively in variety of products like low calorie sugar substitute , diet soft drinks , low calorie confectionaries ,weight loss supplements , sports supplements, ready made low calorie sweets for diabetics etc. It is found in more than 6,000 products, including soft drinks, chewing gum, candy, yoghurt, tabletop sweeteners and some pharmaceuticals such as vitamins and sugar-free cough drops\textsuperscript{47}. Dietary surveys, performed among APM consumers, have shown that the average APM daily intake in the general population ranged from 2 to 3 mg/kg b.w. and was even more in children and pregnant women The Acceptable Daily Intake (ADI) both 50 to 40 mg/kg\textsuperscript{48}. In rodents and humans, APM is metabolised in the gastrointestinal tract into three constituents: aspartic acid, phenylalanine and methanol\textsuperscript{49}. And aspartic acid is an excitatory neurotransmitter whose metabolism is very similar to glutamate. However, a recent study found that female rats fed aspartame developed more lymphomas and leukemias than controls, in a dose-dependent manner, starting from a dose that may be relevant to human intake as low as 20 mg per kg body weight\textsuperscript{50,51}.

4. Domoic acid (DMA): It is an glutamate analog and an agonist of ionotrophic glutamate
receptors like AMPA & NMDA. It is found in marine algae and filter feeder organisms like shellfish accumulate them in their bodies. As these shellfish are consumed DMA reaches human being it leads to increase calcium influx via sustained activation of iGluR causes seizure activity esp of limbic system & direct excitotoxic damage to CA3 cells of cerebral cortex. Lesions produced are extensive neuronal loss in bilateral hippocampus, dentate gyrus, amygdales, thalamus, insula & subfrontal cortex. In fact, experimental administration of AMPA antagonist like 2,3-dihydroxy-6-nitro7sulphamoylbenzoquinoxaline dione (NBQX) can prevent all its effects except of CA3 cell damage.

5. **β-N-oxalylamino-L-alanine (BOAA):** It is found in chickpeas called kesar dal and is a selective agonist of AMPA receptors. It is found to be responsible for Neurolathyrism which is a paralytic disorder characterized by loss of upper motor neurons specifically in malnourished people. However, BOAA does not cause disease in healthy people as is evidenced from animal studies. Later it was found that it causes pathology in man only when associated with multivitamin deficiencies that impair mitochondrial metabolism and render neurons vulnerable for it.

6. **β methylamino –L-alanine (BMAA):** It is found in fruits of cycad plant that grows in Guam region and is responsible for a disorder called Amyotrophic lateral sclerosis of Guam. The toxin becomes active only in presence of bicarbonate ions and the exact mechanism is not known.

7. **Malonate:** It is a mitochondrial toxin which impairs electron transport chain in neurons and makes them sensitive to even low doses of endogenous excitotoxins.

8. **3-Nitropropionic acid (3NPA):** It is a secondary metabolite of fungi of Arthirium sp. which grows on sugarcane. It inhibits succinate dehydrogenase and impairs electron transport chain. It alters the membrane potential and brings about reversal of Mg ion block causing increased susceptibility of neurons to glutamate esp in striatum producing lesions very similar to Huntington’s disease.

9. **Methyl mercury and ammonia:** Their mechanism of action is not clear but involves disruption of interactions between neurons and oligodendrocytes.

10. **Casein:** There are several genetically-determined variants of β-casein, the protein which constitutes about 25-30% of cows’ milk proteins. One variant, A1 β-casein, has been implicated as a potential etiological factor in type 1 diabetes mellitus (DM-1), ischaemic heart disease (IHD), schizophrenia, and autism. Studies show that antibodies against A1 β-casein are increased in patients with DM-1. It was also demonstrated that the relationships between milk protein consumption and IHD mortality rates were much stronger. The hypothesis that links neurological disorders such as schizophrenia and autism to A1 β-casein is that, in genetically susceptible individuals, dietary components like casein and gluten are cleaved in the gut to produce peptide fragments with opioid characteristics (gluteomorphines and casomorphines). These compounds enter the circulation, cross the blood brain barrier and influence neurological functioning.

**EVIDENCE OF INVOLVEMENT OF EXCITOTOXINS IN NEURODEGENERATIVE DISORDERS:**

1. **Amyotrophic lateral sclerosis (ALS):** Anti glutamate strategies like use of drug called Riluzol slows the disease and decreases the mortality. Two fold increase in CSF glutamate levels were found in patients of ALS. The CSF of ALS patients induced apoptosis in cultured spinal motor neurons.
AMPA receptors on spinal motor neurons cause increase in calcium influx and are involved in their excitotoxic degeneration. AMPA antagonist GYK1 52466 prevents increased glutamate induced degeneration of cultured spinal motor neurons.

2. Huntington’s disease (HD): Expansion of CAG repeat sequence is found in first exon on the gene of chromosome 4p16.3 coding a protein called as Huntington in the genome of patients affected. CAG is a codon for glutamate and the normal range of glutamate in a polyglutamate tract is between 6 to 34 whereas in HD cases it is more than 40. Huntington protein interferes with binding of a scaffold protein PSDP-95 which has guanylate cyclase activity at special areas called as PDZ domains on NMDA receptors & KaGlur that normally plays a pivotal role in synaptogenesis and regulation of synaptic plasticity ultimately making NMDA receptors hypersensitive to glutamate which increase calcium influx & cause apoptosis of neurons via stimulation of MLK2 gene transcription.

3. Addison’s disease (AD): Physiologically, NMDA receptors serve as gating switch for the modification of major forms of synaptic plasticity involved in learning & consolidation of short term memory to long term memory. Neuronal damage due to excitotoxic effects of glutamate via NMDA receptors caused by increased glutamate levels due to dysfunction of cystine-glutamate antiporter is seen in cases of AD. Memantine a weak glutamate antagonist increases learning and memory in animal models & in patients of AD.

4. Multiple sclerosis (MS): Local elevated concentrations of glutamate are found in MS patients. Glutamate antagonist amantadine reduces the relapse rate in patients of MS. Malfunctioning of astrocytes and downregulation of enzymes which bring down the level of glutamate like glutamate dehydrogenase & glutamate synthase causing axonal and oligodendrocyte damage is seen in such patients.

5. Parkinson’s disease (PD): There is a mitochondrially encoded defect in complex I of electron transport chain enhancing the susceptibility of neurons for excitotoxic damage. Substantia nigra pars compacta neurons have NMDA receptors & sustained activation of which via increased calcium influx coexistent with impaired energy metabolism leads to PD. Studies have shown 3-NPA & malonate via NMDA receptors loss of dopaminergic neurons in mesencephalic neuronal cultures.

6. Cerebral ischaemia & traumatic brain injury: Positive modulation of NMDA receptor activity by group I receptors mGluR1,5 potentiates neuronal excitotoxicity. Group II receptors (mGluR2/3) exert a protective effect possibly through presynaptic inhibition of Glutamate release. NMDA and AMPA receptor antagonists have been shown to be powerful neuroprotective agents in animal models of stroke. In permanent or reversible occlusion of the middle cerebral arteries, these antagonists consistently reduce the volume of cortex that is infarcted. AMPA receptor antagonists reduce cortical and white matter damage in vitro and in vivo. AMPA antagonist if given close to the time of onset of the ischemia gives optimum neuroprotection against excitotoxic damage. Glutamate excitotoxicity, oxidative stress, and acidosis are primary mediators of neuronal death during ischemia and reperfusion& astrocyte are critical determinant of neuronal survival in the ischemic penumbra.

7. Epilepsy & status epilepticus: Brain damage from repeated seizures can occur independent of cardiopulmonary & systemic metabolic changes suggesting that local factors in brain can lead to neuronal death. Excitatory glutamatergic mechanisms are involved during both acute,transient, evoked
seizures and long-term, adaptive cellular plasticity associated with seizure activity as in chronic epilepsy animal models such as amygdala-kindled rats or rats with spontaneous, recurring seizures after an episode of induced status epilepticus.\textsuperscript{96} NMDA and AMPA receptor antagonists are powerful anticonvulsants in a wide range of animal models of epilepsy.\textsuperscript{97} Astrocytes have a direct role in the regulation of synaptic strength and neuronal excitability and a dysfunction in glial cells, and not in neurons or synapses is the initiating cause of epilepsy.\textsuperscript{100} & robust reactive gliosis is necessary to induce posttraumatic epilepsy.\textsuperscript{101}

SUMMARY AND CONCLUSION

Specific excitotoxins like DMA, MOAA, BMAA, 3NPA are found in specific foodstuffs and can be easily avoided by not eating marine shellfish, cycad fruit, chickpea, fungated sugarcane. However, the other exogenous excitotoxins like MSG, aspartame are being consumed in large amounts under the disguise of appetizing, processed food products and if coexistent with genetic predisposition or ageing or any other factor causing oxidative metabolic stress inevitably, lead to an auto stimulatory glutamnergic vicious cycle causing apoptosis and necrosis of specific neurons and development of neurodegenerative disorders. It should also be appreciated that the effects of excitotoxic food additives generally are not dramatic and are subtle and develop over a long period of time. But they certainly can precipitate these disorders and worsen their pathology. Likewise, foodborne excitotoxins may be harmful to those suffering from strokes, head injury and HIV infection, and certainly should not be used in a hospital setting.

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