Mini Review

The Role of Excitotoxic Programmed Necrosis in Acute Brain Injury

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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.

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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 shrunk, 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 cannot be translated to humans.

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,
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 Ca²⁺-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 Ca²⁺ 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].
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. 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].

![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].]
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 Ca2+ 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 Ca2+-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+-Ca2+ exchanger, which leads to build up of intracellular Ca2+ [59], so this is a source of increased intracellular Ca2+ 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...
Bax induces cytochrome c (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. 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].
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 PARP-1 activates calpain I, which activates Bax, which translocates to mitochondrial membranes, resulting in tAIF release and translocation to cellular nuclei [72]. They subsequently showed that tAIF, 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].

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 αI1-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 Glasgow 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 (TdT) mediated dUTP nick end labeling (TUNEL)).

From Ref. [73].
to translocate to cytoplasm. cleaved PARP, but only full-length PARP, which one would not expect p17 cleaved fragment of casp-3. Finally, they did not show data on neurons. They showed no data regarding α-casp-3 IR despite describing and cytoplasm that resemble morphologically necrotic, not apoptotic, Fig. 1 the authors showed neurons with PARP IR in nuclei or nuclei and cytoplasm after cleavage by α-casp-3, they assumed that this represented an apoptotic process[78]. In their sampled neurotically necrotic[8,13,14,23,25]. Moreover, subsequent animal research has found that neither neuronal apoptosis nor α-casp-3 IR occur in neurons in the adult rodent brain following status epilepticus (SE)[23,25,33] and cerebral ischemia[19]. The last human study following TBI showed poly(ADP-ribose) poly-merase (PARP) IR in both nuclei and cytoplasm in contused 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 α-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 morphologi-cally necrotic[8,13,14,23,25].

The last human study following TBI showed poly(ADP-ribose) glycohydrolase cleaves PAR into ADP-ribose molecules that translocate to TRPM-2 channels at the plasma mem-brane and calpain I cleavage of the Na+–Ca2+ exchanger. Elevated in-tracellular Ca2+ is also produced by ADP-ribose molecules that translocate to TRPM-2 channels at the plasma membrane and calpain I cleavage of the Na+–Ca2+ exchanger. Elevated intracellular Ca2+ activates Ca2+–dependent calpain I and nNOS. Calpain I cleaves cytosolic Bax and Bid, which translocate from cytosol to mito-chondrial membranes, producing tAIF, endoG, and DNase II translocate to neuronal nuclei within the first 60 min of status epilepticus. Calpain I also cleaves the plasma membrane Na+–Ca2+ exchanger, with resultant buildup of intracellular Ca2+. Neuronal NOS (nNOS) forms NO from L-arginine (not shown); NO reacts with superoxide (O2−) to form peroxynitrite (ONOO−), which 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 Ca2+ influx into cells. Finally, NOX1 (NADPH)–1 generates O2− at cellular membranes, which contributes to ischemic neuronal death.

6. Summary and Outlook

In acute neuronal injury of whatever cause, elevated extracellular glutamate from excessive neuronal depolarization and reversal of astro-cytic glutamate uptake results in excessive NMDA-receptor activation and excessive Ca2+ influx through NMDA-receptor-operated cation channels. Elevated intracellular Ca2+ is also produced by ADP-ribose molecules that translocate to TRPM-2 channels at the plasma membrane and calpain I cleavage of the Na+–Ca2+ exchanger. Elevated intracellular Ca2+ activates Ca2+-dependent calpain I and nNOS. Calpain I also targets cytosolic Bax and BID and DNase II from lysosomes. tAIF, endo G, and cytochrome c release from mitochondria. Calpain I also triggers cathepsin B, D and DNase II from lysosomes. tAIF, endo G, cytochrome c, cathepsin B, 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 mito-chondrial 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 tol-erated in humans. There is an ongoing Canadian trial in which an inhibitor of the NMDA receptor to post synaptic 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 com-pleted 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 neoprotective 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.

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