Recent Biochemical Advances in White Matter Ischaemia

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Abstract
Stroke is one of the leading causes of death and disability in the developed world and as a result is the focus of intensive research. Historically, investigators in the field have focused on the effects of energy deprivation on the neuronal population, but, in recent times, as imaging techniques have become more advanced, a greater appreciation of the extent of non-neuronal injury has emerged. Initial investigations into the pathophysiology of white matter ischaemia reported damage to central myelinated axons via reversal of the Na⁺-Ca²⁺ exchange protein due to Na⁺ loading and ischaemia-induced membrane depolarisation. The latter also gates voltage-sensitive Ca²⁺ channels that contribute to the Ca²⁺ overload both directly and indirectly via Ca²⁺ release from intracellular stores. Excitotoxicity, once thought the unfortunate preserve of neurons, also contributes to white matter damage via both N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors. However, important physiological differences are apparent in these receptors when compared to those present at the synapse, leading researchers to ask whether the molecular diversity of glutamate receptors will provide successful therapeutic interventions in the future. This brief review aims to summarise recent progress in the field of white matter ischaemia.

Introduction
Ischaemic injury of central nervous system (CNS) white matter (WM) is of great clinical interest and has justly been the focus of much attention in recent years; however, this has not always been so. Although WM of the CNS is at risk of injury throughout life, from periventricular leukomalacia in the neonate to stroke and vascular dementia in later life, research into the pathophysiology of such insults has traditionally lagged behind that of grey matter. A possible reason for this might be the long-held belief that WM is less susceptible to ischaemic injury than grey matter [1], however, there has been a rapid expansion of literature demonstrating the vulnerability of WM to energy deprivation [2, 3].

CNS Imaging
The development of computerised tomography and magnetic resonance imaging (MRI) revolutionised the way in which stroke patients are investigated. The extension of MRI technology in the form of proton magnetic resonance spectroscopy has provided another string to the clinician’s investigative bow, by permitting the measurement of N-acetyl containing compounds such as N-acetylaspartate and lactate. With regard to WM, such data can provide information on tract integrity (via N-acetylaspartate loss) and metabolic compromise [4].
In addition, diffusion tensor imaging is a recently developed CNS imaging technique that generates quantitative information on both the degree (via the apparent diffusion coefficient) and directionality (isotropic vs. anisotropic) of water diffusion, allowing accurate 3-dimensional descriptions of anisotropic WM tracts [5]. Further to this, a manipulation of diffusion tensor imaging data known as tractography is now available, which allows WM tracts to be visualised using different colours to describe fibre direction, and colour intensity to describe the degree of anisotropic diffusion [6].

**Injury Pathways during Ischaemia**

An interruption to the flow of blood supplying the CNS results in a rapid loss of ATP and the cessation of ion pumps that maintain ionic homeostasis; thus [K⁺], rises, membrane depolarisation occurs, and a cascade of potentially lethal events is set in motion. In the much studied isolated hypoxic rat optic nerve (a WM tract) model energy deprivation leads to a rapid conduction block attributable to the increase in [K⁺], and ensuing membrane depolarisation [7]. Initial investigations revealed that a toxic Ca²⁺ influx mediated via reversal of the Na⁺-Ca²⁺ exchange protein due to Na⁺ loading, via a non-inactivating Na⁺ conductance and membrane depolarisation, was responsible for loss of function [7]. Some later studies also found a protective role for specific voltage-gated Ca²⁺ channel (VGCC) antagonists [8, 9] that was possibly missed in the earlier studies due to the non-specific actions of some VGCC blockers, which may include inhibition of the release of neuroprotective substances such as γ-amino-butyric acid and adenosine [10].

More recently, writing in the journal Neuron, Ouardouz et al. (2003) [11] reported for the first time the ‘Trojan horse’ of intra-axonal Ca²⁺ release [12]. Working with rat dorsal columns, the authors found that removal of bath Ca²⁺ did not improve post-ischaemic recovery of the tract. Subsequent Ca²⁺ imaging experiments demonstrated that a rise in [Ca²⁺], still persisted in such experiments, indicating ischaemia-induced release of intra-axonal Ca²⁺. Efforts to block Ca²⁺ release from the endoplasmic reticulum proved successful in preventing irreversible injury, leading the authors to describe a mechanism similar to the excitation-contraction coupling seen in skeletal muscle, in which ischaemic depolarisation sensed by L-type VGCCs activates ryanodine receptors on the endoplasmic reticulum, causing release of damaging amounts of Ca²⁺. Later work from the same group on the effects of in vitro chemical ischaemia, this time on the rat optic nerve, also demonstrated a significant amount of intra-axonal Ca²⁺ release, this time dependent upon Na⁺ loading [13]. Thus, preventing Ca²⁺ influx alone may not be sufficient to avoid a permanent loss of function and, interestingly, different WM tracts (dorsal columns vs. optic nerve) may respond differently to the same insult, although the heavy Na⁺-dependence of both Ca²⁺ influx and release from internal stores may provide a common therapeutic target.

In addition to reports on the effects of energy deprivation on the functional unit of WM, the axon, there has been much attention focused on the injury of the myelinating cells of the CNS – the oligodendrocytes. Early studies reported cell damage mediated by overstimulation of ionotropic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors (a process termed excitotoxicity) [14–15], but 3 recently published papers demonstrated the presence of N-methyl-D-aspartate (NMDA) receptors on oligodendrocytes [16–18]. Traditionally, these receptors were thought to exist only in grey matter, where it is established that they play important roles in learning, memory and neuronal excitotoxicity [19–20]. Writing in Nature, Salter and Fern [16], Káradóttir et al. [17] and Micu et al. [18] all independently reported the presence of functional NMDA receptors on oligodendrocytes with the potential to cause injury.

Salter and Fern [16] used a transgenic mouse model in which green fluorescent protein expression is under the control of the oligodendrocyte-specific 2′,3′-cyclic nucleotide 3′-phosphodiesterase promoter. Using confocal microscopy, they demonstrated a loss of fluorescence of both oligodendrocyte somata and processes under conditions of oxygen and glucose deprivation. AMPA/kainate receptor block conferred protection to cell bodies, while, surprisingly, NMDA receptor antagonism provided process protection [16]. Similarly, using WM precursor oligodendrocytes from the cerebellum and corpus callosum, Káradóttir et al. [17] simulated the energy deprivation that contributes to periventricular leukomalacia, the most common neuropathology associated with cerebral palsy [21]. NMDA receptor-mediated currents were then recorded in the whole cell clamp configuration which, interestingly, displayed an uncharacteristically low level of block by Mg²⁺ at the resting potential of the cells. This is a potentially important observation, as it would render the cell more susceptible to a toxic Ca²⁺ influx in the event of prolonged periods of increased extracellular glutamate levels [17].
Finally, Micu et al. [18] studied the glutamate receptor expression of oligodendrocytes in the adult rat optic nerve, by loading the \( \text{Ca}^{2+} \) indicator X-rhod-1 into both oligodendrocyte somata and the cytoplasmic compartment of the myelin sheath. The authors found that while AMPA/kainate antagonists ameliorated ischaemia-induced \( \text{Ca}^{2+} \) rises in cell bodies, NMDA receptor blockade was required to abolish the increase in \( \text{Ca}^{2+} \) signal in myelin. In all 3 papers it was reported that the NMDA receptors may contain an unusual subunit combination of NR1, NR2C and/or -D and NR3 subunits [16–18]. Micu et al. [18] suggest this may have important consequences due to a heightened sensitivity of the receptors to glutamate and glycine, which would facilitate inter-cellular signalling but also predispose the tissue to excitotoxic damage.

**Therapeutic Considerations**

There has been little progress in the treatment of stroke since the approval of tissue plasminogen activator (tPA) a decade ago and, while effective, tPA is limited by the short window of opportunity that exists for administration [22]. There have been several attempts to augment the beneficial effects of tPA with other agents and, although results have been disappointing, progress may not be too far away. For example, the phase III Stroke-Acute Ischemic NXY Treatment (SAINT I) trial provided evidence that free radical scavenging agents may be one possible adjunct [23].

In the context of cell-specific neuroprotection, much optimism surrounded the progression of both NMDA and non-NMDA glutamate receptor antagonists into clinical trials several years ago, but results have been disappointing thus far. The development and subsequent approval of memantine, an uncompetitive NMDA receptor antagonist noted for its novel kinetics, for moderate-to-severe Alzheimer’s disease, may therefore be seen as a step forward in this respect [24]. However, recent work suggests that it may, in fact, only be truly neuroprotective at doses that produce side-effects common to other NMDA receptor antagonists and that the beneficial effects seen in Alzheimer’s disease patients may be due to interference with other neurotransmitter systems [25]. In their papers Salter and Fern [16] and Káradóttir et al. [17] point out that the unusual subunit composition of WM NMDA receptors may facilitate the development of more specific NMDA antagonists and, with regard to this, it has been noted that no selective agonists or antagonists currently exist for NR3 subunits [26].

**Conclusion**

Recent advances in science and technology mean that workers in the field of WM ischaemia have access to a mountain of information upon which to draw plans for future therapeutic strategies. Advances in imaging should enable early identification of those at risk, whilst also allowing careful, accurate monitoring of any interventions developed as a result of recent laboratory research. That clinical trials in the field have produced disappointing results makes such discoveries of utmost importance if clinicians are to one day have effective treatments against ischaemic brain injury at their disposal.

**References**


