Ca^{2+} Signals and Neuronal Death in Brain Ischemia

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Abstract—Although Ca^{2+} signals are necessary for cell communication and survival, abnormal cellular Ca^{2+} load can trigger different cell death programs. Ca^{2+} mediates cell death by activating proteases (ie, calpains), by reinforcing signals leading to caspase activation or by triggering other catabolic processes mediated by lipases and nucleases. Failure in the clearance of excitatory amino acid is a critical determinant of neuronal loss in the ischemic brain. Glutamate activates glutamate-ionotropic receptors at synaptic and extra-synaptic sites, causing prolonged neuronal depolarization and triggering deregulation of cellular ion homeostasis, mainly intracellular calcium and sodium. The mechanisms leading to the sustained calcium deregulation in excitotoxic conditions are only in part elucidated. Recently, we have shown that calpains mediate the inhibition of calcium efflux in primary dissociated neurons challenged with excitotoxic glutamate concentrations. Calpains cleave the sodium-calcium exchanger (NCX) and inhibit its capability to remove calcium accumulated as a consequence of the excitotoxic stimulus. Our findings highlight the link between calcium-dependent proteases, calcium overload and neuronal degeneration after an excitotoxic insult. **(*Stroke*. 2007; 38[part 2]:674-676.)**

Key Words: calcium transporter ■ cell cultures ■ neurochemistry ■ neuroprotection

It is well established that genetically encoded programs decide the fate of individual cells or organs during development and in normal tissue-cell turnover. However, in recent years it has also become clear that cells execute one or more biochemical programs to signal or execute cell death, particularly under pathological conditions. In many instances, developmental cell death and death under pathological conditions share similar morphological features as well as signals and execution systems. For example, the characterization of the main signals, modulators and executioners of programmed cell death in *Caenorhabditis elegans* has led to the understanding of very important death pathways in pathological cell death of mammalian organisms. This is not a singularity of the death program that we call apoptosis, because the concept can be extended to other paradigms of cell death. For example, autophagic cell death, whose main feature is the presence of cytoplasmic lysosomes–derived vacuoles, is frequent in both neuronal development and neurodegenerative disease.[1] The existence of conserved biochemical pathways to signal and execute cell death has somewhat reduced the emphasis on the morphological characterization of cell death. Despite efforts to define individual forms of cell death based on their appearance, it is clear that in all circumstances cell disassembly involves nuclear fragmentation/dissolution, organelle disruption (sooner or later) and eventually membrane lysis and phagocytosis. Thus, condensation and eventual fragmentation of the nucleus occurs in apoptosis as well as in necrosis and autophagic cell death. In apoptosis, as well as in necrosis, the mitochondria can be partially or totally damaged and, again, in both apoptosis and necrosis the cytoskeletal structure is compromised and molecules are exposed at the cell surface to promote recognition and scavenging. Therefore, the concept of a death program is not necessarily linked to a morphological appearance. It seems more likely that several death executing routines may be activated at once within injured cells, one or the other becoming predominant depending on the stimulus and the tissue metabolic conditions. For example, there is increasing evidence that apoptotic-like features can also be found when the main executioners of apoptosis, the caspases, are inhibited,[2] whereas caspase-mediated cleavage of relevant substrates may be involved in cell lysis/ necrosis.[3] Thus, under pathological conditions several protease families may cooperate to disassemble cells targeting different organelles or cellular substructures. It is, for example, remarkable that caspases and calpains share many substrates. Notably the cleavage sites for the 2 protease families are in several cases located on very close regions within the same protein.[3] Whereas the predominance of one or another death executing mechanism may be dictated by factors as different as energy requirement, signaling molecules and the intensity of a given insult, in many instances the differentiation program within a given cell tissue dictates the way to die. This is particularly true of neurons, where spatial selectivity of death signals and promiscuity of execution systems can result in the complex and relatively slow demise, which occurs in neurodegenerative disease. The promiscuity of death subroutines is evident in brain ischemia, where the intensity of the insult and the death triggers

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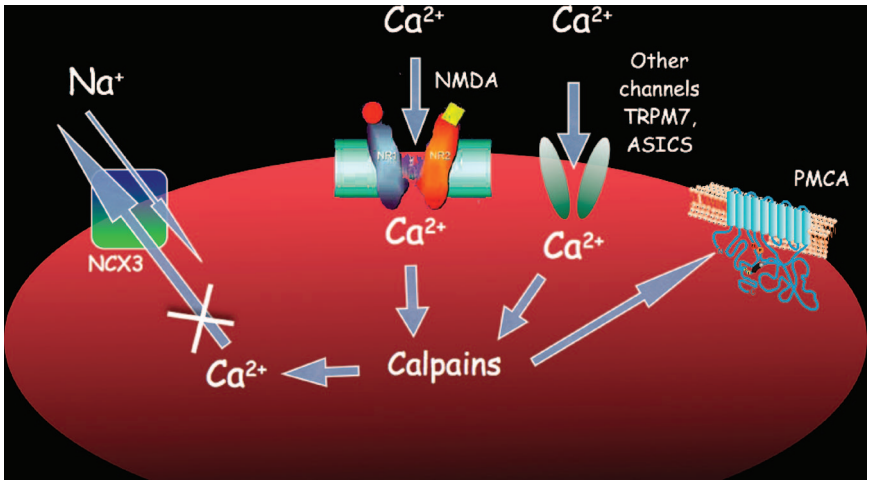

Ca^{2+} entry through NMDA and possibly other channels triggers the excitotoxic Ca^{2+} overload. Calpains are activated and cleave NCX3 precluding the possibility to restore Na/Ca^{2+} exchange. This ultimately leads to an irreversible build-up of the intracellular Ca^{2+} concentration.

activate both caspase-dependent and independent death pathways. The latter involve the Ca^{2+} activation of the calpain family of proteases.

Death Signals in Brain Ischemia: Calpains Amplify Lethal Ca^{2+} Signals

Failure in the clearance of excitatory amino acids causes sustained gating of receptor-operated channels,[4] with prolonged neuronal depolarization and calcium overload. In ischemic regions, excitotoxic stimulation can trigger an inflammatory response and apoptotic or necrotic cell death, depending on the intensity of the insult.[5,6] Subsequent work has shown that synaptic glutamate overstimulation may predominantly elicit apoptosis,[7] whereas more widespread excess glutamate causes predominantly necrosis. Thus, treatments that reduce ion imbalance by blocking gating of glutamate-ionotropic receptor and voltage gate channels can protect neurons and reduce brain infarct.[8,9] However, as shown recently, after an ischemic insult, other plasma membrane channels can contribute to neuronal depolarization and Ca^{2+} accumulation.[10,11] Hence, the study of downstream events triggered by unphysiological calcium increase and involved in neuronal excitotoxic degeneration can potentially provide a pharmacological target to prevent extensive neuronal demise during stroke. Increasing evidence suggests that unregulated calcium-dependent proteolysis is a key process during excitotoxicity and suppression of calpains activity ameliorates postischemic damage.[12,13] We have recently shown that calpains can cleave one of the major isoforms (isoform 3) of the sodium-calcium exchanger (NCX3) that operate Ca^{2+} efflux from neurons. Lentiviral delivery of calpastatin, the endogenous calpain inhibitor, prevented cleavage of NCX3 and prevented the secondary Ca^{2+} overload linked to neuronal demise.[14] Ca^{2+} overload was also prevented in the majority of neurons by overexpressing a functional NCX isoform (NCX2), whereas it was exacerbated by siRNA downregulation of NCX3. Replacement of NCX3 with the functional isoform NCX2 also protected neurons from excitotoxicity. Our findings suggest a mechanism that can be downstream of the initial Ca^{2+} influx through different plasma-membrane channels (Figure) and, as such, is potentially interesting for pharmacological intervention aimed to prevent an irreversible Ca^{2+} overload.

As shown by others, NCX is a fundamental player during ischemia[15,16] and neuronal degeneration[17] and its downregulation leads to massive calcium overload and neuronal demise. Interestingly, NCX3 is required also in the maturation of dissociated neurons[18] and in the control of intracellular Ca^{2+} in skeletal muscle fibers.[19] Not surprisingly, sustained and unregulated protease activity has been linked with a wide number of pathological conditions, including neuronal degeneration.[20] As shown previously, caspases can affect the activity of the other major Ca^{2+} extruding system, the plasma membrane Ca^{2+} ATPase (PMCA), which is internalized after cleavage.[3] Recent work has extended these observations showing that during glutamate-mediated neuronal death, PMCAs can be internalized, which contributes to Ca^{2+} deregulation and further supports a fundamental role for Ca^{2+} transporters in neuronal demise.[21]

In conclusion, it is apparent that different death programs/routines have in common at least 2 fundamental features: (a) they are evolutionarily conserved. This may be explained by a dual role of many components as life and death signals; (b) they involve the activation of one or more families of proteases and possibly other degradative enzymes. The diverse actions of intracellular Ca^{2+} signals provide an optimum example of the fact that the same signal may be physiological or detrimental depending on threshold and cellular conditions. Tight homeostatic mechanisms regulate intracellular Ca^{2+} concentration to spatially and temporally localize Ca^{2+} signals[22] and to allow multiple Ca^{2+}-mediated signaling cascades to occur independently within the same cell. However, excessive Ca^{2+} influx, release from intracellular stores or impairment in the Ca^{2+}-extruding machinery can overcome Ca^{2+}-regulatory mechanisms and lead to cell death.[20] Ca^{2+} signals can also reinforce the execution of Ca^{2+}-independent death subroutines.[20] Redistribution of Ca^{2+} within intracellular stores can amplify apoptotic signals,[23] but as shown above can also initiate cell death execution by calpains.[24]

Disclosures

None.

References

1. Yuan J, Lipinski M, Degterev A. Diversity in the mechanisms of neuronal cell death. *Neuron*. 2003;40:401–413.

2. Volbracht C, Leist M, Kolb SA, Nicotera P. Apoptosis in caspase-inhibited neurons. *Mol Med*. 2001;7:36–48.
3. Schwab BL, Guerini D, Didszun C, Bano D, Ferrando-May E, Fava E, Tam J, Xu D, Xanthoudakis S, Nicholson DW, Carafoli E, Nicotera P. Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. *Cell Death Differ*. 2002;9:818–831.
4. Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu Rev Neurosci*. 1990;13:171–182.
5. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci U S A*. 1995b;92:7162–7166.
6. Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron*. 1995;15:961–973.
7. Leist M, Volbracht C, Kühnle S, Fava E, Ferrando-May E, Nicotera P. Caspase-mediated apoptosis in neuronal excitotoxicity triggered by nitric oxide. *Mol Med*. 1997;3:750–764.
8. Chen HS, Pellegrini, JW Aggarwal SK, Lei SZ, Warach S, Jensen FE, Lipton SA. Open-channel block of *N*-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci*. 1992;12:4427–4436.
9. Choi DW, Koh JY, Peters S. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J Neurosci*. 1988;8:185–196.
10. Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonald JF, Tymianski M. A key role for TRPM7 channels in anoxic neuronal death. *Cell*. 2003;115:863–877.
11. Xiong ZG, Zhu XM, Chu XP, Minami M, Hey J, Wei WL, MacDonald JF, Wemmie JA, Price MP, Welsh MJ, Simon RP. Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell*. 2004;118:687–698.
12. Higuchi M, Tomioka M, Takano J, Shirotani K, Iwata N, Masumoto H, Maki M, Itohara S, Saido TC. Distinct mechanistic roles of calpain and caspase activation in neurodegeneration as revealed in mice overexpressing their specific inhibitors. *J Biol Chem*. 2005;280:15229–15237.
13. Lee KS, Frank S, Vanderklish P, Arai A, Lynch G. Inhibition of proteolysis protects hippocampal neurons from ischemia. *Proc Natl Acad Sci U S A*. 1991;88:7233–7237.
14. Bano D, Young KW, Guerin CJ, Lefeuvre R, Rothwell NJ, Naldini L, Rizzuto R, Carafoli E, Nicotera P. Cleavage of the plasma membrane Na+/Ca2+ exchanger in excitotoxicity. *Cell*. 2005;120:275–285.
15. Pignataro G, Gala R, Cuomo O, Tortiglione A, Giaccio L, Castaldo P, Sirabella R, Matrone C, Canitano A, Amoroso S, Di Renzo G, Annunziato L. Two sodium/calcium exchanger gene products, NCX1 and NCX3, play a major role in the development of permanent focal cerebral ischemia. *Stroke*. 2004a;35:2566–2570.
16. Pignataro G, Tortiglione A, Scorziello A, Giaccio L, Secondo A, Severino B, Santagada V, Caliendo G, Amoroso S, Di Renzo G, Annunziato L. Evidence for a protective role played by the Na+/Ca2+ exchanger in cerebral ischemia induced by middle cerebral artery occlusion in male rats. *Neuropharmacology*. 2004b;46:439–448.
17. Wang T, Xu H, Oberwinkler J, Gu Y, Hardie RC, Montell C. Light activation, adaptation, and cell survival functions of the Na+/Ca2+ exchanger CalX. *Neuron*. 2005;45:367–378.
18. Gomez-Villafuertes R, Torres B, Barrio J, Savignac M, Gabellini N, Rizzato F, Pintado B, Gutierrez-Adan A, Mellstrom B, Carafoli E, Naranjo JR. Downstream regulatory element antagonist modulator regulates Ca2+ homeostasis and viability in cerebellar neurons. *J Neurosci*. 2005;25:10822–10830.
19. Sokolow S, Manto M, Gailly P, Molgo J, Vandebrouck C, Vanderwinden JM, Herchuelz A, Schurmans S. Impaired neuromuscular transmission and skeletal muscle fiber necrosis in mice lacking Na/Ca exchanger 3. *J Clin Invest*. 2004;113:265–273.
20. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol*. 2003;4:552–565.
21. Pottorf WJ, Johanns TM, Derrington SM, Strehler EE, Enyedi A, Thayer SA. Glutamate-induced protease-mediated loss of plasma membrane Ca pump activity in rat hippocampal neurons. *J Neurochem*. 2006;98:1646–1656.
22. Criddle DN, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R. Ethanol toxicity in pancreatic acinar cells: mediation by non-oxidative fatty acid metabolites. *Proc Natl Acad Sci U S A*. 2004;101:10738–10743.
23. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science*. 2003;300:135–139.
24. Nicotera P, Hartzell P, Baldi C, Svensson S-A, Bellomo G, Orrenius S. Cystamine induces toxicity in hepatocytes through the elevation of cytosolic Ca2+ and the stimulation of a nonlysosomal proteolytic system. *J Biol Chem*. 1986;261:14628–14635.

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