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Review article

Calpains and neuronal damage in the ischemic brain: The swiss knife in synaptic injury

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ABSTRACT

The excessive extracellular accumulation of glutamate in the ischemic brain leads to an overactivation of glutamate receptors with consequent excitotoxic neuronal death. Neuronal demise is largely due to a sustained activation of NMDA receptors for glutamate, with a consequent increase in the intracellular Ca^{2+} concentration and activation of calcium- dependent mechanisms. Calpains are a group of Ca^{2+}-dependent proteases that truncate specific proteins, and some of the cleavage products remain in the cell, although with a distinct function. Numerous studies have shown pre- and post-synaptic effects of calpains on glutamatergic and GABAergic synapses, targeting membrane- associated proteins as well as intracellular proteins. The resulting changes in the presynaptic proteome alter neurotransmitter release, while the cleavage of postsynaptic proteins affects directly or indirectly the activity of neurotransmitter receptors and downstream mechanisms. These alterations also disturb the balance between excitatory and inhibitory neurotransmission in the brain, with an impact in neuronal demise. In this review we discuss the evidence pointing to a role for calpains in the dysregulation of excitatory and inhibitory synapses in brain ischemia, at the pre- and post-synaptic levels, as well as the functional consequences. Although targeting calpain-dependent mechanisms may constitute a good therapeutic approach for stroke, specific strategies should be developed to avoid non-specific effects given the important regulatory role played by these proteases under normal physiological conditions.

Abbreviations: ADAR2, adenosine deaminase acting on RNA, type 2; AIF, apoptosis inducing factor; AMPAR, α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid (AMPA) receptors; AP- 2, adaptor protein complex 2; ARMS, ankyrin repeat-rich membrane spanning; BAR domain, Bin/amphiphysin/Rvs domain; BDNF, brain- derived neurotrophic factor; C2L, C2-like domain; $[Ca^{2+}]_i$, intracellular free calcium concentration; cain/cabin 1, calcineurin inhibitor/calcineurin-binding protein 1; CaMKII, Ca^{2+}/calmodulin- dependent protein kinase II; CDK5, cyclin- dependent kinase 5; CF, cytosolic fraction; CREB, cAMP response element-binding protein; CTD, C-terminal domain; EAAT, excitatory amino acid transporter; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; FOXO, forkhead box protein O; $GABA_AR$, $GABA_A$ receptors; GAD, glutamic acid decarboxylase; GAT, GABA transporter; GDNF, glial cell line-derived neurotrophic factor; GRIP, glutamate receptor interacting protein; GSK3, Glycogen synthase kinase 3; HDAC, histone deacetylase; IAP-LC–MS/MS, immunoaffinity purification coupled to liquid chromatography/mass spectrometry; JNK, c-Jun N-terminal kinase; KAR, kainate receptors; KCC2, potassium chloride cotransporter 2; Kidins220, Kinase D-interacting substrate of 220 kDa; MAGUK, membrane-associated guanylate kinase; MCAO, middle cerebral artery occlusion; MEF2D, myocyte enhancer factor 2D; mGluR, metabotropic glutamate receptors; MLK3, mixed-lineage protein kinase 3; MMF, microsomal and membrane fraction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide; MUPP1, multi-PDZ domain protein 1; Naspm, 1-naphthyl acetyl spermine; NLS, nuclear localization signal; NMDAR, *N*-methyl-D-aspartate (NMDA) receptors; NMF, nuclear and mitochondrial fraction; nNOS, neuronal nitric oxide synthase; OGD, oxygen and glucose deprivation; PC, protease core domain; PDZ, PSD95/Drosophila disc large tumor suppressor (DlgA)/ZO-1 protein; PEF, penta-EF hand; PH LPP1, protein phosphatase PH domain and leucine-rich repeat protein phosphatase 1; PIKE-L, long form of phosphoinositide 3-kinase enhancer; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PML, promyelocytic leukemia; PSD95, postsynaptic density 95; Ptyr, phosphotyrosine; SAP97, synapse-associated protein 97; SFK, Src family of kinases; SH3, Src homology 3; SNAP-25, synaptosomal-associated protein of 25 kDa; STEP, striatal enriched protein tyrosine phosphatase; TARP2, transmembrane AMPA receptor regulatory protein; UPS, ubiquitin-proteasome system; VDCC, voltage-dependent calcium channels; VGAT, vesicular GABA transporter; VGLUT, vesicular glutamate transporter; 4-VO, four-vessel occlusion.

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Contents

1. Introduction

Brain ischemia arises from a disturbance in the blood supply to the brain, mainly due to cardiac arrest or to occlusion of a blood vessel. A therosclerotic or thrombotic blockade of blood vessels limits blood flow to a discrete area of the brain, while cardiac arrest affects the whole brain. The resulting decrease in oxygen supply and the consequent impairment in the metabolic activity affects the balance between excitatory and inhibitory neurotransmission, due to an upregulation of the glutamatergic activity and a downregulation of the GABAergic neurotransmission (Choi, 1987; Schwartz-Bloom and Sah, 2001). The extracellular accumulation of glutamate leads to an overstimulation of glutamate receptors, with a consequent intracellular free calcium concentration ($[Ca^{2+}]_i$) overload, which plays a key role in neuronal demise (excitotoxicity). The toxic effects of glutamate-evoked $[Ca^{2+}]_i$ dysregulation are at least in part mediated by activation of calpains, a group of calcium- dependent proteases present in different neuronal compartments. This review will first describe the calpain- calpastatin system and the mechanisms responsible for the activation of calpains in ischemic and excitotoxic conditions. We will also discuss the impact of calpain activation on glutamatergic and GABAergic neurotransmission in the ischemic brain, and the potential role of calpain inhibition as a tool for neuroprotection.

In addition to the effects on the neuronal proteome mediated by calpains, brain ischemia also alters neuronal proteostasis by inhibition of the ubiquitin-proteasome system and release of cathepsins from the lysosomal compartment. The role of these proteolytic systems in neuronal demise in brain ischemia has been reviewed elsewhere (Caldeira et al., 2014; Yamashima and Oikawa, 2009), and therefore will not be discussed here.

2. The calpain-calpastatin system

2.1. Calpains

Calpains are a family of calcium-dependent neutral cysteine proteases ubiquitously expressed (Goll et al., 2003). When active, these enzymes modify the structure and activity of their protein targets by limited proteolysis. This mechanism is distinct from the complete protein degradation mediated by proteasomes and lysosomes, and regulates different cellular processes such as tissue regeneration, cell development, proliferation, differentiation, gene expression, signal transduction, synaptic plasticity and apoptosis (Goll et al., 2003; Liu et al., 2008; Ono and Sorimachi,