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INVITED REVIEW

Glutamate receptors, neurotoxicity and neurodegeneration

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Abstract Glutamate excitotoxicity is a hypothesis that states excessive glutamate causes neuronal dysfunction and degeneration. As glutamate is a major excitatory neurotransmitter in the central nervous system (CNS), the implications of glutamate excitotoxicity are many and far-reaching. Acute CNS insults such as ischaemia and traumatic brain injury have traditionally been the focus of excitotoxicity research. However, glutamate excitotoxicity has also been linked to chronic neurodegenerative disorders such as amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease and others. Despite the continued research into the mechanisms of excitotoxicity, there are currently no pharmacological interventions capable of providing significant neuroprotection in the clinical setting of brain ischaemia or injury. This review addresses the current state of excitotoxic research, focusing on the structure and physiology of glutamate receptors; molecular mechanisms underlying excitotoxic cell death pathways and their interactions with each other; the evidence for glutamate excitotoxicity in acute neurologic diseases; laboratory and clinical attempts at modulating excitotoxicity; and emerging targets for excitotoxicity research.

Keywords Excitotoxicity · Glutamate · Neuronal cell death · Calcium · Free radical · Neuroprotective drugs · NMDA receptor · TRP channel · Gap junction · Sodium–calcium exchange

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Overview

Glutamate excitotoxicity is a hypothesis that states excessive glutamate causes neuronal dysfunction and degeneration. As glutamate is a major excitatory neurotransmitter in the central nervous system (CNS), the implications of glutamate excitotoxicity are many and far-reaching. Acute CNS insults such as ischaemia and traumatic brain injury have traditionally been the focus of excitotoxicity research. However, glutamate excitotoxicity has also been linked to chronic neurodegenerative disorders including amyotrophic lateral sclerosis (ALS), multiple sclerosis, Parkinson's disease, and others. The focus of this review, however, will be the mechanisms of excitotoxicity, the role of excitotoxicity in acute CNS insults and developing areas of excitotoxic research.

The mechanisms underlying glutamate excitotoxicity are complex. However, especially in the acute pathologies, glutamate excitotoxicity is not thought to be the result of a genetic mutation or structural deficit in the channel. Unlike most of the other topics in this chapter, glutamate excitotoxicity is not considered a channelopathy. Instead, glutamate excitotoxicity can be thought of as a normal physiological response to a CNS insult.

Historical context

The toxic effects of glutamate was first observed by Lucas and Newhouse [123] who described degeneration of the inner layers of the retina following subcutaneous injections of glutamate in infant mice. Olney [153] later coined the term "glutamate excitotoxicity" in a landmark paper describing intracranial brain lesions in response to subcutaneous injections of glutamate in infant and adult mice.

Olney and Sharpe [154] were able to repeat these findings in a primate model shortly thereafter, albeit requiring higher doses of glutamate. In these studies, it was also noted that the hypothalamus and periventricular areas of the brain were particularly sensitive to systemic glutamate. A similar neuroanatomical pattern of degeneration was observed following cerebral anoxia [100], which led some to believe that glutamate excitotoxicity may play a role in ischaemic neuronal death. Supporting evidence was provided by Rothman [172] who demonstrated reduced susceptibility to anoxic insult in hippocampal cell cultures using gamma-D-glutamyglycine, a non-specific postsynaptic excitatory amino acid (EAA) inhibitor. Glutamate excitotoxicity has since been inextricably linked to ischaemic CNS injury and other CNS pathologies thought to have similar mechanisms, such as secondary injury following traumatic brain injury. Chronic over-excitation of neurons elicited by glutamate is newer concept, but has linked glutamate excitoxicity to neurodegenerative processes in ALS, Huntington's disease, Parkinson's disease and Alzheimer's dementia. Chronic excitotoxicity is beyond the scope of this review, which will focus primarily on the mechanisms underlying acute excitotoxicity.

Glutamate receptors

NMDA receptor

Of the glutamate receptor types currently known, none have received more attention than the *N*-methyl-D-aspartate receptor (NMDAR). Named for its affinity for *N*-methyl-D-aspartate, the NMDAR has been implicated in various processes across the neurosciences, from learning and memory to neurodegeneration. The NMDAR channel is made up of a combination of three different subunits, NR1–3. When activated, the NMDAR allows the influx of cations, though most notably calcium. Excessive intracellular calcium concentrations cause the activation of intracellular pathways leading to both physiological (i.e. learning and memory) and pathological processes (i.e. excitotoxic injury). The NMDAR exhibits a complex gating mechanism, requiring not only binding of various ligands but also cellular depolarisation.

The traditional NMDAR is heterotetramer composed of two NR1 subunits and two NR2 subunits. The NR1 subunit is made up of ~938 amino acids and has eight splice variants. Together, two NR1 subunits form the ion channel proper and exhibit all the classical properties attributed to NMDARs, including glutamate activation, magnesium block, zinc inactivation, glycine activation, interactions with polyamines and pH sensitivity. In addition to glutamate binding, Mayer et al. [131] showed that depolarisation of the NMDAR-expressing neuron is necessary to electrostatically remove a magnesium ion normally blocking current entry at the level of the ion channel pore. Zinc can alternatively inhibit the NMDAR-mediated currents elicited by glutamate [130]. Physiologically, magnesium is removed by the activation of other ionotropic glutamate channels (AMPA and kainate, to be discussed later). Glycine was shown by Johnson and Ascher [99] as a mandatory cofactor with glutamate necessary for NMDAR channel opening. The role of polyamines in NMDAR modulation is less clear and may participate in both cell growth and cell death. Spermine, a polyamine, can potentiate NMDAR currents at low concentrations, but also reduce currents in a voltage-dependent manner at higher concentrations [171]. Spermine has also been shown to increase the frequency of channel opening and glycine affinity whilst acting as a voltage-gated NMDAR channel blocker when applied extracellularly [15]. To add to the complexity of the polyamine/NMDAR interaction, many of these effects are dependent on the subunit constituents of the NMDAR channel [221]. As such, the physiological significance of these interactions is still under investigation. Protons inhibit NMDAR current via a direct interaction with the NR1 subunit on a single lysine residue [208] which may be alleviated by spermidine and other polyamines [168]. Physiologically, alterations in pH common after acute neurological insults (such as stroke and traumatic brain injuries) can modulate NMDAR function, but also other acid-sensing ion channels (ASICs, to be discussed later).

NR2 subunits have more of a regulatory and refining role in NMDAR function. Currently, four NR2 subtypes exist (NR2A–D), with NR2A widely distributed in the brain, NR2B expressed primarily in the forebrain, NR2C found predominantly in the cerebellum and NR2D localised to the thalamus [29]. Within the NMDAR complex, NR2 subunits modulate the characteristics of the NR1 ion channel pore mentioned above, though recently, NR2 subunits have been ascribed another role in postsynaptic architecture. Specifically, NR2B subunits have been shown to bind and link postsynaptic proteins, creating specialised postsynaptic microenvironments [177]. This close association is accomplished by postsynaptic densities comprising scaffolding proteins which allow the spatial approximation of intracellular enzymes (e.g. neuronal nitric oxide synthase) with ionic second messengers (e.g. Ca^{2+} influx from NMDARs). The physiological effect of this spatial relation can be dramatic and has been shown in animal studies to reduce histological damage as well as neurological dysfunction after stroke [2].

Recent studies have shown a third subunit associated with the NMDA receptor gene family [33]. The NR3

subunit is expressed in two isoforms: NR3A, which is expressed throughout the CNS, and NR3B, which is expressed primarily in motor neurons. Preliminary evidence suggests that NR1/NR3A and NR1/NR3B complexes are not activated by NMDA or glutamate, but rather elicit an excitatory Ca^{2+}-impermeant response via glycine.

AMPA/kainate receptors

The AMPA and kainate classes of glutamate receptors belong to the same superfamily as the NMDARs and share approximately 25% homology. AMPA receptors (AMPARs) are made up of a combination of four subunits (GluR1–4) and require only glutamate application for activation. The specificity of cation influx of AMPARs is variable, however, and is governed primarily by subunit composition. GluR1, GluR3 and GluR4 all display strong inwardly rectifying current–voltage and calcium permeability, whereas the GluR2 subunit removes calcium permeability [30, 73, 88]. Physiologically, AMPARs are thought to regulate the fast excitation required to remove the magnesium block of nearby NMDARs.

Kainate receptors are made up of subunits from GluR5–7 (also known as GluK5–7) and KA1–2 (also known as GluK1–2). The properties of kainate channels are similar to AMPARs in that they allow ion flux directly following glutamate application, though they are mostly impermeant to calcium ions. Although AMPARs are localised mostly in the postsynaptic membrane, studies have shown that kainate receptors may be localised in both the pre-synapse [36] and post-synapse [32, 213]. Some studies have shown that the application of kainate can stimulate glutamate release [179], whereas others have shown that kainate application inhibits presynaptic glutamate release [68]. Postsynaptically, kainate channels serve a similar purpose as AMPARs in alleviating magnesium block in NMDARs.

Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) are single-peptide seven-transmembrane spanning proteins linked to intracellular G-proteins. It was originally believed that all metabotropic glutamate receptors used G-proteins as a transduction molecule, though recent evidence suggests that G-protein-independent signalling can occur [85]. Currently, eight different mGluRs (mGluR1–8) are known and are classified into three groups (groups I, II and III) based on sequence homology and their intracellular effects.

Group I metabotropic glutamate receptors include mGluR1s and mGluR5s. Activation of group I mGluRs are linked via G-proteins to the activation of phospholipase C whose downstream effects include inositol triphosphate production and subsequent intracellular calcium mobilisation [3, 9]. This group of mGluRs also modulates excitatory postsynaptic potentials at hippocampal synapses via tyrosine kinases in a G-protein-independent fashion [85]. Group II mGluRs include mGluR2s and mGluR3s. These mGluRs cause a decrease in adenylyl cyclase signalling, resulting in downstream inhibition of voltage-dependent calcium channels [34, 202]. These receptors are found at both the pre-synapse and the post-synapse [152]. Since presynaptic calcium is integral to neurotransmission, group II mGluRs modulate neurotransmission via their action on voltage-gated calcium channels. Group III mGluRs include mGluR4s, mGluR6s, mGluR7s and mGluR8s. These mGluRs have similar properties to the group II mGluRs and are also associated with a decrease in adenylyl cyclase signalling, resulting in downstream inhibition of voltage-dependent calcium channels [202]. These mGluRs are also found in both the presynaptic and postsynaptic terminals [22], and similar to group II receptors, these mGluRs modulate neurotransmission by functioning as autoreceptors and modulating calcium channel influxes.

With respect to excitotoxicity, the group I family of mGluRs is associated with the post-synapse and appear to potentiate NMDAR-mediated Ca^{2+} influx [26]. The remaining metabotropic glutamate receptor heterodimers comprising mGluR2,3 and mGluR4,6,7,8 subunits are linked to the inhibition of cAMP formation. These receptors are primarily found in the pre-synapse and reduce Ca^{2+} influx via NMDARs [25].

The above evidence shows that alterations in the amino acid sequences of glutamate receptor subunits could alter calcium permeability or other properties which could lead to worsening excitotoxicity. However, whilst there are currently no known channelopathies attributable to glutamate receptor mutations directly, some recent evidence has suggested that mutations in postsynaptic proteins bound to AMPARs may exert a channelopathy-like effect in epilepsy [69]. Improved knowledge of the pharmacology and distribution of glutamate receptors may also lead to the development of improved receptor antagonists for the treatment of neurologic disease. Finally, the linkage of ionotropic receptors to intracellular enzymes may provide alternative targets for pharmacological neuroprotection following excitotoxic insults.

Mechanisms of excitotoxicity

Despite intense research into the mechanisms of excitotoxicity, the actual intracellular mechanisms responsible for neuronal death are still being elucidated. One major obstacle is in the heterogeneity of neurodegeneration following glutamate application. In neuronal cultures, both apoptosis-like and necrosis-like cell death is seen depend-

ing on the severity of NMDA insult [20]. In vivo, the morphology of cell death may be dependent on the receptor subunit composition of neurons [163]. This heterogenous population of cell death is also apparent in whole animal models of stroke [192] and traumatic brain injury [191].

At one end of the spectrum, neurons displaying necrotic morphology are seen following intense glutamatergic insult [20]. The mechanisms underlying neuronal necrosis are similar to those governing other cell types and include loss of cellular homeostasis with acute mitochondrial dysfunction leading to massive energy failure. Milder glutamatergic insults, however, have been shown to cause cell death ascribed to various cell death pathways. Though these cell death pathways include a gamut of molecular players including cysteine proteases, mitochondrial endonucleases, peroxynitrite, PARP-1 and GAPDH in excitotoxic neurodegeneration, no single pathway has emerged dominant.

Calcium: a key to excitotoxicity

Calcium influx was shown to be essential to glutamate excitotoxicity in a paper by Choi [37]. In this study, glutamate excitotoxicity in neuronal cultures was potentiated in a calcium-rich extracellular solution, whereas a calcium-free extracellular solution markedly reduced neurodegeneration. Subsequent studies by Choi [38] proposed that NMDARs may be primarily responsible for this calcium entry, but it was Tymianski et al. [210] who demonstrated that the path of calcium influx, and not the calcium load, was important in the NMDAR-mediated neurodegenerative process. Sattler et al. [176] later demonstrated higher lethality with lower calcium influxes via NMDARs compared to higher calcium influxes via other calcium-permeant channels. Subsequent work by the same group showed that NMDARs are spatially linked to neuronal nitric oxide synthase (nNOS) which can produce toxic levels of nitric oxide (NO) [177].

Other lines of evidence suggest that the majority of intracellular calcium is sequestered into mitochondria in glutamate excitotoxicity [205, 218]. In these studies, it was demonstrated that the calcium-buffering capacity of neurons was dramatically reduced using a mitochondrial protonophore or removal of sodium from the extracellular solution. These studies also suggested a low affinity, high capacity sodium/calcium exchanger buffering calcium into mitochondrial stores. In this scenario, calcium sequestration can lead to metabolic acidosis and free radical generation via mitochondrial toxicity.

The timing of calcium entry following glutamate application was elucidated by Randall and Thayer [167] who showed three phases of intracellular calcium concentration changes in cultured hippocampal neurons: an initial phase of increased intracellular calcium lasting 5–10 min, a 2-h latent phase with normal calcium concentrations and finally a gradual sustained rise in intracellular calcium associated with cell death. Taken together, increased intracellular calcium causing cell death may involve several mechanisms including activation of nitric oxide synthase, calcium-sensitive proteases and mitochondrial damage.

Nitric oxide: intricately linked to NMDAR-mediated calcium influx

One of the hallmarks of excitotoxic neurodegeneration is the production of nitric oxide. Early work by Dawson et al. [48] supported the role of NO in glutamate-mediated neurodegeneration by preventing cell death in vitro with nitric oxide synthase (NOS) inhibitors. In nNOS knockout mice studies, NMDA-mediated excitotoxicity was markedly reduced, demonstrating that nNOS is the NOS isoform primarily responsible for excitotoxic neurodegeneration [49]. NMDARs and nNOS were eventually connected by a study by Sattler et al. [177] who determined that the postsynaptic density protein of 95 kDa (PSD-95) provided a structural link between nNOS and NMDARs. In these experiments, it was shown that NMDARs are bound to nNOS via a postsynaptic density protein of molecular weight 95 kDa (PSD-95). PSD-95 binds to the C-terminus of the NR2B subunit via a PDZ1 domain and the N-terminus of nNOS via a PDZ2 domain. In this model, a microenvironment is formed in the post-synapse whereby Ca^{2+} entering the neuron preferentially activates nNOS via calmodulin.

Once formed, NO has a number of intracellular targets [193]. However, nitric oxide can also interact with the free radical superoxide to form peroxynitrite, a potent oxidant that can cause protein nitration, protein oxidation, lipid peroxidation and direct DNA damage [164, 165] leading to cell death. More recently, NO has been shown to be directly neurotoxic through an interaction with GAPDH [83].

Free radicals: a mitochondrial contribution to excitotoxicity

The first indirect evidence for the role of free radicals in glutamate excitotoxicity was provided by Dykens [56] who showed that cerebellar neurons cultured in mannitol- or superoxide dismutase-rich medium were resistant to kainate-induced excitotoxicity. Subsequently, cultured cortical neurons overexpressing superoxide dismutase were shown to be resistant to glutamate and ischaemia-induced neurotoxicity [76]. Various groups other have shown neuroprotection against glutamate excitotoxicity in cultures using various antioxidant compounds including nitrone-based scavengers, free radical spin traps and 21-aminosteroids/lazaroids (to be discussed later).

Direct detection of free radical production following excitotoxicity was demonstrated in cerebellar granule cells [110] and cortical cultures [54, 170]. Using paramagnetic resonance imaging, Lafon-Cazal et al. [110] demonstrated a dose-dependent increase in superoxide production following increasing concentration of NMDA application. Reynolds and Hastings [170] further provided cell culture evidence that free radicals were generated in mitochondria by attenuating free radical production with mitochondrial uncouplers.

The mechanism of free radical production was linked to calcium in by Dykens [55], demonstrating that isolated mitochondria exposed to increasing calcium and sodium concentrations result in a feed-forward system of increasing free radical production. In cortical cultures, Dugan et al. [54] showed that the removal of extracellular calcium attenuated free radical production following NMDA application, but was unaffected by nitric oxide synthase inhibitors. Reynolds and Hastings [170] demonstrated a similar reliance on calcium entry.

Taken together, the evidence supports free radical generation in mitochondria secondary to calcium influx via NMDARs. Cytoplasmic free radicals, especially superoxide, can interact with other radicals, such as nitric oxide, to form powerful oxidants [90].

Zinc: another divalent ion in glutamate excitotoxicity

Within neurons, Frederickson [66] proposed dividing zinc into three distinct pools: protein-associated zinc, vesicle-associated zinc and free intracellular zinc. Normally, the majority (~80%) of zinc is protein-bound, with very low levels of free intracellular zinc [155]. Vesicle-associated zinc is largely associated with glutamatergic neurons [128], which can contain up to 300 μM concentrations of zinc [228].

Initial in vivo evidence for zinc involvement in CNS neurodegeneration was demonstrated in kainate-treated rodents [67] where depletion of zinc at presynaptic terminals with simultaneous zinc accumulation at cell soma of degenerating neurons was observed. Following cerebral ischaemia in rats, Tonder et al. [207] reported dentate hilar degeneration in the hippocampus with a similar depletion of presynaptic terminal zinc in mossy fibres coupled with the accumulation in postsynaptic neurons. In addition, zinc accumulation in degenerating neuronal soma was noted in a forebrain ischaemia rodent model where neuroprotection was observed with zinc chelators [107]. Similar histological evidence was provided in rodent models of traumatic brain injury, which also demonstrated neuroprotection with pre-injury zinc chelation [196].

Although the evidence for zinc involvement in acute excitotoxic pathologies is strong, the exact mechanism by which zinc causes neurodegeneration occurs remains elusive. Direct evidence for zinc neurotoxicity was provided by extracellular exposure of cortical cultures to zinc which led to concentration- and time-dependent modes of apoptotic or necrotic neuronal death [39, 228]. Based on the histological changes observed in vivo, it was initially thought that the majority of the postsynaptic neurotoxic zinc was derived from the presynaptic terminals [107]. However, later studies demonstrated zinc accumulation in the postsynaptic soma following insults even without presynaptic zinc stores [118]. Currently, the evidence supports multiple mechanisms of postsynaptic zinc accumulation including presynaptic zinc translocation, extracellular zinc influx, mobilisation of zinc from the protein-bound pool (especially from metallothioneins) via oxidative mechanisms [4] and release from mitochondrial pools [186]. Zinc entry into neurons has been linked to voltage-gated calcium channels, sodium exchangers, NMDARs and AMPA/kainate receptors [185], but most recently, TRMP7 channels have been implicated as a novel route of zinc entry [95].

Increasing intracellular zinc levels have been associated with a number of deleterious effects. Firstly, direct glycolytic dysfunction as a result GAPDH inhibition has been described, resulting in energy failure in mouse cortical neurons [189]. Within mitochondria, zinc has long been known to interfere with the electron transport chain in isolated mitochondria [146] and more recently has been shown to directly inhibit the citric acid cycle [24]. Increasing levels of reactive oxygen species are also a result of increasing zinc levels which can occur through mitochondrial dysfunction directly [187] or overactivation of superoxide-generating enzymes such as NADPH oxidase [148] or LOX-12 [235]. Increasing levels of zinc also cause signalling changes intracellularly, including p38 phosphorylation or ERK 1/2 activation. p38 MAPK phosphorylation leads to increased potassium influx and caspase-mediated cell death [134], whereas ERK 1/2-mediated cell death is caspase-independent [52].

The relationship of zinc to the NMDA/nitric oxide excitotoxic cascade was demonstrated by Bossy-Wetzel et al. [21] who showed zinc accumulation downstream of NO or NMDA application, requiring reactive nitrogen species such as peroxynitrite to release intracellular zinc stores. Under physiological conditions, zinc inhibits neuronal nitric oxide synthase [159], but can increase nNOS activity under pathological conditions [104], possibly potentiating neuronal damage. More recent studies have shown that zinc accumulation following glutamate application is almost completely dependent on calcium entry and subsequent ROS generation [51].

Taken together, the evidence strongly supports the role of zinc in excitotoxic injury in animal and cell culture

models. However, the significance of modulating zinc pathways to human diseases remains to be seen.

Caspases: a classical cell apoptotic pathway

Caspases, a set of cysteine proteases implicated in classical apoptotic death, were initially shown to play a role in delayed excitotoxic injury in cerebrocortical [203] and cerebellar cultures [53]. In these studies, the pre-application of various caspase inhibitors provided neuroprotection from NMDA-mediated neurodegeneration. These studies also suggested that caspase activation occurs downstream of calcium influx and mitochondrial dysfunction as caspase inhibitors did not affect these events. Subsequent studies by Tenneti and Lipton [204] further elucidated on the downstream effects of caspases by demonstrating cytosolic activation of caspases at 20 min post-NMDA application with caspase activity in the nucleus at 18–24 h post-insult. Nuclear caspase activity is indicative of cleaved ICAD, a protein inhibiting the activity of caspase-3-activated DNase (CAD) and results in DNA fragmentation and cell death [57].

Peroxynitrite: simple, but powerful

Nitric oxide can interact with a huge number of proteins [194], but is also capable of forming highly oxidative molecules when combined with superoxide. Superoxide, an oxygen molecule with a free electron, is produced physiologically during normal cellular respiration, which is then catalysed by superoxide dismutase into oxygen and hydrogen peroxide. In glutamate excitotoxicity, this buffering system is overwhelmed (see above), and superoxide spills into the cytoplasm possibly via a number of anion channels including the voltage-dependent anion channel in the mitochondria [81]. The reaction between NO and superoxide is essentially diffusion-limited [90] and, like nitric oxide, can cause protein nitrosylation via direct [211] and indirect [180] mechanisms. However, peroxynitrite is also a powerful oxidative molecule additionally capable of causing lipid peroxidation, direct DNA damage and protein dysfunction. Specific interactions of peroxynitrite with proteins include protein oxidation [164] and protein nitration of tyrosine residues [14, 96], though protein oxidation occurs at higher rates than nitrosylation [8]. Peroxynitrite has also been demonstrated to directly oxidise and damage genetic material in plasmids [175] and in cells [174], leading to either to modified bases or DNA strand cleavage. Finally, peroxynitrite can induce lipid peroxidation [165], directly damaging the plasma membrane or causing the production of damaging aldehydes.

Physiologically, peroxynitrite has been reported to inhibit the mitochondrial electron transport chain at complex I and complex II [166]. Moreover, peroxynitrite can inhibit the normal function of cytochrome c in the electron transport chain [145] as well as manganese and iron superoxide dismutase in scavenging superoxide [96] via protein nitration [227]. This interaction can potentiate caspase-mediated cell death and an eventual apoptotic cell death. Peroxynitrite-mediated DNA damage can cause overactivation of poly(ADP)-ribose polymerase (PARP-1), a nuclear repairing enzyme requiring NAD [237], leading to energy failure and necrotic cell death. Peroxynitrite can also interact with proteases in other cell death pathways [115, 137] (to be discussed later).

Similar to NMDA application, the intensity of peroxynitrite insult dictates the observed neurodegenerative morphology [20]. Thus, peroxynitrite is an intriguing candidate to simply and eloquently explain the heterogeneity of neurodegeneration observed in excitotoxicity.

Calpains/PARP-1/AIF: still under investigation

As described above, evidence suggests that calcium is sequestered relatively quickly following glutamate application. However, transient increases in cytoplasmic calcium do occur, and as such, calpains, cytoplasmic calcium-sensitive cysteine proteases have been implicated in the pathogenesis of excitotoxicity. Brorson et al. [23] demonstrated modest neuroprotection in hippocampal cell cultures from NMDA insults with calpain inhibitors even when treated as late as 1 h post-insult. However, unlike the caspase family of proteases, the role of calpains remains less clear and may actually play a reparative role in axons with low, sublethal doses of NMDA [60].

Recently, mu-calpain proteolytic activity has been shown to be necessary in the cleavage and release of apoptosis-inducing factor (AIF) from mitochondria in a cell-free system [162]. Further support for the calpain/AIF pathway in excitotoxicity comes from studies in neuronal cultures subjected to oxygen–glucose deprivation which showed that calpain inhibition prevented AIF translocation and subsequent neuronal death [31]. AIF release from the mitochondria and translocation into the nucleus causes chromatin condensation, DNA fragmentation and cell death [199]. An alternative excitotoxic mechanism proposes that the activation of PARP-1, a nuclear DNA repair enzyme, causes the release of AIF in excitotoxicity. In these studies, cortical cultures derived from PARP-1 knockout mice demonstrated markedly reduced AIF translocation and neurodegeneration after NMDA treatment [230]. Moreover, the PAR polymer generated by overactivation of PARP-1 is required to release AIF [229]. PARP-1 itself appears to require NO formation to become overactivated [233], perhaps through an intermediate like peroxynitrite. Recent-

ly, a potential link between the calpain/AIF and PARP-1/AIF pathways has been proposed. In these studies, it was shown that PARP-1 activation is essential for calpain activation in cells treated with a DNA alkylating agent [141], but the mechanism by which calpains are activated remains unclear. However, more recent studies have suggested that calpains cause the release of an inactive form of AIF and may not be required at all PAR-mediated cell death [219]. These studies showed that the inhibition of calpains does not prevent AIF translocation following NMDA application.

Taken together, these studies suggest a model in which glutamate excitotoxicity causes calcium-mediated NO production and mitochondrial failure. The superoxide produced by mitochondrial dysfunction interacts with NO to produce peroxynitrite, a highly oxidant molecule capable of directly damaging DNA. DNA damage causes the overactivation of PARP-1 which releases PAR polymers into the cytoplasm. The PAR polymers activate calpains by some unclear mechanism, leading to AIF release from mitochondria and subsequent cell death. Although the PARP-1/AIF/calpain is a tantalising model, more research is needed to clarify the role of calpains in AIF-mediated cell death.

GAPDH/Siah1 pathway: a novel apoptotic pathway

The GAPDH/Siah1 pathway is a novel pathway linking nitric oxide to a form of apoptotic-like death [83]. GAPDH is a ubiquitous housekeeping enzyme that, under normal conditions, participates in glycolysis. However, with increasing intracellular concentrations, NO can nitrosylate GAPDH and bind Siah1, an ubiquitin ligase. The newly formed heterodimer translocates to the nucleus by virtue of the Siah1 nuclear translocation domain and enhances p300/CBP-associated acetylation of nuclear proteins within [184]. The downstream activation of nuclear proteins such as p53 leads to pyknotic nuclei and morphological characteristics indicative of apoptosis. To complicate matters, a novel cytosolic protein named GOSPEL has recently been shown to provide neuroprotection by competing with Siah1 binding to GAPDH. However, the GAPDH-GOSPEL binding is also mediated by NO [183]. Currently, it is unclear whether NO is capable of (and how if it is) selectively targeting GAPDH or GOSPEL.

Finally, it is interesting to note that the interaction of GAPDH with nitric oxide also causes a functional loss [212]. As GAPDH is an active participant in glycolysis, it is possible that this same mechanism can result in necrotic morphology via energy failure. Like peroxynitrite, the GAPDH-Siah1 cascade remains an interesting candidate to explain some degree of the heterogeneity observed in glutamate excitotoxicity.

Excitotoxicity in neurologic disease

Various lines of evidence have supported the role of NMDARs and excitotoxicity in ischaemia Initial evidence supporting the role of excitotoxicity in ischaemia comes from Jorgensen and Diemer [100] who recognised a similar pattern of neuroanatomical degeneration compared to rats treated systemically with monosodium glutamate [153]. Subsequent studies demonstrated increased extracellular glutamate concentrations in response to ischaemia as measured by microdialysis [74], whereas still other studies demonstrated an association in regional sensitivity to ischaemic damage with NMDAR distribution in developing rats [91]. The increase in glutamate concentrations were observed in the striatum, hippocampus, cortex and thalamus, though interestingly, only the hippocampus was significantly damaged by the 10-min global ischaemia [75]. Focal ischaemic models demonstrated similar patterns of elevated extracellular glutamate [87].

Perhaps the most clinically significant evidence comes from studies showing neuroprotection from ischaemic injury by antagonising glutamate receptors. Both competitive and non-competitive NMDA antagonists are effective in focal models of ischaemia, but show little effect in global models of ischaemia where non-NMDA antagonists appear more effective. Metabotropic glutamate receptors have also been implicated in the pathogenesis of global ischaemia; group I antagonists and group II agonists have been shown to be neuroprotective in gerbils subjected to transient global ischaemia [105]. A different line of evidence was provided by Aarts et al. [2] who showed direct evidence of excitotoxic involvement in ischaemic injury with disruption of the NMDAR-PSD-95-nNOS complex. Rats subjected ischaemic stroke were treated with a polypeptide designed to disrupt the NMDAR-nNOS interaction, and marked neuroprotection was observed. Other indirect evidence comes from the neuroprotection observed using various effectors of glutamate excitotoxicity in ischaemia, including caspase inhibition [58], calpain inhibition [89], free radical scavengers and others.

The role of excitotoxicity in traumatic brain injuries has also received considerable experimental support. Concussive brain injury results in marked increases in extracellular glutamate concentrations in animals [61, 147] and in humans [11, 108], directly demonstrating excessive glutamate levels in vivo. Glutamate receptor inhibition studies have also demonstrated neuroprotection from traumatic injuries, further supporting the role of excitotoxicity in traumatic brain injuries. MK-801, a NMDAR channel blocker, reduced edema following fluid percussion brain injury in rats [133], whereas Riluzole, a sodium channel blocker and glutamate release inhibitor, also provided

histological benefits [232]. Treatments aimed at downstream mechanisms of excitotoxicity were also shown to be beneficial in animals. Kynurenate and indole-2-carboxylic acid reduce cerebral edema and improve cognitive and motor dysfunction induced by trauma whilst protecting against hippocampal cell loss induced by fluid percussion injury [86]. Other indirect lines of evidence include the detection of caspases and their characteristic proteolytic fragments [82, 225], calpain activation and their fragments [101], AIF translocation into the nucleus [31, 234], protein nitration [78] and other effectors implicated in excitotoxicity. These studies demonstrating the neuroprotective efficacy of glutamate receptor antagonists directly, and effector inhibition indirectly, support the role of excitotoxicity in traumatic brain injury.

Modulating excitotoxicity

NMDAR antagonists

Since the association of glutamate excitotoxicity with stroke and trauma, various attempts have been made to attenuate neuronal damage by glutamate receptor function. The NMDAR in particular has a number of sites to exploit pharmacologically, including the ion channel pore, the glutamate-binding site, the glycine-binding site and the polyamine interaction site as described above.

MK-801 (dizolcipine), Memantine, Cerestat, dextromethorphan and its metabolite dextrorphan are all drugs that block the NMDAR at the level of the channel pore, thereby reducing calcium entry. Each of these drugs has been shown in animal models to provide histological and behavioural neuroprotection following focal ischaemia [18, 156, 182, 195]. Many other studies exist expounding upon the effect of these drugs on cell death pathways in various animal models, but it would be cumbersome to outline all the evidence to supporting their use in stroke. In the end, most of the clinical development was abandoned for safety concerns, except Memantine. Dizocilpine provided marked reductions in infarct volumes in cat models of ischaemia [156], but subsequent work in rats showed that subcutaneous injections of >5 mg/kg showed retrosplenial cortical vacuolisation and necrosis [64]. Koek et al. [106] showed in three different animal species (pigeons, rats and rhesus monkeys) that MK-801 induced PCP-like effects, including cataplexy, locomotor disturbances and reduced spatial learning. Moreover, they estimated the potency of MK-801 at about two to ten times stronger than PCP. Transient hypotension [157] and decreased level of consciousness [27] were also concerns with Dizocilpine, which eventually resulted in the discontinuation of clinical development. Similarly, an initial small trial using dextrorphan demonstrated a variety of symptoms in patients including nystagmus, nausea, vomiting, somnolence, hallucinations and agitation. High rates of infusion loading in the 200-mg/h range resulted in severe hypotension, and high rates of maintenance infusion caused apnea and stupor in 3 of 22 subjects [5]. The clinical development of dextrorphan has since been abandoned. More recently, the efficacy of Cerestat (CNS 1102) was tested in a multicentre trial involving 628 patients with low-dose, high-dose, and placebo treatments. No neurological benefits were observed at 90 days post-treatment, but was stopped because of a lack of effect as well as a potential imbalance in mortality with high-dose Cerestat compared to placebo [7]. Memantine, a low-affinity drug, has shown a much better side effect profile in humans [10], but has yet to be clinically developed for stroke and is currently approved for use only in moderate to severe Alzheimer's disease [169].

Competitive NMDA antagonists include Selfotel (CGS 19755) and D-CPP-ene. Both compounds have been shown to reduce hippocampal damage in gerbil models of ischaemia, though CGS 19755 exhibited more neuroprotection even with longer delays in administration [19]. Although promising at first, two phase III trials were abandoned after showing increased rates of mortality in patients with severe stroke [44]. Clinical trials in severe head injury were similarly abandoned when no difference in mortality between treatment arms was observed [140].

Other non-competitive NMDAR blockers fared no better. Clinical development on ACEA 1021, a compound aimed at inhibiting glycine binding to the NMDAR, was halted on phase I in 1997, citing "crystals of ACEA 1021 in the urine of some subjects" despite an otherwise well-tolerated drug [6]. Another glycine antagonist, Gavestinel, followed through to phase III clinical trials, only to demonstrate no difference in morbidity or mortality rates following treatment [119]. Ifenprodil and eliprodel, drugs aimed at the NMDAR polyamine site, were abandoned after phase III clinical trials in 1997 for similar reasons [92].

Taken together, the clinical experience with glutamate receptor manipulation has been poor. Toxicity remains a large obstacle with the ion channel and competitive antagonists, though low-affinity ion channel blockers (i.e. Memantine) appear more tolerable. The same applies to the non-competitive inhibitors as they are generally tolerated well. Unfortunately, even though animal models demonstrate dramatic histological and behavioural neuroprotection, no pharmacological intervention has yet been shown to provide benefit in humans [142].

AMPAR and kainate receptor antagonists

Other research has focused on the non-NMDA glutamate receptors. In contrast to NMDAR antagonists, AMPA receptor antagonists, such as NBQX, initially appeared more effective in preventing neuronal loss in animal models of global ischaemia [188]. Specific for AMPARs, NBQX was later to shown to be effective in reducing histological damage in focal ischaemia models as well by approximately 30% [28]. YM872, another AMPAR antagonist, demonstrated similar neuroprotective effects in animal models of focal ischaemia [200] and traumatic brain injury models [70]. However, tolerability trials in humans showed sedation and euphoria in the elderly, with phase III trials in ischaemic stroke abandoned due to a lack of efficacy in 2006.

Glutamate release blockers

After the failure of several NMDAR antagonists, compounds aimed at inhibiting presynaptic release of glutamate were developed. Lamotrigine is a sodium channel blocker currently approved for use in bipolar disorder and epilepsy. Its proposed mechanism of action suggests inhibiting presynaptic voltage-dependent sodium channels, reducing overall excitability and neurotransmitter release [117]. This study also demonstrated reduced glutamate, GABA, and acetylcholine release in cortical slices with lamotrigine, though glutamate release inhibition was two and five times more potent, respectively. In animal models, lamotrigine failed to provide neuroprotection in focal ischaemia [209], but showed a reduction of CA1 hippocampal loss of 50% following global ischaemia [42]. BW619C89, a derivative of lamotrigine, underwent phase II trials that showed various neuropsychiatric effects (reduced consciousness, agitation, confusion, visual perceptual disturbance or frank hallucinations) in 16 of 21 stroke patients [143]. Clinical development was halted in 2001. Riluzole is another drug in the same class that has been shown to reduce infarct volumes and improve behavioural outcomes in ischaemic injury [124, 216] and traumatic brain injury [217, 232]. Riluzole is currently indicated for use in ALS [136], but no clinical trials for stroke or traumatic brain injury are currently underway.

Free radical scavengers and antioxidants

Other approaches to neuroprotection moved intracellularly to the generation of free radicals. There are three major classes of free radical scavengers under development: free radical spin traps (nitrone-based), the 21-aminosteroids (also known of lazaroids) and glutathione peroxidase mimics. Unfortunately, like the compounds described above, despite promising animal studies, these drugs have not yet been proven efficacious in humans.

Alpha-phenyl-*N*-*tert*-butyl-nitrone (PBN) and *N*-*tert*-butyl-alpha-(2-sulfophenyl)-nitrone) (S-PBN) are related spin trap scavengers, with S-PBN being less permeable through the blood–brain barrier. PBN was shown early on to be effective in preventing hippocampal damage following global ischaemia [41, 231]. Moreover, studies in traumatic brain injury demonstrated histological and behavioural neuroprotection with the cortical contusion model, though interestingly, no difference in effect was seen between S-PBN and PBN, suggesting that its effect may be extracerebral [126]. Subsequent studies in the weight-drop model [71] and fluid percussion injury models [125] demonstrated similar neuroprotective effects. PBN treatment also appears to have a wider therapeutic window than glutamate antagonists, with efficacy in cell cultures even 6 h post-insult [181]. Of the nitrone-based antioxidants, none of the blood–brain barrier-permeant scavengers were tested in stroke. However, there has been considerable research around NXY-059 (also known as Cerovive), a nitrone-based antioxidant that does not permeate the blood–brain barrier. Although this property suggests that NXY-059 does not act directly on intracellular neuronal oxidants, animal studies have shown neuroprotective efficacy in trauma [40] and stroke [109]. In clinical trials, the SAINT1 trial examining the efficacy of NXY-059 in clinical stroke was promising [120], but a subsequent SAINT2 trial showed no clinical benefit with treatment [190].

Another group of compounds which held promise as a free radical scavenger are the 21-aminosteroids or lazaroids. Although these compounds demonstrated cell culture [63] and animal [80, 132] neuroprotection in traumatic brain injury models, human clinical trials with Tirilazad in head injury resulted in decreased mortality only in males with severe head injury patients and subarachnoid haemorrhages [127]. Subsequent trials with Tirilazad in the treatment of subarachnoid haemorrhages [113, 114] showed a reduction in mortality only with the highest grades on arrival. Recent meta-analysis of Tirilazad in the treatment of subarachnoid aneurysmal haemorrhage demonstrated no clinical outcome differences, but reduced symptomatic vasospasm [97]. A similar story exists with ischaemic strokes and Tirilazad. Despite efficacy in many animal models [79, 224], a Cochrane Review demonstrated increased death or disability in stroke victims treated with Tirilazad compared to placebo in a review of six clinical trials without any statistically significant difference in overall mortality [13]. Studies in ischaemic stroke using Tirilazad have since been abandoned.

A final class of antioxidants is derived from a family of enzymes called glutathione peroxidase which rely on a selenocysteine moiety to reduce hydrogen peroxide into water. Ebselen is a glutathione peroxidase mimic also capable of interacting with peroxynitrite and inhibiting enzymes involved in inflammation [178]. In animal studies, Ebselen has been shown to reduce cortical infarct size in a number of rodent models of focal ischaemia [47, 93, 201]. However, clinical trials in Japan demonstrated no significant differences in 3-month clinical outcome in patients with complete middle cerebral artery occlusion [226] and no significant improvement in clinical outcome despite a reduction in infarct size [150]. Phase III clinical trials in Japan focused on patients with cortical infarctions are currently underway.

Nitric oxide synthase inhibitors

Initial studies examining general NOS inhibitors in animal models were complicated by the opposing effects of the different isoforms of NOS. These studies showed that general NOS inhibition using L-NAME at high doses of 30 mg/kg resulted in no reduction in cortical infarcts following focal ischaemia [46], but later reported histological neuroprotection with 3 mg/kg L-NAME [45]. Other groups showed that the intravenous application of L-arginine, a nitric oxide precursor, was neuroprotective following focal ischaemia [138], which was later explained by its effect on the eNOS isoform [139]. Attempts to localise NOS inhibition led to the development of nNOS-specific inhibitors such as 7-nitroindazole (7-NI). Animal studies with 7-NI has shown neuroprotection in global [149] and focal ischaemia [59]. 7-NI has also been shown to reduce neurological deficits in animals following traumatic brain injuries [215], though currently, there are no attempts at clinical development.

A novel class of drugs named membrane-associated guanylate kinase (MAGUK) inhibitors has recently been proposed in the treatment of excitotoxicity. These peptides are competitive antagonists designed to bind postsynaptic scaffolding proteins. Specifically, the MAGUK inhibitor NA1, aimed at dissociating the spatial relationship between nNOS and NMDARs, has demonstrated neuroprotection in various animal models of stroke [2, 197]. In theory, NA-1 can reduce NO production from nNOS specifically without otherwise affecting NMDAR or nNOS function which may avoid much of the toxicity observed previously. NA1 is currently undergoing phase II clinical trials.

Future directions of excitotoxicity

Despite the intensive research into excitotoxic mechanisms, very few pharmacologic treatments have been shown to be successful in related disorders. This failure has been recently suggested to be a result of an overly simplistic NMDA-AMPA model of excitotoxicity [16]. As such, alternative targets for attenuating excitotoxic injury have recently been the focus of considerable attention.

Sodium–calcium exchangers

The sodium–calcium exchanger (NCX) is a transmembrane protein that exchanges one calcium ion per three sodium ions. The direction of ion flux is dependent on a number of factors including pH, sodium concentrations, Ca^{2+} concentrations and ATP levels. Under physiological conditions, the major driving force of these exchangers is the Na^+ gradient created by the Na^+/K^+ ATPase. However, the direction of ion flux is reversible, and high intracellular Na^+ levels can cause Ca^{2+} influx with Na^+ extrusion. Increasing intracellular Ca^{2+} can lead to cell death through mechanisms described above. In cells, the NCX is found in the plasma membrane, mitochondria and the endoplasmic reticulum. Various isoforms of NCX exist, including NCX1, NCX2, NCX3 and NCX4. These isoforms present with different expression profiles in neurons, with forebrain neurons expressing NCX1 and cerebellar neurons expressing NCX3 [103].

In excitotoxic insults, NMDAR-mediated Na^+ influx may be sufficient to reverse NCX function, causing calcium accumulation in neurons. However, this phenomenon occurs only in the presence of Na^+/K^+ ATPase dysfunction [43]. These results suggest that the NCX plays a dual role in ischaemia. In severe insults, where the Na^+/K^+ ATPase is dysfunctional, inhibition of the NCX may prevent Ca^{2+} entry into the cell as a result of excessive depolarisation. However, in milder insults, the NCX may be necessary to maintain calcium homeostasis so long as the Na^+/K^+ ATPase continues to maintain the Na^+ gradient. Accordingly, animal studies employing NCX inhibitors have shown both neuroprotection [129] and worsening of the infarct area after stroke [161]. Whether dissociating the neuroprotective and neurodegenerative effects of NCX are possible remains to be determined.

Hemichannels and gap junctions

Gap junctions are ubiquitously expressed in neurons and glia and are formed by the binding of hemichannels to one another. Hemichannels themselves are made up of connexins. Gap junctions also appear to have a dual role in both neuroprotection and neurodegeneration following excitotoxic insults [62]. In the neuroprotective model (or the Good Samaritan effect), opening of gap junctions allows astrocytes to remove toxic extracellular substances. In the neurodegenerative model (or the bystander effect), opening of gap junctions causes the dissipation of ionic accumula-

tion and/or cytotoxic substances from compromised cells into otherwise unaffected cells, leading to neurodegenerative spreading [122].

Although gap junctions have been shown to open in response to ischaemia [206], their overall role in excitotoxic injury remains controversial. Animal models of focal and global ischaemia have shown neuroprotection with connexin knockdown animals [65] and gap junction inhibitors [50]. However, consistent with its proposed dual role, connexin 43 knockout mice demonstrated increased focal and global ischaemia damage [151]. Similar to the NCX, the net effect of gap junction inhibition is still unclear and requires further investigation.

Acid-sensing ion channels

ASICs are found in the plasma membrane of neurons and open in response to low pH, mechanical stretch, lactate, arachidonic acid and decreased extracellular calcium [16]. ASIC3, a calcium-impermeant isoform, binds Ca^{2+} at an extracellular site which can be removed by H^+ binding or lactate-induced decreases in extracellular calcium [94]. Although most isoforms are sodium channels, some isoforms (i.e. ASIC1a) are also Ca^{2+}-permeant [223].

NMDAR activation has been shown to increase Ca^{2+} influx via ASIC1a channels [72]. This Ca^{2+} influx and subsequent cell death in hippocampal cultures was prevented with NMDAR antagonists and CaMKII inhibitors. Animal models of stroke have shown that inhibiting ASICs can provide significant neuroprotection for up to 7 days post-middle cerebral artery occlusion [160]. Inhibition of ASICs also increased the therapeutic window for NMDA inhibition in rodent models of stroke. These results suggest that ASIC inhibition may be beneficial in neuroprotection following stroke or as an adjunct to other pharmacological interventions.

Transient receptor potential channels

Transient receptor potential (TRP) family of channels is ubiquitously expressed in a variety of tissues. TRP channels are generally permeant to sodium, calcium and magnesium. Of particular importance in excitotoxicity are the TRPM (melastatin) and TRPC (canonical) subfamilies. TRPM7 [1] and TRPM2 [35] have both been implicated in the third phase of excitotoxic calcium influx observed by Randall and Thayer [167]. TRPM7 currents are increased by free radicals, PIP2 hydrolysis [173] and MgATP levels [144] intracellularly, whereas TRPM2 currents are enhanced by reactive oxygen [220] and nitrogen species [84]. Recently, TRPM7 has also been implicated in zinc-mediated neurotoxicity [95].

Both TRPM7 and TRPM2 knockdown have been shown to be neuroprotective in cell cultures. TRPM7 knockdown using siRNAs in cortical cultures provides neuroprotection following oxygen–glucose deprivation [1], whereas TRPM2 siRNA knockdown demonstrated neuroprotection in cortical cell cultures treated with hydrogen peroxide [102]. Conversely, TRPC channels have been shown to be neuroprotective in cerebellar granule cell cultures possibly via BDNF and CREB [98]. Most recently, virally mediated TRPM7 knockdown in adult animals subjected to global ischaemia was shown to provide both hippocampal and behavioural neuroprotection [198]. However, the role of TRP channels in clinical stroke remains to be determined.

Cross talk cell death mechanisms

Certain groups have demonstrated conflicting data with respect to which cell death pathway takes precedence in cell cultures and in vivo. Despite the readily available evidence for the importance of various effectors in excitotoxicity, it is becoming increasingly evident that cell death pathways do not exist independently of each other. Cell death pathway interactions may be either synergistic or competitive, and these interactions may play a large role not only in determining the efficacy of neuroprotective drugs but also in the development of successful drug therapeutics to attenuate damage mediated by excitotoxins.

Calpains cause an inhibition of caspase-3 activation via the proteolytic cleavage of procaspase-9, rendering it inactive [214]. Similar evidence exists using a Huntington's disease model of neurodegeneration in rats [17]. In this model using 3-nitroproprionic acid, it was determined that mu-calpains could proteolytically cleave and inactivate caspase-9 and caspase-3. Calpains can also convert cells otherwise committed to classical apoptosis to a caspase-independent form of cell death [112]. NO-mediated nitrosylation of caspases [137] and calpains [135] cause their inhibition, though peroxynitrite appears to be a more potent inhibitor. Li et al. [121] expanded these initial findings into six other members of the caspase family. However, the overall effect of oxidative inhibition of caspases in neurons still results in neuronal death [236]. Caspases can inhibit PARP-1 [116] and inactivate its DNA repairing ability and thus its role in PARP-1/AIF-mediated cell death. Calpain-mediated cleavage and inactivation of nNOS [111] and NMDARs [77] can also occur. Cleavage of NMDARs by calpains causes reduced NMDAR-mediated currents [222]. Calpain activation may thus reduce NO and peroxynitrite levels. Some of the novel targets of neuroprotection have also been shown to interact with the effectors of excitotoxicity outlined above. TRPM channels have been shown to be sensitive to reactive oxygen and nitrogen species, and TRPM2 can be activated by ADP-ribose, a substrate of

PARP [158]. Finally, calpains have been shown to inactivate NCX, leading to further calcium dysregulation [12].

Taken together, these studies demonstrate only some of the known interactions of cell death pathways. A better appreciation of the complexity of the intracellular effector mechanisms occurring after excitotoxicity may prove useful in further pharmacological developments for excitotoxic injury.

Conclusions

Our understanding of glutamate excitotoxicity has come a long way since its proposal in the 1950s. Even within the last few decades, there has been an exponential growth in research outlining not only excitotoxic mechanisms but also the manipulation of these pathways in animal systems. Unfortunately, we have yet to fully understand all the complexities involved in excitotoxicity as evidenced by our failure to provide neuroprotection in the clinical setting. Whether this is a problem in the animal to human translation of stroke and brain injury models or overly complex cell death pathway interactions remains to be determined. Despite these setbacks, there are exciting new avenues of research outlining new cell death pathways/interactions laterally and new receptor targets downstream of NMDAR activation.

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