Neurobiology of Disease 60 (2013) 61–79

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Role of calpains in the injury-induced dysfunction and degeneration of the mammalian axon

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ARTICLE INFO

Article history:
Received 27 March 2013
Revised 17 July 2013
Accepted 8 August 2013
Available online 19 August 2013

Keywords:
Axon
Calcium
Calpain
Ischemia
Neurofilament
Node
Protease
Synapse
Trauma
Wallerian degeneration

ABSTRACT

Axonal injury and degeneration, whether primary or secondary, contribute to the morbidity and mortality seen in many acquired and inherited central nervous system (CNS) and peripheral nervous system (PNS) disorders, such as traumatic brain injury, spinal cord injury, cerebral ischemia, neurodegenerative diseases, and peripheral neuropathies. The calpain family of proteases has been mechanistically linked to the dysfunction and degeneration of axons. While the direct mechanisms by which transection, mechanical strain, ischemia, or complement activation trigger intra-axonal calpain activity are likely different, the downstream effects of unregulated calpain activity may be similar in seemingly disparate diseases. In this review, a brief examination of axonal structure is followed by a focused overview of the calpain family. Finally, the mechanisms by which calpains may disrupt the axonal cytoskeleton, transport, and specialized domains (axon initial segment, nodes, and terminals) are discussed.

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Abbreviations: AAD, acute axonal degeneration; AIS, axon initial segment; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; ankG, ankyrinG; β-APP, β-amyloid precursor protein; CAM, cell adhesion molecule; CAP, compound action potential; Caspr, contactin-associated protein; Cav, voltage-gated Ca^{2+} channel; CMAP, compound muscle action potential; CNS, central nervous system; CSS1, common small subunit 1; CSS2, common small subunit 2; DIC, differential interference contrast; DNA, deoxyribonucleic acid; DNase I, deoxyribonuclease I; DRG, dorsal root ganglia; dSarm, sterile α/Armadillo/Toll–Interleukin receptor homology domain protein; EDL, extensor digitorum longus; EGTA, ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid; GDC, granular disintegration of the axonal cytoskeleton; GFP, green fluorescent protein; HRP, horseradish peroxidase; IA, intra-arterial; IP, intraperitoneal; IV, intravenous; Kv, voltage-gated K^+ channel; MAP, microtubule-associated protein; MCAO, middle cerebral artery occlusion; Nav, voltage-gated Na^+ channel; NCX, Na^+–Ca^{2+} exchanger; NFH, neurofilament heavy; NFL, neurofilament light; NFM, neurofilament medium; Nmat-1, nicotinamide mononucleotide adenyl transferase 1; NMJ, neuromuscular junction; NrCAM, neuronal cell adhesion molecule; OGD, oxygen glucose deprivation; PNS, peripheral nervous system; SCG, sympathetic superior ganglia; SCI, spinal cord injury; TAI, traumatic axonal injury; TBI, traumatic brain injury; TTX, tetrodotoxin; Ube4b, ubiquitination factor E4B; UPS, ubiquitin–proteasome system; YFP, yellow fluorescent protein; ZNRF1, zinc and ring finger 1.

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Available online on ScienceDirect (www.sciencedirect.com).

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http://dx.doi.org/10.1016/j.nbd.2013.08.010

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Introduction

A growing body of work over the past 20 years has causally linked the calpain family of Ca^{2+} dependent cysteine proteases to axonal dysfunction and degeneration. Axons, which may reach over 1 m in length in humans, are elegantly designed for anterograde and retrograde transport, conduction of electrical impulses, and release of neurotransmitters at synapses. To assist in these critical functions, axons have specialized domains and a unique subaxolemmal and central cytoskeleton, which are disrupted following injury in various experimental models. Strategies to injure axons, such as transection, stretch, ischemia, and complement activation, are known to activate intra-axonal calpains. In this review, I describe the findings from disparate lines of investigations and present a more unified narrative of the pathologic roles that calpains play within the mammalian axon.

Axonal dysfunction and degeneration underlie common neurologic diseases

Traumatic brain injury

Worldwide, traumatic brain injury (TBI) leading to hospitalization or death is estimated to afflict over 10 million people annually and is predicted to surpass many diseases as the major cause of death and disability by the year 2020 (Hyder et al., 2007). The high incidence does not include most patients with mild TBI, which comprises the majority of all brain injuries, as these patients typically do not seek medical treatment. There are no currently approved therapies that specifically target the molecular cascades acutely triggered by TBI. A significant contribution to the morbidity and mortality is from traumatic axonal injury (TAI; Gennarelli et al., 1982; Povlishock, 1992; Smith and Meaney, 2000). In blunt TBI, rotational and shearing forces disproportionately affect long white matter tracts. Except in the most severe cases, where axons tear at the moment of trauma (primary axotomy), injured axons initially show no overt disconnection. However, these injured axons may swell, which is likely due to a disruption of axonal transport, and/or subsequently lose continuity and degenerate (secondary axotomy; Jafari et al., 1997; Maxwell and Graham, 1997; Povlishock and Katz, 2005; Saatman et al., 2003). Better understanding of the molecular cascades that lead to axonal injury and secondary axotomy may allow for targeted therapies for TBI.

Spinal cord injury

In the United States, there are over 250,000 people living with spinal cord injury (SCI; Ray et al., 2011). Like TBI, the victims of SCI are mostly young adults. The only approved therapy for SCI is the steroid methylprednisolone, but its efficacy is highly controversial (Ray et al., 2011). The neurological deficits are predominantly caused by the disruption of ascending and descending tracts (Medana and Esiri, 2003). Immediately after SCI in humans, a proportion of injured axons are not transected (Kakulas, 1999). These axons are swollen and immunoreactive for β-amyloid precursor protein (β-APP), which indicates a disruption of fast anterograde transport. Interestingly, these findings are also commonly found after TBI and TAI.

Traumatic injury to peripheral nerves

Traumatic injury to peripheral nerves results in considerable worldwide disability (Robinson, 2000). Even though traumatic nerve injuries are commonly classified into three types (neuropraxia, axonotmesis, and neurotmesis), injury is probably often mixed; some axons are transected, while others suffer conduction block, which is likely due to focal ischemia and/or demyelination (Robinson, 2000). Similar to axons in the brain and spinal cord, a subset of peripheral nervous system (PNS) axons may not be physically disrupted when subjected to mechanical forces. While some axons may undergo repair, there may be molecular triggers or cascades that push some to subsequently degenerate. Some of these molecules may be intrinsic to axons and be shared by the PNS and central nervous system (CNS).

Cerebral ischemia

The worldwide incidence and burden of strokes and cardiac arrests, which cause global cerebral ischemia, are high (Berdowski et al., 2010; Bramlett and Dietrich, 2004; Gustavsson et al., 2011; Kim and Johnston, 2011). About 50% of the volume of the human brain is white matter, which is almost always injured to some degree in cardiac arrest and stroke (Goldberg and Ransom, 2003). Axons are sensitive to ischemia and hypoxia (Pantoni et al., 1996; Underhill and Goldberg, 2007) even when the ischemic injury does not involve their parental cell bodies (Medana and Esiri, 2003). Ischemia triggers multiple deleterious pathways in axons, some of which are also prominent after mechanical injury to axons. Therefore, therapeutic interventions to mitigate disruption of central conducting pathways may positively impact morbidity and mortality after both ischemia and trauma.

Other diseases

Axonal dysfunction and degeneration are likely important contributors to the morbidity and mortality caused by many other human diseases such as multiple sclerosis, amyotrophic lateral sclerosis, infections (e.g., human immunodeficiency syndrome and cerebral malaria), and peripheral neuropathies (Coleman, 2005; Coleman and Perry, 2002; Glass, 2004; Medana and Esiri, 2003; Shy et al., 2002). A good example is multiple sclerosis, which was initially characterized by multifocal demyelination with relative preservation of axons (Medana and Esiri, 2003). Later studies demonstrated that axon loss occurs earlier than