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Molecular Mechanisms of Glutamate Receptor-Mediated Excitotoxic Neuronal Cell Death

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Abstract

Excitotoxicity is one of the most extensively studied processes of neuronal cell death, and plays an important role in many central nervous system (CNS) diseases, including CNS ischemia, trauma, and neurodegenerative disorders. First described by Olney, excitotoxicity was later characterized as an excessive synaptic release of glutamate, which in turn activates postsynaptic glutamate receptors. While almost every glutamate receptor subtype has been implicated in mediating excitotoxic cell death, it is generally accepted that the N-methyl-D-aspartate (NMDA) subtypes play a major role, mainly owing to their high calcium (Ca^{2+}) permeability. However, other glutamate receptor subtypes such as 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA) or kainate receptors have also been attributed a critical role in mediating excitotoxic neuronal cell death. Although the molecular basis of glutamate toxicity is uncertain, there is general agreement that it is in large part Ca^{2+}-dependent. The present review is aimed at summarizing the molecular mechanisms of NMDA receptor and AMPA/kainate receptor-mediated excitotoxic neuronal cell death.

Index Entries: Neurotoxicity; calcium; glutamate receptor; postsynaptic density.

Introduction

L-glutamate is the major excitatory transmitter in the vertebrate CNS. In addition to its action as a synaptic neurotransmitter, it produces long-lasting changes in neuronal excitability, synaptic organization, neuronal migration during development, and neuronal viability *(1)*. The excitatory responses of this endogenous excitatory amino acid (EAA) are mediated by a number of pharmacologically and functionally distinct cell-membrane receptors,

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i.e., the ionotropic N-methyl-D-aspartate (NMDA), kainate, and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA) receptors as well as metabotropic receptors *(2).* Intensive research in the field of EAA receptors has focused on the identification of the molecular structure of the receptor complexes, the molecular mechanisms of receptor activation and the characterization of receptor-mediated gene expression, which led to a fuller appreciation of the importance of EAA receptors in physiological and pathological neuronal functioning.

At the same time that glutamate was becoming appreciated as the most prevalent excitatory neurotransmitter in the CNS, Lucas and Newhouse *(3)* revealed that glutamate may be a potent neurotoxin. They found that systemic injections of L-glutamate into immature mice destroyed the inner neural layers of the retina. Ten years later, the idea of glutamate as a neurotoxin was widely acknowledged when Olney confirmed the retinotoxicity of glutamate and showed further that the structurally related compound kainate produced brain lesions in immature animals that did not possess a fully developed blood-brain barrier (BBB) *(4,5).* Olney's observations led to the term "excitotoxicity," whereby excitatory amino acids produced neurodegeneration *(4).* Since then, excitotoxicity has been considered to be part of a common pathologic pathway in many human disease states such as cerebral ischemia (stroke), nervous system trauma, epilepsy, and chronic neurodegenerative disorders.

Excitotoxicity is believed to be mediated by an excessive synaptic release of L-glutamate and the consequent overstimulation of glutamate receptors, as attenuation of synaptic transmission and application of glutamate receptor antagonists were shown to be neuroprotective *(6,7).* Nearly every glutamate receptor subtype has been implicated in mediating neurotoxicity *(8–10).* Although the molecular basis of glutamate-mediated toxicity is still uncertain, studies of neurotoxicity in cultured neurons have established important pathological roles for intracellular ionic changes caused by glutamate, especially the influx of Ca^{2+}, Na^{+} and recently Zn^{2+}, as well as K^{+} efflux *(11–15).* However, as initially proposed by Berdichevsky et al. *(16),* Choi and colleagues emphasized the role of Ca^{2+} influx during glutamate neurotoxicity *(17,18).* Based on these and other studies, it was generally accepted that the NMDA subtypes of glutamate receptors play a key role in mediating at least certain aspects of glutamate neurotoxicity, possibly owing to their high Ca^{2+} permeability *(9,11).*

The role of AMPA receptors in triggering excitotoxicity is also important as AMPA receptors antagonists apparently provide better protection than NMDA receptor antagonists in animal models of global cerebral ischemia (reviewed in ref. *19*). This review will summarize and discuss the most recent findings and hypotheses on the molecular mechanisms of excitotoxicity, initiated by excessive activation of both NMDA and AMPA receptors. We will emphasize the role of Ca^{2+} influx in triggering neurotoxic processes as Ca^{2+} is still recognized as a predominant mediator of excitotoxicity.

Calcium and Neurotoxicity

Neuronal activity can lead to a marked increase in the concentration of cytosolic Ca^{2+}, which then functions as a second messenger that mediates a wide range of cellular responses *(10,20).* Excessive influx of extracellular Ca^{2+} together with any Ca^{2+} release triggered from intracellular stores can elevate neuronal cytosolic free Ca^{2+} concentrations to levels that exceed the capacity of intracellular Ca^{2+}-regulatory mechanisms and can lead to metabolic derangements such as the formation of free radicals and cell death (extensively reviewed in refs. *8,10,12,21*). Although cellular Ca^{2+} overload is unlikely to be a common pathway mediating all forms of neuronal cell death, several lines of evidence support a close relationship between excessive Ca^{2+} influx and neuronal injury in the adult mammalian nervous system *(9–11).* The repeated observation

of a requirement for Ca^{2+} in neurodegeneration had given rise to the "calcium hypothesis" of neurotoxicity, which states that "neuronal Ca^{2+} overload leads to subsequent neuronal damage." Based on this hypothesis, efforts to develop effective treatments for excitotoxicity have focused on decreasing the deleterious effects of glutamate by limiting its release or by blocking glutamate receptors, which in turn blocks excessive increase in free cytosolic Ca^{2+}. However, it is now clear that glutamate receptor blockade interferes with normal brain functions and can produce substantial neurological side effects in clinical trials (*see* ref. *22* or visit www.stroketrials.org for trials on NMDA receptor antagonists).

Therefore, extensive research has not only addressed the question of the precise relationship between Ca^{2+} overload and neurotoxicity but in addition, attention has shifted towards the identification of intracellular signaling and regulatory pathways triggered by glutamate receptor overactivation. It became apparent that in addition to changes in intracellular Ca^{2+} levels, the route of Ca^{2+} entry and its intracellular localization give rise to the activation of specific biochemical signaling pathways that mediate particular biological responses, physiological as well as pathological (*see* ref. *23*, also *24–27*). This opens up new questions as to whether and how the activation of the different types of glutamate receptors during an excitotoxic insult will result in varying degrees of neuronal cell death.

In most neurons of the CNS there are at least two major classes of Ca^{2+} permeable channels that are activated by glutamate, either directly or indirectly: the ionotropic glutamate receptors, (NMDA subtypes as well as Ca^{2+} permeable AMPA and kainate subtypes), and voltage-sensitive Ca^{2+} channels (VSCCs). VSCCs are regulated by the membrane potential and are divided into a number of subtypes based on electrophysiological and pharmacological criteria *(28).* The different subtypes have distinct gating characteristics and kinetics of inactivation and show distinct distributions within individual neurons *(29).* These subtype specific characteristics are proposed to be responsible for increases in intracellular Ca^{2+} in specific subcellular compartments upon synaptic activation *(23).* The direct role of VSCCs in excitotoxicity is still unclear. As excitotoxicity causes cell-membrane depolarization, VSCCs will be activated. However, the degree to which Ca^{2+} entry through VSCCs contributes to neuronal damage remains controversial. We will therefore focus for the remainder of this review on the molecular mechanisms by which Ca^{2+} entry through NMDA and Ca^{2+} permeable AMPA/kainate receptors triggers excitotoxicity.

AMPA/Kainate Receptor Mediated Neurotoxicity

AMPA Receptor Structure and Molecular Machinery

AMPA receptors are generally heteromers of subunits encoded by four genes, GluR1-4 (GluR-A-D) and exhibit a higher affinity to AMPA and glutamate compared to kainate *(2,30).* The mRNAs for GluR1, 2, and 3 are expressed broadly throughout the CNS, whereas GluR4 shows a more restricted spatial and temporal expression pattern *(31).* AMPA receptor subunits have four hydrophobic membrane domains with an extracellular N-terminal domain and a cytoplasmically disposed C-terminal tail *(2,30).* A wide variety of functionally distinct receptor isoforms are present in neuronal tissues due to alternatively spliced exonic sequences in the respective mRNA as well as to RNA editing of the different subunits *(2,30).* The GluR2 subunit is unique in that it can undergo RNA editing to encode a positively charged arginine (R) residue in the membrane-associated segment 2 of the subunit, while unedited subunits contain a neutral glutamine (Q) residue at this position *(32,33).* This editing, which occurs with a very high efficiency (over 99%), determines the Ca^{2+} permeability of GluR2 containing AMPA receptor complexes and makes

GluR2(R) containing AMPA receptors impermeable to Ca^{2+} or other divalent cations *(34).*

AMPA receptor subunits also show divergence from each other in the sequence and length of their C-terminal tail, a region that is now known to be responsible for interaction with different intracellular proteins, which are considered to regulate receptor function, targeting, and trafficking *(35–37).* Briefly, GluR2 and GluR3 subunits share a common C-terminal sequence (-SVKI) that interacts with glutamate receptor interacting proteins 1 and 2 (GRIP1, GRIP2), as well as AMPA receptor binding protein (ABP). These proteins contain multiple protein-protein interacting domains, so called PDZ domains (for review *see* refs. *36,38*), which suggests that they have the capacity to assemble and regulate macromolecular protein signaling complexes around the AMPA receptor. GRIP1 and 2 bind signaling proteins such as the Eph receptor and its ligand ephrin-B1 as well as GRASP1, a novel neuron-specific Ras guanine nucleotide-exchange factor *(39,40).* Other interacting proteins of GluR subunits are PICK1 (GluR2/3), NSF (GluR2), SAP97/hdlg (GluR1), and Scr related nonreceptor tyrosine kinase Lyn (GluR2) (reviewed in refs. *36,37,41*). The interaction of these proteins with AMPA receptors has only recently been proposed to govern the receptors' pharmacological properties *(42),* activity-dependent and -independent receptor targeting and trafficking *(37,43),* as well as synaptic transmission and plasticity *(44,45).* Whether or not these interactions play a role in mediating AMPA receptor dependent excitotoxicity is unclear (*see* below).

Kainate Receptor Structure and Molecular Machinery

Kainate receptors are made up of subunits that are moderately homologous to AMPA receptor subunits: GluR5–7, KA1, and KA2 *(2,46).* These receptors exhibit the highest affinity for kainate, followed by glutamate, and have very low affinity for AMPA *(46).* GluR5 and 6 are edited in a similar fashion to the GluR2 subunit and undergo Q/R editing *(47,48),* with GluR6(Q) being the more predominant species, rendering GluR6 containing kainate receptors Ca^{2+} permeable *(49).*

As for receptor interacting proteins, GluR6 and KA2 were shown to bind to PSD95/SAP90 and SAP102, postsynaptic density (PSD) proteins originally identified to specifically interact with the NR2 subunit of NMDA receptors as well as voltage-gated potassium channels *(50,51).* Similar to the significance of AMPA receptor interacting proteins, it is believed that kainate receptor interacting proteins play an important role in receptor-mediated signaling and possibly kainate-induced neurotoxicity (*see* below).

AMPA/KA Receptor-Specific Calcium-Dependent Neurotoxic Pathways

Until recently, AMPA receptors were considered to be impermeable to Ca^{2+}. Consequently, their proposed role in glutamate-mediated excitotoxicity was to induce membrane depolarization upon ligand binding and concomitant Na^{+} entry. This is turn opens VSCCs and allows Ca^{2+} entry through NMDA receptors by releasing the voltage-dependent Mg^{2+} block of the channel. Therefore, early attention was focused on the characterization of NMDA receptor-mediated Ca^{2+} dependent neurotoxic mechanisms. while mechanisms of AMPA-mediated cell death remained less well-described. Choi *(8)* stated in a 1988 review "perhaps [AMPA's] neurotoxicity is meditated primarily by a build-up of cytosolic Ca^{2+}, occurring from both external sources (through voltage dependent channels and exchange for intracellular Na^{+}) and internal sources (triggered by inositol 1,4,5-triphosphate)."

With the subsequent cloning of the first AMPA receptors and identification of the molecular structures and subunits *(2),* it became apparent that there exist Ca^{2+} permeable AMPA/KA receptors, which is dependent on their subunit composition *(52).* These findings, together with the observation that AMPA receptor antagonists showed efficient neuroprotection from excitotoxicity when applied in

vivo (reviewed in ref. *19*), has raised interest in examining the cellular and molecular processes of AMPA/KA receptor-mediated neuronal cell death. Studying the molecular mechanisms of AMPA receptor-dependent excitotoxicity has been difficult mainly due to their subunit specific expression patterns (spatial and developmental), the mechanisms of RNA splicing and editing, as well as the recent identification of subunit specific targeting and trafficking of AMPA receptors upon activation (*see* above, and reviewed in refs. *43,53*).

Role of the GluR2 Subunit of AMPA Receptors

Due to its influence on receptor Ca^{2+} permeability, the GluR2(R) subunit has attracted significant interest in studies of excitotoxicity. GluR2 mRNA is widely expressed in mammalian neurons that are highly vulnerable to excitotoxic damage, such as hippocampal pyramidal and granule neurons *(2)* as well as cortical neurons *(54),* rendering the majority of these AMPA receptors Ca^{2+} impermeable. Despite this generally low Ca^{2+} permeability, AMPA receptor toxicity is likely to be, at least in part, mediated by Ca^{2+} ions.

Neurons expressing Ca^{2+}-permeable AMPA-gated channels can be identified by kainate induced Co^{2+} uptake and have been shown to be present in many regions of the brain, though at very low levels (8–15%) *(25,55,56).* This subpopulation of neurons was selectively destroyed in a Ca^{2+}-dependent manner after AMPA or kainate exposures *(56).* In addition, Brorson and colleagues *(57)* showed that Ca^{2+} entry via Ca^{2+}-permeable AMPA/KA receptors was sufficient to induce excitotoxicity in cerebellar Purkinje cells. Surprisingly, GluR2 containing AMPA receptors do show low Ca^{2+} and other divalent cation permeability, especially in cells expressing low levels of GluR2 relative to other AMPA receptor subunits *(58–60).* Whether this low Ca^{2+} permeability is solely responsible for all AMPA receptor-mediated excitotoxic cell death is unclear. In motorneurons, for example, selective vulnerability to AMPA receptor agonists is not determined exclusively by the relative Ca^{2+} permeability of receptors. By combining whole-cell patch-clamp electrophysiology and single-cell reverse transcription-polymerase chain reaction (RT-PCR), Vandenberghe et al. *(61)* examined fractional expression of GluR2 and Ca^{2+} permeability of AMPA receptors from motorneurons as compared to dorsal horn neurons, the former being more vulnerable to AMPA-mediated neurotoxicity. Their analysis showed that mean values of GluR2 mRNA expression levels and whole-cell relative Ca^{2+} permeability did not differ significantly between the two cell populations, which means that selective vulnerability of motoneurons to AMPA toxicity cannot be explained by an increased Ca^{2+} permeability of their AMPA receptors.

Further indication that Ca^{2+} permeability alone may not determine neuronal vulnerability to AMPA receptor-mediated excitotoxicity comes from studies using mutant mice lacking a functional GluR2 gene. Despite increased Ca^{2+} influx in CA1 neurons, these mice did not exhibit neuropathological lesions suggestive of excitotoxicity *(62).* Using cortical neuronal cultures obtained from GluR2-deficient mice, it was recently demonstrated that elevated Ca^{2+} permeability in these neurons did not correlate with excitotoxicity *(63).* Neurons with reduced or absent levels of GluR2 exhibited increased kainate potency and larger ionic currents. Thus vulnerability to similar concentrations of kainate was higher in GluR2-deficient neurons. However, insults using equi-effective kainate concentrations were equally neurotoxic, despite eliciting higher Ca^{2+} elevations in neurons lacking GluR2. That same study showed that in vivo vulnerability of CA1 hippocampal neurons to stereotactic kainate injections, and CA3 neurons to intraperitoneal kainate administration, was independent of the levels of GluR2. While such data certainly does not exclude a role for Ca^{2+} ions in AMPA receptor-mediated excitotoxicity, it strongly suggests that AMPA receptor mediated excitotoxic signaling is not solely dependent on high levels of Ca^{2+} influx mediated by the absence of the GluR2 subunit. Instead, GluR2 may govern other aspects of AMPA receptor function that may cause neurotoxicity when absent, such as

effects on agonist potency, ionic currents, and synaptic reorganization.

Similar results were observed in mouse mutants with targeted AMPA receptor GluR-2 subunit alleles functionally expressed at different levels and deficient in Q/R-site editing *(64).* Despite increased Ca^{2+} permeability in these mutants, there was no evidence for ongoing cytotoxicity. In addition, Kask and colleagues *(65)* created a mouse with a partial reduction in GluR2 expression levels (30%) and showed that in spite of increased AMPA receptor-mediated Ca^{2+} influx into pyramidal cells, there was no cytotoxicity noticeable in hippocampal neurons. In contrast, heterozygous mice carrying a modified GluR2 allele, which are rendered 100% editing-deficient, also expressed AMPA receptors with increased Ca^{2+} permeability *(66).* However, these mice developed seizures and died prematurely. The conflicting results between the GluR2 knockout mice and the mutant mice may be explained by differences in genetic background, or the ability of AMPA receptors with or without GluR2 to interact with cellular proteins. The presence of GluR2 may enhance the formation of an AMPA receptor complex with distinct postsynaptic density proteins (*see* above and below), which could be important in triggering signaling cascades, some of which may be capable of inducing cell death under conditions of increased Ca^{2+} permeability. Furthermore, different degrees of toxicity among mutant GluR2 mice (Feldmeyer vs Kask vs Brusa) may be explained by a Ca^{2+} threshold that is necessary for triggering events leading to AMPA receptor-mediated damage *(63).* If some threshold Ca^{2+} level is necessary to trigger intracellular second-messenger cascades, similar to triggering an action potential in an all or none fashion, then different degrees of GluR2 expression levels may determine whether or not incoming Ca^{2+} ions will be sufficient to induce toxicity. Any subsequent Ca^{2+} loading would then have no additional effect on neuronal survival and increased Ca^{2+} entry caused by decreased levels of GluR2 would not be responsible for enhanced toxicity.

Zukin and colleagues provided additional evidence for the importance of the GluR2 subunit in excitotoxic cell death (summarized in ref. *67*). They initially showed that brief forebrain or global brain ischemia triggers a specific decrease in the expression of the GluR2 subunit in hippocampal neurons of the CA1 region, a region of the hippocampus highly vulnerable to ischemic damage *(68).* The decreased expression levels of GluR2 were associated with an increased Ca^{2+} permeability of AMPA receptors in these cells *(69).* They further showed that antisense oligonucleotides targeted to mRNA of GluR2 injected into rats and gerbils induced a delayed cell death in CA1 and CA3, which was blocked by AMPA receptor channel blockers *(70).* Unfortunately, these in vivo studies do not allow for a clear distinction as to whether the enhanced neurotoxicity is solely due to increased Ca^{2+} entry through AMPA receptors or to other GluR2-linked mechanisms similar to those discussed earlier. Presently, other studies examining expression of multiple AMPA receptor subunits after ischemia *(71)* or seizures *(72,73)* have not confirmed a preferential reduction in GluR2 expression in ischemia. Interestingly, Ying et al. *(74)* showed that a brief conditioning exposure to sublethal oxygen-glucose deprivation shifted AMPA receptor subunit expression in individual cultured hippocampal neurons to increasing GluR4 (flop) expression, resulting in increased abundance of GluR4 mRNA relative to GluR1 and GluR2 mRNA, whereas GluR2 expression levels were unchanged. However, these changes in AMPA receptor subunit expression did cause an increase in AMPA or kainate evoked free and total intracellular Ca^{2+} concentration and selectively increased vulnerability to kainate-induced cell death. The authors propose that the increase in GluR4 could contribute to lowering the relative abundance of GluR2, a change that might increase formation of AMPA receptors, which lack a GluR2 subunit and have enhanced Ca^{2+} permeability.

Based on these studies, it is still unclear whether or not AMPA receptor-mediated excitotoxicity is solely dependent on the degree of Ca^{2+} entry through the receptors or whether other factors are responsible for triggering neuronal cell death upon receptor activation. One interesting alternative hypothesis is that influx of Zn^{2+} through Ca^{2+}-permeable AMPA/KA receptors contributes to selective neuronal injury (reviewed extensively in refs. *13,14*). Further, as mentioned earlier, AMPA receptor subunits specifically interact with postsynaptic density proteins and form distinct macromolecular protein signaling complexes at synaptic junctions. We have previously shown that the concept of a lattice of proteins at the excitatory synapse governs one mechanism of NMDA receptor-mediated Ca^{2+}-dependent neurotoxicity (*26, see* below). Thus, AMPA receptor-mediated ion fluxes are also likely to be coupled to neurotoxic second messengers via protein-protein interactions.

None of the AMPA receptor interacting proteins identified thus far have been shown to play a role in AMPA receptor mediated excitotoxicity. As mentioned earlier, several proteins have been identified to specifically interact with AMPA receptor subunits. Most of these interactions have been ascribed a role in the dynamic regulation of the postsynaptic structure by regulating receptor trafficking, a molecular concept that is thought to underlie cellular models of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) *(37,43).* Therefore, the link of AMPA receptors to not just physiologic, but also pathologic, downstream signaling cascades via interacting proteins is an attractive hypothesis.

For example, the interaction of GRIP 1, a GluR2 interacting protein, with GRASP-1 may couple AMPA receptors to Ras signaling *(40).* Interestingly, GRASP-1 is a neuronal substrate for Caspase-3 *(40)* and is cleaved in apoptotic neurons during development and ischemia (Ye and Huganir, personal communication). Although the consequence of the Caspase-cleavage of GRASP-1 is unclear, it seems to uncouple the C-terminal GRIP-binding domain of GRASP-1 from the rasGEF domain and may thereby disrupt the regulation or targeting of the GEF. Further, GRASP-1 has been suggested to serve as a scaffold protein for the c-jun N-terminal kinase (JNK) signaling pathway (Ye and Huganir, unpublished observation) and its overexpression in non-neuronal cell lines (HEK293T) activates the JNK pathway. The JNK pathway is known to be involved in stress responses and cell death *(75,76),* which may indicate a role of GRASP-1 in AMPA receptor-mediated cell death. Such possibilities have yet to be determined.

An alternative role of GluR2 interacting proteins in excitotoxicity may be that the presence of GluR2 is necessary to maintain synaptic structure and organization. Thus, toxicity in GluR2-deficient neurons may be related to effects on synaptic function and organization in the brain rather than due to altered Ca^{2+} permeability. An interesting candidate for this role may be the GluR2 interacting protein N-ethylmaleimide-sensitive fusion protein (NSF), a protein involved in membrane-fusion events *(77–79).* The NSF-GluR2 interaction is suggested to be required for the surface expression of GluR2-containing AMPA receptors and disruption of the interaction leads to the functional elimination of AMPA receptors at synapses *(77,80).* Interestingly, NSF expression in the PSD has been shown to be upregulated after an ischemic insult *(81).* It is not clear at this point whether an increase in NSF will lead to an increase in surface expression of existing GluR2-containing AMPA receptors upon ischemic stimulation. One might speculate that increased surface expression of GluR2 will in turn: 1) decrease Ca^{2+} permeability through AMPA receptors; and 2) restore synaptic organization, which together may act as a feedback mechanism to protect neurons from further degeneration.

Role of the GluR1 Subunit of AMPA Receptors

While the GluR1 subunit has attracted interest in studies of synaptic plasticity and organi-

zation *(43)*, very little is known about its role in excitotoxic neuronal cell death. Very recently, a few studies reported a specific involvement of GluR1 expression and/or synaptic localization in neurodegenerative disorders. For example, one group found in postmortem brain tissues of patients with Alzheimer's disease (AD) an abnormal expression of GluR1 and its interacting protein SAP97 *(82)*. Also, Lissin and colleagues *(83)* reported that application of glutamate or AMPA to cultured neurons evoked a rapid and selective redistribution of GluR1 subunits away from synaptic sites independent of NMDA receptor activation. While this activity-dependent redistribution of GluR1 could be induced under conditions that caused relatively little neuronal death, the authors suggested that the redistribution is unlikely to be a direct consequence of excitotoxicity. It is possible, however, that rapid redistribution of GluR1-containing AMPA receptors functions physiologically in the context of excitotoxicity, perhaps as a mechanism to protect neurons from excessive stimulation by high concentrations of glutamate.

Role of Kainate Receptor Subunits

Similar to studies on AMPA receptor-mediated cell death, the role of kainate receptors in excitotoxicity was overshadowed by the action of NMDA receptor activation on neuronal survival, and therefore very little was known about direct neurotoxic functions of kainate receptors in neurodegeneration. Further, it is difficult to differentiate between AMPA and kainate receptors, as kainate mediates a fast, desensitizing response at kainate receptors *(84)* while it incompletely desensitizes AMPA receptors *(85)*. Only the recent development of selective antagonists of AMPA receptors has allowed for progression in our understanding of kainate receptor function, physiological as well as pathological *(86–88)*. For example, kainate receptors have been suggested to modulate synaptic transmission by both pre- and postsynaptic mechanisms *(89)* and were recently shown to be involved in synaptic plasticity at the mossy-fiber synapse in the hippocampus *(90,91)*. Regarding disease, kainate receptors are considered to contribute to temporal lobe seizures *(92–95)*. GluR6-mediated excitotoxicity has also been attributed a role in the pathogenesis of Huntington's disease (HD) *(96–99)*.

As mentioned earlier, the GluR5 and GluR6 subunit of kainate receptors undergo Q/R site RNA editing, which is subject to developmental and regional regulation *(100)*, thereby reducing the Ca^{2+} permeability of GluR5- and/or GluR6-containing kainate receptors. The generation of mice with disruption of kainate receptor subunits allowed for the genetic dissection of the role of each subunit in synaptic physiology, as well as in excitotoxic processes. While GluR5(Q636R) kainate receptor mutant mice exhibited no obvious effects on kainate-induced seizures or behavior in mice *(101)*, mice deficient in GluR6 Q/R site editing showed increased kainate receptor-mediated Ca^{2+} influx and enhanced seizure vulnerability *(102)*. Furthermore, overexpression of the GluR6 subunit through transduction with herpes simplex virus vectors in neurons of the CA3 region of the hippocampus, produced extensive cellular damage and induced limbic seizures as well as spontaneous nonsynaptic bursting in vitro *(103,104)*. Interestingly, GluR6 deficient mice exhibited decreased sensitivity to kainate in hippocampal neurons of CA3. Furthermore, these mutant mice showed decreased vulnerability to kainate-induced seizures as measured by the onset of seizures and by the activation of immediate early genes in the hippocampus *(94)*. The authors did not explicitly address the question of Ca^{2+} permeability through those GluR6 lacking kainate receptors, and it is not clear whether the neuroprotective effects were due to decreased kainate sensitivity of the GluR6-deficient receptors or due to other GluR6-dependent mechanisms.

Similar to AMPA and NMDA receptors, one may speculate that specific interactions of kainate receptor subunit C-terminal tails will play a role in triggering excitotoxic cell death. Garcia and colleagues *(51)* showed that SAP90/PSD-95 and SAP102 interact with both

KA2 and GluR6, while SAP97 binds to GluR6. When co-expressed in non-neuronal cell lines, SAP90/PSD-95 altered GluR6 or KA2/GluR6 receptor function by reducing desensitization. The same group further demonstrated that the interaction of PSD-95 to GluR6 links kainate receptors to JNK activation by anchoring upstream activators of JNKs to the receptor complex *(105).* It is yet to be determined whether activation of this pathway requires Ca^{2+} influx through the kainate receptors.

NMDA Receptor-Mediated Neurotoxicity

NMDA Receptor Structure and Molecular Machinery

Studies using different molecular cloning techniques have resulted in the identification of five NMDA receptor subunits, NR1 and NR2A-D *(2,30).* Each subunit is structured similar to AMPA type receptors with four membrane domains, an extracellular amino terminal domain, and an intracellular carboxy terminal tail *(2).* The NR1 subunit has been ascribed eight functional splice variants (NR1a-h) and one nonfunctional truncated splice variant *(106),* while among NR2 subunits only NR2D has been shown to exhibit splice variants *(107).* In regard to receptor stoichiometry, the NMDA receptor is either a hetero-tetramer or pentamer *(30,108).* The Ca^{2+} permeability of the receptor is controlled by an asparagine residue (N598) in the NR1 subunit within the channel pore loop structure of the second membrane domain, in a position homologous to the Q/R sites of GluR1-4 *(109,110).* This residue also determines the voltage-dependent Mg^{2+} block of NMDA receptors *(111).* Further, N598 has been shown to control gating properties, potentiation and block by polyamines, inhibition by protons and Zn^{2+}, and affinity to glutamate and glycine *(112,113).*

The NR1 subunit is believed to be necessary to form a functional NMDA receptor and co-expression of NR1 together with NR2 subunits leads to the formation of ion channels that have distinct functional and pharmacological properties very similar to those of NMDA receptors in neurons *(114,115).* NR1 knockout mice do not survive past the first few postnatal days even though there are only minimal changes in the structure or function of neurons of the brain *(116–118),* while knockout mice for NR2A survive and develop normally *(119).* Due to the large number of splice variants in the NR1 subunits, different combinations of NR1 and NR2 subunits can result in a wide array of receptor complexes with differing affinities for ligands. For example, NR1 splice variants can clearly show distinct sensitivities to agonists, antagonists, Ca^{2+}, Zn^{2+}, polyamines as well as phosphorylation by protein Kinase C (PKC) (reviewed in ref. *106*). In addition, each NR2 subunit confers a unique set of characteristics upon the resultant NMDA receptor, such as sensitivity to Mg^{2+} block, glycine and glutamate affinity, and single-channel conductance *(114,115,120).* This makes the study of NMDA receptor-mediated excitotoxicity challenging and may explain the modest information that is known thus far about the molecular mechanisms of these events.

Similar to the AMPA receptor subunits, NMDA receptor subunits show divergence in the sequence of their C-termini, especially the very C-terminal regions. These C-terminal domains allow for interaction of the NMDA receptor with numerous intracellular synaptic and cytoskeletal proteins, which leads to the formation of large multiprotein complexes associated with the receptor (extensively reviewed in refs. *41,121*). While still very little is known about the functional significance of these protein-protein interactions in physiological synaptic transmission and organization, there is even less evidence for a role of NMDA receptor-associated proteins in excitotoxicity. However, given the diversity of receptor subunit composition and subsequent distinct binding possibilities to synaptic protein complexes, it is very likely that targeting ion fluxes to receptor-specific downstream effectors activates specific intracellular signaling pathways.

NMDA Receptor-Specific Calcium Dependent Neurotoxic Pathways

As mentioned earlier-NMDA receptors have been ascribed a dominant role in mediating excitotoxicity, mainly due to their high Ca^{2+} permeability *(9,18).* However, as a result of an increasing interest in the molecular mechanisms of NMDA receptor-mediated excitotoxicity, it became more obvious that it is not only the high influx rate of Ca^{2+} ions through the channel pore that determines neuronal survival, but also that individual receptor subunits may exhibit distinct excitotoxic potentials that could dictate susceptibility to toxicity.

Role of NR1 Subunit of NMDA Receptors

The presence of the NR1 subunit is essential to form a fully functional receptor *(114).* Whether this is due to the role of NR1 in regulating Ca^{2+} permeability and Mg^{2+} block of the receptor, or whether it is due to the large number of C-terminal tail splice variants and their involvement in NMDA receptor function, or whether it is simply due to the necessity of NR1 subunits to ensure receptor assembly, is still uncertain. The contribution of the NR1 subunit to excitotoxicity is difficult to determine. For example, cultured neurons obtained from mutant mice lacking the NR1 subunit were resistant to NMDA and glutamate-induced rapidly triggered cell death *(122).* Similar results were obtained in vitro and in vivo by application of antisense oligonucleotides *(123).* In this study, treatment with antisense oligonucleotides to NR1 prevented NMDA-induced neurotoxicity in neuronal cultures and reduced focal ischemic infarction produced in vivo. However, this only indicates that functional NMDA receptors are necessary for triggering receptor-dependent excitotoxicity and does not confer NR1 a specific role in causing cell death. Mice expressing a mutant form of NR1 in the site critical for Ca^{2+} permeation (N598Q and N598R) show disturbed NMDA receptor-mediated signaling and fail to develop autonomic functions such as feeding and breathing *(124).* The authors concluded that disturbed NMDA receptor signaling mediates a variety of neurological phenotypes, while the effects of this mutation on NMDA receptor-mediated neurotoxicity are still to be elucidated. Unlike NR1 null mice, mutant mice expressing only 5% of normal levels of NR1 do survive to adulthood and display behavioral abnormalities that seemed similar to animal behaviors in models of schizophrenia *(125).*

Another target of investigation for the involvement of the NR1 subunit in excitotoxicity are the different splice variants (*see* Fig. 1). Eight functional splice variants of the NR1 subunit are created by alternative splicing in exons 5, 21 and 22 (*see* earlier refs. and *see* review in *(106)).* Exon 5 encodes a splice cassette of the amino terminal domain (N1), while exons 21 and 22 encode two independent carboxy terminal domain splice cassettes (C1 and C2). Previous reports have demonstrated that the occurrence of alternative splicing depends on the developmental stage *(126,127)* and the particular brain region examined *(128,129).* However, little information is given about factors that might induce changes in alternative splicing of the NR1 gene in neurons of the adult brain. Most attention was given to the role of these splice variants in the context of synaptic transmission and organization as well as NMDA receptor physiology (e.g., refs *130–134*) while little is known about their contributions to excitotoxicity. Kreutz and colleagues *(135)* studied the effects of axonal trauma of the optic nerve on expression of alternatively spliced NR1 variants in the retinal ganglion cell layer. The NR1-2b (presence of N1 and C2 cassettes) and NR1-4b (presence of N1 cassette only) isoforms were preferentially expressed between 2 d and 1 wk after injury, whereas expression for all other isoforms remained either unchanged or decreased to barely detectable levels within 4 wks. The authors further demonstrated that the increased expression of NR1-4b is crucial for neuronal survival after partial axonal trauma, as decreasing NR1-4b expression levels using antisense oligonucleotides significantly decreased cell survival of retinal ganglion cells. The authors propose

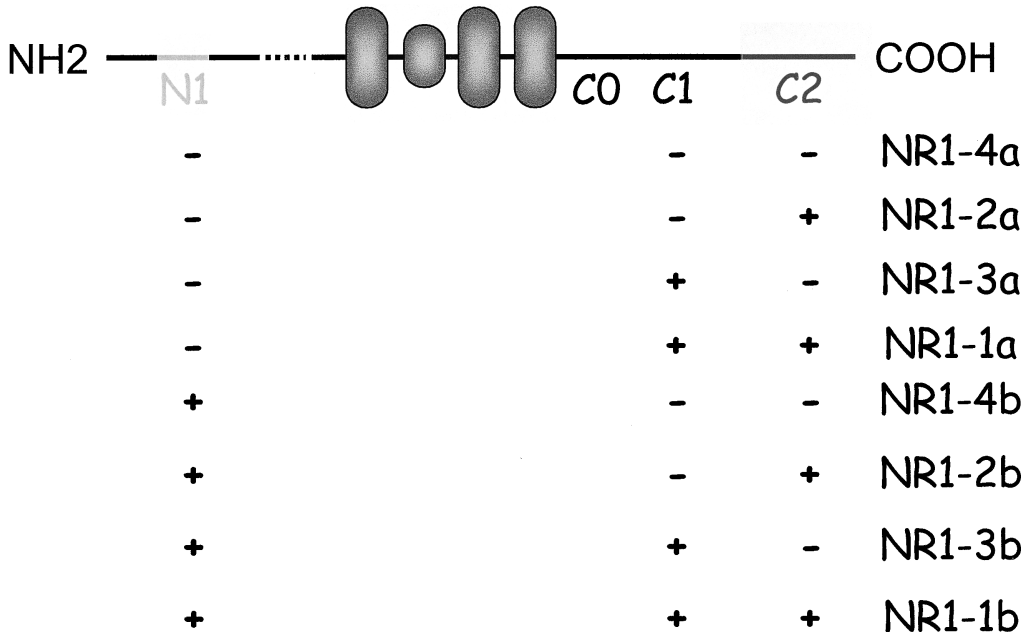

Fig. 1. Schematic depiction of the NMDA receptor subunit NR1 splice variants. The nomenclature of the NR1 subunits used in the text corresponds to the presence (+) or absence (–) of the three alternatively spliced exons of NR1. Note, C0 represents the very membrane-proximal segment of the NR1 cytoplasmic tail and does not undergo mRNA splicing.

that altered splicing leads to a different composition of the native NMDA receptor and different responses to glutamate activation.

Recently, Rameau and colleagues *(136)* have demonstrated a role for some of the NR1 splice variants in NMDA receptor toxicity in heterologous cells and primary neuronal cultures. NR1 pore mutations that block Ca^{2+} entry and NR1 C-terminal tail deletions decreased NMDA-induced neurotoxicity significantly when transfected into CHO cells. The authors further showed that expression of these constructs in primary cortical neurons protected cells from NMDA receptor-dependent cell death. Their study suggests that while the N1 cassette of the amino terminus exhibits very low toxicity on its own, C1 and C2 enhance NMDA receptor toxicity. The C-terminal domain of NR1 has gained interest over the past years due to its interaction with several different cytoplasmic proteins (extensively reviewed in ref. *41*); however, little is known about the role of NR1 interacting proteins in NMDA receptor-mediated excitotoxicity.

The C0 domain of NR1, which represents the very membrane-proximal segment of the NR1 cytoplasmic tail, interacts with an actin binding protein, α actinin *(137).* This interaction has been suggested to mediate the anchoring of NMDA receptors to cytoskeletal elements within the synapse as depolymerization of F-actin causes a redistribution of NMDA receptor clusters to nonsynaptic sites *(138–140).* The binding of α-actinin to the NMDA receptor is directly antagonized by Ca^{2+}/calmodulin *(133,141).* Ca^{2+}/calmodulin binds to both the C0 and the C1 segment of NR1 and its binding inhibits NMDA receptor opening and reduces mean channel open time *(133,134).* Therefore, Ca^{2+} influx through the NMDA receptor may lead to the displacement of α-actinin through its binding to Ca^{2+}/calmodulin and may induce release of the receptor from the actin

cytoskeleton and in turn redistribution of the receptor away from the synapse. Localization of glutamate receptor clusters in spines is critical for function and efficacy of physiological neuronal synaptic transmission *(35,142).* Excitotoxicity, on the other hand, produces a rapid and profound loss of dendritic spines in cultured neurons *(143),* mimicking the loss in dendritic spine synapses in neurological conditions including epilepsy, schizophrenia, aging, and prion protein-related diseases *(144–146).* This suggests that receptor localization at synapses might be critical to excitotoxicity, and that dendritic spines constitute the subcellular sites that govern neuronal vulnerability to excitotoxicity. However, NMDA receptors are also found at extrasynaptic sites *(147–149),* raising the possibility that the synaptic and extrasynaptic subsets of NMDA receptors play different physiological and pathological roles in the cell. Therefore, the synaptic anchoring of NMDA receptors via an NR1-α-actinin interaction may be important for the toxic potential of NMDA receptor activation. We tested this hypothesis by perturbing F-actin in primary cultured cortical neurons, which selectively reduced the activity of synaptically activated NMDA receptors *(150).* This allowed us to functionally separate the effects of synaptic and extrasynaptic receptors on excitotoxicity and neuronal Ca^{2+} homeostasis. Our data indicate that NMDA receptors can trigger excitotoxicity both within and outside of synapses; in other words, synaptically and extrasynaptically activated NMDA receptors are equally capable of excitotoxicity. However, their relative contributions vary with the location of extracellular excitotoxin accumulation, a factor governed by the mechanism of extracellular neurotransmitter accumulation, not the location of the receptor itself.

NR1 subunits containing C1 specifically interact with two other proteins, Yotiao *(151)* and neurofilament L *(152).* This may explain the C1-dependent clustering of NR1 in heterologous cells *(153)* and may provide further evidence that the NR1 subunit of NMDA receptors is important for the synaptic localization of the receptor. The role of these proteins in NMDA receptor function is still unclear. Yotiao binds to both protein kinase A and protein phosphatase 1, but it is not known whether these proteins are directly involved in NMDA receptor-mediated neurotoxicity.

Role of NR2 Subunit of NMDA Receptors

It has been thoroughly described that neuronal cells in culture exhibit a developmental profile of NMDA receptor-mediated glutamate neurotoxicity in which younger cultures are less vulnerable than older cultures *(17,154–156).* This phenomenon seems to be complemented by a developmental switch in NMDA receptor subunit NR2 expression, which may underlie the increase in susceptibility to excitotoxicity over time. The expression of NR2B and NR2D begins at least are early as E14, whereas NR2A and NR2C are first detected perinatally *(157–159).* The developmental change in NMDA receptor subunit expression has been shown to be dependent on neuronal activity *(160–162).* Bessho and colleagues *(163)* showed that K^+-induced depolarization in cerebellar granule cells, which initially promotes neuronal survival *(164,165),* upregulates NR2A subunit mRNA via Ca^{2+} influx through VSCCs. Interestingly, these cells become more vulnerable to NMDA-mediated toxicity after prolonged K^+ depolarization, which the authors suggest to be partially due to the increased levels of NR2A and an enhanced NMDA receptor-mediated Ca^{2+} influx.

Few studies have directly addressed the question of the relation between developmental expression of NR2 subunits and vulnerability to NMDA receptor-mediated neurotoxicity. Mitani and colleagues *(166)* studied functional changes of NMDA receptors related to susceptibility to NMDA toxicity in developing neurons in the pontine nucleus. They directly injected NMDA into the pontine nucleus of animals of different ages. The susceptibility to NMDA neurotoxicity peaked near postnatal day 15, and NMDA-induced currents showed prominent reduction of the voltage-dependent Mg^{2+} block near postnatal day 15 with a con-

comitant distinct expression of NR2C. They concluded that there is a possibility that functional changes in NMDA receptor channels play a crucial role in the occurrence of developmentally specific neuronal injury. Mizuta et al. *(167)* demonstrated that while cultures of cortical neurons were not affected by glutamate on culture day 7–9, the cells exhibited increased glutamate sensitivity on day 11. Using Western-blot analysis, they detected levels of NR2B and NR1 on both days (8 and 11), while NR2A protein levels were hardly detectable on either day 8 or day 11. The authors concluded that glutamate neurotoxicity was mainly mediated by a heteromeric NR1/NR2B receptor. Cheng and colleagues *(168)* showed that NR1 and NR2A mRNA levels increased continuously over time in neuronal cultures, whereas NR2B mRNA increased dramatically during the first 10 d and subsequently remained stable. The time-course of of NR2B mRNA increase most closely followed the increase in glutamate-stimulated changes in the intracellular Ca^{2+} concentration and neuronal injury. These authors also concluded that NR2B expression might be a critical determinant of glutamate neurotoxicity. However, both groups agree that the correlation between changes in either mRNA or protein levels and toxicity still do not exclusively demonstrate a relationship between NMDA receptor subunit composition and neuronal vulnerability to excitotoxicity. It could just purely be an increase in the number of functional channels, or the newly expressed subunit may not be incorporated into functional receptors at the plasma membrane. More detailed studies are necessary to address these issues.

Results obtained from heteromeric NR1-NR2 expression systems in non-neuronal cell lines indicated that co-transfection of NR1 and NR2A resulted in more cell death than transfections of NR1 with NR2B, while NR1-NR2C receptors did not induce toxicity at all *(169,170).* However, how these results correlate with the situation in neurons is uncertain, as non-neuronal cell lines obviously do not provide the cellular machinery present in neurons. The creation of mutant mice has provided further insight into the functional role of distinct NMDA receptor subunits. While several NMDA receptor mutant and knockout mice have been created (summarized in ref. *171*), very few of these mice have been investigated in regard to their vulnerability to excitotoxicity. Morikawa and colleagues *(172)* investigated the role of NR2A and NR2B subunits in a model of brain ischemia subjected to mutant mice deficient in NR2A *(119,173)* and double-mutants deficient in NR2A and NR2B. They showed that lack of NR2A resulted in significant reduction in injury volume, which could not be further reduced by the additional elimination of NR2B. The authors carefully propose that NR2A plays a pivotal role in glutamate neurotoxicity. However, these results could be explained by a simple decrease in the number of functional NMDA receptors as NR2A knockout mice show decreased NMDA receptor channel activity in CA1 *(119,173).* Mice lacking NR2C have also been subjected to an animal model of cerebral ischemia and were shown to exhibit reduced injury *(174).* To draw further conclusions from these studies, it would be helpful to analyze and control the mutant mice for the total number of receptor, receptor localization (synaptic vs nonsynaptic), membrane localization or receptor subunit composition.

An alternative to a knockout mouse is the use of mutant mice expressing truncated receptors. Most of these mutants seem to form gateable receptors that are synaptically activated, but are defective in intracellular signaling and synaptic localization *(175–177).* This indicates that the C-termini of NR2 subunits provide a physical linkage to interacellular components, which mediate the triggering of cellular responses of transient Ca^{2+} ions entering through the NMDA receptor channel.

A large number of cytoplasmic PSD proteins have been identified that distinctly bind to specific NMDA receptor subunits (reviewed in refs. *36,37,41*). Considering that Ca^{2+} ions entering the channel diffuse rapidly *(178)* and therefore the amplitude of Ca^{2+} transients is

best monitored directly at the intracellular face of the ion channel pore, a physical coupling of the receptor channel to a Ca^{2+} signaling machinery seems essential. This idea has been supported by a number of studies showing that different physiological Ca^{2+}-dependent processes, including synaptic plasticity and gene expression, are separately regulated through distinct signaling pathways linked to specific routes of Ca^{2+} influx *(23,27,179,180).*

Previous data have suggested that such distinct Ca^{2+} signaling pathways would also exist for NMDA receptor-mediated neurotoxic Ca^{2+} influx. We showed that Ca^{2+} dependent neurotoxicity is triggered most efficiently when Ca^{2+} influx occurs through NMDA receptors and cannot be reproduced by loading neurons with equivalent quantities of Ca^{2+} through non-NMDA receptors or VSCCs *(24,25).* Thus we hypothesized that lethal Ca^{2+} signaling by NMDA receptors is determined by the molecules with which they interact. However, very few of the identified NMDA receptor interacting proteins have been ascribed a functional role in channeling Ca^{2+} signals to intracellular second-messenger cascades, physiological or pathological. One major family of PSD proteins that bind to NR2 subunits is the PSD-95/SAP90 subfamily of the membrane-associated guanylate kinase (MAGUK) superfamily (reviewed in refs. *181,182*). PSD-95/SAP90 is the best-characterized protein of this family and binds to the C-terminal tail of NR2A and NR2B *(50).* PSD-95 also interacts with other intracellular signaling molecules, including neuronal nitric oxide synthase (nNOS; *183–185*), an enzyme that participates in NMDA receptor-mediated nitric oxide (NO) signaling pathways *(186,187).* We therefore sought to test the hypothesis that PSD-95 acts as a scaffolding protein that links Ca^{2+} ions coming through the NMDA receptor to intracellular signaling molecules, such as nNOS, and that this link is responsible for preferential triggering of neurotoxic cascades by NMDA receptor activation *(24,25).*

We studied the role of PSD-95 in NMDA receptor-mediated excitoxicity by suppressing the expression of PSD-95 in cultured neurons using antisense oligonucleotides *(26)* PSD-95 deficient neurons were selectively protected against NMDA receptor-mediated cell death, but not against excitotoxicity evoked by other glutamate or Ca^{2+} channels. NMDA receptor function was unaffected as receptor expression, NMDA currents, and Ca^{2+} loading via NMDA receptors were unchanged. This suggested that the loss of PSD-95 modified downstream events of NMDA receptor activation rather than NMDA receptor function. This was further confirmed as we were able to show that suppressing PSD-95 selectively blocked Ca^{2+}-activated NO production by NMDA receptors, but not by other pathways, without affecting nNOS expression or function *(26).* Thus PSD-95 is required for the efficient coupling of NMDA receptor activity to NO toxicity, and imparts specificity to NMDA receptor-mediated excitotoxic Ca^{2+} signaling. The concept of PSD-95 acting as a scaffolding protein rather than a modulatory protein was consistent with data obtained from a mutant mouse expressing a truncated form of PSD-95 in which synaptic NMDA receptor currents, subunit expression, localization, and synaptic morphology were all unaffected by the mutation *(188)* However, the frequency function of NMDA-dependent LTP and LTD was shifted to produce enhanced LTP at different frequencies of synaptic stimulation.

The idea of PSD-95 acting solely as a scaffolding protein that brings intracellular signaling molecules in close vicinity to the NMDA receptor is apparently contradictory to one recent study carried out by Yamada et al. *(189).* By injecting PSD-95 cRNA into *Xenopus* oocytes expressing the NMDA receptor (NMDAR) the authors showed a decreased sensitivity of the receptor channel to glutamate as well as inhibition of PKC-mediated potentiation of the NMDAR channel. They suggest that PSD-95 functionally modulates the channel activity of NR1/NR2B and that PSD-95 may play a protective role against neuronal excitotoxicity. The contradiction of these results, in which PSD-95 was designated a NMDAR-modulatory role, to the aforementioned results and those of other *(175,188–190)*

might be explained by the differences in the culture systems and their distinct underlying cellular and molecular machinery.

The interaction of NR2A and NR2B with PSD-95 may bring more Ca^{2+}-sensitive second messengers into close vicinity to the NMDA receptor similar to nNOS, including SynGAP, a synaptic GTPase protein for Ras *(191,192)* or Citron, a Rho effector in the brain *(193,194).* In addition, PSD-95 is linked to Fyn, a Src family protein tyrosine kinase (PTK) as well as other Src family PTKs such as Src, Yes, and Lyn *(195).* Their role in NMDA receptor-mediated excitotoxicity is yet to be determined.

Interestingly, ischemic challenges have been described to induce biochemical and morphological changes in the PSD *(81,196–198).* In regard to NMDA receptor subunits, Tagaki and colleagues *(197)* showed a significant decrease in the solubility of NMDA receptors and PSD-95, which was most prominent in the vulnerable CA1 region of the hippocampus. The authors propose that the basis for ischemia-induced alterations in the properties of PSD-95 and its associated proteins may be complex and multifaceted, involving changes in Ca^{2+} concentrations, phosphorylation and/or dephosphorylation reactions, activation of proteases, oxidative reactions, and other changes in the postsynaptic cell. They conclude that these modulations of PSD structure may have significant consequences for synaptic function and neuronal survival.

Concluding Remarks

It is apparent that as we slowly gain increasing knowledge regarding the functional role of glutamate receptor subtypes in neurotoxic processes, we are still challenged with the identification of specific neurotoxic pathways that are linked to the activation of distinct receptors subtypes and subunits. At present we know a great deal about downstream processes that are triggered during an excitotoxic insult, such as activation of endonucleases, proteases, phosphatases, kinases, or the formation of free radical species and NO (extensively reviewed in refs. *8,9,12,21*). However, we have very little knowledge about how precisely Ca^{2+} ions (or other mediators) are channeled in order to distinctly activate these enzymes. One hypothesis, which is mentioned throughout this review, is that the interaction of glutamate receptor subunits with distinct intracellular scaffolding proteins allows for the formation of a multimolecular protein complex at the site of neuronal activation, namely the PSD. The NMDA receptor-PSD-95-nNOS complex is one such example, suggesting that similar complexes exist and that they can lead to distinct triggering of neurotoxic signaling cascades *(26).* Revealing specific signal-transduction pathways involved in excitotoxicity may consequently lead to the identification of molecular targets whose pharmacological or genetic manipulation might produce improved therapies for neuronal cell death in many neurodegenerative diseases.

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