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Pathobiochemical mechanisms of excitotoxicity: the role of astrocytic metabolic failure

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Abstract. This work analyzes the pathobiochemical mechanisms of excitotoxicity arising from the impairment of the astrocytic glutamate-glutamine cycle (GGC). It has been established that dysfunction of key astrocytic proteins—the EAAT2 transporter and the enzyme glutamine synthetase (GS)—constitutes a central link in the pathogenesis of a wide spectrum of neurological disorders, ranging from acute ischemia to chronic neurodegeneration. Four primary biological initiators of this process have been recognized: energy depletion (ATP deficiency), oxidative stress, transcriptional interference, and direct toxic inhibition. It is demonstrated that the loss of astrocytic control over extracellular glutamate triggers a lethal cascade in neurons, which includes NMDA receptor hyperactivation, calcium overload, mitochondrial dysfunction, and the activation of lytic enzymes.

Keywords: *glutamate-glutamine cycle, astrocytes, excitotoxicity, EAAT2, glutamine synthetase, ischemia, neurodegeneration.*

Introduction. Glutamate is the dominant excitatory neurotransmitter in the central nervous system (CNS), playing a fundamental role in synaptic transmission, plasticity, learning, and memory. However, this vital molecule is a "double-edged sword": when it escapes strict physiological control, glutamate transforms into a potent neurotoxin [1]. Maintaining the delicate balance between excitation and inhibition is a critical condition for brain homeostasis [2].

Astrocytes play a central, if not the primary, role in regulating extracellular glutamate levels [3]. For a long time, these glial cells were considered merely a passive structural "scaffold" for neurons. However, it is now proven that astrocytes are active and indispensable participants in

CNS function [3, 4, 5]. They control the blood-brain barrier, regulate ion homeostasis (particularly K+ buffering), provide neurons with metabolic substrates [4], and modulate synaptic activity.

In the context of neurotransmission, their key function is forming an integral metabolic unit with neurons [6]. At the core of this interaction lies the astrocytic glutamate-glutamine cycle (GGC)—a biochemical mechanism ensuring glutamate detoxification and metabolic recycling [6, 7]: astrocytes uptake >90% of synaptic glutamate, convert it into non-toxic glutamine, and return it to neurons as a precursor for neurotransmitter synthesis [6].

Dysfunction of this vital cycle is not merely a consequence but a key trigger of pathobiochemical cascades in a wide range of severe neurological pathologies [3]. It underlies acute injuries such as ischemic stroke [8] and traumatic brain injury (TBI) [9]. Additionally, a growing body of research indicates that the pathophysiology of common neurodegenerative diseases, including Parkinson's disease [11, 12, 5], Alzheimer's disease [10], other tauopathies [6], and hepatic encephalopathy [13], is significantly influenced by GGC failure and the resulting chronic excitotoxicity.

Understanding the precise biochemical mechanisms leading to GGC failure and the neuronal death cascade it initiates is critical for developing new therapeutic strategies. This report aims to provide a comprehensive analysis of the pathobiochemical cascade of excitotoxicity, beginning with primary disruptions in astrocytic metabolism (loss of EAAT2 and GS) and concluding with the terminal pathways of neuronal death (calcium dysregulation, mitochondrial collapse, and necroptosis).

The Physiological Cascade: biochemical homeostasis of Glutamate. In a healthy brain, maintaining ultra-low (micromolar) glutamate concentrations in the synaptic cleft is a key condition for preventing neuronal hyperexcitation [7, 3]. This homeostasis is ensured by the coordinated operation of the glutamate-glutamine cycle (GGC) [6, 7]. The process begins during synaptic transmission when the presynaptic neuron releases glutamate into the synaptic cleft. To terminate the signal, astrocytes immediately uptake >90% of glutamate [6] via the EAAT2 (GLT-1) transporter [8, 14, 15]. Biochemically, EAAT2 is a secondary active transporter driven by the powerful electrochemical gradient of Na+ ions directed inward [7]. The transport stoichiometry

MEDICINE AND PHARMACY

of one glutamate molecule (Glu^-) requires the co-transport of three Na+ ions and one proton (H+) inward, in exchange for one K+ ion outward.

Glutamate uptake and Na+ influx via EAAT2 directly stimulate Na+/K+-ATPase activity [16]. The ATPase immediately begins pumping out the entered Na+, consuming ATP. This tight biochemical coupling [16] is why glutamate clearance is one of the most energy-demanding processes in the brain [4, 7]. Inside the astrocyte, glutamate plays a dual role. The main pathway involves conversion to glutamine via the exclusively astrocytic enzyme glutamine synthetase (GS) [17, 13], which catalyzes an ATP-dependent reaction where glutamate combines with ammonia and ATP to form glutamine. Alternatively, glutamate can enter the Krebs cycle (TCA) via a reaction catalyzed by glutamate dehydrogenase (GDH), converting into α-ketoglutarate, which allows the astrocyte to use glutamate as an energy substrate [18, 19]. Ultimately, the astrocyte releases the resulting non-toxic glutamine, which is then absorbed by neurons and transformed back into glutamate by the enzyme glutaminase, therefore restocking the vesicular neurotransmitter pool [6, 7].

Breaking the Cascade: pathobiochemical triggers of dysfunction. GGC failure results from one or more biochemical triggers affecting the astrocyte. The first mechanism, energy collapse (ATP deficit), is characteristic of acute ischemia [8]. Cessation of oxygen supply leads to an instant drop in ATP synthesis. Since glutamate uptake and ATPase function are tightly linked [16], the drop in ATP instantly stops Na+/K+-ATPase [4], leading to a rapid accumulation of Na+ ions inside the astrocyte. Consequently, the Na+ gradient, which is the sole driving force for EAAT2 [7], disappears. The transporter's biochemical thermodynamics reverse, and high intracellular Na+ and glutamate concentrations force EAAT2 to operate in reverse mode, actively pumping glutamate from the astrocyte back into the synaptic cleft and exacerbating excitotoxicity. Additionally, the glutamine synthetase reaction is ATP-dependent [13] and also halts.

Another crucial trigger involves oxidative stress and inflammation, characteristic of traumatic brain injury (TBI) [9] and ischemia-reperfusion injury [20]. In response to injury, astrocytes transition into a reactive state [9, 21]. Activation of inflammatory pathways, such as NF-κB [9], stimulates NADPH oxidase and iNOS, leading to the generation of superoxide and nitric oxide. Their interaction instantly

forms peroxynitrite (ONOO-). As an ultra-potent oxidant, peroxynitrite attacks key GGC proteins [22] by irreversibly nitrating critical tyrosine residues in the structure of EAAT2 and GS, altering their conformation and inactivating them. It also oxidizes sensitive thiol (SH-) groups in cysteine residues, further leading to loss of enzyme function [22]. Furthermore, oxidative stress affects chaperone proteins such as HSP90β, which normally stabilizes EAAT2; upon its damage, the EAAT2 protein becomes unstable and rapidly degrades [20].

Chronic excitotoxicity in neurodegenerative illnesses can be explained by transcriptional and synthesis disruption, which is frequently the cause of chronic failure [6, 10, 11]. EAAT2 synthesis is controlled by the Hippo-YAP signaling pathway [14, 8]. In many pathologies, including ischemia [8] and ALS [14], the Hippo pathway is activated, leading to the inactivation of the transcriptional co-activator YAP, preventing it from entering the nucleus and suppressing EAAT2 gene transcription. Additionally, NF-κB (specifically its p65 subunit) can act as a transcriptional repressor by binding to the EAAT2 gene promoter (SLC1A2) and physically blocking its readout during reactive astrogliosis [9]. The Hippo-YAP pathway also regulates glutamine synthetase expression [17], and EAAT2 expression can be further suppressed by microRNAs activated during reactive astrogliosis [21].

Finally, hepatic encephalopathy (hyperammonemia) provides a striking example of direct toxic inhibition, sometimes known as the "Trojan Horse" theory [13]. Astrocytes are the only brain cells that detoxify ammonia (NH4+) via GS [13]. However, this leads to a biochemical "vicious cycle" [21]: cytosolic GS massively synthesizes glutamine from NH4+ and glutamate. This "non-toxic" glutamine is transported into astrocytic mitochondria ("Trojan Horse"), where the enzyme glutaminase cleaves ammonia from glutamine, releasing NH4+ directly into the mitochondrial matrix. NH4+ acts as a protonophore, destroying the H+ gradient and causing mPTP opening and oxidative stress. Simultaneously, massive glutamine synthesis depletes the cytosolic glutamate pool needed by the astrocyte for conversion to α-ketoglutarate for its own Krebs cycle. As a result, the astrocyte falls into its own energy deficit and mitochondrial dysfunction, which secondarily inhibits ATP-dependent EAAT2 and GS [21].

Consequences of the Breakdown: the neuronal cascade of excitotoxicity. Glutamate accumulation in the synaptic cleft, caused by astrocyte dysfunction, triggers a final and lethal

cascade of events directly in the postsynaptic neuron. Excess glutamate concentrations continuously stimulate ionotropic receptors on the postsynaptic membrane, particularly AMPA receptors (AMPAR) and N-methyl-D-aspartate receptors (NMDAR) [1, 12]. This leads to the key event of excitotoxicity: excessive NMDAR activation. This removes the magnesium block from the NMDAR channel, opening the way for a massive, uncontrolled influx of calcium ions into the neuronal cytosol [1, 12].

Following this, a critical moment described as the "triad of excitotoxicity" occurs, linking glutamate, calcium, and mitochondria [1]. Neuronal mitochondria attempt to compensate for the calcium shock by sequestering excess ions into their matrix [1]. However, this overload provokes the opening of the mitochondrial permeability transition pore (mPTP), leading to the collapse of the mitochondrial membrane potential, cessation of ATP synthesis in the electron transport chain, and massive generation of reactive oxygen species (ROS) [1].

Excess cytosolic calcium subsequently acts as an alarm signal, activating a series of lytic enzymes. Calcium-dependent proteases (calpains) begin destroying cytoskeletal proteins, while phospholipases (Phospholipase A2) attack cell membrane lipids, releasing arachidonic acid. Neuronal NO-Synthase (nNOS), also calcium-dependent, synthesizes nitric oxide (NO). The reaction of NO with mitochondrial superoxide forms peroxynitrite (ONOO-), an ultra-potent oxidant that damages DNA, nitrates proteins, and causes lipid peroxidation [1, 12, 22]. Ultimately, the neuron finds itself in a state of irreversible energy deficit and oxidative stress. If ATP remains, apoptosis is triggered. However, under conditions of severe ATP deficit characteristic of excitotoxicity, an alternative, ATP-independent pathway of programmed death is activated—necroptosis [23].

Conclusions. The analyzed pathobiochemical cascade demonstrates that the dysfunction of the astrocytic glutamate-glutamine cycle is a central, unifying link in the pathogenesis of a wide spectrum of neurological disorders. We observe how pathological triggers, entirely different in nature—acute ischemia (energy collapse and EAAT2 reversal) [8, 16], traumatic brain injury (oxidative stress and protein nitration) [9, 22], neurodegeneration (transcriptional failure) [14, 10, 5], and hepatic failure («Trojan Horse» hypothesis) [13, 21]—converge at one point: the impairment of

two key astrocytic proteins, EAAT2 and GS. This single point of failure—the loss of astrocytic control over extracellular glutamate—diverges into an irreversible neuronal cascade of excitotoxicity, the biochemical pillars of which are Ca2+ dysregulation, mitochondrial collapse (mPTP, ROS), and lytic enzyme activation [1]. Thus, pathobiochemical analysis proves that neuronal death in excitotoxicity is often a secondary phenomenon. The primary event is the biochemical and metabolic insufficiency of the astrocyte.

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MEDICINE AND PHARMACY

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