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EXCITOTOXICITY AND CELL DAMAGE - A REVIEW

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ABSTRACT: Excitotoxicity refers to the pathological process by which nerve cells are damaged & killed by glutamate or related excitatory amino acid under conditions such as after intense exposure. This occurs when receptor for the excitatory neurotransmitter such as N-methyl-D-aspartate (NMDA) or AMPA are over activated. Such excitotoxic neuronal death may take part in the pathogenesis of brain or spinal cord injury associated with several human disease states. Various mechanisms involving excitotoxicity have been proposed to explain the neuronal cell death characteristic of neurodegenerative diseases, including elevation of intracellular calcium, accumulation of oxidizing free radicals, impairment of mitochondrial function and activation of apoptotic programs.

INTRODUCTION: The negative effect of glutamate upon the CNS were first observed in 1954 by T. Hayashi, a Japanese scientist who noted that direct application of glutamate to the CNS caused seizure activity, though this report went unnoticed for several years.

The toxicity of glutamate was then observed by D. R. Lucas and J.P. Newhouse in 1957, when the subcutaneous injection of monosodium glutamate to newborn mice destroyed the neurons in the inner layers of the retina [1]. Later, in 1969, John Olney discovered the phenomenon was not restricted to the retina, but occurred throughout the brain, and coined the term excitotoxicity [2].

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Excitotoxicity is the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the NMDA receptors and AMPA receptors are over activated. Excitotoxins like NMDA and kainic acid which binds to these receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions (Ca^{2+}) to enter the cell [3].

Ca^{2+} influx into cells activates a number of enzymes, including phospholipases, endonucleases and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA. Excitotoxicity may be involved in spinal cord injury, stroke, traumatic brain injury, hearing loss (through noise overexposure or ototoxicity) and in neuro-degenerative diseases of the central nervous system (CNS) such as multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS),

Parkinson's disease, alcoholism or alcohol withdrawal, and Huntington's disease [4, 5]. Other common conditions that cause excessive glutamate concentrations around neurons are hypoglycemia [6] and status epilepticus [7].

Excitatory Amino acid Neurotransmitters: Excitatory effects of amino acids on neurones were first reported by Curtis *et al.* who described the depolarising effect of glutamic acid on spinal neurones of the rat, using the newly discovered technique of iontophoresis. Apart from glutamic acid itself, this class includes another constitutive amino acid, aspartic acid, as well as exogenous compounds of natural (quisqualic, kainic, and domoic acids) or synthetic origin (*N*-methyl-D-aspartic acid [NMDA]).

Followings are excitatory amino acid neurotransmitters;

- Glutamic acid
- Aspartic acid
- Sulphur-containing excitatory amino acids
- Quinolinic acid
- N-Acetylaspartylglutamic acid [8].

RECEPTOR INVOLVED IN EXCITATORY RESPONSE [9]

Description	NMDA	AMPA	Kainate	Metabotropic
Family	Ion channel	Ion channel	Ion channel	G-protien linked
Structure	Oligomeric	Oligomeric	Oligomeric	Monomeric 7-TM domain
Subunits/subtypes	1 NR1 subunit 4 NR2 subunits (A-D)	4GluR subunits (1-4)	3GluR-subunits (5-7) 2KA-subunits (1,2)	8 subtypes Known
Unitary conductance	Mainly 40-50 pS	Mainly 10-20 pS	Mainly <10 pS	
Ionic selectivity	Na^+,K^+,Ca^{2+}	Na^+,K^+	Na^+,K^+	
Desensitisation	Slow	Rapid (AMPA) Slow (kainite)	Rapid (kainite)	
Selective agonist	NMDA Quinolinic acid Ibotenic acid	Quisqualic acid AMPA Kainic Acid	Kainic acid Domoic-acid	Trans-ACPD Ibotenic acid Quisqualic acid
Selective antagonist	2-APV Selfotel MK801(noncopetetive)	CNQX GYKI 52466 (noncopetetive)	CNQX NS 102 SYM2081	Phenylglycines
Regulatory sites	Glycine Polyamine	Thiazide		

Mechanisms of Excitotoxicity

- **Ionotropic and metabotropic glutamate receptors:** There is an excess of glutamate and glutamatergic activity in certain neurodegenerative diseases. The excitatory effects of glutamate are exerted via the activation of three major types of ionotropic receptors and several classes of metabotropic receptors linked to G-proteins. The major ionotropic receptors activated by glutamate are commonly referred to as the *N*-methyl-*D*-aspartic acid (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and kainic acid (KA) receptors.

These ionotropic receptors are ligand-gated ion channels permeable to various cations [10]. Continuous activation of large numbers of NMDA receptors (especially the NR1/NR2B-subtype) leads to increases in intracellular calcium loads and catabolic enzyme activities, which can trigger a cascade of events eventually leading to apoptosis or necrosis [11]. These downstream effects include mitochondrial membrane depolarization, caspase activation, production of toxic oxygen and nitrogen free radicals, and cellular toxicity [12, 13]. AMPA-type glutamate receptors have also been implicated in excitotoxicity because assemblies of these receptors are highly permeable to $Ca^{2+}$and

possibly contribute to the delayed neuronal cell death processes induced by Ca^{2+}overload. The Ca^{2+} permeability of the AMPA receptor is determined by the presence or absence of the GluR2 subunit in the receptor complex. Low expression of GluR2 permits the construction of AMPA receptors with high Ca^{2+} permeability and contributes to neuronal degeneration in ischemia. Surprisingly, decreasing GluR2 levels or selective blockage of Ca^{2+}-permeable AMPA receptors was also shown to protect against neurodegeneration [14].

- **Excitotoxicity and ions (**Na^+, Cl^-, and $Ca2^+$): Acute excitotoxicity is thought to be mediated by excessive depolarization of the postsynaptic membrane. This results in an osmotic imbalance when countered by an influx of Na^+, Cl^-, and water, leading to the eventual rupture of cell membranes [15]. Numerous reports indicate that acute excitotoxic neurodegeneration following glutamate receptor activation is dependent on Na^+andCl^- entry. Accordingly, removal of extracellular Na^+ or Cl^- abolishes NMDA-mediated neurodegeneration [16]. Sustained Ca^{2+} influx through glutamate receptor channels is thought to represent a common pathway of neuronal cell death [17].

- **Excitotoxicity and oxidative stress:** Oxidative stress is a major player in the pathology of neurodegenerative disorders. The relationship between oxidative stress and neuronal death has been extensively investigated. Oxidative stress damages nucleic acids, proteins and lipids and potentially opens the mitochondrial permeability transition pore, which in turn can further stimulate ROS production, worsen energy failure and release proapoptotic factors such as cytochrome *c* into the cytoplasm [18].Generation of high levels of ROS and downregulation of anti-oxidant mechanisms result in neuronal cell death in neurodegenerative diseases [19].

- **Excitotoxicity and mitochondrial mediated apoptosis:** Mitochondria represent the energy powerhouses and buffering sinks of the cell. Mitochondria not only function as the site of oxidative phosphorylation and cellular respiration, but also play a critical role in maintaining a low concentration of calcium in the cytosol. Changes in either of these critical functions of mitochondria have formidable consequences and often determine the cell's fate in survival/death signalling pathways. In particular, excessive uptake of calcium or generation of ROS induces activation of the mitochondrial permeability transition and subsequent release of calcium and proapoptotic factors into the cytosol [20, 21].

Excitotoxicity and Neurodegenerative Disease: Neurodegenerative diseases are a major cause of morbidity in the elderly and thus, an important issue in public health. Excitotoxicity provides an elegant hypothesis to explain the pathogenesis of these diseases, and, potentially, a rationale for the development of appropriate therapies.

- **Excitotoxicity in Alzheimer's disease:** Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system associated with progressive cognitive and memory loss. Molecular hallmarks of the disease are extracellular deposition of the β-amyloid peptide (Aβ) in senile plaques, the appearance of intracellular neurofibrillary tangles (NFT), cholinergic deficit, extensive neuronal loss, and synaptic changes in the cerebral cortex, hippocampus and other areas of brain essential for cognitive and memory functions [22].

  According to the amyloid cascade hypothesis, AD pathogenesis is initiated by the overproduction and extracellular deposition of Aβ and the intracellular deposition of NFT. These depositions serve as initiating factors for multiple neurotoxic pathways, which may include excitotoxicity, oxidative stress, energy depletion, inflammation and apoptosis [23]. Interestingly, recent studies have shown that glutamatergic signalling is compromised by Aβ-induced modulation of synaptic glutamate receptors in specific brain regions, paralleling early cognitive deficits [24].

- **Excitotoxicity in Huntington's disease:** Huntington's disease (HD) is an inherited neurodegenerative disorder that affects cognition, motor function and mood. Neuropsychiatry changes are caused by the dysfunction or death of specific neuronal cell types in the brain. GABAergic projections of

medium-size spiny neurons (MSNs) of the neostriatum are the most severely affected [25]. Notably, increasing GABA receptor function has been shown to promote neuronal survival after ischemia by depressing the overall excitability of the cell [26, 27].

- **Excitotoxicity in Parkinson's disease:** Parkinson's disease (PD) is a neurological disorder that is caused by the degeneration of nigral dopaminergic neurons and the consequent massive drop of dopamine (DA) content in the striatum [28]. The concept of excitotoxicity has also been applied to PD. Studies have demonstrated that parkin (hereditary Parkinson disease PARK2 gene product) regulates the function and stability of excitatory glutamatergic synapses.

  Postsynaptic expression of parkin dampens excitatory synaptic transmission and causes a marked loss of excitatory synapses in hippocampal neurons. Conversely, knockdown of endogenous parkin or expression of PD-linked parkin mutants profoundly enhances synaptic efficacy and triggers a proliferation of glutamatergic synapses. This proliferation is associated with increased vulnerability to synaptic excitotoxicity [29].

  The resulting excessive glutamatergic drive could be a source of excitotoxicity in the nigra. As described above, persistent activation of NMDA receptor increases intracellular calcium levels. A role for elevated intracellular calcium in the events leading to cell death in PD is supported by the observation that dopaminergic neurons expressing the calcium-binding protein calmodulin may be selectively preserved in PD [30].

CONCLUSION: Glutamic acid is the major excitatory neurotransmitter in the mammalian CNS, being responsible for rapid synaptic transmission in the major afferent and efferent pathways of the brain and spinal cord, as well as in numerous local circuits. Excitotoxicity is associated mainly with activation of NMDA receptors, but other types of excitatory amino acid receptor also contribute. Excitotoxicity results from a sustained rise in intracellular Ca^{2+} concentration i.e. Ca^{2+}-overload. Raised intracellular Ca^{2+} causes cell death by various mechanisms, including activation of proteases, formation of free radicals, and lipid peroxidation. Formation of nitric oxide and arachidonic acid are also involved. Various mechanisms act normally to protect neurons against excitotoxicity, the main ones being Ca^{2+} transport systems, mitochondrial function and the production of free radical scavengers. Oxidative stress refers to conditions e.g. hypoxias in which the protective mechanisms are compromised, reactive oxygen species accumulate, and neurons become more susceptible to excitotoxic damage.

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