HYPOTHESIS

Insights & Perspectives

A beneficial role for elevated extracellular glutamate in Amyotrophic Lateral Sclerosis and cerebral ischemia

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Abstract
This hypothesis proposes that increased extracellular glutamate in Amyotrophic Lateral Sclerosis (ALS) and cerebral ischemia, currently viewed as a trigger for excitotoxicity, is actually beneficial as it stimulates the utilization of glutamate as metabolic fuel. Renewed appreciation of glutamate oxidation by ischemic neurons has raised questions regarding the role of extracellular glutamate in ischemia. Is it detrimental, as suggested by excitotoxicity in early in vitro studies, or beneficial, as suggested by its oxidation in later in vivo studies? The answer may depend on the activity of N-methyl-D-aspartate (NMDA) glutamate receptors. Early in vitro procedures co-activated NMDA receptors (NMDARs) containing 2A (GluN2A) and 2B (GluN2B) subunits, an event now believed to trigger excitotoxicity; however, during in vivo ischemia D-serine and zinc molecules are released and these ensure only GluN2B receptors are stimulated. This not only prevents excitotoxicity but also initiates signaling cascades that allow ischemic neurons to import and oxidize glutamate.

KEYWORDS
ALS, cerebral, excitotoxicity, glutamate, ischemia, receptor

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease of the upper and lower motor neurons.[1] Although intensely studied for many years its etiology is currently unknown. Numerous hypotheses have been proposed, including ischemia,[2] neuroinflammation,[3] mitochondria dysfunction,[4] and oxidative stress.[5] Perhaps the most well known, and studied, hypothesis is glutamate excitotoxicity.[6] This occurs when ion channel glutamate receptors are overstimulated by the abnormally high concentration of extracellular glutamate that occurs in ALS. The result of this over-stimulation is a rapid influx of a large number of calcium (Ca) ions, which has a detrimental impact on neuron function. These ions activate enzymes that degrade proteins, lipids, and nucleic acids. Their rapid uptake by mitochondria leads to membrane depolarization, a reduced rate of adenosine triphosphate production and an increased rate of reactive oxygen species generation in these organelles. This influx of Ca ions culminates in neuron degeneration and death.[6,7]

What triggers the increase in extracellular glutamate seen in ALS? There is no simple answer as the quantity of glutamate depends on the rate at which it flows into and out of the extracellular space. Glutamate flows into this area from both neurons and astrocytes. Neurons release glutamate into the synapse after an action potential sweeps through the axon, while astrocytes release it into the extrasynaptic space via the cystine glutamate antiporter (sys xc–).[9] This transporter system, located in the plasma membrane of astrocytes,[9] links the export of glutamate to the import of cystine on a one-to-one basis.[10] Glutamate is removed from the extracellular space of the cortex by three excitatory amino acid transporters (EAATs): EAAT1/GLAST, EAAT2/Glt-1, and EAAT3/EAAC1.[11] EAAT1/GLAST and EAAT2/Glt-1 are located in astrocytic processes that surround the neuronal synapse,[11] while EAAT3/EAAC1 is located in the dendrites of neurons, but positioned outside the synaptic cleft.[12] EAAT2/Glt-1 has the most impact as...
it quickly removes the majority of extracellular glutamate;\[11\] however, EAAT1/GLAST also has an important role as it participates in the glutamate–glutamine cycle.\[12,14\] In this cycle, glutamate, imported by astrocytes through EAAT1/GLAST, is converted into glutamine, via glutamine synthase. This glutamine is then released back into the extracellular space and imported by neurons, who convert it back to glutamate, via glutaminase. This glutamate is then packaged into vesicles for synaptic release.\[13,14\] The glutamate–glutamine cycle can, therefore, supply neurons with needed glutamate without an accompanying increase in extracellular glutamate concentration. Under normal conditions astrocytic glutamate transporters and the glutamate–glutamine cycle ensure that the concentration of extracellular glutamate remains very low; however, astrocytes in ALS brain and spinal cord possess an unusually low number of EAAT2/Glt-1 transporters\[15\] and the resulting decrease in the rate of glutamate removal is one factor contributing to the elevated level of extracellular glutamate that characterizes this disease.\[6,7\] There is currently no evidence for a decreased number of EAAT1/GLAST transporters in ALS; therefore, the glutamate–glutamine cycle may function normally in this disease.

Numerous studies have explored the role of glutamate excitotoxicity in ALS; however, other studies have focused on larger scale features of the disease. One such feature is cortical thinning. This reduction in cortical thickness, which progresses over the course of the disease,\[16\] can reach values of between 8 and 12%\[17\] and, contrary to popular belief, is present in all four cerebral lobes.\[16–18\] Spinal cord thinning, reflected by a decrease in cord cross sectional area, also occurs in ALS patients.\[19\] The underlying cause of thinning in the cortex and spinal cord is unknown.

The Hypothesis

As discussed above, ALS is characterized by both cortical thinning and an unusually high concentration of extracellular glutamate. These features are typically viewed and studied as distinct entities. The etiologic model outlined in this manuscript proposes that they are cause and effect. Cortical thinning is hypothesized to reflect tissue compression, resulting from a gradual buildup of cerebrospinal fluid (csf) in the subarachnoid space. This compression would decrease the rate of blood flow, subjecting neurons to ischemic stress. As intense ischemia is known to stimulate an increase in extracellular glutamate\[20,21\] cortical thinning, with its associated ischemia, is proposed to cause the increased extracellular glutamate in ALS.

As discussed above, this elevated concentration of extracellular glutamate is believed to trigger a toxic influx of Ca ions. The hypothesis outlined in this manuscript proposes that this does not actually occur in vivo. The concept of glutamate excitotoxicity arose from the observation that the addition of glutamate to tissue cultures triggered an influx of Ca, which led to neuronal death, and that this could be prevented by blocking glutamate receptors\[22\] however, these in vitro results may not accurately reflect what occurs in vivo. In addition, recent studies, discussed below, reveal that under ischemic conditions extracellular glutamate may actually play a beneficial role by serving as metabolic fuel. As ischemia appears to be an important component of ALS, the abundant extracellular glutamate in this disease is proposed to play a similar role. This hypothesis, however, goes one step further and suggests that during ischemia the extracellular glutamate stimulates the signaling cascades that initiate this metabolic shift from glucose to glutamate. The remaining portion of this manuscript discusses this hypothesis in more detail, noting portions supported by existing literature and aspects that require additional study.

Cortical Thinning in ALS May Reflect Tissue Compression Caused by a Buildup of CSF in the Subarachnoid Space

This hypothesis proposes that cortical thinning in ALS reflects the gradual compression of cortical tissue, triggered by an increase of csf in the overlying subarachnoid space. This buildup of fluid may result from a problem in the csf circulatory system, which begins with the formation of csf by the choroid plexus in the lateral ventricles.\[23\] Once formed it flows through the third and fourth ventricles and then into the subarachnoid space surrounding the brain and spinal cord.\[24\]

CSF exits the subarachnoid space along the superior surface of the brain via arachnoid villi, invaginations of the arachnoid membrane into the overlying dural vein.\[24\] Flow thru villi is driven by a pressure gradient between the subarachnoid space and dural vein.\[25\] It is proposed that in ALS there is reduced outflow from the subarachnoid space due to an increase in outflow resistance,\[26\] possibly caused by cell proliferation, inflammation or fibrosis in the villi,\[27\] or an increase of pressure in the dural vein.\[28\] Over time the decreased rate of outflow would cause csf to backup throughout the subarachnoid system, compressing the brain and spinal cord.

Evidence for Increased CSF Volume and Outflow Resistance in ALS

There is very little information regarding csf volume in ALS. A few papers contain limited observations, made during studies focused on other topics. One noted increased csf volume in limb onset ALS patients (206 vs. 153 ml in control subjects), but did not consider this statistically significant.\[29\] Another observed enlarged lateral, third and fourth ventricles in ALS patients.\[30\] Detailed descriptions of the arachnoid membrane and villi in ALS are similarly lacking. Studies of the arachnoid villi and csf volume in ALS are needed to confirm or refute the idea of a pathological accumulation of this fluid.

Evidence for Cortical Compression in ALS

Although support for increased csf volume in ALS is limited there is evidence for compression in ALS cortex and spinal cord. Compression changes the physical characteristics of neurons in the parenchyma. It decreases soma size,\[31\] dendrite length, and field span.\[32,33\] It also increases neuron density.\[31,33\] These features are present in neuronal tissue of ALS patients. Neuron soma in the motor cortex\[34–36\]
and spinal cord are smaller in ALS than in control samples. Apical dendrites of Betz cells in ALS are distally disintegrated with vacuoles, while neurons in ALS spinal cord possess unusually short and thin dendrites. There is also an increase in neuron density in deep cortical layers of ALS brain with extensive Betz cell loss. The fact that the decrease in neuron size occurs in all size brackets and in Onuf’s nucleus, which does not experience neuron loss in ALS suggests that the smaller neurons in ALS are not simply a result of the selective death of larger neurons.

Tissue compression also decreases blood vessel diameter and, therefore, blood flow. A decrease in the rate of blood flow and an increase in mean transit time are features of ALS cortex. The fact that decreased perfusion occurs early in the course of ALS and correlates with disease severity is indicative of the critical role played by ischemia.

Lastly, chronic compression of neuronal tissue leads to changes in functional characteristics. It triggers an increase in neuron excitability, reflected by a decrease in threshold current. A similar reduction in threshold current is seen in ALS neurons. Compression also decreases the synthesis and release of γ-aminobutyric acid (GABA) by inhibitory neurons and a reduced number of GABA neurons is characteristic of ALS cortex. Given the above, cortical and spinal tissue in ALS display histological and functional characteristics indicative of chronic compression. This provides initial support for the hypothesis that thinning of the cortex and spinal cord in ALS is the result of tissue compression.

**ISCHEMIC NEURONS STIMULATE THE INCREASE IN EXTRACELLULAR GLUTAMATE**

As discussed above, a reduced rate of blood flow through compressed cortical tissue exposes neurons to ischemic stress and ischemia is a known stimulator of increased extracellular glutamate. A literature review reveals that this accumulation of glutamate is initiated by signals arising from the ischemic neurons. When neurons experience ischemia they release proteins, known as alarmins, to alert microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1), which, although normally bound to DNA, leaves the nucleus and is released from the neuron following ischemic stress. Once in the extracellular space it activates microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1), which, although normally bound to DNA, leaves the nucleus and is released from the neuron following ischemic stress. Once in the extracellular space it activates microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1), which, although normally bound to DNA, leaves the nucleus and is released from the neuron following ischemic stress. Once in the extracellular space it activates microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1), which, although normally bound to DNA, leaves the nucleus and is released from the neuron following ischemic stress. Once in the extracellular space it activates microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1), which, although normally bound to DNA, leaves the nucleus and is released from the neuron following ischemic stress. Once in the extracellular space it activates microglia and astrocytes to the fact that they are in trouble. One alarmin is high mobility group box 1 (HMGB1)

**FIGURE 1** Proposed mechanism by which ischemic neurons initiate the increase in extracellular glutamate, D-serine and zinc that activates GluN2B receptors. The resulting signaling cascades are proposed to stimulate an influx of glutamate via the EAAT3. See text for additional discussion. EAAT2/Glt-1 - Excitatory Amino Acid Transporter 2/Glutamate Transporter 2, GluN2B - N-methyl-D-aspartate glutamate receptor containing 2B subunits, GSH - Glutathione, HMGB1 - High Mobility Group Box 1, IL-1B - Interleukin 1 beta, sys x− - Cysteine Glutamate Antiporter, TNFα - Tumor Necrosis Factor alpha

and activated microglia in ALS tissue, along with a recently documented increase in sys x− activity in ALS spinal cord supports this possibility. There is, however, a lack of information regarding the expression of IL-1B in ALS brain or spinal cord. This same signaling cascade, characterized by increased expression of HMGB1, TNFα, and IL-1B along with increased activity of sys x− is also present in cerebral ischemia.

**ISCHEMIC NEURONS UTILIZE GLUTAMATE AS AN ALTERNATIVE SOURCE OF FUEL**

Per the above, neurons essentially request an increase in extracellular glutamate when faced with severe ischemic stress. This suggests that they need this amino acid for some reason. A unique feature of glutamate may explain why. Although most studies emphasize glutamate’s role as a neurotransmitter, it has been known since 1963 that it can also serve as metabolic fuel when glucose is unavailable. More recent studies confirm that neurons oxidize glutamate when...
faced with ischemia\(^{[68-70]}\) as long as oxygen levels are adequate to support mitochondrial function.\(^{[69]}\) Given that neurons possess this ability it is proposed that those present in ALS cortex, or impacted by ischemia/stroke, metabolize glutamate in their battle against severe ischemic stress.

**EXTRACELLULAR GLUTAMATE MAY STIMULATE ISCHEMIC NEURONS TO IMPORT AND OXIDIZE GLUTAMATE**

Ischemic neurons may utilize glutamate as a source of fuel; but, before it can be oxidized it must be imported into the neuron and moved into the mitochondria. These activities may be stimulated by specific signaling cascades initiated during ischemia. As glutamate is a neurotransmitter, and its extracellular concentration is increased during ischemia, stimulation of glutamate receptors may initiate this process. In other words, glutamate may stimulate its own uptake.

### Ion channel glutamate receptors

To consider this possibility we must look briefly at the most abundant glutamate receptors in the adult brain. There are two types of receptors: ion channels and metabotropic G protein coupled receptors. Our current interest lies with the ion channel receptors of which there are two main types: the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid (AMPA) receptor (AMPAR) and the N-methyl-D-aspartate (NMDA) receptor (NMDAR).\(^{[71]}\) AMPARs are tetramers, composed of various combinations of subunits GluA1–GluA4. When activated by glutamate they can transmit Ca, sodium (Na), or zinc (Zn) ions\(^{[72]}\) with the subunit GluA2 dictating transmission capability. Receptors that contain GluA2 transmit Na, while receptors that lack GluA2 transmit Ca (Ca-AMPARs)\(^{[72,73]}\). NMDARs are tetramers that transmit Ca. Common NMDARs in adult brain contain two 2A subunits (GluN2A) or two 2B subunits (GluN2B) coupled to two GluN1 subunits. These receptors differ with respect to functional characteristics and location.\(^{[71]}\) GluN2A receptors have faster activation/deactivation kinetics and are located mainly in the synapse, while GluN2B receptors have a higher affinity for glutamate\(^{[71]}\) and are located mainly outside the synapse.\(^{[74,75]}\) The difference in location is important as it means GluN2A receptors are quickly activated by glutamate released during a typical action potential while extrasynaptic GluN2B receptors are not activated unless glutamate spills out of the synapse following intense synaptic stimulation, a decrease in glutamate uptake by astrocytes or a release of glutamate by astrocytes.\(^{[74,75]}\)

### Stimulation of GluN2B and Ca-AMPARs may lead to activation of Akt/Protein Kinase B (Akt/PKB)

Per the above, ischemic stress appears to shift activity toward the Ca-AMPAR and GluN2B receptors; therefore, these could play a role in stimulating the switch to glutamate metabolism. They may perform this function by initiating signaling cascades that activate Akt/PKB, as this kinase appears to play an important role in the uptake and oxidation of glutamate (discussed in section “Activation of Akt/PKB may stimulate the importation of glutamate into neurons, its transport into the mitochondria and its oxidation in the mitochondria matrix”). There is, however, uncertainty with regard to receptor identity. Although two signaling pathways initiated by NMDARs are hypothesized to activate Akt/PKB (see below), there is currently little experimental evidence indicating that these are composed of GluN2B subunits. This point is addressed further in section “Additional studies are needed to confirm that stimulation of GluN2B and Ca-AMPARs trigger the activation of Akt/PKB”.

As noted, two NMDAR signaling cascades may activate Akt/PKB. The first is initiated by neuronal nitric oxide synthase (nNOS), an enzyme colocalized with NMDAR via the scaffolding protein PSD-95. The rate at which Ca flows through the NMDAR determines the activity of nNOS.\(^{[87]}\) A low rate of Ca flux activates calcium-calmodulin protein kinase II (Ca/CaMKII) and this phosphorlylates, and hence...
FIGURE 2  Proposed signaling cascades initiated by stimulation of GluN2B and Ca-AMPAR receptors trigger the activation of Akt/PKB. This phosphorylates several proteins that assist in the neuronal importation and oxidation of glutamate. See text for additional discussion. Akt/PKB - Akt/Protein Kinase B, Aralar/AGC1 - Aralar/Aspartate Glutamate Carrier 1, BDNF - Brain Derived Neurotrophic Factor, Ca-AMPAR - Calcium permeable alpha-amino-3-hydroxyl-5-methyl-4-isoxazule-propionic acid receptor, CREB - cAMP Response Element Binding protein, EAAT3 - Excitatory Amino Acid Transporter 3, IP3 - Inositol P3 (1,4,5), IP3R - Inositol P3 receptor, nNOS - neuronal Nitric Oxide Synthase, NO - Nitric Oxide, PDK1 - Pyruvate Dehydrogenase Kinase 1, PI3K - Phosphatidylinositol 3 Kinase, PKG - Protein Kinase G, PLC- Phospholipase C, TrkB - Tropomyosin Related Kinase B, VDCC - Voltage Dependent Calcium Channel

inactivates, nNOS. A higher rate of Ca flux activates the phosphatase calcineurin and this removes the phosphate group from nNOS, triggering its activation[87]; therefore, if the NMDAR is composed of GluN2B subunits, which are hypothesized to experience a significant Ca flux during ischemia, then ischemic stress would activate nNOS. Once activated it would form nitric oxide (NO). This then activates guanyl cyclase and the formation of cyclic guanosine monophosphate (cGMP), which leads to the activation of protein kinase G (PKG).[88,89]

PKG then activates the phosphatidylinositol 3 kinase (PI3K) -Akt/PKB pathway[89–91] (Figure 2). Increased expression of nNOS[92,93] and the increased activity of PI3K[94] and Akt[95] in ALS spinal cord suggests that this signaling cascade may be active in this disease. Increased activity of nNOS[96] and the PI3K/Akt pathway[97] is also present in cerebral ischemia.

The second NMDAR signaling cascade that may activate Akt/PKB utilizes brain derived neurotrophic factor (BDNF). Stimulation of NMDARs triggers the post synaptic release of BDNF.[98] This interacts, in an autocrine manner, with post synaptic tropomyosin related kinase B (TrkB) receptors[99] and their activation stimulates the PI3K-Akt/PKB pathway[99,100] (Figure 2). Increased TrkB mRNA[101,102] and protein[103] in ALS spinal cord, along with the increased activity of PI3K and Akt noted above, suggest that this signaling cascade may be active in ALS; however, the quantity of BDNF and its role in ALS is still uncertain.[103] With regard to cerebral ischemia, increased levels of BDNF and TrkB and increased stimulation of PI3K/Akt help protect hippocampal area CA3 during periods of oxygen glucose deprivation.[99]

Stimulation of Ca-AMPAR receptors may also trigger signaling cascades leading to Akt/PKB activation. When stimulated they also initiate the post synaptic release of BDNF[104] and, per the above, this activates the Akt/PKB pathway (Figure 2). Ca-AMPARs may also indirectly activate Akt/PKB by assisting in the activation of metabotropic glutamate receptor 5 (mGluR5),[105] which then activates the PI3K - Akt/PKB pathway[106,107] (Figure 2). Stimulation of mGluR5 is also important as it activates phospholipase C (PLC), which triggers the formation of inositol P3 (IP3) and subsequent release of Ca from the endoplasmic reticulum (ER) via IP3 receptors (IP3Rs).[105] (Figure 2). An increased quantity of mGluR5 is present in ALS brain.[108] All of the above suggests that two signaling cascades initiated by NMDARs and two initiated by Ca-AMPARs may stimulate the activation of Akt/PKB.

Additional studies are needed to confirm that stimulation of GluN2B and Ca-AMPARs trigger the activation of Akt/PKB

Per the above, stimulation of NMDARs appears to initiate two signaling cascades leading to the activation of Akt/PKB, but the precise composition of these receptors is currently unknown. There is indirect evidence that they are composed of GluN2B subunits, such as the fact that Ca/CaMKII, involved in the control of nNOS, binds GluN2B[109] and that NO, formed by NMDAR activation, reduces Ca flux through GluN2A receptors[110]; however, additional studies are needed to confirm that it is the stimulation of GluN2B receptors that leads to the activation of Akt/PKB. Also many of the referenced studies, particularly those involving Ca-AMPARs, were performed on hippocampal neurons. Additional studies may be needed to verify that the same signaling cascades occur in cortical neurons.

Activation of Akt/PKB may stimulate the importation of glutamate into neurons, its transport into the mitochondria and its oxidation in the mitochondria matrix

Per the above, it is hypothesized that stimulation of GluN2B and Ca-AMPARs leads to the activation of Akt/PKB. This kinase may allow ischemic neurons to switch their metabolism from the oxidation
FIGURE 3  The upper figure displays the traditional tricarboxylic acid cycle that oxidizes pyruvate after it has been converted to Acetyl CoA by PDH. The lower figure displays a modified cycle that oxidizes glutamate when the traditional cycle has been deactivated by Akt/PKB phosphorylation of PDK1 and the PDK1 phosphorylation/deactivation of PDH. See text for additional discussion. GOT - Glutamate Oxaloacetate Transaminase, PDH - Pyruvate Dehydrogenase Complex

of glucose to the oxidation of glutamate by stimulating transport of glutamate across the plasma membrane and assisting with its importation into the mitochondria. Once glutamate is in the mitochondria matrix Akt/PKB may ensure that it, and not pyruvate, is oxidized.

Akt/PKB plays a role in the neuronal importation of glutamate and its subsequent transport into the mitochondria. It stimulates movement of glutamate into neurons by increasing the expression and activity of the neuronal glutamate transporter EAAT3/EAAC.[111] It then helps transport glutamate into the mitochondria by phosphorylating, and hence activating, the transcription factor cAMP response element binding protein (CREB),[112] which stimulates transcription of the Aralar/Aspartate Glutamate Carrier 1 (Aralar/AGC1) gene.[113] The Aralar/AGC1 protein, located in the inner mitochondrial membrane,[114] transports glutamate into the mitochondria matrix.

Ca plays an important role in the transport of glutamate by Aralar/AGC1. The portion of Aralar/AGC1 extending into the mitochondrial inter-membrane space contains several Ca binding sites.[115] When Ca, released from nearby ER, binds to these sites glutamate is moved into the mitochondria matrix, but the Ca ions remain outside.[116] The Aralar/AGC1 carrier is very sensitive to Ca, as it is activated when the Ca concentration outside the mitochondria membrane is very low.[116,117] As discussed below, a low concentration of Ca outside[117] and inside the mitochondria helps stimulate glutamate oxidation.

Once glutamate is inside the mitochondria Akt/PKB ensures that it, and not pyruvate, is oxidized. One way it does this is by entering the mitochondria[118] and phosphorylating/activating pyruvate dehydrogenase kinase 1 (PDK1).[119] Once activated this kinase phosphorylates, and hence deactivates, the pyruvate dehydrogenase complex (PDH).[119] PDH is the enzyme complex that converts pyruvate into acetyl coenzyme A (acetyl CoA), allowing it to enter the tricarboxylic acid (tca) cycle[120] (Figure 3). Akt/PKB, by activating PDK1 and indirectly deactivating PDH, ensures that pyruvate is not converted into acetyl CoA and, therefore, does not enter the tca cycle (Figure 3). If PDH is inactive this allows glutamate to enter a modified cycle via the activation of glutamate oxaloacetate transaminase (GOT).[67] This enzyme transfers the amino group from glutamate to oxaloacetate transforming glutamate into α-ketoglutarate,[120] which can be oxidized in the modified cycle[67] (Figure 3).
A second way Akt/PKB ensures the oxidation of glutamate is by indirectly controlling the concentration of Ca in the mitochondria matrix. As noted, deactivation of PDH occurs when it is phosphorylated. This is only possible when the Ca concentration in the mitochondria matrix is very low because a high Ca concentration activates pyruvate dehydrogenase phosphatase, which removes the phosphate group from PDH allowing it to become active. The concentration of Ca inside the mitochondria matrix is dictated by its concentration outside the membrane and this is determined, to a great extent, by the rate at which Ca is released from nearby ER, via outflow through IP3Rs. A high rate of flow increases the Ca concentration outside the mitochondria and this opens the mitochondria calcium uniporter (MCU), allowing Ca ions to flood into the mitochondria matrix. This would indirectly activate PDH (via pyruvate dehydrogenase phosphatase) allowing oxidation of pyruvate. A low concentration of Ca outside the mitochondria prevents the opening of the MCU but allows the continued transport of glutamate as Aralar/AGC1 functions at very low Ca concentrations. Activated Akt/PKB ensures a low Ca concentration outside the mitochondria membrane by phosphorylating the IP3Rs on the ER, as this decreases the rate of Ca flow through these channels. It therefore, indirectly, ensures that Ca concentration inside the mitochondria remains very low and that glutamate is oxidized. Given the above, activated Akt/PKB allows neurons to oxidize glutamate by stimulating its importation into the neuron, by assisting in its transport into the mitochondria and by ensuring that glutamate, and not pyruvate, is oxidized in the mitochondria matrix (Figure 2).

This metabolic shift from pyruvate to glutamate oxidation is proposed to occur in ALS and cerebral ischemia; however, there is currently a lack of support for its activity in the former as little is known concerning the expression of EAAT3/EAAC1, the quantity of acetyl CoA or activity of PDH, PDK1, or GOT in ALS tissue. There is more support for the metabolic shift in cerebral ischemia as this condition is associated with an increase in the expression of EAAT3/EAAC1, a decrease in the activity of PDH and an increase in the activity of GOT. There is, however, a lack of information regarding the quantity of acetyl CoA.

**ACTIVATION OF ASTROCYTIC $\text{sys} \text{x}_c^{-}$ HELPS PROTECT ISCHEMIC NEURONS FROM OXIDATIVE STRESS**

Per the above, stimulation of astrocytes by IL-1B during ischemia increases the activity of sys $\text{x}_c^{-}$ and this triggers the export of glutamate and the import of cystine. We have seen that the glutamate may assist neurons by serving as metabolic fuel (Figure 1). The astrocytic influx of cystine is also beneficial to neurons, but in a less direct manner. Astrocytes use cystine, imported via sys $\text{x}_c^{-}$, to synthesize glutathione (GSH), an important antioxidant. Much of this GSH is subsequently exported by the astrocytes and chemically reduces extracellular cystine to cysteine or it is broken down by extracellular enzymes to release cysteine. Cysteine is critically important to mature neurons as they do not possess sys $\text{x}_c^{-}$ and, therefore, must import cysteine, via EAAT3/EAAC1 (Figure 1), to synthesize GSH. Without astrocytes to supply them with cysteine neurons would be unable to produce this vital antioxidant. The astrocytic sys $\text{x}_c^{-}$, therefore, plays two important roles during cerebral ischemia: it supplies neurons with metabolic fuel and protects them against oxidative stress (Figure 1).

**Activity of sys $\text{x}_c^{-}$ under conditions of increased extracellular glutamate**

There have been questions raised regarding the function of sys $\text{x}_c^{-}$ under conditions of increased extracellular glutamate. Early studies revealed that extracellular glutamate competes with cystine for importation via sys $\text{x}_c^{-}$; therefore, an unusually high concentration of extracellular glut!amate, such as that in ALS or cerebral ischemia, may reduce the influx of cystine and synthesis of GSH, exposing both astrocytes and neurons to oxidative stress in a process known as oxidative glutamate toxicity. This toxicity can, however, be prevented by co-activation of the EAAT3/EAAC1 glutamate transporters, possibly because this leads to a localized decrease in extracellular glutamate concentration. As the hypothesis outlined in the current manuscript proposes that ischemic conditions stimulate a significant influx of glutamate through EAAT3/EAAC1 transporters it is likely that the astrocytic sys $\text{x}_c^{-}$ would continue to function in this environment.

**THE SHIFT FROM GluN2A to GluN2B RECEPTORS DURING IN VIVO ISCHEMIA MAY PREVENT EXCITOTOXICITY**

The postulated in vivo shift from GluN2A to GluN2B receptors, triggered by ischemic stress, may prevent the onset of glutamate excitotoxicity. Our improved understanding of the role of glutamate receptors in this pathological process may help explain why. In early in vitro studies exposure of a tissue culture to glutamate resulted in excitotoxicity and neuron death, which could be reduced by blocking NMDARs. Subsequent studies suggested that stimulation of GluN2B receptors triggered excitotoxicity while stimulation of GluN2A receptors led to normal neuron function; however, a study published in 2013 found that targeted stimulation of GluN2B receptors did not trigger excitotoxicity. Instead, the pathological influx of Ca occurred only after an extended period of GluN2A and GluN2B co-activation. This observation could explain the excitotoxicity and neuron death noted in the early in vitro studies. As these were performed prior to the identification of molecules that could block specific NMDARs and under conditions that did not impose oxygen and glucose deprivation (OGD) (OGD, like ischemia in vivo, may inhibit the activity of specific receptors) any glutamate added to the culture would stimulate both GluN2A and GluN2B receptors. Also, little is known concerning the activity of the glutamate–glutamine cycle in vitro. If it does not function in a tissue culture this may also lead to an increase in extracellular glutamate and co-activation of GluN2A and GluN2B receptors, triggering excitotoxicity and neuron death. The observations made by
Zhou et al. emphasize the critical role played by D-serine and Zn, released in vivo following ischemic stress and possibly in vitro following OGD. By inhibiting GluN2A receptors these molecules ensure that only GluN2B receptors are stimulated and hence excitotoxicity does not occur. All of this suggests that glutamate excitotoxicity may not be a normal physiological function but the result of an artificial co-activation of GluN2A and GluN2B receptors.

CONCLUSIONS

The proposed model suggests that the elevated concentration of extracellular glutamate in ALS and cerebral ischemia initiates a metabolic switch to ensure neuron survival when glucose is limited. Under normal in vivo conditions glutamate is quickly removed from the synapse so only GluN2A receptors are stimulated and neurons oxidize glucose. With the onset of severe ischemia neurons release HMGB1, which initiates an inflammatory response as it stimulates microglia to secrete TNFα and IL-1β. Together these factors trigger an increase in extracellular glutamate and D-serine that, in combination with an increase in extracellular Zn, shifts activity from synaptic GluN2A receptors to extrasynaptic GluN2B receptors. It is proposed that these extrasynaptic receptors initiate intracellular signaling cascades that activate Akt/PKB, which then stimulates the importation and oxidation of glutamate. The onset of ischemia may, therefore, enable glutamate to stimulate its own uptake and oxidation.

These ideas have significant implications with regard to treatment of cerebral ischemia/stroke. If stimulation of GluN2B, and Ca-AMPARs, is beneficial during periods of ischemia these receptors should not be blocked as part of a treatment strategy. In fact, supplemental glutamate may benefit ischemic neurons by increasing receptor stimulation and possibly the rate of glutamate influx and oxidation.

Successful treatment of ALS may require a different approach. If ischemia in ALS results from tissue compression due to a build-up of csf, as proposed in this manuscript, treatment should focus on the removal of excess csf. This would alleviate compression on the brain and spinal cord allowing for increased blood flow and reduced ischemic stress.

CONFLICT OF INTEREST

The author has no conflict of interest with regard to the submitted manuscript.

DATA AVAILABILITY STATEMENT

Data requests are to be made to the corresponding author.

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