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Excitotoxic mechanisms in stroke: An update of concepts and treatment strategies

Alan S. Hazell *

Department of Medicine, University of Montreal, Montreal, Quebec, Canada

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Abstract

Cerebral damage as a consequence of glutamate-mediated excitotoxicity represents a major consequence of stroke. However, the development of effective clinical treatments for this potentially devastating condition has been largely unsuccessful to date, despite promising basic research. This review will focus on the latest advances in our understanding of the excitotoxic process including the release of glutamate as a neurotransmitter and the potential contribution of complexins, the important role of astrocytes, including its involvement in glutamate uptake, alterations in glutamate transporter levels, reversed glutamate uptake, and the vesicular release of glutamate. Recent progress in our understanding of the involvement of excitotoxicity in white matter injury following ischemic insults is also discussed, as is oxidative stress and ischemic tolerance, along with an update on the use of treatment strategies with potential therapeutic benefit including stimulation of neurogenesis. Such key issues are at the heart of future interventions directed at limiting the extent of the excitotoxic process, and remain a viable consideration for effective stroke management.

Keywords: Glutamate transporter; Cerebral ischemia; Excitotoxicity; Therapeutic; Complexin; Astrocyte; Glutamate; Neurogenesis

1. Introduction

A complex series of events underlie the pathophysiology of stroke, a leading cause of death and disability worldwide, and which can be either ischemic or hemorrhagic in nature. Most commonly, the phenomenon arises as a consequence of permanent or prolonged occlusion of a cerebral artery (Ameriso and Sahai, 1997). Immediately following interruption of blood flow to brain tissue the injury process is initiated. Without recovery of normal blood flow within a short period of time, death of all cells within the ischemic territory is typically the final outcome (Pulsinelli, 1997). While complete details of this pathway of destruction remain unclear, considerable advances in our knowledge of this process have been made over the last 20 years. Substantial evidence indicates that glutamate-mediated excitotoxicity is a major contributor to the resulting neuropathology. Survival of the affected area is then dependent on its ability to respond to this insult. This review will discuss major events associated with the process of excitotoxicity, therapeutic strategies, and current limitations in the treatment of stroke.

2. Complexins and neurotransmitter release

Rapidly following cessation of blood flow, energy metabolism is compromised in the affected territory of a stroke, quickly leading to large increases in neuronal activity and a resulting enhancement in glutamate release (Benveniste et al., 1984). Recent studies indicate that complexins are important regulators of neurotransmitter release. Complexin I and complexin II are two genes with a high degree of homology which are differentially expressed in human brain (Harrison and Eastwood, 1998). The products of these two genes are 15–16 kDa cytosolic proteins that each contain an α-helical middle domain of approximately 58 amino acids (Pabst et al., 2000). Complexin I is expressed in axosomatic (inhibitory) synapses, while complexin II is localized in axodendritic and axospinous synapses, of which the majority are excitatory (Harrison and Eastwood, 1998; Yamada et al., 1999). At the presynaptic terminal, complexins compete with the chaperone protein α-SNAP

* Correspondence address: NeuroRescue Laboratory, Hôpital Saint-Luc (CHUM), 1058 St-Denis, Montreal, Quebec, Canada H2X 3J4.
Tel.: +1 514 890 8310x35740/35758; fax: +1 514 412 7314.
E-mail address: alan.stewart.hazell@umontreal.ca.

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(soluble *N*-ethylmaleimide-sensitive factor-attachment protein) for binding to SNAP receptors (SNAREs) (Pabst et al., 2000). These SNAREs consist of the synaptic vesicle protein synaptobrevin as well as the synaptic membrane proteins SNAP-25 and syntaxin 1 (McMahon et al., 1995; Pabst et al., 2000). Complexins have been shown to rapidly bind to the SNARE complex in an anti-parallel configuration and with high affinity (Pabst et al., 2002). Prior to vesicle release into the synaptic cleft, these membrane proteins form a stable core complex; interaction of complexins with the SNARE complex influence its stability (Pabst et al., 2000) by promoting the direct assembly of all three SNARE proteins, and which involves an interaction of the transmembrane regions of both syntaxin and synaptobrevin (Hu et al., 2002).

Recently, studies indicate that release of complexins from the SNARE complex by its competition with another synaptic vesicle protein, synaptotagmin 1, triggers fast exocytosis and may explain the speed and efficiency of this process (Tang et al., 2006). Current evidence therefore support an important role for complexins in the modulation of the neurotransmitter release process and the maintenance of normal synaptic function, with alternations in their levels being associated with brain injury and psychiatric illness (Eastwood and Harrison, 2005; Hazell and Wang, 2005; Yi et al., 2006). However, the effects of alterations in complexin levels on neurotransmitter release remain unclear. Increased expression following injection of recombinant complexin II inhibits neurotransmitter release while decreased activity following anti-complexin II antibody treatment results in increased release (Ono et al., 1998), suggesting that the protein may inhibit neurotransmitter release. On the other hand, reports have also demonstrated that loss of complexins leads to reduced neurotransmitter release efficiency (Reim et al., 2001) and loss of hippocampal long-term potentiation (Takahashi et al., 1995), evidence of a facilitatory role. Thus, although the exact role of complexins in synaptic vesicle exocytosis is still unresolved, current evidence suggest that loss of complexin II is likely to destabilize synaptic terminal release of glutamate, contributing to dysfunction of circuitry with possible pathological consequences. Alterations in the function and/or expression of complexins may therefore be a complicating factor contributing to the excessive sustained release of glutamate following acute stroke.

3. Glutamate receptors and excitotoxicity

Neuronal release of glutamate following cerebral ischemia activates several types of pre- and post-synaptic glutamate receptors. The consequent rise in intracellular calcium concentration may lead to mitochondrial dysfunction, generation of reactive oxygen species, and the activation of proteases, phospholipases, and endonucleases, leading to cell death.

3.1. Ionotropic glutamate receptors

There are three known types of ionotropic glutamate receptors based on their pharmacological properties; the *N*-methyl-D-aspartate (NMDA) receptor, AMPA receptor, and the kainate receptor. AMPA and kainate receptors have fast kinetics and are permeable to Na^+ and K^+ and also to low levels of Ca^{2+} (Hollmann et al., 1991). NMDA receptors possess voltage-dependent divalent and monovalent cation channels that are more permeable to Ca^{2+} ions. Because of the higher permeability towards Ca^{2+} and the voltage-dependent fluxes, NMDA receptors are thought to play an important role in development of excitotoxicity. In fact, glutamate receptor agonists such as NMDA, kainate, or quisqualate are shown to be far more potent neurotoxins than glutamate itself when infused into the brain (Obrenovitch et al., 1994). This is due to the presence of efficient uptake mechanisms for glutamate but not for these agents. The neuroprotective influence exerted by NMDA receptor antagonists, as well as by AMPA and kainate receptor antagonists following brain injury in animal models further supports the notion that excessive glutamate receptor stimulation contributes to damage via an excitotoxic process.

It is now generally accepted that Ca^{2+} overload and the activation of Ca^{2+}-dependent enzymes following excessive overstimulation of ionotropic glutamate receptors are key factors determining excitotoxicity. Studies have long established that removal of extracellular Ca^{2+}, but not Na^+, reduces cell death in response to addition of glutamate in cultured neurons (Choi, 1987). The importance of source-specific excitotoxic Ca^{2+} entry mediated via ionotropic receptors was emphasized when it was later shown that Ca^{2+} loads produced by voltage-sensitive Ca^{2+} channels were not damaging but a similar increase in intracellular Ca^{2+} via NMDA receptors was detrimental (Tymianski et al., 1993).

3.2. Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors that produce their effects via signalling mechanisms involving phosphoinositide-dependent processes, cyclic AMP or protein kinase C. Recent studies have identified mGluRs as a way in which neural cells regulate the release of glutamate and its uptake. Three groups of mGluRs have been characterized to date. Group I mGluR agonists have been reported to cause a downregulation of the EAAT1 transporter, while the Group II agonist DCG IV upregulates its expression (Gegelashvili et al., 2000). Group II mGluRs are found on both pre- and post-synaptic membranes as well as glial cells (Petralia et al., 1996), are negatively coupled to cyclic AMP, and regulate glutamate release via presynaptic Group II autoreceptors (Glaum and Miller, 1994). Group III mGluRs are also negatively coupled to cyclic AMP. Cerebral ischemia results in decreased ATP levels (Folbergrová et al., 1992), a source of cyclic AMP via activity of adenylate cyclase. Thus, it is conceivable that loss of glutamate transporter regulation occurs as a consequence of changes in activity of mGluRs due to the declining ATP status.

4. Astrocytes and glutamate transporters

Astrocytes are responsible for many key processes in brain including buffering of K^+, inactivation of released