Computational and Structural Biotechnology Journal 13 (2015) 212–221

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

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

Mini Review

The Role of Excitotoxic Programmed Necrosis in Acute Brain Injury

Denson G. Fujikawa

David Geffen School of Medicine, University of California at Los Angeles, VA Greater Los Angeles Healthcare System, United States

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 19 March 2015
Accepted 21 March 2015
Available online 28 March 2015

Keywords:
Excitotoxicity
Necrosis
Programmed cell death
NMDA receptor

ABSTRACT

Excitotoxicity involves the excessive release of glutamate from presynaptic nerve terminals and from reversal of astrocytic glutamate uptake, when there is excessive neuronal depolarization. *N*-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptor, are activated in postsynaptic neurons, opening their receptor-operated cation channels to allow Ca^{2+} influx. The Ca^{2+} influx activates two enzymes, calpain I and neuronal nitric oxide synthase (nNOS). Calpain I activation produces mitochondrial release of cytochrome *c* (cyt *c*), truncated apoptosis-inducing factor (tAIF) and endonuclease G (endoG), the lysosomal release of cathepsins B and D and DNase II, and inactivation of the plasma membrane Na^{+}–Ca^{2+} exchanger, which add to the buildup of intracellular Ca^{2+}. tAIF is involved in large-scale DNA cleavage and cyt *c* may be involved in chromatin condensation; endoG produces internucleosomal DNA cleavage. The nuclear actions of the other proteins have not been determined. nNOS forms nitric oxide (NO), which reacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$). These free radicals damage cellular membranes, intracellular proteins and DNA. DNA damage activates poly(ADP-ribose) polymerase-1 (PARP-1), which produces poly(ADP-ribose) (PAR) polymers that exit nuclei and translocate to mitochondrial membranes, also releasing AIF. Poly(ADP-ribose) glycohydrolase hydrolyzes PAR polymers into ADP-ribose molecules, which translocate to plasma membranes, activating melastatin-like transient receptor potential 2 (TRPM-2) channels, which open, allowing Ca^{2+} influx into neurons. NADPH oxidase (NOX1) transfers electrons across cellular membranes, producing O_2^-. The result of these processes is neuronal necrosis, which is a programmed cell death that is the basis of all acute neuronal injury in the adult brain.

Contents

E-mail address: dfujikaw@ucla.edu.

http://dx.doi.org/10.1016/j.csbj.2015.03.004

1. Introduction

"Excitotoxicity" is a word coined in the 1970s by John Olney, based upon his studies of the *in vivo* effects of glutamate and its analogues on neurons [1,2]. Olney proposed the excitotoxic hypothesis, namely, that glutamate and aspartate, the principal excitatory neurotransmitters in the central nervous system, are responsible for the excitotoxic death of neurons [3]. A subsequent study showed that glutamate activates a subtype of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor, which opens its receptor-operated cation channels and allows influx of Ca^{2+} ions [4]. An NMDA-receptor antagonist was shown to protect hippocampal neurons following transient forebrain ischemia *in vivo* [5]. NMDA-receptor antagonists were also shown to protect neurons from glutamate excitotoxicity in cortical neuronal culture [6], which requires the presence of extracellular Ca^{2+} [7]. Excessive Ca^{2+} influx into neurons activates two Ca^{2+}-dependent enzymes, calpain I and neuronal nitric oxide synthase (nNOS), which in turn activates programmed cell death pathways that result in neuronal death. Excitotoxicity underlies all types of acute brain injury, including prolonged epileptic seizures, cerebral ischemia, traumatic brain and spinal cord injuries, and hypoglycemia [8].

1.1. General Considerations Regarding the Studies Cited

1.1.1. In Vitro Models of Excitotoxic Programmed Necrosis

In vitro models of acute neuronal injury, specifically those utilizing cell cultures, are useful because the blood–brain barrier is bypassed, allowing direct exposure of cells to specific concentrations of agents, and establishment of EC_{50} or IC_{50} concentrations. However, the cells are usually dispersed in a monolayer bed, with or without an astrocytic layer beneath it, and are bathed in artificial CSF. Moreover, 18-day-old fetal neurons are commonly used, and even if they "mature" for several weeks in culture, they are not comparable to adult neurons *in vivo*. Also, the artificial two-dimensional geometry of dispersed neurons and the lack of the supporting three-dimensional tissue matrix can lead to misleading results. For example, for many years it was thought that cellular necrosis is produced by a severe insult that leads to rapid cell swelling and lysis. However, *in vivo*, seizure-, ischemia- and hypoglycemia-induced necrotic neurons are shrunken, with pyknotic, condensed nuclei, swollen, irreversibly damaged mitochondria and plasma membrane disruption, with swelling of surrounding astrocytic processes [9–15].

1.1.2. In Vivo Models of Excitotoxic Programmed Necrosis

Compared to *in vitro* models, *in vivo* models maintain the 3-dimensional structure of the brain. Mechanisms can be probed, using protein or enzyme inhibitors or protein or enzyme knockouts or conditional knockouts. Moreover, *in vitro* studies have to be coupled with an *in vivo* model, commonly oxygen–glucose deprivation in neuronal culture with a focal or global cerebral ischemia model, in order to establish relevance. A major limitation is that many agents have poor penetration of the blood–brain barrier. To bypass this problem, agents are commonly injected directly into a brain region to determine their efficacy. This is clearly an artificial situation with little translational relevance if one is looking for an agent that can be given orally. Another major limitation of *in vivo* models is that rodents are most often used, and results in rodents for focal cerebral ischemia, for example, have not been borne out in human studies.

2. The Major Types of Cell Death

In 1990 Peter Clarke described three types of developmental cell death: apoptosis, necrosis and autophagy [16]. Autophagic cell death,

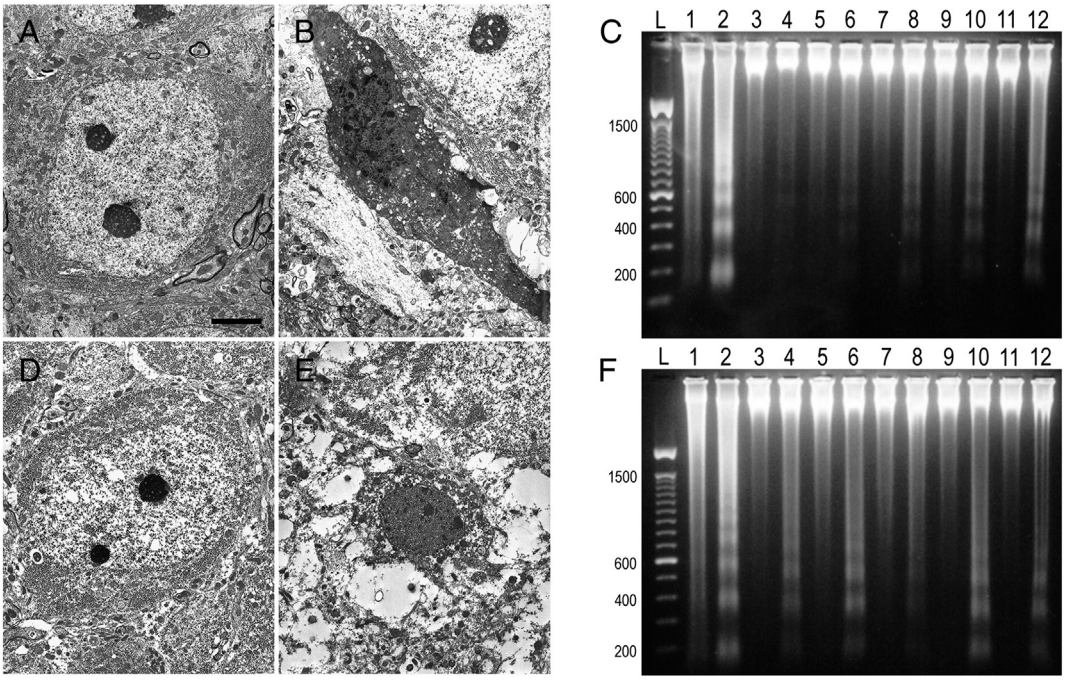

Fig. 1. Necrotic neurons show internucleosomal DNA cleavage, a programmed process that requires endonuclease activity. A and D show electron photomicrographs of normal neuronal nuclei in the ventral hippocampal CA1 region. B and E show necrotic neurons in the same region 24 and 72 h after 3-hour lithium-pilocarpine-induced status epilepticus. The necrotic neurons are shrunken and electron-dense, with pyknotic (shrunken) nuclei and at 24 h irregular, scattered chromatin clumps and cytoplasmic vacuoles. Between 24 and 72 h the neuronal nucleus became even more pyknotic and the cytoplasm more disrupted. C and F show agarose gel electrophoresis of DNA in the same brain region (lanes 5 and 6) and four additional regions: dorsal hippocampus (lanes 3 and 4), neocortex (lanes 7 and 8), amygdala–piriform cortex (lanes 9 and 10) and entorhinal cortex (lanes 11 and 12) 24 and 72 h after status epilepticus. There is a DNA ladder showing 1500 to 200 base pair bands. Lanes 1 and 2 are control and methamphetamine-treated rat splenic tissue; the methamphetamine-treated splenic tissue shows DNA laddering at 200 base-pair intervals and is a control for cellular apoptosis. Lanes 3, 5, 7, 9 and 11 are the above-mentioned brain regions in control rats at 24 h (C) and 72 h (F), and lanes 4, 6, 8, 10 and 12 are the same brain regions in seizure rats. Two-hundred base-pair DNA laddering can be seen in the brain regions of seizure rats at 24 and 72 h following seizures. This indicates that DNA laddering occurs in necrotic neurons.
This figure was obtained from Ref. [82]; the electron photomicrographs were previously unpublished, but the DNA agarose gel electrophoresis results were originally published in Ref. [13].

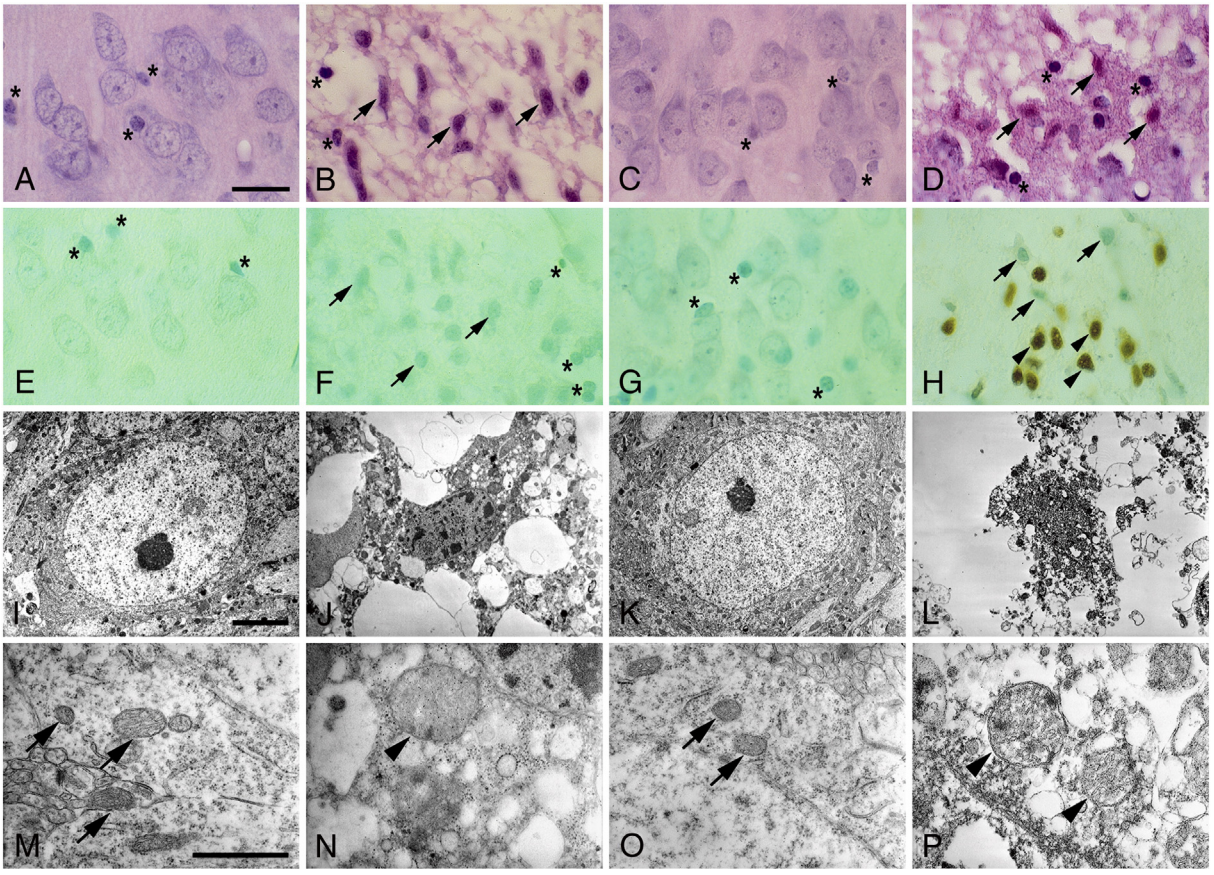

Fig. 2. Acidophilic neurons by light microscopy develop TUNEL-positive nuclei, indicating double-stranded DNA breaks, and are morphologically necrotic by electron microscopy. A and E and C and G show hematoxylin and eosin (H & E)-stained control normal neurons (A and C) and TUNEL-stained neurons with methyl green counterstain in layer 2 of the piriform cortex 24 and 72 h after normal saline injection i.p. (E and G). The asterisks indicate astrocytes. E and G are TUNEL-negative. B and D and F and H show the left piriform cortex 24 and 72 h after 3-h lithium-pilocarpine status epilepticus (LPCSE), stained with H & E (B and D) and TUNEL with methyl green counterstain (F and H). In B and D arrows point to some of the acidophilic neurons and asterisks indicate astrocytes. Arrows point to TUNEL-negative shrunken neurons in F and H, and arrowheads in H point to some of the TUNEL-positive pyknotic nuclei. I and K are low-magnification electron photomicrographs of control normal neurons in the right piriform cortex of the same rat brains as A, C, E and G 24 and 72 h after normal saline injection. J and L are low magnification electron photomicrographs showing necrotic neurons in the right piriform cortex of the same rat brains as B, D, F and H 24 and 72 h after 3-h LPCSE. Nuclei are shrunken, with scattered chromatin clumps and disrupted cytoplasm with numerous vacuoles. The necrotic neurons are surrounded by swollen astrocytic processes. M and O are high magnification electron photomicrographs showing normal mitochondria (arrows point to some). N and P show swollen mitochondria (arrowheads) with disrupted cristae; these are irreversibly damaged. The scale bar in A is 20 μm, in I it is 4 μm and in M it is 1 μm.
This figure was obtained from Ref. [14].

which classically occurs during starvation, when cells turn inward to consume intracellular proteins [17], is beyond the scope of this review and will not be discussed.

Naturally occurring neuronal apoptosis peaks in the first postnatal week in rats and is negligible by postnatal day 21 [18]. The cysteine protease family of caspases are activated in immature neurons and contribute to apoptotic death. However, in cerebral ischemia caspase-3, the central effector caspase, is activated during the first postnatal week, but this gradually decreases as rats get older, and by postnatal day 60 there is no evidence that it is activated in neurons destined to die [19–22].

In acute neuronal injury in the adult rat brain, irreversibly damaged neurons are morphologically necrotic (Figs. 1–3) [10–14,23,24], and undergo a caspase-independent programmed cell death [23,25]. This caspase-independent programmed pathway is associated with activation of the Ca^{2+}-dependent cysteine protease calpain I [26–28]. Other intracellular enzymes also contribute to necrotic neuronal death and will be discussed later.

There was confusion about the role of apoptosis in acute neuronal death when it was shown that apoptosis involves internucleosomal DNA cleavage [29], implicating activation of an endonuclease, and double-stranded DNA fragmentation, visualized with *in situ* terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL stain) [30]. In addition, initial reports were that caspase-3 was activated in the adult brain in cerebral ischemia and focal status epilepticus, so the neuronal death was presumed to be apoptotic [31,32]. However, we showed that both TUNEL-positive nuclei and internucleosomal DNA cleavage (DNA "laddering") occur in brain regions with ultrastructural evidence of morphologically necrotic neurons [13,14,23] (Figs. 1 and 2). Also, apoptotic and necrotic neurons can be distinguished easily, even at the light-microscopic level [14] (Figs. 2 and 4), and no morphologically apoptotic neurons have been shown to occur in the adult brain following acute neuronal injury. Subsequently, caspase activation was not found in neurons following status epilepticus (SE) [23,25,26,33] and cerebral ischemia [19–22], which is in keeping with developmental studies of cerebral ischemia [20–22].

3. Synaptic vs. Extrasynaptic NMDA Receptors

It was assumed until recently that excessive glutamate release presynaptically activated an excessive number of post-synaptic NMDA receptors, triggering excitotoxic neuronal death by allowing excessive Ca^{2+} influx through receptor-operated cation channels. However, in 2002 Hardingham and colleagues showed that extrasynaptic NMDA receptors were responsible for excitotoxic neuronal death, with synaptic NMDA receptor actually serving a neuroprotective function [34]. This novel idea only recently became addressed by investigators, who corroborated this finding and added new twists [35–37].

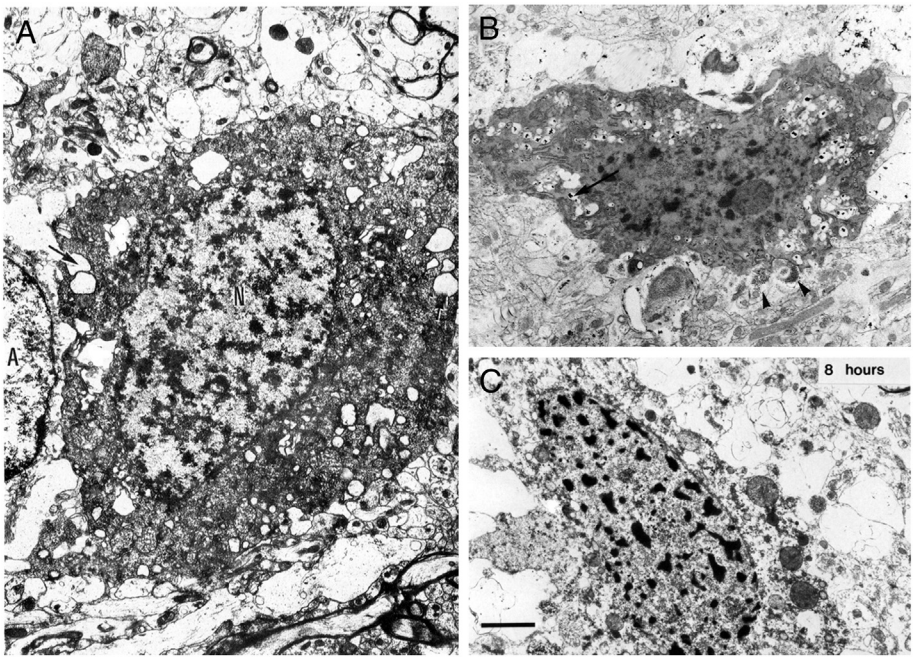

Fig. 3. Acutely injured neurons are morphologically necrotic, which was first shown over 40 years ago but not recognized until relatively recently. These electron photomicrographs show necrotic neurons following cerebral ischemia (A), status epilepticus (B) and hypoglycemia (C). The nuclei are shrunken, with scattered chromatin clumps, with cytoplasmic vacuoles in A and B and swollen mitochondria in C. This illustrates the fact that damaged neurons are morphologically necrotic in all three conditions.
The photomicrograph in A is from Ref. [11], that from B is from Ref. [24] and C is from Ref. [10].

However, it was discovered over 30 years ago that synapses are necessary for hypoxic neuronal death in cell culture [38]. More recently, it was shown that synaptic NMDA receptors in hippocampal neuronal culture can mediate excitotoxic neuronal death [39]. Finally, suppressing the expression of the NMDA-receptor scaffolding protein postsynaptic density-95 (PSD-95) selectively attenuates excitotoxicity in cortical neuronal culture by reducing nitric oxide (NO) production [40]. This last finding has been extended into a treatment for stroke by infusing a PSD-95 inhibitor 1 h after a 90-min middle cerebral artery occlusion in the macaque monkey, with resultant

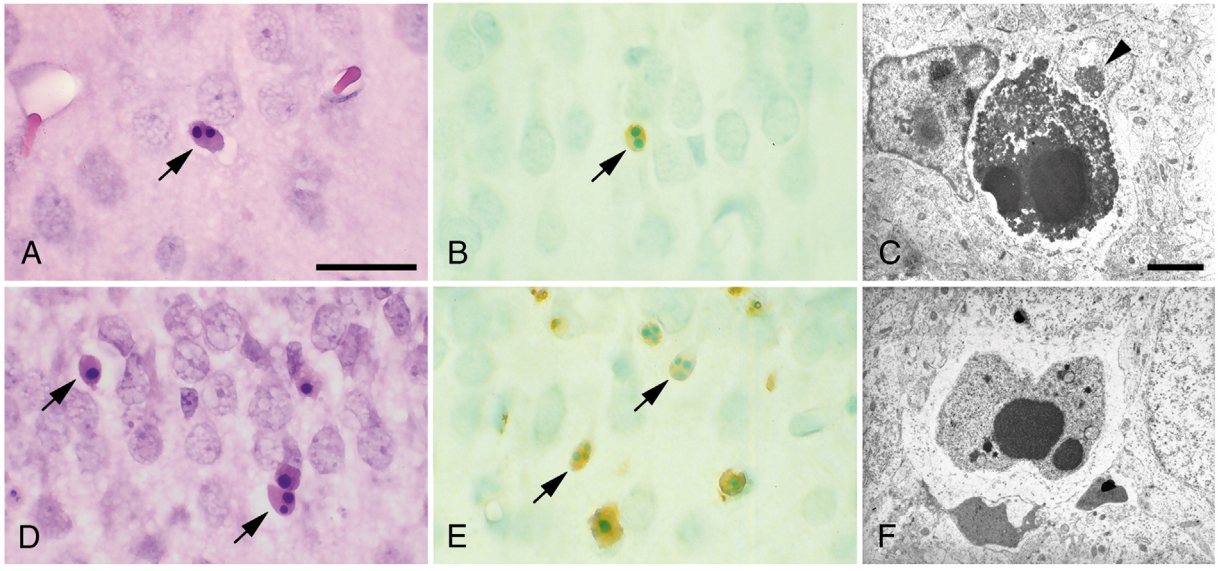

Fig. 4. Apoptotic neurons can be identified by light microscopy. A and B show naturally occurring apoptotic neurons in the retrosplenial cortex of postnatal day 8 rat pups, stained with H & E (A) and TUNEL with methyl green counterstain (B). Note their small size compared with normal neuronal nuclei and their characteristic appearance, with large, round chromatin clumps. In B the large chromatin clumps stain with methyl green, not TUNEL, whereas the remainder of the cell is lightly stained. TUNEL staining appears in the rest of the cell because of early disruption of the nuclear membrane. Arrows point to the apoptotic neurons. C is an electron-microscopic image of a naturally occurring apoptotic neuron in the opposite hemisphere of the same rat pup, being phagocytosed by an astrocyte. Its large chromatin clumps are clearly visible; the moth-eaten appearance of the rest of the cell suggests that it is in an advanced stage of degeneration. The arrowhead points to an apoptotic body pinched off from the cell. D and E show apoptotic neurons in a postnatal day 8 rat pup's retrosplenial cortex, stained with H & E (D) and TUNEL with methyl green counterstain (E). The rat pup was given the NMDA-receptor antagonist MK-801, which increases the number of apoptotic neurons, 24 h earlier. Arrows point to some of the apoptotic neurons. F shows an apoptotic neuron in the rat pup's opposite hemisphere in a relatively early stage of degeneration. The scale bar for A, B, D and E is 20 μm; for C and F it is 2 μm.
From Ref. [14].

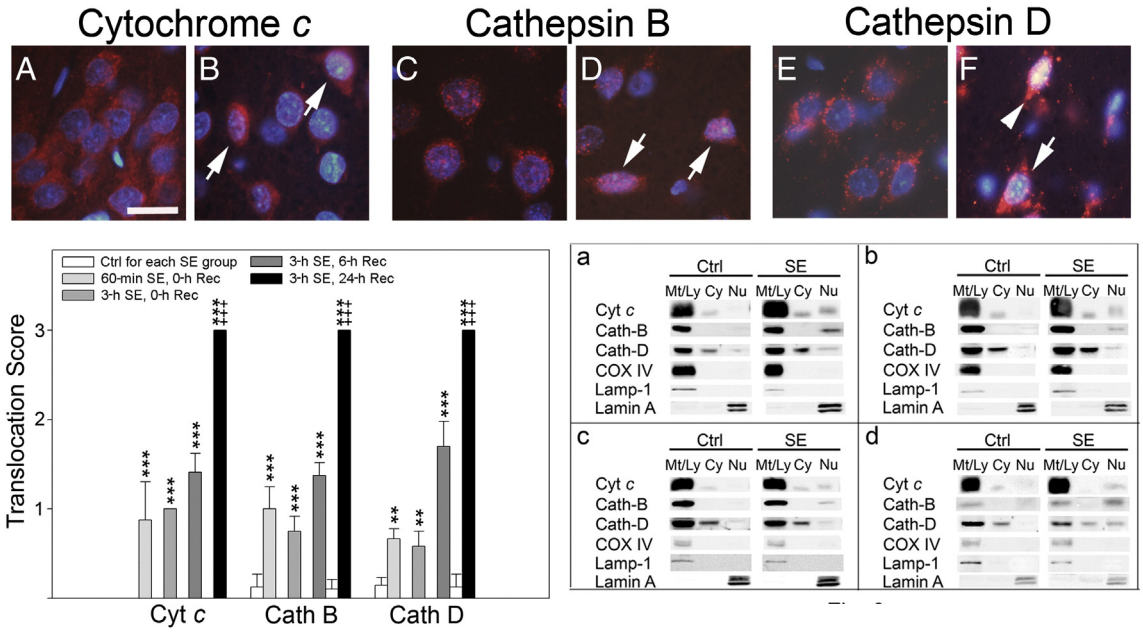

Fig. 5. Mitochondrial cytochrome *c* and lysosomal cathepsins B and D translocate to neuronal nuclei within 60 min after the onset of prolonged seizures. The immunofluorescence photomicrographs (A, C and E) show control mitochondrial cytochrome *c* and lysosomal cathepsins B and D (stained red) in piriform cortical neurons; nuclei are stained blue with DAPI. Panels B, D and F show nuclear translocation of cytochrome *c* and cathepsins B and D 60 min after the onset of generalized seizures. Arrows point to relatively normal appearing purplish nuclei showing nuclear translocation, and the arrowhead in F points to a shrunken neuron with a white, pyknotic nucleus showing nuclear translocation. The scale bar is 10 μm. The bar graph shows semi-quantitative results of nuclear translocation of cytochrome *c* (cyt *c*), cathepsin B (cath-B) and cathepsin D (cath-D) at 60 min and later time points, with the most nuclear translocation evident 24 h after 3-h seizures (**$p < 0.01$, ***$p < 0.001$ compared to controls, many with 0 scores; +++$p < 0.001$ compared to the three earlier groups). Panels a–d show Western blots of subcellular fractions of cyt *c*, cath-B and cath-D after 60-min (a) and 3-h seizures with 0-h (b), 6-h (c) and 24-h (d) survival. There is nuclear translocation of cyt *c*, cath-B and cath-D at all time points. COX-4, LAMP-1 and Lamin A are mitochondrial, lysosomal and nuclear markers.
Modified from Ref. [61].

reduction in infarct volume and preservation of neurological function [41].

It has long been known that extracellular glutamate elevation can occur from reversal of astrocytic glutamate uptake, in addition to excessive presynaptic glutamate release. This occurs, for example, when there are excessive neuronal depolarizations from seizures, causing elevation of extracellular K^+, the concentration of which is normally low. One glutamate molecule is transported into astrocytes and presynaptic terminals with three Na^+ cations, and one K^+ cation is extruded under baseline conditions [42]. When the extracellular K^+ is elevated, the process is reversed, and K^+ is taken up by astrocytes and presynaptic terminals, and glutamate and Na^+ are released [42]. The excessive extracellular glutamate can then activate an excessive number of NMDA receptors, whether synaptic or extrasynaptic, with resultant massive Ca^{2+} influx into neurons, triggering their enzymatic destruction.

4. Programmed Cell Death Pathways

There are two major programmed cell death pathways: the caspase-dependent and the caspase-independent pathways.

4.1. The Caspase-Dependent Pathway

The caspase-dependent pathway is actually composed of two pathways: the intrinsic mitochondrial pathway and the extrinsic death receptor pathway. Since neither is relevant to acute neuronal injury in the adult rodent brain I will provide only a brief overview. Both pathways are activated by a cellular insult which results in cell death.

The intrinsic mitochondrial pathway involves the initiator caspase, caspase-9, which, when activated, forms an "apoptosome" in the cytosol, together with cytochrome *c*, which translocates from mitochondria, Apaf-1 and dATP [43]. The apoptosome activates caspase-3, the central effector caspase, which in turn activates downstream factors that are responsible for the apoptotic death of a cell [43]. The extrinsic death receptor Fas pathway is activated by Fas ligand interaction with Fas complexes to form a death-inducing signaling complex (DISC), which contains the Fas-associated death domain protein (FADD) and caspases 8 and 10, which, in turn initiate apoptosis [44].

4.2. The Caspase-Independent Pathway

4.2.1. Calpain I Activation

In acute neuronal injury in the adult rodent brain, there were early reports of caspase-3 activation in cerebral ischemia [45,46] and SE [32,47], resulting in neuronal death, but subsequent studies were unable to find caspase-3 activation in cerebral ischemia [19–22] and SE [23,25,26,33]. This is consistent with the observation that caspase-3 activation occurs in neonatal brain but its expression becomes negligible in the adult rat brain [20–22]. On the other hand, calpain I, the Ca^{2+}-dependent cysteine protease, has been shown to be activated in cerebral ischemia [27,28], SE [26,48,49], and traumatic brain injury (TBI) [50–52], resulting in neuronal death, with no contradictory studies to date.

Activated calpain I cleaves the death-promoting Bcl-2 family members Bid [53,54] and Bax [55], which translocate to mitochondrial membranes, resulting in release of truncated apoptosis-inducing factor (tAIF) [53], cytochrome *c* (cyt *c*) [56] and endonuclease G (endoG) [54] in the case of Bid and cyt *c* in the case of Bax [55]. tAIF translocates to neuronal nuclei, and together with cyclophilin A and phosphorylated histone H2AX (γH2AX) is responsible for large-scale, 50 kb-pair DNA cleavage [57], a feature of programmed necrosis. EndoG produces internucleosomal (180 base pair) DNA cleavage [58]. Activated calpain I has also been shown to cleave the plasma membrane Na^+–Ca^{2+} exchanger, which leads to buildup of intracellular Ca^{2+} [59], so this is a source of increased intracellular Ca^{2+} other than through glutamate activation of the NMDA receptor and opening of its receptor-operated cation channel.

Cytochrome *c* (cyt *c*) in cellular apoptosis is a component of the apoptosome [43], as mentioned previously. Calpain I through Bid or

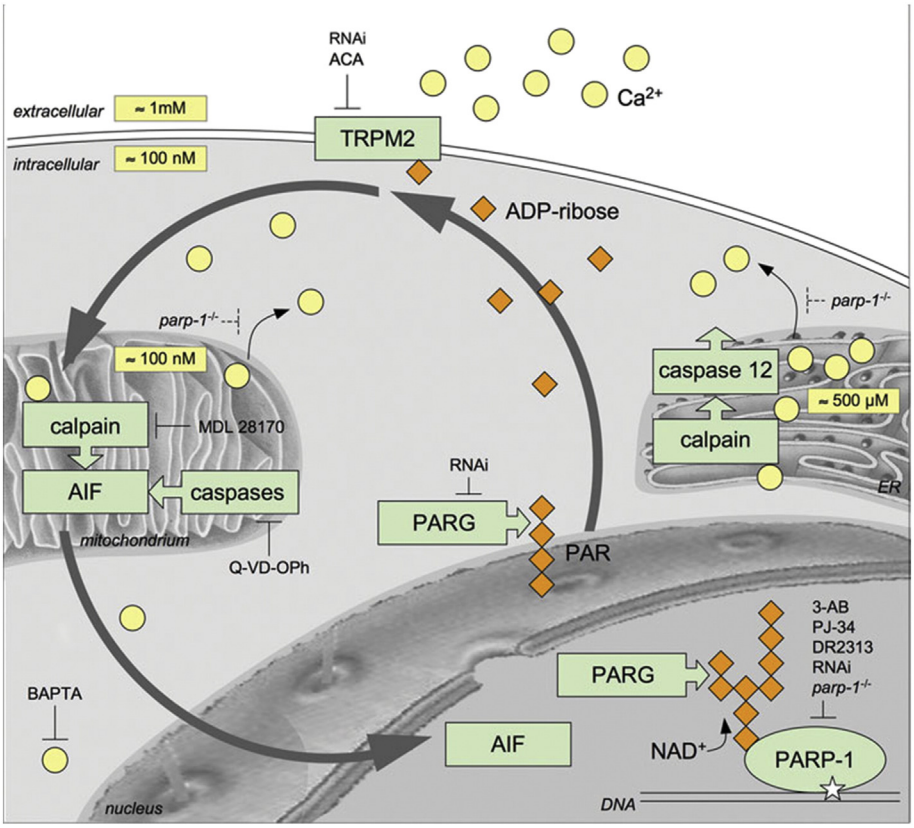

Fig. 6. In mouse embryonic fibroblasts, ADP-ribose opens plasma membrane TRPM2 channels to permit Ca^{2+} influx into neurons. Poly(ADP-ribose) polymerase-1 (PARP-1) produces poly(ADP-ribose) polymers (PAR) (chains of orange diamonds), which are hydrolyzed by poly(ADP-ribose) glycohydrolase (PARG) into ADP-ribose molecules (single orange diamonds). When formed in excess, these ADP-ribose molecules translocate to the plasma membrane to activate melastatin-like transient receptor potential-2 (TRPM-2) channels, which open and allow excessive Ca^{2+} (yellow circles) to enter neurons.
From Ref. [70].

Bax induces cyt *c* release from mitochondria [55,56]. In HeLa cell culture, following exposure to UV irradiation, cyt *c* has been shown to translocate to nuclei and be associated with cytosolic translocation of acetylated histone H2A [60]. In addition, isolated nuclei treated with cyt *c* and a cytosolic extract of UV-irradiated cells produced chromatin condensation [60]. Whether these actions of cyt *c* occur *in vivo* in acute neuronal injury is not known, but we have shown that cyt *c*, together with lysosomal cathepsins B and D and DNase II and mitochondrial AIF and endoG, translocates to neuronal nuclei with the first 60 min of generalized SE (Fig. 5) [61]. Apoptosis-inducing factor is involved in 50 kb-pair DNA cleavage, and DNase II and/or endonuclease G could be responsible for the 180 base-pair internucleosomal DNA cleavage we have found following SE [13,14,23]. Cathepsins B and D could hydrolyze nuclear proteins (such as histones and lamin A), as they do in lysosomes, but whether this occurs, as well as the roles played by cyt *c*, DNase II and endoG, in acute neuronal necrosis remain to be determined.

Yamashima and colleagues found that in primate global cerebral ischemia, the cathepsin B inhibitor CA-074 protects against neuronal death from lysosomal rupture and release of cathepsins into the cytosol of neurons in the hippocampus, which is the basis of their "calpain-cathepsin hypothesis" [62]. They subsequently found that hydroxynonenal (HNE) phosphorylates heat shock protein 1 (HSP-1), which is responsible for permeabilization of lysosomal membranes, allowing release of cathepsins into the cytosol [63] and DNase II into the nucleus [64]. The ultrastructural features of the dead neurons was necrotic [64].

4.2.2. Neuronal Nitric Oxide Synthase (nNOS) Activation

Neuronal nitric oxide synthase (nNOS) is a Ca^{2+}-dependent cytosolic enzyme that forms nitric oxide (NO) from L-arginine, and NO reacts with the free radical superoxide (O_2^-) to form the toxic free radical peroxynitrite ($ONOO^-$) [65]. Free radicals such as $ONOO^-$, O_2^- and hydroxyl radical (OH^-) damage cellular membranes and intracellular proteins, enzymes and DNA [66].

4.2.3. Poly(ADP-Ribose) Polymerase-1 (PARP-1) Activation

DNA damage activates nuclear poly(ADP-ribose) polymerase-1 (PARP-1), a DNA repair enzyme. PARP-1 forms poly(ADP-ribose) polymers, to repair DNA, but when DNA damage is extensive, PAR accumulates, exits neuronal nuclei and travels to mitochondrial membranes, where it, like calpain I, is involved in AIF release from mitochondria [67–69].

In mouse embryonic fibroblasts, nuclear poly(ADP-ribose) glycohydrolase (PARG) hydrolyzes PAR polymers into ADP-ribose molecules, which, when excessive, translocate to the plasma membrane to activate melastatin-like transient receptor potential 2 (TRPM-2) channels, which open to allow Ca^{2+} influx into neurons [70] (Fig. 6).

4.2.3.1. Is "Parthanatos" Really a Separate Category of Neuronal Death?. The Dawson laboratory has called their data on MNNG-, H_2O_2— or NMDA-induced PARP-1 activation, translocation of its substrate poly(ADP-ribose) (PAR), mitochondrial release of full-length or truncated apoptosis-inducing factor (AIF), with its translocation to nuclei, "parthanatos" [71]. But a study of double-knockout mouse embryonic fibroblasts (MEFs) in culture exposed to the DNA alkylating agent MNNG showed sequential steps involving PARP-1, calpain I, Bax and AIF, which they called "programmed necrosis" [72]. This was based upon data showing that DNA alkylation-induced cell death is caspase-independent and necrotic rather than apoptotic [72]. "Programmed necrosis" is a commonly accepted term for the response to acute cell injury. Moreover, the nuclear pyknosis that the Dawson lab showed in their

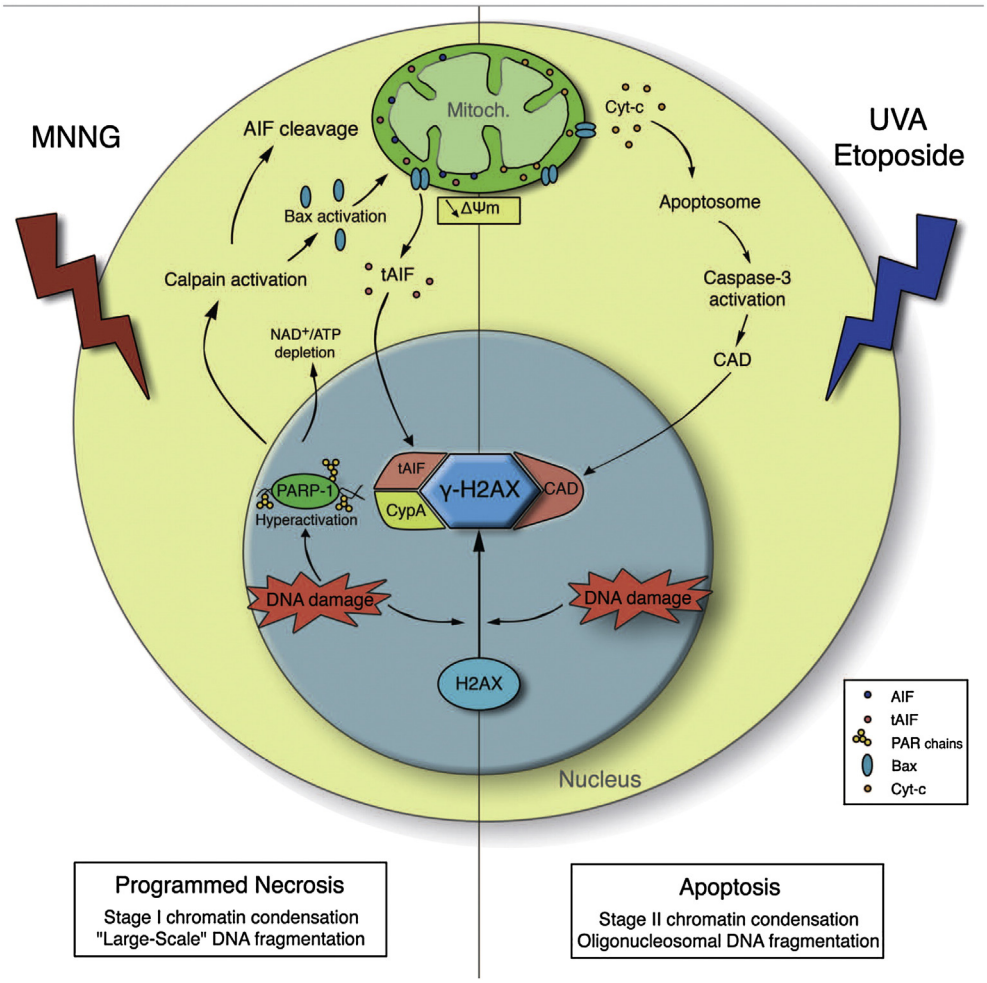

Fig. 7. In mouse embryonic fibroblasts, DNA damage (from MMNG) results in PARP-1 activation, which activates calpain I, which in turn activates Bax and promotes cleavage of AIF (tAIF). tAIF translocates to nuclei, and together with phosphorylated histone H2AX (γH2AX) and cyclophilin A (CypA), cleaves DNA into large-scale DNA fragments (DNA damage). These events constitute programmed necrosis (left side of the diagram). The right side, which concerns mechanisms promoting cellular apoptosis, is not relevant to acute neuronal injury in the adult brain.
From Ref. [73].

Figs. 1–3 are compatible with necrotic neurons [69], so there is no reason to postulate a separate category of neuronal death.

4.2.3.2. PARP-1 Activates Calpain I to set off tAIF Release From Mitochondria. Using double-knockout mouse embryonic fibroblasts (MEFs), Santos Susin's group has shown that that PARP-1 activates calpain I, which activates Bax, which translocates to mitochondral membranes, resulting in tAIF release and translocation to cellular nuclei [72]. They subsequently showed that tAIF, phosphorylated histone H2AX (γH2AX) and cyclophilin A together produce large scale (50 kb) DNA cleavage [57,73] (Fig. 7). It has also been shown in primary neuronal culture that PARP-1 is necessary for calpain I activation [28]. We know that calpain I is activated by intracellular Ca^{2+}, but the mechanism by which PARP-1 activates calpain I is not known.

4.2.4. NADPH Oxidase (NOX1) Transfers Electrons Across Cell Membranes, Producing O_2^-

The NOX family of NADPH oxidase transfers electrons across cellular membranes. The electron acceptor is O_2 and the product of the transfer is superoxide (O_2^-). NOX1 contributes to ischemic neuronal death through generation of O_2^- [74,75].

5. Human Studies of Programmed Cell Death

Compared to *in vitro* cell culture and *in vivo* animal studies, there are relatively few human studies on programmed mechanisms of acute neuronal injury. There is a study of resected human hippocampal tissue in patients with temporal lobe epilepsy (TLE), with post-mortem controls, in which caspase-activated DNase (CAD) levels were higher in neuronal nuclei in TLE patients compared to controls, and cleaved (activated) caspase-3 immunoreactivity (a-casp-3 IR) was greater in the hippocampi of TLE patients as well [76]. However, there was limited translocation to nuclei of apoptosis-inducing factor (AIF) and when it appeared, it did not occur in neurons with a-casp-3 IR. Moreover, these findings did not occur in the setting of acute injury. So the meaning of these findings is not clear.

The first two of four human studies of human brain tissue following severe traumatic brain injury (TBI) showed activation of calpain I, based on the appearance of calpain I-cleaved αII-spectrin 150 and 145 kDa breakdown products (SBDPs) in subjects' CSF [50,52]. Increased SBDP-150 and SBDP-145 levels were positively correlated with worse Glascow Coma Scale scores [50,52] and with longer elevations of intracranial pressure ≥ 25 mm Hg [50].

A third TBI study has shown increase in Bcl-2 and cleavage (activation) of caspase-1 (a-casp-1) and casp-3 (a-casp-3) IR and double-stranded DNA fragmentation (terminal deoxynucleotidyl transferase

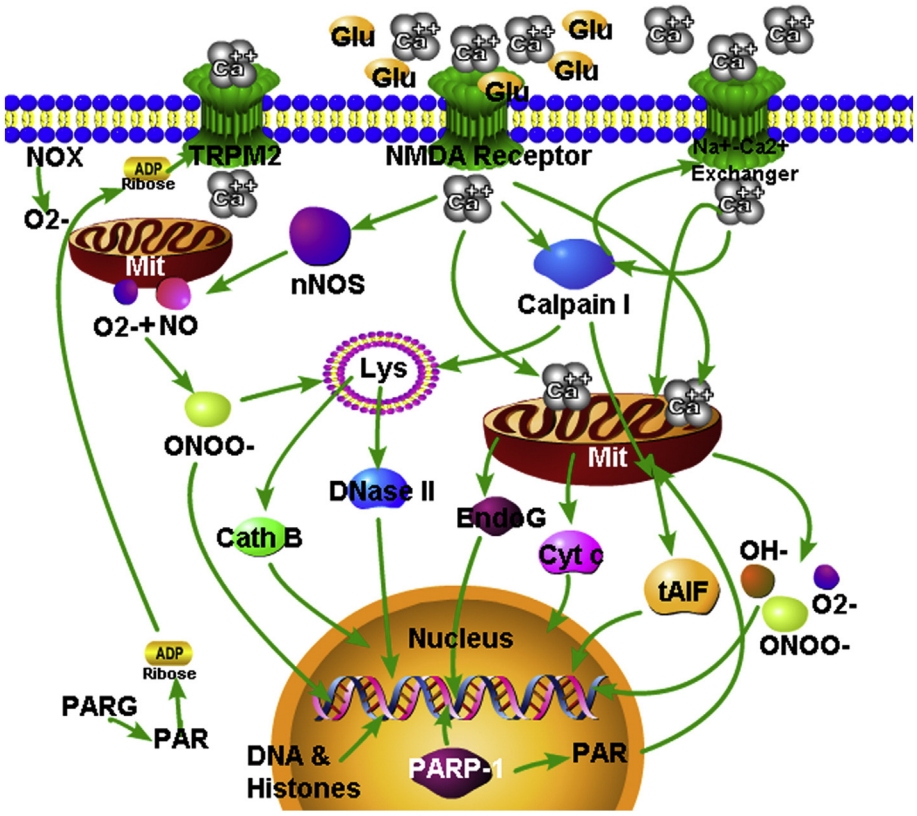

Fig. 8. A summary of the programmed cell death mechanisms contributing to acute neuronal necrosis. Activation of the NMDA receptor by excessive extracellular glutamate opens its receptor-operated cation channel, resulting in excessive Ca^{2+} influx. The Ca^{2+}-dependent enzymes calpain I and neuronal nitric oxide synthase (nNOS) are activated. Calpain I activation cleaves cytosolic Bax and Bid, which translocate to mitochondrial membranes (not shown); calpain I also translocates to mitochondrial (Mit) membranes, and Bax, Bid and calpain I all contribute to truncated AIF (tAIF) and cytochrome *c* (cyt *c*) release from mitochondrial membranes, whereas calpain I and Bid contribute to the mitochondrial release of endonuclease G (endoG). The tAIF translocates to neuronal nuclei and participates in large-scale (50 kb) DNA cleavage, and endoG translocates to nuclei, producing internucleosomal (180 base pair) DNA cleavage. Calpain I also permeabilizes lysosomal (Lys) membranes, resulting in release of cathepsin B (Cath B), cathepsin D (not shown) and DNase II, which all translocate to neuronal nuclei. EndoG, together with mitochondrial cytochrome *c* (Cyt *c*) and AIF and lysosomal cathepsins B and D and DNase II, all translocate to neuronal nuclei within the first 60 min of status epilepticus. Calpain I also cleaves the plasma membrane Na^{+}–Ca^{2+} exchanger, with resultant buildup of intracellular Ca^{2+}. Neuronal NOS (nNOS) forms NO from L-arginine (not shown); NO reacts with superoxide (O_2^-) to form peroxynitrite ($OONO^-$). Peryoxynitrite, O_2^- and hydroxyl radical (OH^-) damage DNA, which results in PARP-1 activation. PARP-1 forms poly(ADP-ribose) (PAR) polymers, which exit neuronal nuclei and translocate to mitochondrial membranes, where they also participate in AIF release from mitochondrial membranes. Poly(ADP-ribose) glycohydrolase (PARG) may hydrolyze PAR into ADP-ribose molecules in neurons. The ADP-ribose molecules translocate to the plasma membrane and activate melastatin-like transient receptor potential-2 (TRPM-2) receptors, with resultant Ca^{2+} influx into cells. Finally, NOX1 (NADPH-1) generates O_2^- at cellular membranes, which contributes to ischemic neuronal death.

dUTP nick-end labeling [TUNEL stain]) in brain tissue removed for surgical decompression for acute intracranial hypertension [77]. The authors mistakenly interpreted some neurons with TUNEL-positive pyknotic nuclei as being apoptotic and a non-pyknotic nucleus as necrotic (their Fig. 2), and *in vivo* animal studies have since shown that a-casp-3 IR is found in neurons in the immature brain following cerebral ischemia, but is gone by postnatal day 60 [20–22]. Other studies have found that neither neuronal apoptosis nor a-casp-3 IR occur in neurons in the adult rodent brain following status epilepticus (SE) [23,25,33] and cerebral ischemia [19]. Moreover, subsequent animal research has focused on caspase-independent neuronal death, which is morphologically necrotic [8,13,14,23,25].

The last human study following TBI showed poly(ADP-ribose) polymerase (PARP) IR in both nuclei and cytoplasm in contused brain tissue sampled ≤4 h after trauma, and based on experimental studies showing cleaved PARP translocation to cytoplasm after cleavage by a-casp-3, they assumed that this represented an apoptotic process [78]. In their Fig. 1 the authors showed neurons with PARP IR in nuclei or nuclei and cytoplasm that resemble morphologically necrotic, not apoptotic, neurons. They showed no data regarding a-casp-3 IR despite describing in the Materials and Methods section that they used an antibody to the p17 cleaved fragment of casp-3. Finally, they did not show data on cleaved PARP, but only full-length PARP, which one would not expect to translocate to cytoplasm.

6. Summary and Outlook

In acute neuronal injury of whatever cause, elevated extracellular glutamate from excessive neuronal depolarization and reversal of astrocytic glutamate uptake results in excessive NMDA-receptor activation and excessive Ca^{2+} influx through NMDA-receptor-operated cation channels. Elevated intracellular Ca^{2+} is also produced by ADP-ribose molecules that translocate to TRPM-2 channels at the plasma membrane and calpain I cleavage of the Na^{+}–Ca^{2+} exchanger. Elevated intracellular Ca^{2+} activates Ca^{2+}-dependent calpain I and nNOS. Calpain I activates cytosolic Bax and Bid, which translocate from cytosol to mitochondrial membranes, producing tAIF, endo G and cyt *c* release from mitochondria. Calpain I also triggers cathepsin B, D and DNase II from lysosomes. tAIF, endo G, cyt *c*, cathepsins B and D and DNase II translocate to neuronal nuclei, where tAIF, together with γH2AX and cyclophilin A, cause 50 kb DNA cleavage. Internucleosomal (180 bp) double-stranded DNA cleavage occurs following cerebral ischemia and SE, likely produced by endo G and DNase II (caspase-activated DNase, or CAD, may also be responsible). nNOS production of NO, which forms $ONOO^-$, and other free radicals that exit mitochondria and are also produced at plasma membranes by NOX1, damage DNA, triggering PARP-1 activation, excessive PAR formation and its translocation to mitochondrial membranes, with tAIF or AIF release. Poly(ADP-ribose) glycohydrolase cleaves PAR into ADP-ribose molecules that translocate

to TRPM-2 channels at the plasma membrane, resulting in excessive Ca^{2+} influx. These mechanisms are summarized in Fig. 8.

To date there have been no successful trials in which various agents found to be neuroprotective in animals have shown efficacy or been tolerated in humans. There is an ongoing Canadian trial in which an inhibitor of the coupling of the NMDA receptor to postsynaptic density-95 (PSD-95), which inhibits nNOS production of NO, is being used. Inhibitors of PSD-95 have been shown to be neuroprotective in rodent and primate stroke models [79,80].

Magnesium blocks the NMDA-receptor operated cation channel, thereby preventing Ca^{2+} entry, the first step in the excitotoxic cascade of reactions producing programmed necrosis. However, a recently completed trial of using a loading dose of $MgSO_4$ in the pre-hospital setting and continuing a maintenance dose for 24 h did not show reduction in the degree of disability at 90 days [81].

Finally, although PARP-1 and calpain I inhibition have been shown to be neuroprotective in acute neuronal injury in animals, to date there have been no human clinical trials of either inhibitor.

Conflict of Interest

I have no conflict of interest and nothing to disclose.

References

[1] Olney JW. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. An electron microscopic study. J Neuropathol Exp Neurol 1971;30:75–90.
[2] Olney JW, Rhee V, Ho OL. Kainic acid: a powerful neurotoxic analogue of glutamate. Brain Res 1974;77:507–12.
[3] Olney JW. Excitatory transmitters and epilepsy-related brain damage. In: Smythies JR, Bradley RJ, editors. International Review of Neurobiology. Orlando: Academic Press, Inc.; 1985. p. 337–62.
[4] MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 1986;321:519–22.
[5] Simon RP, Swan JH, Griffiths T, Meldrum BS. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science 1984;226:850–2.
[6] Choi DW, Maulucci-Gedde M, Kriegstein AR. Glutamate neurotoxicity in cortical cell culture. J Neurosci 1987;7:357–68.
[7] Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci 1987;7:369–79.
[8] Fujikawa DG, editor. Acute Neuronal Injury: The Role of Excitotoxic Programmed Cell Death Mechanisms. New York: Springer; 2010 [306 pp.].
[9] Auer RN, Kalimo H, Olsson Y, Siesjo BK. The temporal evolution of hypoglycemic brain damage. II. Light- and electron-microscopic findings in the hippocampal gyrus and subiculum of the rat. Acta Neuropathol (Berl) 1985;67:25–36.
[10] Auer RN, Kalimo H, Olsson Y, Siesjo BK. The temporal evolution of hypoglycemic brain damage. I. Light- and electron-microscopic findings in the rat cerebral cortex. Acta Neuropathol (Berl) 1985;67:13–24.
[11] Brown AW, Brierley JB. Anoxic–ischaemic cell change in rat brain light microscopic and fine-structural observations. J Neurol Sci 1972;16:59–84.
[12] Colbourne F, Sutherland GR, Auer RN. Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. J Neurosci 1999;19:4200–10.
[13] Fujikawa DG, Shinmei SS, Cai B. Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internucleosomal DNA fragmentation in adult rats. Eur J Neurosci 1999;11:1605–14.
[14] Fujikawa DG, Shinmei SS, Cai B. Kainic acid-induced seizures produce necrotic, not apoptotic, neurons with internucleosomal DNA cleavage: implications for programmed cell death mechanisms. Neuroscience 2000;98:41–53.
[15] Griffiths T, Evans M, Meldrum BS. Intracellular calcium accumulation in rat hippocampus during seizures induced by bicuculline or L-allylglycine. Neuroscience 1983;10:385–95.
[16] Clarke PGH. Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol 1990;181:195–213.
[17] Uchiyama Y, Shibata M, Koike M, Yoshimura K, Sasaki M. Autophagy—physiology and pathophysiology. Histochem Cell Biol 2008;129:407–20.
[18] Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler V, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–4.
[19] Gill R, Soriano M, Blomgren K, Hagberg H, Wybrecht R, Miss MT, et al. Role of caspase-3 activation in cerebral ischemia-induced neurodegeneration in adult and neonatal brain. J Cereb Blood Flow Metab 2002;22:420–30.
[20] Hu BR, Liu CL, Ouyang Y, Blomgren K, Siejö BK. Involvement of caspase-3 in cell death after hypoxia–ischemia declines during brain maturation. J Cereb Blood Flow Metab 2000;20:1294–300.
[21] Liu CL, Siesjö BK BK, Hu BR. Pathogenesis of hippocampal neuronal death after hypoxia–ischemia changes during brain development. Neuroscience 2004;127:113–23.
[22] Zhu C, Wang X, Xu F, Bahr BA, Shibata M, Uchiyama Y, et al. The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia–ischemia. Cell Death Differ 2005;12:162–76.
[23] Fujikawa DG, Ke X, Trinidad RB, Shinmei SS, Wu A. Caspase-3 is not activated in seizure-induced neuronal necrosis with internucleosomal DNA cleavage. J Neurochem 2002;83:229–40.
[24] Griffiths T, Evans MC, Meldrum BS. Status epilepticus: the reversibility of calcium loading and acute neuronal pathological changes in the rat hippocampus. Neuroscience 1984;12:557–67.
[25] Fujikawa DG, Shinmei SS, Zhao S, Aviles Jr ER. Caspase-dependent programmed cell death pathways are not activated in generalized seizure-induced neuronal death. Brain Res 2007;1135:206–18.
[26] Araújo IM, Gil JM, Carreira BP, Mohapel P, Petersen A, Pinheiro PS, et al. Calpain activation is involved in early caspase-independent neurodegeneration in the hippocampus following status epilepticus. J Neurochem 2008;105:666–76.
[27] Cao G, Xing J, Xiao X, Liou AK, Gao Y, Yin XM, et al. Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury. J Neurosci 2007;27:9278–93.
[28] Vosler PS, Sun D, Wang S, Gao Y, Kintner DB, Signore AP, et al. Calcium dysregulation induces apoptosis-inducing factor release: cross-talk between PARP-1- and calpain-signaling pathways. Exp Neurol 2009;218:213–20.
[29] Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980;284:555–6.
[30] Gavrielli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
[31] Cao G, Minami M, Pei W, Yan C, Chen D, O'Horo C, et al. Intracellular Bax translocation after transient cerebral ischemia: implications for a role of the mitochondrial apoptotic signaling pathway in ischemic neuronal death. J Cereb Blood Flow Metab 2001;21:321–33.
[32] Henshall DC, Chen J, Simon RP. Involvement of caspase-3-like protease in the mechanism of cell death following focally evoked limbic seizures. J Neurochem 2000;74:1215–23.
[33] Narkilahti S, Pirtillä TJ, Lukasiuk K, Tuunanen J, Pitkänen A. Expression and activation of caspase 3 following status epilepticus. Eur J Neurosci 2003;18:1486–96.
[34] Hardingham GE, Bading H. Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. Biochim Biophys Acta 2002;1600:148–53.
[35] Tu W, Xu X, Peng L, Zhong X, Zhang W, Soundarapandian MM, et al. DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. Cell 2010;140:222–34.
[36] Wang Y, Briz V, Chishti A, Bi X, Baudry M. Distinct roles for mu-calpain and m-calpain in synaptic NMDAR-mediated neuroprotection and extrasynaptic NMDAR-mediated neurodegeneration. J Neurosci 2013;33:18880–92.
[37] Zhou X, Ding Q, Chen Z, Yun H, Wang H. Involvement of the GluN2A and GluN2B subunits in synaptic and extrasynaptic N-methyl-D-aspartate receptor function and neuronal excitotoxicity. J Biol Chem 2013;288:24151–9.
[38] Rothman SM. Synaptic activity mediates death of hypoxic neurons. Science 1983;220:536–7.
[39] Wroge CM, Hogins J, Eisenman L, Mennerick S. Synaptic NMDA receptors mediate hypoxic excitotoxic death. J Neurosci 2012;32:6732–42.
[40] Sattler R, Xiong Z, Lu WY, Hafner M, MacDonald JF, Tymianski M. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. Science 1999;284:1845–8.
[41] Cook DJ, Teves L, Tymianski M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. Nature 2012;483:213–7.
[42] Nicholls D, Attwell D. The release and uptake of excitatory amino acids. Trends Pharmacol Sci 1990;11:462–8.
[43] Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997;90:405–13.
[44] Wajant H. The Fas signaling pathway: more than a paradigm. Science 2002;296:1635–6.
[45] Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, et al. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J Neurosci 1998;18:4914–28.
[46] Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, et al. Inhibition of interleukin-1-beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A 1997;94:2007–12.
[47] Kondratyev A, Gale K. Intracerebral injection of caspase-3 inhibitor prevents neuronal apoptosis after kainic acid-evoked status epilepticus. Mol Brain Res 2000;75:216–24.
[48] Wang S, Wang S, Shan P, Song Z, Dai T, Wang R, et al. Mu-calpain mediates hippocampal neuron death in rats after lithium-pilocarpine-induced status epilepticus. Brain Res Bull 2008;76:90–6.
[49] Siman R, Noszek JC, Kegerise C. Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. J Neurosci 1989;9:1579–90.
[50] Brophy GM, Pineda JA, Papa L, Lewis SB, Valadka AB, Hannay HJ, et al. AlphaII-spectrin breakdown product cerebrospinal fluid exposure metrics suggest differences in cellular injury mechanisms after severe traumatic brain injury. J Neurotrauma 2009;26:471–9.
[51] McGinn MJ, Kelley BJ, Akinyi L, Oli MW, Liu MC, Hayes RL, et al. Biochemical, structural, and biomarker evidence for calpain-mediated cytoskeletal change after diffuse brain injury uncomplicated by contusion. J Neuropathol Exp Neurol 2009;68:241–9.
[52] Mondello S, Robicsek SA, Gabrielli A, Brophy GM, Papa L, Tepas J, et al. AlphaII-spectrin breakdown products (SBDPs): diagnosis and outcome in severe traumatic brain injury patients. J Neurotrauma 2010;27:1203–13.

[53] Polster BM, Basanez G, Etxebarria A, Hardwick JM, Nicholls DG. Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. J Biol Chem 2005;280:6447–54.
[54] Takano J, Tomioka M, Tsubuki S, Higuchi M, Nobuhisa Iwata N, Itohara S, et al. Calpain mediates excitotoxic DNA fragmentation via mitochondrial pathways in adult brains: evidence from calpastatin mutant mice. J Biol Chem 2005;280: 16175–84.
[55] Gao G, Dou QP. N-terminal cleavage of bax by calpain generates a potent proapoptotic 18-kDa fragment that promotes bcl-2-independent cytochrome C release and apoptotic cell death. J Cell Biochem 2000;80:53–72.
[56] Mandic A, Viktorsson K, Strandberg L, Heiden T, Hansson J, Linder S, et al. Calpain-mediated Bid cleavage and calpain-independent Bak modulation: two separate pathways in cisplatin-induced apoptosis. Mol Cell Biol 2002;22:3003–13.
[57] Artus C, Boujrad H, Bouharrour A, Brunelle MN, Hoos S, Yuste VJ, et al. AIF promotes chromatinolysis and caspase-independent programmed necrosis by interacting with histone H2AX. EMBO J 2010;29:1585–99.
[58] Ishihara Y, Shimamoto N. Involvement of endonuclease G in nucleosomal DNA fragmentation under sustained endogenous oxidative stress. J Biol Chem 2006;281: 6726–33.
[59] Bano D, Munarriz E, Chen HL, Ziviani E, Lippi G, Young KW, et al. The plasma membrane Na^+/Ca^{2+} exchanger is cleaved by distinct protease families in neuronal cell death. Ann N Y Acad Sci 2007;1099:451–5.
[60] Nur-E-Kamal A, Gross SR, Pan Z, Balklava Z, Ma J, Liu LF, et al. Nuclear translocation of cytochrome c during apoptosis. J Biol Chem 2004;279:24911–4.
[61] Zhao S, Aviles Jr ER, Fujikawa DG. Nuclear translocation of mitochondrial cytochrome c, lysosomal cathepsins B and D, and three other death-promoting proteins within the first 60 minutes of generalized seizures. J Neurosci Res 2010;88:1727–37.
[62] Yamashima T, Kohda Y, Tsuchiya K, Ueno T, Yamashita J, Yoshioka T, et al. Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. Eur J Neurosci 1998;10:1723–33.
[63] Sahara S, Yamashima T. Calpain-mediated Hsp70.1 cleavage in hippocampal CA1 neuronal death. Biochem Biophys Res Commun 2010;393:806–11.
[64] Tsukada T, Watanabe M, Yamashima T. Implications of CAD and DNase II in ischemic neuronal necrosis specific for the primate hippocampus. J Neurochem 2001;79: 1196–206.
[65] Singh IN, Sullivan PG, Hall ED. Peroxynitrite-mediated oxidative damage to brain mitochondria: protective effects of peroxynitrite scavengers. J Neurosci Res 2007;85: 2216–23.
[66] Mattiasson G, Sullivan PG. The emerging functions of UCP2 in health, disease, and therapeutics. Antioxid Redox Signal 2006;8:1–38.
[67] Andrabi SA, Kim S-W, Wang H, Koh DW, Sasaki M, Klaus JA, et al. Poly(ADP-ribose) (PAR) polymer is a death signal. Proc Natl Acad Sci U S A 2006;103:18308–13.
[68] Yu S-W, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, et al. Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci U S A 2006;103:18314–9.
[69] Yu S-W, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002;297:259–63.
[70] Blenn C, Wyrsch P, Bader J, Bollhalder M, Althaus FR. Poly(ADP-ribose)glycohydrolase is an upstream regulator of Ca^{2+} fluxes in oxidative cell death. Cell Mol Life Sci 2011;68: 1455–66.
[71] David KK, Andrabi SA, Dawson TM, Dawson VL. Parthanatos, a messenger of death. Front Biosci 2009;14:1116–28.
[72] Moubarak RS, Yuste VJ, Artus C, Bouharrour A, Greer PA, Menissier-de Murcia J, et al. Sequential activation of poly(ADP-ribose) polymerase 1, calpains, and Bax is essential in apoptosis-inducing factor-mediated programmed necrosis. Mol Cell Biol 2007;27:4844–62.
[73] Baritaud M, Boujrad H, Lorenzo HK, Krantic S, Susin SA. Histone H2AX: the missing link in AIF-mediated caspase-independent programmed necrosis. Cell Cycle 2010;9: 3166–73.
[74] Kahles T, Kohnen A, Heumueller S, Rappert A, Bechmann I, Liebner S, et al. NADPH oxidase Nox1 contributes to ischemic injury in experimental stroke in mice. Neurobiol Dis 2010;40:185–92.
[75] Tang XN, Cairns B, Kim JY, Yenari MA. NADPH oxidase in stroke and cerebrovascular disease. Neurol Res 2012;34:338–45.
[76] Schindler CK, Pearson EG, Bonner HP, So NK, Simon RP, Prehn JH, et al. Caspase-3 cleavage and nuclear localization of caspase-activated DNase in human temporal lobe epilepsy. J Cereb Blood Flow Metab 2006;26:583–9.
[77] Clark RS, Kochanek PM, Chen M, Watkins SC, Marion DW, Chen J, et al. Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. FASEB J 1999;13:813–21.
[78] Ang BT, Yap E, Lim J, Tan WL, Ng PY, Ng I, et al. Poly(adenosine diphosphate-ribose) polymerase expression in human traumatic brain injury. J Neurosurg 2003;99: 125–30.
[79] Aarts M, Liu Y, Liu L, Besshoh S, Arundine M, Gurd JW, et al. Treatment of ischemic brain damage by perturbing NMDA receptor–PSD-95 protein interactions. Science 2002;298:846–50.
[80] Cook DJ, Tymianski M. Nonhuman primate models of stroke for translational neuroprotection research. Neurotherapeutics 2012;9:371–9.
[81] Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, et al. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. N Engl J Med 2015; 372:528–36.
[82] Fujikawa DG. Neuroprotective strategies in status epilepticus. In: Wasterlain CG, Treiman DM, editors. Status Epilepticus: Mechanisms and Management. Cambridge, MA: MIT Press; 2006. p. 463–80.