

Review Article

Sources of extracellular glutamate in developing white matter

Robert Fern

Abstract

Neurotransmitters mediate synaptic communication between neurons and are therefore fundamental to such essential human characteristics as learning, memory, cognition and persona. Recent work indicates that neurotransmitters and their receptors are also used for communication between non-synaptic elements of the nervous system and may be involved in glial-glia, glia-axon and glial-neuronal information transfer and processing. We have recently found evidence that glial cells in developing white matter, which contains no synapses or neuronal somata, express a wide variety of neurotransmitter receptors, including the NMDA-type glutamate receptor that had long thought to be exclusively neuronal. At the point when white matter is laying down myelin and glial cells are forming their long-term morphological arrangement with axons, NMDA receptors mediate post-synaptic-potential input onto glia and may be crucially involved in stabilizing glial-axonal cyto-architecture. There is also strong evidence that glutamate receptors on glial cell membranes greatly heighten the cells susceptibility to injury, a phenomenon that may explain the selective damage of developing white matter found in common human birth disorders such as cerebral palsy. There are many potential sources of extracellular glutamate in ischemic white matter including axons, oligodendrocytes, reverse glutamate transport, loss of astrocyte processes and astrocyte swelling. These potential pathways for glutamate release are described here.

Keywords

Astrocyte; Axon; Glia; Glutamate transport; Ischemia; glial cells; NMDA receptors; glutamate; ischemic white.

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Introduction

Cerebral palsy (CP) is the most common human birth disorder, having a prevalence of 2-3:1000 live births in the West (and much higher in the 3rd world).¹ CP is manifest in the first years of life and continues unabated throughout the lifetime of the patient; there are currently no effective remedies or prophylactic strategies.² In the majority of cases, CP involves an apparently ischemic lesion "periventricular leukomalacia" (PVL) located within developing white matter (WM) structures such as those adjacent to the ventricles,^{2,3} or "periventricular white matter injury" (PWMI) which is centered upon these structures but has a more diffuse distribution.⁴ Patients suffer a variety of symptoms including motor, sensory and cognitive deficits.^{2,4} CP is an incurable lifelong disorder of high prevalence and deserves high priority within medical research. The development of an effective intervention to protect developing WM is an important goal and is dependent upon an understanding of the basic cellular mechanisms involved in the early stages of the injury. Interventions might be effective both in the fetus at risk identified by recent developments in fetal screening or in very premature infants who may suffer WM injury post-partum.⁵⁻⁷ This paper will examine the mechanisms underlying the central pathophysiological event in ischemic injury of developing WM: glutamate release.

PVL is characterized by damage to axons and death of glia, features that may be apparent within 4 hours of the onset of injury.⁸ Severe cases exhibit focal injury described as "coagulating necrosis" while diffuse cases of PWMI feature widespread loss of oligodendrocytes (OLs) and marked delayed hypomyelination.⁹ The location of the damage in periventricular WM is related in part to a low security of blood flow in this region at this age,² and in part to an extremely high vulnerability to ischemia in developing WM OLs.^{10,11} The majority of cases arise during mid-term,¹⁰ and coincide with a key point in OL development. At this age, OL precursor cells are

progressing to the immature OL stage.¹² In cell culture, immature OLs are more sensitive to ischemic injury than any other CNS cell type examined,¹³ and *in situ* they are at least twice as sensitive to acute ischemia as astrocytes or axons.¹³⁻¹⁵ OLs are the myelinating elements of the CNS, and are essential for saltatory conduction of action potentials. The high vulnerability to ischemia of the somata of immature OLs is partly a consequence of non-NMDA glutamate receptor (GluR) expression,¹⁶⁻¹⁹ while the processes of these cells express NMDA GluRs which make them even more susceptible to ischemic-type injury. The physiological significance of GluR expression on OLs is not well understood. However, ischemia evokes a rapid elevation in extracellular glutamate in the developing nervous system, including developing WM,²⁰⁻²³ gating GluRs and producing a toxic influx of Ca^{2+} .^{11,13-15}

The build-up of extracellular glutamate responsible for OL GluR activation is central to the injury process and may originate from a variety of sources (Figure 1). Axons are one potential source of extracellular glutamate (Figure 1, "1"),^{2,11} and mature spinal cord axons contain glutamate that may be lost under ischemic conditions.²⁴ OLs also contain glutamate *in situ*,²⁴ and *in vitro* studies suggest that immature OLs release glutamate during OGD (Figure 1, "2").¹³ However, immature OLs *in situ* show robust accumulation of glutamate during OGD rather than the depletion that would accompany glutamate release.^{14,15} Differences in excitatory amino acid transport (EAAT) expression in OLs *in situ* and *in vitro* may explain this paradox.²⁵ Astrocytes are an important source of glutamate release during ischemia in the mature CNS, with release mediated by several potential mechanisms.^{26,27} Glutamate release from mature gray matter astrocytes is partly mediated by excitatory amino acid transporters (EAATs), which are also expressed in developing WM astrocytes (Figure 1, "3").²⁵ In addition to potential transport-mediated release, astrocytes in developing WM are subject to loss of cell processes (clasmotodendrosis), which will liberate intracellular glutamate into the extracellular space (Figure 1, "4"). The mechanism of acute astrocyte injury involves Na-K-Cl-cotransport (NKCC) and cell swelling,^{14,15} and swelling-mediated astrocyte glutamate release represents a further potential source of extracellular glutamate (Figure 1, "5").^{28,29} Additionally, glutamate release from hemi-channels,^{30,31} cystine-glutamate exchange³² and P2X7 receptor³³ has to be considered. There are therefore a number of potential pools of glutamate available for release in ischemic developing WM, and a variety of possible mechanisms to mediate escape from these pools.

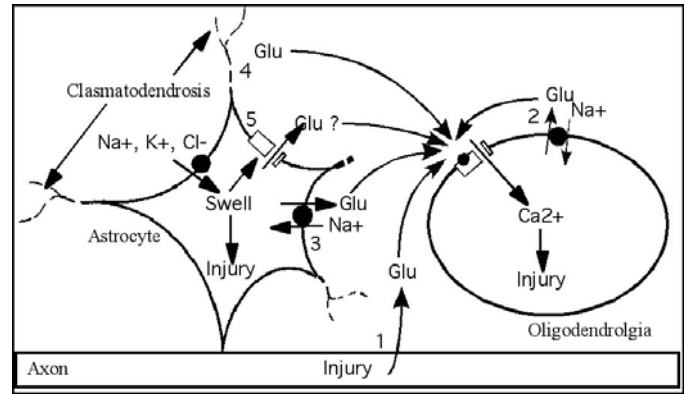


Figure 1. Potential sources of glutamate release in developing white matter (see text for details).

Following the onset of ischemia modeled as oxygen-glucose withdrawal (OGD), aliquots collected from isolated perfused post-natal day 10 rat optic nerve (RON) reveal delayed rises in aspartate and glutamate from a stable baseline (Figure 2, top panel). The non-transmitter amino acids serine and threonine (Figure 2, bottom) were also assayed and their delayed release may correlate with the cell membrane breakdown that is ongoing in the P10 RON after 30-40 min.^{13-15,34} The extra-cellular space of the isolated RON is in communication with the bathing medium and measuring glutamate levels in the perfusate during control perfusion and during OGD shows net glutamate release from all the elements within this model WM structure. A similar approach has recently been used in mature mouse optic nerve.³⁰

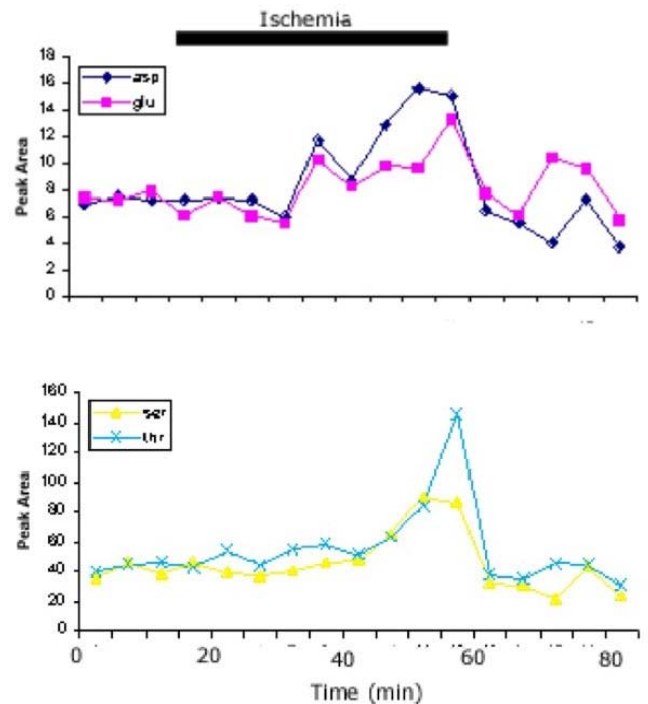


Figure 2. Release of amino acids from P10 rat optic nerve.

EAATs are responsible for clearing synaptically released glutamate from the extracellular space of the CNS.^{26,35,36} Of the five EAATs that have been cloned, three are widely distributed in the mature CNS.³⁵⁻³⁸ GLAST and GLT-1 are generally expressed at high levels and are located largely in glial cells, while EAAC1 is expressed at lower levels and is predominantly neuronal. The voltage and Na⁺-dependence of these glutamate transporters can result in the reversal of transport under ischemic conditions.^{26,39} EAAC1, GLAST and GLT-1 are expressed in mature WM,^{38,40-42} although the cellular distribution of these transporters is controversial.^{41,42} Little is currently known about the cellular distribution of these transporters in neonatal WM. Recent studies have shown that interruption of glutamate transport in WM leads to excitotoxic injury of axons and glia,⁴³ highlighting the importance of glutamate clearance in WM structures. We have used transgenic mice where GFP expression is under the control of glial cell type specific promoters, coupled to standard immuno-histochemistry (IHC) to examine EAAT expression in the neonatal WM.⁴⁴ This approach removes concerns about antibodies cross-reacting during double labeling and results show GLT-1 expression segregated to astrocytes in P10 optic nerve in GFAP-GFP animals with EAAC1 expression absent in these cells. Little GLAST expression was seen in the astrocytes. The IHC studies were coupled to staining for the uptake of exogenously perfused D-aspartate (D-Asp), which confirmed that the transporter expression detected by IHC is functional. D-Asp is a non-metabolizable glutamate analogue taken-up and released from glia via the same mechanisms as glutamate, it is by far the best characterized glutamate analogue for this purpose.^{28,45}

In addition to reverse uptake, astrocyte swelling is also associated with glutamate release.⁴⁶ Two pathways for swelling-induced glutamate release have been identified in cultured astrocytes. Swelling induced by high [K⁺]_o can evoke a transient early phase of release that is mediated by EAATs.^{28,46} A more prominent delayed (~5 min) phase of release is sensitive to block by anion channel inhibitors such as L-644711 and NPPB, and is probably mediated by glutamate-permeable volume-sensitive anion channels (VSAC).^{26,28,47,48} In cultured astrocytes, delayed glutamate release can also be partially prevented by inhibition of NKCC with bumetanide or by NKCC obliteration, while NKCC activation is required for high [K⁺]_o induced astrocyte swelling.^{47,48} Swelling induced by hypotonic media produces glutamate release that is insensitive to bumetanide, but sensitive to L-6644711 and other anion channel blockers.⁴⁹ Cell swelling is therefore associated with delayed glutamate release in an anion channel blocker sensitive fashion, while NKCC activation

produces astrocyte swelling and recruitment of this mechanism. A role for VSAC-mediated glutamate release is indicated by some *in vivo* ischemia studies of gray matter.⁵⁰ Other studies have shown VSAC activation block when intracellular ATP levels fall,⁵¹ but it appears that astrocytes retain elevated cytoplasmic ATP for some time following the onset of ischemic conditions.⁵²

Confocal imaging was used to collect 3-D image stacks of individual cells in the optic nerve and corpus callosum slice from P10 GFP-GFAP mice. The image stacks were rendered and volume changes assessed. Figure 3 shows flattened image-stacks of a single GFP-astrocyte in a brain slice collected in this fashion, and underneath is a plot of the soma volume change (cell swelling) of this astrocyte during and after 30 min of OGD. Significant soma swelling is apparent during OGD which subsequently recovers. This preliminary data suggest that astrocyte swelling may be a significant source of glutamate release in developing white matter during ischemia.

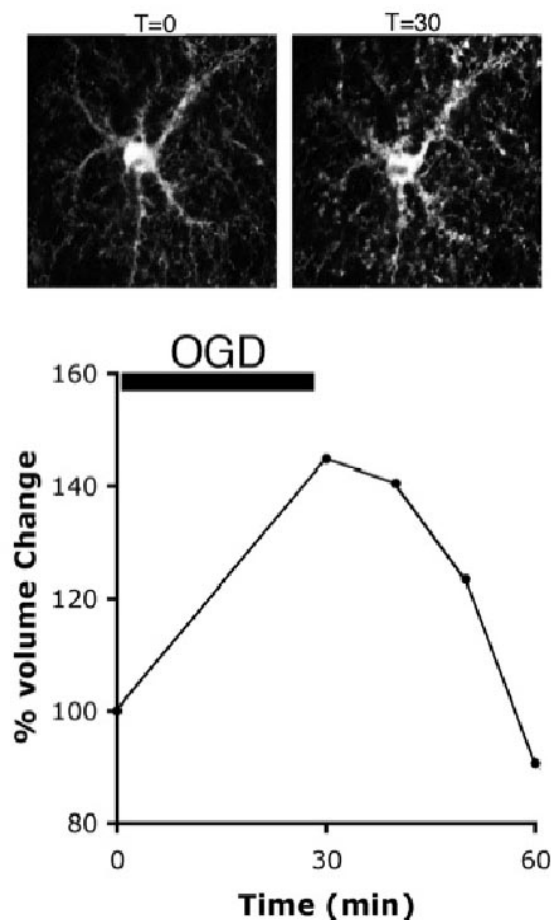


Figure 3. Astrocyte volume changes during OGD in P10 mouse optic nerve (see text for details).

We have shown that developing WM astrocytes die rapidly during OGD,^{14,15} and that loss of astrocyte processes (clasmotodendrosis) is an early feature of developing white matter ischemia.⁵³ Loss of astrocyte processes under ischemic conditions is rapid, being evident in model systems within 15 min.^{53,54} The detached processes rapidly lose membrane integrity, meaning that the relatively high concentration of glutamate in these structures will escape to the ECS.^{14,15} The importance of clasmotodendrosis in WM injury has not been established, but it seems likely that the rapid disintegration of glutamate-rich processes in the neighborhood of oligodendroglia has some role in the subsequent injury of those cells. The rapid shedding of processes following exposure to ischemic conditions does not involve the immediate lysis of the processes, which may maintain membrane integrity for several minutes. It appears therefore that clasmotodendrosis occurs in parallel with acute swelling of the somata, but is more rapid and proceeds via a different mechanism.

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