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The role of lysosomal rupture in neuronal death

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ABSTRACT

Apoptosis research in the past two decades has provided an enormous insight into its role in regulating cell death. However, apoptosis is only part of the story, and inhibition of neuronal necrosis may have greater impact than apoptosis, on the treatment of stroke, traumatic brain injury, and neurodegenerative diseases. Since the "calpain–cathepsin hypothesis" was first formulated, the calpain- and cathepsin-mediated regulation of necrotic cascades observed in monkeys, has been demonstrated to be a common neuronal death mechanism occurring from simpler organisms to humans. However, the detailed mechanism inducing lysosomal destabilization still remains poorly understood. Heat-shock protein-70 (Hsp70) is known to stabilize lysosomal membrane and protect cells from oxidative stress and apoptotic stimuli in many cell death pathways. Recent proteomics approach comparing pre- and post-ischemic hippocampal CA1 neurons as well as normal and glaucoma-suffered retina of primates, suggested that the substrate protein upon which activated calpain acts at the lysosomal membrane of neurons might be Hsp70. Understanding the interaction between activated calpains and Hsp70 will help to unravel the mechanism that destabilizes the lysosomal membrane, and will provide new insights into clarifying the whole cascade of neuronal necrosis. Although available evidence is circumferential, it is hypothesized that activated calpain cleaves oxidative stress-induced carbonylated Hsp70.1 (a major human Hsp70) at the lysosomal membrane, which result in lysosomal rupture/permeabilization. This review aims at highlighting the possible mechanism of lysosomal rupture in neuronal death by a modified "calpain–cathepsin hypothesis". As the autophagy–lysosomal degradation pathway is a target of oxidative stress, the implication of autophagy is also discussed.

Contents

Abbreviations: CA1, cornu *Ammonis* 1; ER, endoplasmic reticulum; Hsp, heat-shock protein; Hsc, heat-shock constitutive protein; HNE, 4-hydroxy-2-nonenal; Lamp, lysosome-associated membrane protein; MALDI-TOF/TOF, matrix-assisted laser desorption ionization-time of flight/time of flight; MS/MS, mass spectrometry/mass spectrometry; ROS, reactive oxygen species; 2D DIGE, two-dimensional differential in-gel electrophoresis; DNPH, 2,4-dinitrophenylhydrazine; DNP, 2,4-dinitrophenylhydrazone; 2D Oxyblots, two-dimensional gel electrophoresis with immunoblot detection of carbonylated proteins; V-ATPase, vacuolar-type proton ATPase.

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1. Background: calpain activation (Fig. 1)

During embryonic development, neurons are pruned by apoptosis with excess ones being removed to ensure proper and precise synaptic connections. In contrast, during adult life, neurons prematurely die mostly by necrosis when subject to acute or chronic neurotoxic insults. Typically, neuronal necrosis is prominent in ischemic brain injury, and it underlies the pathology of also traumatic brain injury and neurodegenerative diseases. Nowadays, the incidence of brain ischemia is dramatically increasing in Western countries, becoming thus a major cause of chronic physical and/or cognitive disability (Flynn et al., 2008). Accordingly, detailed molecular analysis of ischemic neuronal death should have important clinical implications. Ischemic neuronal death develops selectively in cells most vulnerable to hypoxic damage, such as hippocampal CA1 neurons, medium-sized neurons in the striatum, and Purkinje cells in the cerebellum (Rami et al., 2008). From rodents to primates, transient brain ischemia is well known to cause delayed neuronal death in the hippocampal CA1 neurons. As ischemic CA1 neuronal death gradually develops within 5–7 days after the insult, it can provide an appropriate time window for studying the underlying mechanism of neuronal death. In the past two decades, substantial efforts focusing on CA1 have been made to elucidate the biochemical and molecular mechanisms of ischemic neuronal death. However, many basic questions remain unanswered.

The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) pump as well as extrusion of calcium through the plasma-membrane Ca^{2+}-ATPase, maintains free cytosolic Ca^{2+} concentrations at approximately 100–200 nM, which is orders of magnitude less than extracellular levels. Excessive NMDA receptor activation induces Ca^{2+} influx and its release from the intracellular stores. Such disruption of Ca^{2+} homeostasis plays a key role in neuronal death, and the intracellular Ca^{2+} mobilization initiates neuronal death (Fig. 1). This, in turn, leads to autophagic death, apoptosis, or necrosis, depending upon the severity of the insult. Ca^{2+} overload triggers lethal downstream cascades, including calpain and caspase activation, and can also lead to mitochondrial dysfunction. The disappointing results of clinical trials using various Ca^{2+} blockers validate the importance of elucidating the downstream cascade of Ca^{2+}-mobilization in order to develop a novel neuroprotective strategy.

The molecular cascades of the cell death are diverse, and have been categorized into two main types, apoptosis or necrosis. In the embryonic brain, apoptosis plays an integral part for its anatomic and functional maturation. Intracellular ATP levels are a primary determinant of apoptosis or necrosis. In the adult brain, the apoptotic route will predominate when and where ATP is plentiful, whereas the necrotic route will predominate when and where ATP is depleted (Rami et al., 2008). Necrosis typically occurs following ischemia, hypoxia, stroke or trauma, but it has also been reported in neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's disease. While a mild Ca^{2+} increase preferentially induces apoptosis, an abrupt and severe Ca^{2+} increase initiates necrosis. During stroke, for instance, the core area being immediately and drastically affected by the restricted blood flow usually suffers from necrosis. On the contrary, many neurons undergo apoptosis in the surrounding penumbra area which is less

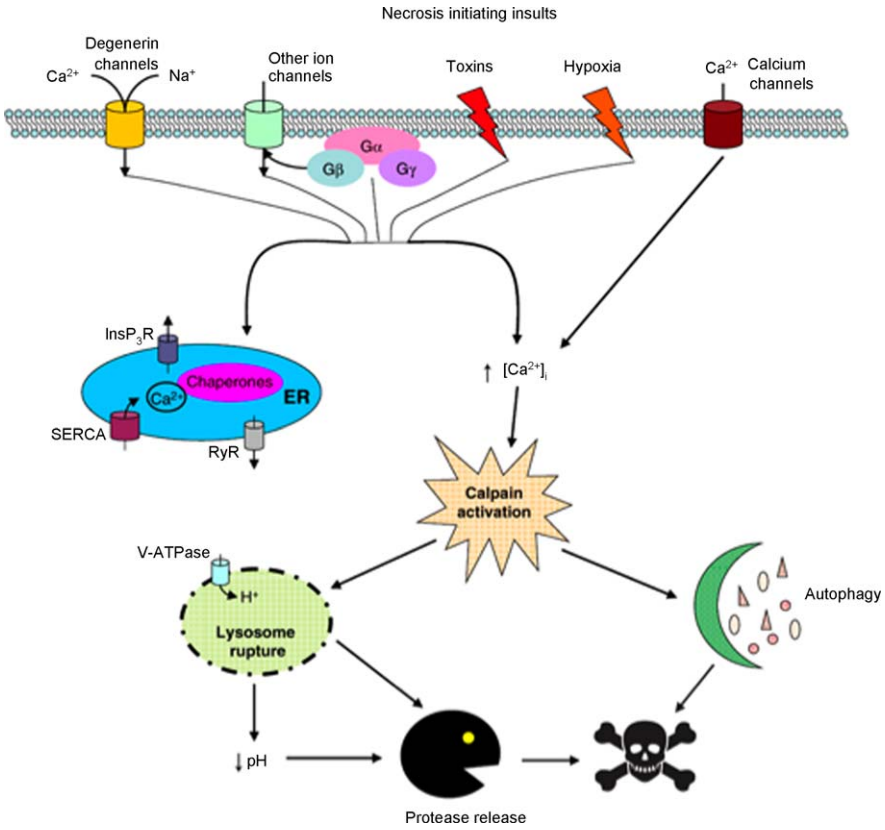

Fig. 1. A flow chart of calpain–cathepsin and autophagy pathways from necrosis initiating insults to cell death. Upon induction of necrotic insults, intracellular Ca^{2+} is increased mainly by release from ER, which activates μ-calpain. Parallel to activated calpain-induced lysosomal rupture, autophagy is upregulated directly and/or through calpain activation, but eventually synergizes with extra-lysosomal cathepsins to mediate cell death. A cup-formed phagophore (also called isolation membrane) surrounds cytosolic substrates thereby creating an autophagosome. As the cytoplasmic pH is reduced after the lysosomal rupture, this increases the potential for extra-lysosomal cathepsins to degrade cell constitutive proteins at an optimal pH. $[Ca^{2+}]_i$, cytoplasmic calcium concentration; ER, endoplasmic reticulum; $InsP_3R$, inositol triposphate receptor; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; V-ATPase, vacuolar H^+-ATPase (cited from Kourtis and Tavernarakis, 2009).