8 Calpain and Caspase in Ischemic and Traumatic Brain Injury

Kevin K.W. Wang

CONTENTS

8.1 Two Different Forms of Neuronal Cell Death: Apoptosis and Oncosis ............
8.2 Apoptosis and Oncosis in Neurodegeneration ...........................................
8.3 Activation of Calpain 1 and 2 in Oncotic Death ........................................
8.4 Caspase Cascade in Apoptosis ........................................................................
8.5 Calpain activation in Certain Apoptosis Systems ...........................................
8.6 Caspase-3 and Caspase 1 and 2. What They Have in Common ....................
   8.6.1 Caspase-3 and Calpain 1 and 2 Share Many Common or Related Substrates
   8.6.2 Tenzing Exx-Things Worse ....................................................................
8.7 Calpain-Caspases Crosstalk ...........................................................................
8.8 Perspective .......................................................................................................
8.9 Acknowledgments .........................................................................................
8.10 References .....................................................................................................

8.1 TWO DIFFERENT FORMS OF NEURONAL CELL-DEATH: APOPTOSIS AND ONCOSIS

Programmed cell death or apoptosis is cell death generally characterized by the presence of DNA fragmentation at the nuclear linkage region and DNA condensation, as well as cell shrinkage giving way to the formation of apoptotic bodies.1 Apoptosis occurs physiologically during development and other stages of its lifetime to eliminate unwanted cells. Unscheduled apoptosis also occurs in a large number of pathological or injurious conditions.2 Oncosis (or necrotic necrosis) occurs when cells were injured acutely and severely to the point that is beyond repair. It is characterized by the presence of massive ions (Ca²⁺ and Na⁺) influx, mitochondria and cell swelling, massive multi-site DNA breakage and plasma membrane bursting. Both forms of cell death have been well documented in acutely injured neurons. Necrosis is a term that means “mutilating,” which occurs in the end stage of both apoptosis and oncosis, thus is non-selecting and should be avoided.3

© 2001 by CRC Press LLC
181
8.2 APOPTOSIS AND ONCOSIS IN NEURODEGENERATION

Not very long ago it was commonly believed that acute neurological cell death is necrotic of nature. That changed when newer evidence for apoptosis such as the presence of DNA laddering and nuclear DNA condensation in dying/dead neurons were identified in models of cerebral ischemia and excitotoxicity. It was followed by the evidence for apoptotic neuronal death in experimental TBI as well as spinal cord injury (SCI). In fact, apoptotic neuronal death has been reported in a large number of chronic neurodegenerative conditions, such as Parkinsonism, Huntington’s disease, Alzheimer’s disease and amyotrophic lateral sclerosis (ALS). More recently, various researcher groups have pointed out that apoptosis is likely a less dominant form of neuronal death. Thus it’s more likely both apoptotic and necrotic neuronal death are manifested in these disorders.

8.3 ACTIVATION OF CALPAIN 1 AND 2 IN ONCOTIC DEATH

In ischemic and traumatic brain injury, it is now well established that sustained extracellular free calcium reveals results from calcium in flux via the ionotropic glutamate receptors (NMDA, AMPA) and kainate receptors as well as through voltage gated calcium channels. This leads to calpain-mediated proteolysis of various cellular proteins and contributes to excitotoxic neuronal death. Calpain overactivation was also found in myocardial infarct and ischemic renal injury. At least in neuronal culture models, calpain activation can be mimicked by treatment with glutamate, hypoxia, A23187 and calcium channel opener, mirenclon. Calpain is generally an inactive pro-enzyme form in a resting cell but becomes overactivated under extreme conditions that result in transglutaminase [Ca2+] elevation, which is generally associated with necrotic oncosis (Table 8.1). Instances include calcium-ionophore treated cells (such as A23187-Molt 4 cells), or glutamate-treated glutamatergic central neurons. In the first case, calcium rushes in through the pore-forming A23187. In the latter case, calcium comes in through the transmembrane glutamate-receptors, which also function as ligand gated calcium/sodium channels. Similarly, mirenclon, a potent neurotoxin that opens both voltage- and ligand-gated calcium channels also induces rapid and massive calcium influx and subsequently calpain activation. We investigated the form of cell death with FITC treatment and found no DNA laddering or cell swelling. General DNA degradation and dissolution rather than condensation were observed. These are properties that are associated with necrotic oncosis. More recently, we found that by elevating the extracellular calcium concentration from 0.8 mM to 5.8 mM alone is sufficient to create calcium influx and calpain activation in the calcium-treated cells. For evidence of calpain activation, we found the specific fragmentation pattern for alpha-spectrin (280 kDa), forming immunoreactive fragments of 150 kDa and 145 kDa, identical to those forms by in vitro digestion of control cell lysate with purified calpain. Furthermore, calpain inhibitors such as calpain inhibitor I, and PD1,059,006 inhibit the formation of these fragments. Calpain 1 and 2 activities are also regulated by endogenous protein inhibitor calpastatin.
Calpain and Caspase in ischemic and traumatic brain injury

<table>
<thead>
<tr>
<th>TABLE 8.1 Compare and Contrast: Caspase-3 and Calpain 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caspase-3</strong></td>
</tr>
<tr>
<td>Proteinase class</td>
</tr>
<tr>
<td>Exogenous inhibitors</td>
</tr>
<tr>
<td>Reacting mode</td>
</tr>
<tr>
<td>Activation mode</td>
</tr>
<tr>
<td>Preferred cleavage site</td>
</tr>
<tr>
<td>Endogenous substrates</td>
</tr>
<tr>
<td>Consequence of substrate proteolysis</td>
</tr>
<tr>
<td>Cell death involvement</td>
</tr>
<tr>
<td>Inhibitors are antiproteases:</td>
</tr>
</tbody>
</table>

Calpain 1 and 2 are ubiquitous mammalian proteins and are enriched in CNS neurons. They do not have strict recognition sequence in substrate cleavage site but prefer Val-Ile or Leu in the P2 position (second residue N-terminal to cleavage site) (Table 8.1). Only a small subset of cellular proteins are susceptible to calpain attack while the majority of cellular proteins are resistant. Calpains tend to cleave substrates large “limited fragments” without further degradation. Calpain 1 and 2 substrates include cytoskeletal proteins (e.g., alpha-spectrin, beta-spectrin, MAP2, neurofilaments H and M), membrane receptors (EGF receptor, PDGFR receptor) and G-proteins (alpha subunits). Signal transduction enzymes, protein kinase C isozymes and many cell adhesive-dependent enzymes (e.g., CaMKII, L-type calcium pump, calcineurin and neuronal nitric oxide synthase) and PEST-containing proteins. A number of transcription factors have been shown to be calpain-regulated (e.g., fos, c-jun, c-myc, c-mos and NF-kappaB). When calpains 1 and 2 are uncontrollably activated the destruction and/or modification of these cellular proteins could prove to be detrimental to the host cells.

8.4 CASPASE CASCADE IN APOPTOSIS

Caspases are a family of related cysteine proteases discovered based on the initial discovery that a C. elegans apoptosis-linked protein CED-3 is homologous to the mammalian caspase-1 (then called interleukin-1-beta converting enzyme or ICE). This led to the discovery of a large number of ICE-like proteases (now renamed caspases) and
their roles as mediator of apoptosis in a wide range of cells.⁶⁶ Caspase-3 and caspase-7 appear to be common downstream apoptosis effectors. First, caspase-3/7 can be activated intrinsically or extrinsically. The intrinsic pathway is mitochondria-dependent. Upon an apoptotic trigger, Bax or Bax-like proteins translocate to the mitochondrial outer membrane, dimerize with Bcl-2, which allows the release of cytochrome C into the cytosol. Then, cytochrome C-complex with Apaf-1 and caspase-9 leads to autolytic activation and the processing/activation of caspase-3 and 7. The extrinsic pathway involves ligand binding to the so-called death-domain containing receptors (such as TNF-R, Fas). The ligand-bound receptor recruits adapter protein(s), which also contains death domains (FADD for Fas, TRADD-FADD protein pair for TNF-R1). FADD then induces caspase-9 molecules to co-localize and thus the autolytic activation of the associated caspase-8 or caspase-10. Like caspase-9, caspase-8/10 processes and activates caspase-3. Caspase-7 is highly homologous to caspase-3 and shares identical substrate cleavage site requirement Ap-Xaa-Xaa-Aaa. Thus it might substitute for caspase-3 in most cell types and tissues. However, in the CNS, no mRNA for caspase-7 was detected.⁶⁷ Consistent with that, caspase-3-knockout mice showed normal development except the CNS, in which too many neurons were found.⁶⁸ Therefore, it seems that caspase-3 is more important in neurodegeneration and we will focus our discussion on caspase-3. Caspases activity can also be directly suppressed by endogenous inhibitors (CIAP, 2, XIAP, NABP)⁷⁰ (Table 8.1).

Caspase-3 also has a finite number of cellular protein substrates.⁷¹ The key specificity determinant is the Asp (D) in the P1 and P4 positions, identified using synthetic peptides as substrates (Table 8.1). Like caspase, caspase-3 tends to produce limited fragments of its substrates, leaving them as fingerprints for caspase-3 activation. The list of substrate has grown rapidly. They can be classified into the following classes: (i) cytoskeletal proteins (such as actin, α and β spectrin, GSK-3), (ii) signal transduction enzymes (protein kinase C delta and beta isoforms, CAMPT-2a and IV, PAK, focal adhesion kinase), phosphatases C, P21-activated kinase, MEK5), (iii) cell cycle proteins (PITSLGE kinase, Rb), and (iv) nuclear substrates, such as DNA PKcs, PARP-1, JNK and NubMA and Caspase-activated DNase which is responsible for DNA fragmentation in the inter-nucleosomal regions.⁷² Caspase-3 also activates caspase-6, which degrades lamin A, B1, B2 and C.

Three biochemical markers have been used extensively to detect caspase-3 activation in neurological or neurodegenerative disorders: (i) processing of intact 52 kDa procaspase-3 to the 17 kDa and 12 kDa dimeric forms,⁷³ (ii) fragmentation of the 115 kDa poly (ADP-ribosyl) polymerase (PARP) to a 89 kDa form,⁷⁴ and (iii) processing of nonerythroid alpha spectrin (280 kDa) into α560 kDa and 120 kDa spectrin breakdown product (SBP-D120).⁷⁵ In fact, caspase-3, PARP and alpha-spectrin processing have been reported in several neuronal apoptosis models, such as cultured neurons or neuronal cells subjected to stereotrope as well as cerebellar granule neurons subjected to potassium deprivation.⁷⁶ Further, similar evidence has been observed in vivo models of ischemia-hypoglycemia, excitotoxicity and MPTP toxicity (in cultured primary neurons). Antibodies specifically detect the activated caspase-3 (17 kDa), the 89-kDa PARP fragment and the
Calpain and Caspase in Ischemic and Traumatic Brain Injury

SBP120 have recently been reported, making immunohistochemical studies possible. More recently, there is a large body of literature identifying the presence of caspase-3 activation and proteolytic activity in vivo models for cerebral ischemia, excitotoxicity, TBI and SCI. Caspases appear to be centrally involved in any apoptotic cascade in various cell types, in caspase inhibitors such as Z-D-DCB, Z-VAD(fmk) and Ac-DEVD-CHO almost universally protect against any forms of apoptosis. Similarly these agents are found to be excellent neuronal apoptosis inhibitors. Also, they were ineffective against necrotic toxicity. Recently several inhibitors of this class have been used in suppressing the "apoptotic" component in a cerebral ischemia and TBI.

6.5 CALPAIN ACTIVATION IN CERTAIN APOPTOSIS SYSTEMS

Calpain activation was first identified in thymocytes and T-cell apoptosis. As measured by calpain assays, calpain inhibits apoptosis and that calpain inhibitors protect against apoptosis in immune cells. We then showed that calpain is also activated in murine prostate cancer neuroblastoma SH-SY5Y cells, in NCI-deprived rat PC-12 cells and in low potassium-treated rat cerebellar granule neurons (CGN). Based on detection of calpain activity and specific caspase fragments generated by calpain (SBP150 and SBP145) (see below), Caspase-8 appears to be centrally involved in any apoptotic cascade as caspase inhibitors (Z-D-DCB, Z-VAD-fmk and Ac-DEVD-CHO) almost universally protect against any forms of apoptosis. Unlike caspases, calpain inhibitors protect against a subset of apoptotic conditions.

8.5 CASPASE-3 AND CALPAIN 1 AND 2: WHAT THEY HAVE IN COMMON

8.6.1 CASPASE-3 AND CALPAIN 1 AND 2 SHARE MANY COMMON OR RELATED SUBSTRATES

Table 8.1 illustrates the many aspects in which the calpain 1 and 2 are similar to caspase-3, including that they are both cysteine proteases, have endogenous protein inhibitors, produce limited fragments of selected protein substrates and are involved in cell death. Here we illustrate that they share many identical or related endogenous inhibitors (Table 8.2). Alpha II-spectrin (alpha-fodrin) has been recognized as a preferred substrate for calpain 1 and 2. Calpain mediates alpha II-spectrin breakdown to 150 kDa and 145 kDa doublet (SBP150 and SBP145). Several groups also found that alpha II-spectrin was degraded to a 120 kDa fragment.
### TABLE B.2 Common or Related Substrates for Calpain and Caspase-7

<table>
<thead>
<tr>
<th>Substrate Protein</th>
<th>Major Fragments Produced by Calpain</th>
<th>Major Fragments Produced by Caspase-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS (38 kDa)</td>
<td>150 kDa, 130 kDa</td>
<td>150 kDa, 146 kDa</td>
</tr>
<tr>
<td>PKCα (38 kDa)</td>
<td>140 kDa</td>
<td>110 kDa</td>
</tr>
<tr>
<td>AChE (63 kDa)</td>
<td>35 kDa</td>
<td>40-42 kDa</td>
</tr>
<tr>
<td>Vimentin (54 kDa)</td>
<td>52-64 kDa</td>
<td>44 kDa &amp; others</td>
</tr>
<tr>
<td>Tau (55 kDa)</td>
<td>45 kDa</td>
<td>42 kDa &amp; others</td>
</tr>
<tr>
<td>CAMKII γ (55 kDa)</td>
<td>36 kDa</td>
<td>40 kDa</td>
</tr>
<tr>
<td>CAMKII δ (56 kDa)</td>
<td>36 kDa</td>
<td>37 kDa</td>
</tr>
<tr>
<td>Calexisin A (60 kDa)</td>
<td>45 kDa</td>
<td>42 kDa</td>
</tr>
<tr>
<td>PAR (152 kDa)</td>
<td>82 kDa</td>
<td>90 kDa</td>
</tr>
<tr>
<td>PARP (114 kDa)</td>
<td>69 kDa &amp; 24 kDa</td>
<td>70 kDa &amp; 40 kDa</td>
</tr>
<tr>
<td>DNase I (281 kDa)</td>
<td>140 kDa</td>
<td>140 kDa</td>
</tr>
<tr>
<td>Calmodulin (205 kDa)</td>
<td>75 kDa</td>
<td>Multiple</td>
</tr>
<tr>
<td>Caspase 3 (17 kDa)</td>
<td>Analogy</td>
<td>29 kDa</td>
</tr>
<tr>
<td>Calpain I &amp; II (40 kDa)</td>
<td>Not degraded</td>
<td>Analogy</td>
</tr>
<tr>
<td>PKCα, β, γ (57 kDa)</td>
<td>Not degraded</td>
<td>Analogy</td>
</tr>
<tr>
<td>PKCB, δ (75 kDa)</td>
<td>40 kDa</td>
<td>Not degraded</td>
</tr>
<tr>
<td>Transthyretin (180 kDa)</td>
<td>2 kDa</td>
<td>Degraded</td>
</tr>
<tr>
<td>Phospholipase Cα3 (155 kDa, mammalian)</td>
<td>Not degraded</td>
<td>100 kDa</td>
</tr>
<tr>
<td>Phospholipase A2 (100 kDa, yeast)</td>
<td>70 kDa</td>
<td>Not degraded</td>
</tr>
<tr>
<td>Antiapoptotic protein (65 kDa)</td>
<td>Multiple</td>
<td>Multiple</td>
</tr>
<tr>
<td>ADRD Receptor (110 kDa)</td>
<td>Multiple</td>
<td>Multiple</td>
</tr>
<tr>
<td>p73L1.2</td>
<td>Degraded</td>
<td>Not degraded</td>
</tr>
<tr>
<td>Iγ/γ</td>
<td>Not degraded</td>
<td>Degraded</td>
</tr>
<tr>
<td>Bcl-2 (26 kDa)</td>
<td>25 kDa</td>
<td>Not degraded</td>
</tr>
<tr>
<td>Nex (21 kDa)</td>
<td>Not degraded</td>
<td>18 kDa</td>
</tr>
<tr>
<td>Bcl (22 kDa)</td>
<td>18 kDa</td>
<td>Not degraded</td>
</tr>
<tr>
<td>Bcl-XXL (24 kDa)</td>
<td>15 kDa</td>
<td>Not degraded</td>
</tr>
<tr>
<td>20 kDa</td>
<td>20 kDa</td>
<td></td>
</tr>
</tbody>
</table>
(SRDP20) by caspase-3 during apoptosis in T-cells, neurons and other cell types. We also found that the SRDP120 was observed only in apoptotic neurons but not in astrocytes, which is consistent with the fact that caspase is only activated in apoptosis but not in necrosis. We also observed that cell death-mediated alpha II-spectrin breakdown to 150 kDa and 165 kDa doublets were also present in apoptotic neurons (Fig. 8.1). The SRDP145 and SRDP120 can be easily distinguished on SDS-PAGE. Anti-caspase-produced SRDP150-specific antibodies have been produced by various groups. Taking advantage of the new N-terminal of SRDP120, we recently developed an anti-SRDP120-specific antibody. In fact, using SRDP120 as a marker for apoptosis is convenient for several commercial anti-alpha-spectrin antibodies react with rat (e.g., cerebellar granule neurons) and human alpha-spectrin (e.g., 68 STS cells). This antibody detects apoptotic neurons in culture as well as in vivo models of TBI. Thus the usage of these spectrin and SRDP antibodies are powerful tools in detecting the apoptotic and necrotic neurons.

FIGURE 8.1 Caspase 1 and 2 and caspase-3 in mixed network and synapsis. Upon activation, caspase-3 can activate caspase 1 and 2 in two ways: (1) by degrading cytoskeletal and membrane proteins and thus compromising cell cytoskeletal permeability to C5b; and (2) partial inactivation of caspase-3 by proteolysis. Once activated, caspase joint caspase-3 in degrading a whole range of cytoskeletal, cymoskeletal and nuclear substrates, resulting in microvesicles / synapsis are detected.
New Concepts in Cerebral Ischemia

Besides alpha-II-spectrin, we found that the beta II-spectrin is also simultaneously degraded by calpain and caspase-3 in ischemia and by calpain only in in vitro. Adjacent calpain and caspase-3 cleavage sites lead to 110 kDa beta SB2D1, while caspase-3 cleavage at another major site that produces an 85 kDa. Vimentin is an intermediate filament protein of the cytoskeleton network. It was found to be a calpain sensitive cell. Recently, vimentin degradation by caspase is again observed in sympathetic skin fibroblasts and prostate epithelial cells. Actin, a major spectrin binding protein, has been shown to be degraded by caspase-3 and -9. However, actin is not a particularly good substrate to calpain and is only partially fragmented, which leads to the failure of some researchers to consistently observe its cleavage in cell death. Overall, we speculate that the simultaneous degradation of various cytoskeletal proteins in apoptosis (and on-oxidation) significantly compromise the cytoskeletal network and cell structural integrity and possibly membrane permeability. Tau, a microtubule (MT) binding protein that stabilizes the stability of MT, which is of extreme importance in neurons since protein and other biomolecules transport in axon (axon and dendrites), is supported by MT. Too, is composed of up to 6 isoforms in adult human neurons. Tau aggregates and hyperphosphorylated Tau have been linked to the formation of neurofibrillary tangles in Alzheimer's disease. Sensitivity of Tau to calpain proteolysis has been suggested as playing a role in neuronal degeneration. In fact, multiple fragments are generated with the major fragment of about 30-50 kDa. Costa et al. further showed that in COS-1 cells, Tau is also degraded and is sensitive partially to caspase-3 and calpain inhibitors. In fact, the combination of inhibition of both pro-caspase provided the best protection. This prompted the authors to examine and subsequently confirm the direct susceptibility of Tau to caspase and calpain. More recently, the degradation of Tau by caspase in fixed neuroblastic neurons was confirmed. Amyloid precursor protein (APP) is another key protein associated with AD. Apparently, when APP was improperly cleaved, it produces the b-amyloid peptide 1-40 and 1-42, which form extracellular aggregates (amyloid plaques). Although the responsible proteases have been recently identified as beta-secretase and presenilin, other proteases might contribute to producing truncated forms of APP that are more readily cleaved by BACE1 and presenilin (PS). Calpain can cleave APP at three different sites, all located in the extracellular N-terminal domain. It is therefore likely that internalized APP might be cleaved. The C-terminal cleavage of APP could indeed produce a fragment that contains the whole of the b-amyloid peptides 1-40 and 1-42 (amyloidogenics), which can be further processed by BACE1 and PS. APP is also cleaved by caspase-3 at two sites in the extracellular N-terminal domain and at s 2 site due to the apoptosis. Again, the cleavage in the C-terminal region might help facilitate the production of b-amyloid peptide indirectly.

A subgroup of signal transduction enzymes are sensitive to calpain and/or caspase-3 degradation. We established that CaM-kinase IV is fragmented in casepase-3 and calpain in staurosporine-induced apoptosis, but cleaved by calpain only in MTX-induced necrosis. Here caspase produces an immunoreactive 38 kDa N-terminal fragment while calpain produces a 35 kDa N-terminal fragment. In parallel with its proteolytic duties, we observed a corresponding increase in
Calpain and Caspase in Ischemic and Traumatic Brain Injury

CAMPK activity in these cells. Previously others and we have shown that CAMPK-II is sensitive to calpain proteolysis.90 In one recent study, we found that CAMPK-II is also sensitive to caspase-3 proteolysis in apoptotic cells.91 (Table 8.1). The anti-apoptotic properties of CAMPK-II activity were recently confirmed in control granule neurons by See et al.92 The calcium-dependent uptake, beta- and gamma-isoforms of protein kinase C have been found to be degraded by calpain in vitro and in cell culture under a variety of conditions.93 In fact, calpain is suspected of playing a role in the down regulation of PKC. Interestingly, two calcium-independent PKC isoforms (alpha and beta) have now been shown to be degraded by caspase-3 but not by the calcium-dependent forms.94 PI-specific phospholipase C is also activated by calpain95 whereas phospholipase A2 and phospholipase C are activated by caspase-3 substrates.96 Both phospholipid hydrolases are involved in transmembrane signal transduction. In phagocytes, focal adhesion kinase (PAK), p45/p47(PAK), and p21ras(p21ras) are all 125 kDa non-covalent tyrosine kinase implicated in integrin-mediated signal transduction. It is degraded by calpain during platelet activation to 90 kDa and 45 kDa, 40 kDa fragments.97 Recently, PAK was also found to be degraded by caspase-3/7 during apoptosis in HeLa cells to an 85 kDa fragment.98 IP3 receptors (IP3R) are involved in IP3 signaling. Recent report found that IP3R1 and IP3R3 are degraded by caspase-3 while insensitive to calpain. Conversely, IP3R3 is degraded only by calpain.99

PARP is the most well known nuclear substrate for caspase-3. During apoptosis, the 113 kDa PARP is degraded by caspase to a distinct 89 kDa fragment and 24 kDa fragment.100 But recently, PARP has been found to be cleaved at alternative sites. generating fragments from 70 kDa to 40 kDa during ischemic necrosis.101 Another recent study described the purification of a non-caspase protease that cleaves bovine PARP.102 They identified the protease as bovine in-calpain. The fragmentation of PARP by calpain in vitro again yields fragments ranging from 70-40 kDa, similar to the PARP cleavage pattern observed in necrotic cells.103 Using microsomes (MTX) as a necrotic challenge, we indeed found that PARP is cleaved into 70 kDa and 40 kDa fragments. This process is sensitive to calpain inhibitor I, but the extent of calpain-mediated proteolysis in necrosis is much less than that observed by caspase-3 mediated apoptosis.104 DNA polymerase (a catalytic subunit) is another nuclear protein that is degraded by both calpains and caspase-3.105

In apoptosis, proteolysis is likely to be an important event in shutting down cell function by disabling a number of signal transduction enzymes in disabling the cell's ability to repair its DNA, or going through cell cycle and deactivate the cytochrome network, therefore allowing membrane blushing and the subsequent plaque formation by macrophages. In necrotic necrosis although not necessarily by design, calpain-mediated proteolysis undoubtedly plays a similar role in disabling the cells in the signal transduction, membrane and cytoskeleton integrity loses and nuclear function. Furthermore, calpain might facilitate the apoptotic cell death by aiding caspase in proteolysis of cellular proteins.

\[ \text{Necrosis} \]
B.6.2 TURNING BAX-THINGS WORSE

Another way that caspase and calpain can work together is at the Bel-2/bax protein family level (Figure 8.2). Bel-2, which is a well-studied anti-apoptotic protein associated with the mitochondria, is cleaved by caspase-3 in a positive feedback loop to an N-terminally truncated form (Bel-2) which becomes pro-apoptotic. Similarly, Wood et al. and we[11] reported that Bax are also truncated N-terminally by calpain, but not caspase. Bax is in fact more pro-apoptotic[12] (Figure 8.2). Bid is a neutral molecule but once it is cleaved by caspase-8, it will translocate to mitochondria and induce cytochrome C and Smac release.[13] This has been viewed as a cross-talk from the receptor pathway to the mitochondria pathway of apoptosis. Lastly, Bel-2XL, another anti-apoptotic protein, can be truncated by either calpain or caspase to a pro-apoptotic form[14] (Figure 8.2). Thus, both calpains and caspases are involved in a positive feedback mechanism to further protein degradation and apoptotic cell death.

![Image of Caspase-3 and Bcl-2 cleavage diagram]

**FIGURE 8.2** Caspase-proteolysis regulation of Bcl-2/Bax family proteins. Pro-apoptotic Bax, when truncated by calpain, becomes more active. Bid, a normally neutral molecule, becomes pro-apoptotic when activated by caspase-8. Likewise, anti-apoptotic molecules Bel-2 and Bel-2XL, truncated by caspase-9 and calpain, respectively activate caspase-3 and calpain-3, both of which activate caspase-9 and caspase-3, respectively.

8.7 CALPAIN-CASPASES CROSS TALK

It has been recently reported that calpastatin is degraded by calpain in A23187-treated cells[15] (Table 8.2). We suspect that the caspase pathway might be linked to calpain pathway through calpastatin (Figure 8.1). We indeed found that the 105 kDa HMW form of CAST is in fact very sensitive to caspase proteolysis in apoptotic state. -proteolysed neuroblastoma SY5Y cells. A major fragment of 75 kDa is formed in SY5Y. We concluded that calpain-1 and 3 are the likely proteases...
Capnains and Caspases in Ischemic and Traumatic Brain Injury

involved. However, there is only a two-fold drop of inhibitory activity of CAST upon fragmentation since the major cleavage sites are outside of the inhibitory sequence in each repeat unit. We speculate that the degradation of CAST might have yet other functions in unleashing calpain activity or other calpain-independent roles in drop brain. Again, the susceptibility of CAST to calpains is confirmed by Peters-Aarseth et al. 16

Recently, it was shown by others and us that pro-caspase-3 is a substrate of calpains. 17,18 As it turns out, pro-caspase-3 (31 kDa) is transected by calpain at the N-terminal to a 30 kDa form, which appeared to be less vulnerable to being activated by caspase 8 or caspase 9. Thus, further allowed that caspase-7, 8 and 9 are also transected at the N-terminal. 19 The transection of the upstream caspase-6 (and caspase-8) is even more significant as it abolishes their ability to interact with the mitochondrial-released Apo-1 or with the "death domain" receptors, respectively. We interpret this as a mechanism by which during collapse, when there is high level of calpain activity, the caspase activation cascade and thus apoptotic phenotype would be shut down (Figure 8.3).

On the other hand, Nakagawa et al. recently showed that caspase-12 is in fact processed and activated by calpains. 20 Since caspase-12 is the ER-associated caspase that is involved in swelling A-beta toxicity, it has additional meaning. Lee et al. have shown that A-beta treatment of hippocampal cultures can in fact activate calpains. Interestingly, calpains also process a cell-activating protein p38 (to a truncated p25 form) thereby activating cells. They further showed that calpain and cell-activating p38 in fact could protect against A-beta toxicity, which was also confirmed by others. Together, this would suggest that calpains play a key role in A-beta-induced toxicity.

![Diagram](https://example.com/diagram.png)

**Figure 8.3** Calpains, caspases and neuronal apoptosis and necrosis. Under specific conditions, direct caspase-dependent and calpain-independent apoptosis can occur. However, in most neuronal injury, calcium influx (potentially causes calpains activation as well, which can lead to a mixed oncosis/apoptosis phenotype. Lastly, in severe excitotoxicity and ischemic injury, stress occurs with calpain activation is the most likely phenotype.
8.8 PERSPECTIVE

In this chapter we described the presence of three phenotypic forms of cell death: Caspase-dependent apoptosis, mixed apoptosis-apoptosis with calpain and caspase activation, and caspase-independent necrosis.

Indeed, apoptosis might or might not be associated with Ca\(^{2+}\) overload. In acute PAH-injured JNK1-/- cells, there was little caspase activation (Figure 8.3). The difference could be the rapid onset of apoptosis and the resultant cell death (which takes 3–4 h). Thus, in rare conditions that intracellular calcium elevations occur, a most calpain-independent but caspase-dependent apoptosis would occur. In fact, artificially, we can demonstrate that using long-term treatment (14–48 h) with calcium chelator (EGTA) (Figure 8.3), however, more than likely delayed neuronal injury induced by hypoxia, excitoxocity, etc., calcium level invariably rises and thus, you have a mixed apoptosis-apoptotic phenotype with the activation of both calpain and caspase (Figure 8.3). This is induced by caspase-mediated calcium entry and calcium ionization fragmentation (Figure 8.1). Caspase go on and degrade or modify the large array of similar or identical cellular proteins (Table 8.2), ultimately resulting in neurodegeneration. Therefore follows that calpain and caspase inhibitors have neuroprotective effects, which have been demonstrated by various laboratories. In fact, both calpain and caspase inhibition have been shown to have an extended therapeutic window (5–6 h) delay treatment is still efficacious. Furthermore, Rami et al.\(^{81}\) recently showed that the combination treatment of a calpain inhibitor with a caspase inhibitor provides efficacious neuroprotection in a 4/3 global ischemia model. A third scenario is where neurons are injured acutely with challenges such as severe ischemia (in case of stroke), large and rapid rises in intracellular free calcium levels result in high calcium activation which most likely shut down caspase activation亿元以上 by truncation of caspases and Apaf-1, producing a mixed necrotic phenotype (Figure 8.3).

8.9 ACKNOWLEDGMENTS

I would like to thank my present and former associates Rathna Nai, Sateeshsha Datta, Albert Probert Jr, Dr. Kim McGlinchey, Dr. Raul Postema, and my collaborators, Dr. Ronald Hayas, Dr. Poo-wa Tseng, and Dr. Margaret O'Shea for contributing to the work.

8.10 REFERENCES

ischaemia can cause DNA fragmentation and indicators of apoptosis in the retina, 
Neuroscience, 64 (1-2), 89, 1992.

6. Aronen, A., Paliard, H., Ewasi, F., Maruta, J., Laitinen, B., Ben-Ari, Y. and
Charlton-Merola, C. Regional variability in DNA fragmentation after global ischaemia
induced by combined biochemical and electron microscopy observations in the rat brain. 

7. Fjigglaw, R.J., Hirmola, M., Kerusa, J. and Rautti, P. DNA fragmenta-
tion in rat brain after transenkephalin administration of kainic. 

8. Li, Y., Chi, M.H., Jiang, N. and Zole, K. In situ detection of DNA fragmentation
after focal cerebral ischemia in mice. 

9. Ben-Ari, Y., Krane, D., Anghel, R., Mazur, P. and Logan, J.S. Apop-
tosis and necrosis: two distinct events induced, transiently, by rapid and intense
ischaemia with N-methyl-D-aspartate or nico-

taxide/superoxide in cortical cell cultures. 

10. Chen, J., Jansen, M., Chen, S., Pol, W., Elwagbash, K., Greenberg, P.A. and
Sinha, R.P. Early detection of DNA strand breaks in the brain after transient focal ische-

mica: Implications for rat colchicine damage to apoptotic and neuronal cell death. 

Apoptosis, B.W. Barlow, J. and 

Mintz, T.K. Evidence of apoptotic cell death after experimental traumatic brain injury in the


13. Angellis, P., Vyas, S., Jernv-Aagd, F., Parola, M., Leather, A.P., 
Korkosz, T., Mouatz-Prigent, A., Rubens, M., Hesch, E.C. and 
Agid, Y. Apoptosis and morphology in 
terminal neurones of patients with Parkinson's disease. 

14. Czerny, C.W. Apoptosis, decision criteria and neuronal degeneration in 
Alzheimer's disease. 


scarlina is not induced by motor neurons. 

16. Desil, F., Charlton-Merola, C., Krabak, M. and Ben-Ari, Y. Glutamate-
induced neuronal death is not a programmed cell death in cerebellar culture. 

Neurochirurgia. 23 (4), 107, 1977.

18. MacManus, J.R., Rumbaugh, L., Blanch, M.A., Laitinen, N.B., How, M.B., 
Walker, T. and Mortley, V. Glutamate

-induced rat cortical neuronal cultures die in a way different from the classical apoptosis 


regiosentric system: an apoptosis-necrosis model in the guinea pig. 

20. Wang, K.K. and Yam, P.W. Colchicine inhibition as an overview on its 
therapeutic potential. 


Calpain and Caspase in Ischemic and Traumatic Brain Injury

New Concepts in Cerebral Ischemia


61. Glimcher, S.M., Fink, Z., and Maroto, S., Inhibition of caspases prevents cell death of hippocampal CA1 neurons, but not impairment of hippocampal long-term potentiation following global ischemia, Neuroscience, 95 (4), 1219, 1999.


Calpain and Caspase in Ischemic and Traumatic Brain Injury


72. Cruz, V.A.; Bergqvist, I.; Zhu, H.; Li, H.; and Kumar, S. Specific cleavage of alpha-fodrin during Fas- and ceramide-induced apoptosis is mediated by an inducible beta-converting enzyme (in caspase 3 protease distinct from the poly(ADP-ribose) polymerase protease. J. Biol. Chem., 275 (49), 31277. 1999.


New Concepts in Cerebral Ischemia


Calpain and Caspase in Ischemic and Traumatic Brain Injury


200

New Concepts in Cerebral ischemia.


