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SYMPOSIUM: Neuronal Apoptosis

Apoptotic and Anti-Apoptotic Synaptic Signaling Mechanisms

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Although several prominent morphological features of apoptosis are evident in the cell body (e.g., cell shrinkage, membrane blebbing, and nuclear DNA condensation and fragmentation) the biochemical and molecular cascades that constitute the cell death machinery can be engaged in synaptic terminals and neurites. Initiating events such as oxyradical production and calcium influx, and effector processes such as Par-4 production, mitochondrial alterations and caspase activation, can be induced in synapses and neurites. Several prominent signal transduction pathways in synaptic terminals play important roles in either promoting or preventing neuronal death in physiological and pathological conditions. For example, activation of glutamate receptors in postsynaptic spines can induce neuronal apoptosis, whereas local activation of neurotrophic factor receptors in presynaptic terminals can prevent neuronal death. Factors capable of inducing nuclear chromatin condensation and fragmentation can be produced locally in synaptic terminals and neurites, and may propogate to the cell body. Recent findings suggest that, beyond their roles in inducing or preventing cell death, apoptotic and anti-apoptotic cascades play roles in synaptic plasticity (structural remodelling and long-term functional changes). For example, caspase activation results in proteolysis of glutamate receptor (AMPA) subunits, which results in altered neuronal responsivity to glutamate. Activation of neurotrophic factor receptors in synaptic terminals can result in local changes in energy metabolism and calcium homeostasis, and can induce long-term changes in synaptic transmission. The emerging data therefore suggest that synapses can be considered as autonomous compartments in which both pro- and anti-apoptotic signaling pathways are activated resulting in structural and functional changes in neuronal circuits. A better understanding of such synaptic signaling mechanisms may reveal novel approaches for preventing and treating an array of neurodegenerative conditions that are initiated by perturbed synaptic homeostasis.

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Synaptic life and death signaling: where the action is

The synapse evolved as a highly complex and finely-tuned signal transduction compartment that allows for long-range intercellular communication. From the perspective of short-term function of neuronal circuits, neurotransmitters have been the most heavily studied type of synaptic signal. On the other hand, it is also clear that transduction pathways for a variety of other signalis are concentrated in synaptic terminals including those activated by neurotrophic factors, cytokines and cell adhesion molecules. Activation of neurotransmitter and neurotrophic factor signaling pathways have been shown to play important roles in synaptogenesis (62, 63), fast synaptic transmission (50), long-term changes in synaptic function (1, 22, 44, 56), synaptic degeneration/pruning (63, 79) and cell death (66, 67, 75, 87). It is well-established that activity in neuronal circuits plays a major role in synaptic organization and programmed neuronal death during development of the nervous system, and that synaptic signals involving neurotransmitters and neurotrophic factors are key mediators of such activity-dependent neuronal plasticity (89). Programmed cell death, a form of apoptosis that occurs during development, appears to be controlled by synaptic signaling. Neurons that receive sufficient neurotrophic factor receptor activation survive, whereas those not receiving a threshold level of neurotrophic factor input undergo apoptosis. Conversely, overactivation of glutamate receptors located in postsynaptic dendrites can trigger neuronal apoptosis (66-68, 79). Accordingly, glutamate and neurotrophic factor signaling pathways have been

shown to interact such that neurotrophic factors can protect neurons against glutamate-induced cell death (14, 68). These kinds of data suggest that cell survival is controlled by a balance between opposing trophic and degenerative signaling pathways (74).

Neuronal death that occurs in various age- and injury-related disorders appears to involve many of the same signaling mechanisms involved in natural cell death during development of the nervous system. This concept of a development — adult plasticity — cell death signaling continuum (66) has gained support from studies of experimental models that suggest important roles for neurotrophic factors and neurotransmitters (particularly glutamate) in neurodegenerative disorders (48, 70, 77). Recent studies have begun to elucidate the specific molecular and biochemical mechanisms that mediate synaptic degeneration and neuronal death in various neurodegenerative conditions. In this article I describe some of these new findings, with a focus on "apoptotic" and "anti-apoptotic" signaling pathways and their roles in modulating synaptic plasticity and cell death. The data suggest that neuronal plasticity and survival are regulated by the interactions of "apoptotic" and "anti-apoptotic" signaling pathways activated in synaptic terminals. In this view, neurodegenerative disorders result from imbalances in such normally adaptive regulatory mechanisms (Figure 1).

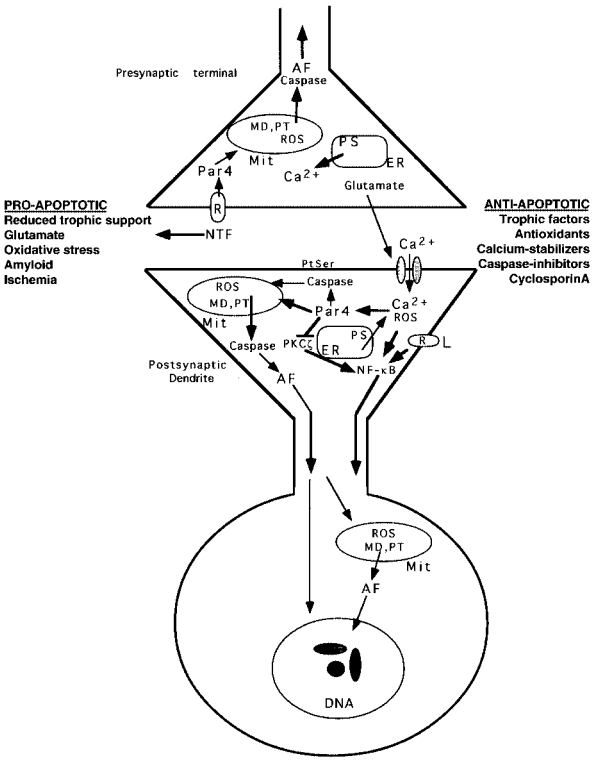

Figure 1. Apoptotic and anti-apoptotic mechanisms operative in synaptic terminals that may play roles in regulating synaptic plasticity, remodeling and cell survival. See text for discussion. Modified from reference 79.

"Synaptic apoptosis"

Recent studies of cultured neurons, synaptosomes and postmortem brain tissue have shown that essentially all of the "cell death machinery" (9) can be activated locally in synaptic terminals and dendrites. For example, exposure of synaptosomes (a preparation consisting of pinched off pre- and post-synaptic terminals) to insults such as staurosporine and amyloid β-peptide (which induce apoptosis in intact neurons) can activate classic apoptotic events including increased Par-4 (prostate apoptosis response-4) production, caspase activation, loss of plasma membrane phospholipid asymmetry, mitochondrial membrane depolarization, and release of factors capable of inducing nuclear DNA condensation and fragmentation (20, 79, 80). Such apoptotic cascades can cause several different changes in synaptic terminals. For example, Par-4 induction and caspase activation promote mitochondrial dysfunction, further caspase activation, and release of factors into the cytosol that are capable of inducing DNA condensation and fragmentation (20, 79). Studies of cultured hippocampal neurons (79) and adult rats exposed the seizure-inducing excitotoxin kainate, have shown that activation of synaptic glutamate receptors can induce apoptotic cascades that are initially localized to the postsynaptic dendritic compartments (20, 79).

Apoptotic cascades in synaptic terminals appear to play important roles in several different neurodegenerative disorders. I use Alzheimer's disease as one example. Synapse loss is a prominent feature of Alzheimer's disease that is tightly correlated with learning and memory deficits. Accumulations of neurotoxic forms of amyloid β-peptide (a 40-42 amio acid fragment of the amyloid precursor protein) are believed to promote neurodegenerative cascades at the level of the synapse by a mechanism involving membrane lipid peroxidation and impairment of ion-motive ATPases, and glucose and glutamate transporters (46, 57-59, 76). Immunostaining of brain sections from Alzheimer's patients with antibodies that selectively recognize the activated form of caspase-3 reveals a pattern of caspase activation consistent with early activation in synaptic terminals and neurites (12). Moreover, Par-4 levels are increased in degenerating neurons in hippocampus of Alzheimer's

patients (35). Exposure of synaptosomes and cultured hippocampal neurons to amyloid β-peptide results in rapid increases in levels of Par-4, caspase activation and mitochondrial dysfunction in synaptic terminals and dendrites (80). Additional studies have shown that cultured cells and transgenic mice expressing mutations in presenilin-1, that cause an early-onset inherited form of Alzheimer's disease, exhibit enhanced activation of caspases and mitochondrial dysfunction following exposure to amyloid β-peptide and other insults relevant to the pathogenesis of Alzheimer's disease (35, 39; and see below)

Although it has been proposed that neuronal apoptosis requires expression of "killer" genes (42), transcriptional-dependence of the cell death process has not been demonstrated in most cases. Most of the evidence that apoptosis is an "active" process comes from studies showing that inhibitors of protein synthesis, such as cycloheximide, can prevent neuronal death (60). However, more recent studies have clearly shown that gene transcription is not required for cell death in many paradigms of neuronal apoptosis, and that protein synthesis is only required in some cases (75). Indeed, depending upon the concentration employed, cycloheximide may prevent neuronal apoptosis either by preventing translation of existing mRNAs that encode "killer" proteins (20) or by inducing the expression of anti-apoptotic proteins such as Bcl-2 (27). Moreover, apoptosis of cultured hippocampal neurons induced by glutamate, iron and glucose deprivation is not prevented by actinomycin D or high concentrations of cycloheximide (27; and unpublished data). Convincing evidence against a major role for transcriptional processes in neuronal apoptosis comes from studies showing that apoptotic biochemical cascades can be induced in synaptosomes, a preparation lacking DNA (20, 79).

Oxyradicals and calcium: major triggers of synaptic apoptosis. Oxidative stress and perturbed neuronal calcium homeostasis are implicated in neuronal degeneration that occurs in many different settings. For example, sustained elevations of intracellular calcium levels and increased oxyradical production occur in neurons in cell culture and in vivo models of excitotoxic and ischemic brain injury (16, 64, 73). Calcium and free radicals are central to the cell death process because agents that stabilize calcium homeostasis and antioxidants can prevent neuronal death in many experimental models. Studies of postmortem brain tissue from patients with neurodegenerative disorders have provided evidence for increased oxidative stress and intracellular calcium levels in degenerating neurons. For example, in Alzheimer's disease activation of calcium-dependent proteases (86) and membrane lipid peroxidation (53) are increased in vulnerable neuronal populations. Studies of cell culture and animal models of Alzheimer's diaease support roles for oxidative stress and dysregulation of calcium homeostasis in the neurodegenerative process (31, 57, 69, 70). Developmental neuronal apoptosis may also involve increased oxidative stress (13) and perturbed calcium homeostasis (68). Indeed, two major mechanisms whereby neurotrophic factors prevent neuronal apoptosis are by stabilizing calcium homeostasis (14, 15, 68, 71) and suppressing oxyradical production (37, 73, 78, 84).

Alterations in several prominent signaling pathways results in disruption of neuronal calcium homeostasis and increased oxyradical production. The excitatory neurotransmitter glutamate, which activates receptors linked to calcium influx, plays a prominent role in many cases. Overactivation of glutamate receptors, particularly under conditions of impaired energy availability and oxidative stress, can result in neuronal calcium overload and either apoptosis or necrosis (3). Activation of glutamate receptors may contribute to neuronal apoptosis during development of the nervous system (68) and in disorders ranging from stroke (51, 55), to Alzheimer's disease (69, 70), to Huntington's disease (6) to ALS (90). The discovery that neurotrophic factors (e.g., bFGF, NGF, BDNF and IGFs) can protect neurons against excitotoxic and oxidative insults (see ref. 75, 77 for review) led to the elucidation of the underlying mechanisms. It was found that neurotrophic factors can modulate the expression of several different calcium-regulating proteins (e.g., calcium-binding proteins and glutamate receptor subunits; 15, 71) and antioxidant enzymes (73, 78). The latter findings suggest that disengagement of neurotrophic factor signaling pathways may promote neuronal death by impairing the ability of the neurons to regulate calcium homeostasis and free radical metabolism. Another emerging signaling pathway that regulates neuronal survival involes membrane proteins called integrins that mediate cell-substrate and cell-cell interactions. It has been known for some time that disengagement of integrin interactions with extracellular matrix and cell adhesion molecules can trigger apoptosis in non-neuronal cells. When integrins are engaged (i.e, when cells are bound to a substrate or other cells) a signaling pathway involving an enzyme called focal adhesion kinase is activated, and downstream survival-promoting pathways are functional. Thus, the integrin signaling pathway is very similar to that of neu-

rotrophic factors and apoptosis results when levels of activation of this survival-promoting pathway are insufficient (30).

How do calcium and oxyradicals induce neuronal apoptosis? Calcium activates proteases including caspases and calpains, which then cleave various substrates resulting in cell degeneration (11). Importantly, calcium and oxyradicals interact in a cross-amplifying manner such that increased oxidative stress disrupts calcium homeostasis, and increased intracellular calcium levels induce oxyradical production (57, 58, 73). Calcium and oxyradicals adversely affect mitochondria resulting in mitochondrial membrane depolarization and generation of apoptotic factors (47, 49). Events leading to such mitochondrial alterations may include translocation of Bax (32) and Par-4 (S. L. Chan and M. P. Mattson, unpublished data) to the mitochondrial membrane.

Par-4: an emerging pivotal player in synaptic apoptosis. Prostate apoptosis response-4 (Par-4) is a 38 kDa protein that was identified because its expression increased rapidly in prostate tumor cells underoging apoptosis following exposure to calcium ionophores and chemotherapeutic agents; the induction of Par-4 was shown to play a key role in the death of those tumor cells (91). We found that Par-4 protein levels are rapidly increased in PC12 cells and primary hippocampal neurons in response to various apoptotic insults including trophic factor withdrawal and exposure to amyloid β-peptide (35). Suppression of Par-4 production using antisense technology prevents neuronal apoptosis (35), demonstrating a requirement for Par-4 in the cell death process. Time course analyses revealed that Par-4 production occurs prior to mitochondrial dysfunction and caspase activation (35). Par-4 appears to play a necessary role in mitochondrial dysfunction and caspase activation in these paradigms of neuronal apoptosis because suppression of Par-4 production or function stabilizes mitochondrial function, inhibits caspase activation and prevents apoptosis (13, 35). Oxidative stress and calcium influx are potent triggers for Par-4 expression and, accordingly, antioxidants and manipulations that reduce levels of intracellular calcium suppress Par-4 expression in neurons exposed to several different apoptotic insults including trophic factor withdrawal (13, 20, 21, 35).

The amino acid sequence of Par-4 reveals both a leucine zipper domain, and a partially overlapping death domain, in the C-terminal region of the protein (91). The leucine zipper domain of Par-4 is required for its pro-apotpotic action because overexpression of Par-4 lacking the leucine zipper domain does not promote apoptosis,

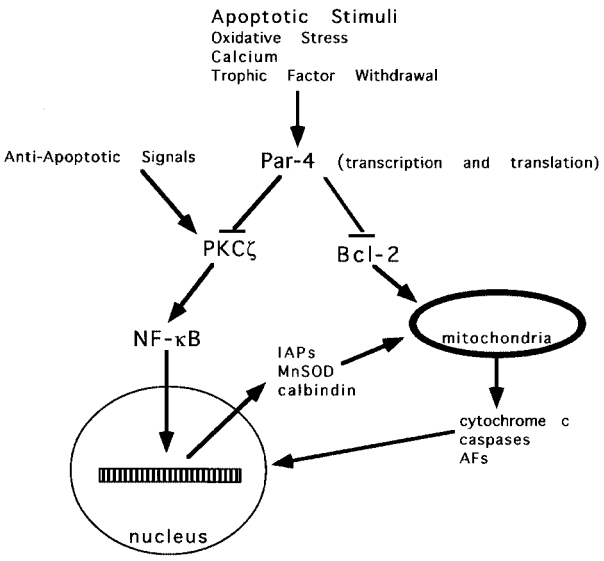

Figure 2. Possible mechanisms whereby Par-4 promotes neuronal apoptosis. See text from description.

and overexpression of the leucine zipper domain alone acts in a dominant-negative manner to prevent apoptosis (35). The requirement of the leucine zipper domain strongly suggests that an interaction of Par-4 with another protein is required for its cell death function. Several proteins have been identified that can interact with Par-4 and may mediate apoptosis. Initial studies of tumor cells identified protein kinase Cζ (18) as a protein that interacts with Par-4. Par-4 interacts with the regulatory domain of PKCζ, and this interaction inhibits the kinase activity. A role for the latter interaction in promoting apoptosis is suggested by data showing that activation of PKCζ can prevent apoptosis (Berra). Consistent with a role for PKCζ in the pro-apoptotic action of Par-4 in neurons, we have found that Par-4 interacts with PKCζ in embryonic hippocampal neurons (Figure 2). The interaction of Par-4 with PKCζ may result in suppression of activation of the anti-apoptotic transcription factor NF-κB (4, 84). Thus, we found that induction of NF-κB activity in PC12 cells exposed to apoptotic stimuli is markedly suppressed in cells overexpressing full-length Par-4, while NF-κB activity is enhanced in cells overexpressing the dominant negative Par-4 leucine zipper (10). NF-κB is present in the cytoplasm of neurons in an inactive form consisting of three subunits, the transcription factor dimer (the prototypical dimer consists of p50 and p65) and an inhibitory subunit called I-κBα. When NF-κB is activated by exposure to cytokines or oxidative stress, for example, I-κB dissociates from the p50/p65 dimer and the dimer translocates to the nucleus and binds

to specific sequences in the enhancer region of target genes. The gene targets of NF-κB that mediate its anti-apoptotic actions include the antioxidant enzyme Mn-SOD, the inhibitor of apoptosis proteins (IAPs), and the calcium-binding protein calbindin D28k.

An additional protein that interacts with Par-4 is Bcl-2 (Figure 2). Bcl-2 is an anti-apoptotic protein that is a member of a protein family that includes both anti- and pro-apoptotic members. Co-immunoprecipitation studies have shown that Par-4 interacts with Bcl-2, but not with Bax (a pro-apoptotic family member), in PC12 cells (S. L. Chan and M. P. Mattson, manuscript in preparation). The interaction of Par-4 with Bcl-2 has interesting implications for positive and negative modulation of the mitochondrial alterations involved in apoptosis. Many studies have shown that Bcl-2 can prevent neuronal apoptosis by a mechanism involving suppression of oxidative stress and stabilization of mitochondrial function. Bcl-2 associates with mitochondrial membranes, and this interaction may be induced by apoptotic stimuli. We have found that Par-4 also associates with mitochondria in neurons following exposure to apoptotic stimuli such as trophic factor withdrawal or exposure to staurosporine (S. L. Chan and M. P. Mattson, unpublished data). The association of Par-4 with mitochondria is greatly decreased in PC12 cells overexpressing Bcl-2. Interestingly, levels of Bcl-2 expression are greatly reduced in PC12 cells overexpressing Par-4, which may be the result of decreased NF-κB activation since the gene encoding Bcl-2 is induced by NF-κB [83]. Consistent with a role for Par-4 in downregulating Bcl-2 expression are data indicating mutually exclusive patterns of cellular expression of Par-4 and Bcl-2 in prostate tumors (8) and neurons in the brains of patients with Alzheimer's disease (35).

Recent findings suggest a particularly important role for Par-4 at the level of the synapse. Exposure of synaptosomes to apoptotic insults resulted in relatively rapid increases (1-2 hours) in Par-4 protein levels (20). Such local increases in Par-4 leves in synaptic terminals were blocked by cycloheximide indicating that protein synthesis was required for Par-4 induction. The latter findings provide direct evidence that expression of a death-related protein can be induced locally in synaptic terminals. Further studies showed that Par-4 induction by apoptotic insults in synaptic terminals can be suppressed using antisense technology, and that such suppression of Par-4 expression results in a marked attenuation of mitochondrial dysfunction and caspase activation (20). Thus, Par-4 appears to play a central role in synaptic apoptotic cascades.

Anti-apoptotic signaling at the synapse

Since the pioneering studies that elucidated roles for nerve growth factor (NGF) in preventing death of peripheral nerves during development of the nervous system, a rapidly growing number of neurotrophic factors have been identified and characterized, and their roles in developmental and pathological neuronal death are being elucidated. One valuable view of neuronal apoptosis is that it occurs only when anti-apoptotic signaling pathways are insufficiently activated or overwhelmed by adverse environmental conditions (75). Recent advances in our understanding of the specific mechanisms whereby anti-apoptotic signals act are enhancing our understanding of both apoptosis and synaptic plasticity. Among anti-apoptotic signaling pathways those activated by neurotrophic factors and cytokines have been the most heavilty studied. Many different neurotrophic factors including NGF, basic FGF, BDNF, NT-3, IGF-1, TGFβ and GDNF can protect cultured neurons against one or more apoptotic insults including exposure to oxidative, metabolic and excitotoxic insults (2, 75, 77). Another class of neuroprotective factors is cytokines including TNF and secreted forms of amyloid precursor protein (15, 36, 72, 84). In general, neurotrophic factors and cytokines exert their beneficial effects by activating transcription-dependent and/or transcription-independent signaling pathways that stabilize cellular calcium homeostasis and suppress oxyradical production). By preventing the calcium overload and oxyradical production the neurotrophic factors and cytokines suppress the apoptotic process at an early step, prior to mitochondrial dysfunction and caspase activation.

In addition to modulating the expression of genes that encode proteins that regulate calcium homeostasis (e.g., calcium-binding proteins and glutamate receptor subunits) and free radical metabolism, neurotrophic factors can exert direct effects in synaptic terminals and neurites. Evidence supporting direct protective effects of trophic factors on synapses comes from studies showing that treatment of synaptosomes with basic FGF, ADNF (activity-dependent neurotrophic factor) and the secreted form of amyloid precursor protein (sAPPα) can protect the synaptosomes against damage caused by exposure to oxidative insults (37, 84). Specifically, the neurotrophic factors can prevent impairment of glucose and glutamate transport induced by exposure to Fe^{2+} and amyloid β-peptide. The signal transduction mechanism of bFGF involves its high-affinity receptor tyrosine kinase, while that of sAPPα involves the second messenger cyclic GMP. The specific mechanism whereby

activation of bFGF receptors protects synaptic terminals is unclear. In the case of sAPPα, it appears that putative sAPPα receptors located in postsynaptic regions of dendrites are linked to cGMP production and activation of cGMP-dependent protein kinase (5, 25, 26, 72). Cyclic GMP-dependent kinase may then phosphorylate a protein phosphatase which, in turn, dephosphorylates and thereby activates a certain type (high conductance and charybdotoxin-sensitive) of potassium channel (25). Phosphorylation of the potassium channels results in membrane hyperpolarization and may thereby reduce neuronal vulnerability to excitotoxic and oxidative insults. The extent to which such local actions of trophic factors in synapses and neurites mediates the well-documented effects of the neurotrophic factors on neurite outgrowth (68) and synaptic plasticity (52) remains to be determined.

Some transcription factors can be activated locally in synaptic terminals and neurites. Exemplary of such transcription factors is NF-κB. NF-κB is present in synaptic terminals wherein it can be activated in response to excitatory neurotransmitter receptor stimulation (43). In a series of studies performed during the past 7 years we have shown that activation of NF-κB in hippocampal neurons protects them against apoptosis induced by various stimuli including oxidative, metabolic and excitotoxic insults (see 83 for review). Studies of the role of NF-κB in neuronal survival began with the observation that TNF can protect cultured hippocampal neurons against cell death induced by glucose deprivation and exposure to glutamate (15). We subsequently showed that TNF activates NF-κB in the neurons and that blockade of NF-κB activation abolishes the neuroprotective effect of TNF (4, 78). Several gene targets of TNF that may contribute to its anti-apoptotic actions have been identified and include Mn-SOD (78), the calcium-binding protein calbindin (15), glutamate receptor (NMDA and AMPA) subunits (29), and inhibitor of apoptosis proteins (17).

In addition to neurotrophic factor signaling, signaling by cell adhesion-related pathways may play important roles in modulating neuronal cell death. Sites of cell adhesion contain high concentrations of membrane receptor and adhesion proteins, prominent among which are integrins. We have found that integrin-mediated signaling can reduce neuronal vulnerability to excitotoixcity and apoptosis by a mechanism involving stabilization of calcium homeostasis (30). The latter studies showed that primary hippocmapal neurons cultured on a laminin substrate exhibit increased resistance to cell death induced by glutamate and oxidative stress, and that the protective effect was abolished by RGD peptide and antibodies against integrins.

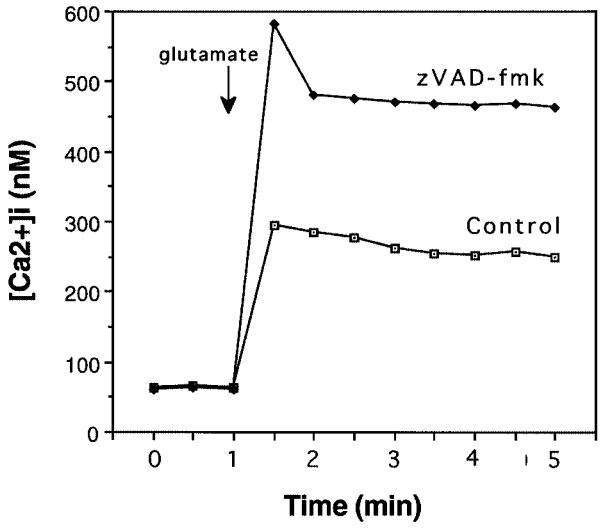

Figure 3. Evidence that caspases modify neuronal responses to glutamate. Intracellular free calcium levels were monitored (by imaging of the calcium indicator dye fura-2) prior to and following exposure to glutamate in hippocampal neurons in a control culture and a culture that had been pretreated with the caspase inhibitor zVAD-fmk prior to exposure to glutamate.

While cell surface receptor-mediated synaptic signaling has received the bulk of attention in the fields of neuronal plasticity and cell death, intriguing new signaling pathways are being identified that act independently of membrane receptors. For example, an anti-apoptotic signaling pathway activated by calcium influx was recently described (24, 28). Calcium activates a protein called gelsolin, which then cleaves actin filaments. Actin depolymerization, in turn, results in reduced calcium influx through NMDA receptors and voltage-dependent calcium channels (28). Apparently actin filaments interact with the channels, probably in an indirect manner involving yet-to-be-determined actin-binding proteins.

Roles for synaptic apoptotic and anti-apoptotic cascades in neuronal plasticity and neurodegenerative disorders

Calcium and reactive oxygen species play important roles in both synaptic plasticity and neuronal apoptosis. Postsynaptic influx of calcium, resulting from glutamate receptor activation, is required for induction of both long-term potentiation and long-term depression (92). Nitric oxide, an oxyradical, may play an important role in maintenance of long-term potentiation, apparently by acting as a retrograde signal that diffuses from postsy-

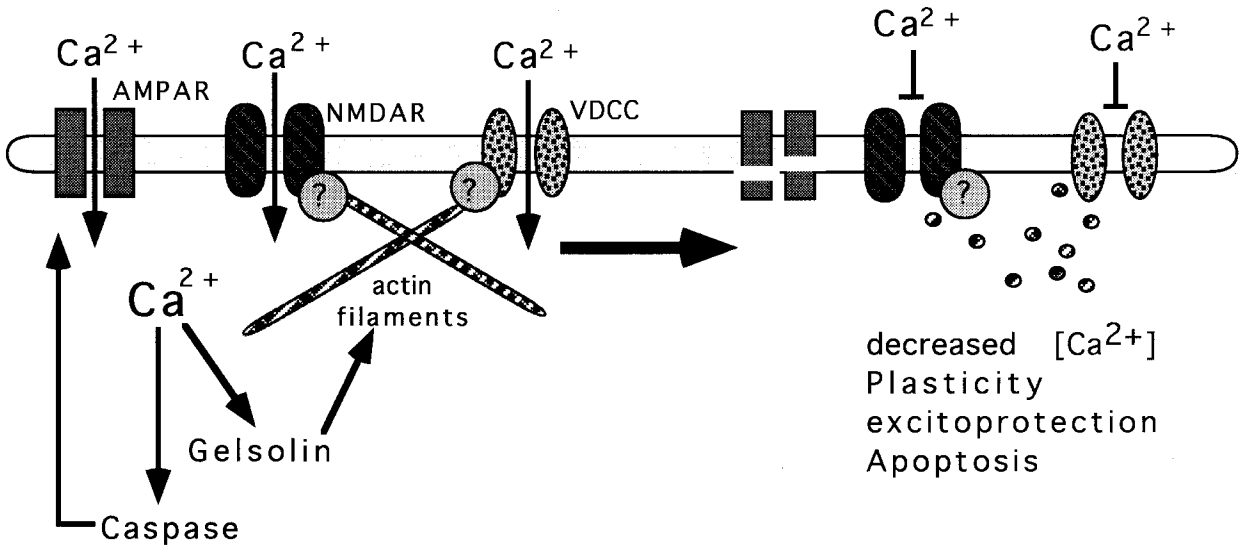

Figure 4. Proposed mechanisms for regulation of calcium influx by caspases and actin filaments. Calcium influx induces activation of caspases which, in turn, cleave AMPA receptor subunits resulting in reduced activation of the receptor channels. Calcium influx also activates the actin-severing protein gelsolin resulting in actin depolymerization. Actin depolymerization results in enhanced rundown of currents through NMDA receptors and voltage-dependent calcium channels. By influencing calcium influx channels caspases and actin filaments may play important roles in modulating synaptic plasticity and, in pathophysiological conditions, determining whether a neuron dies by apoptosis or necrosis.

naptic cells to presynaptic terminals (98). Both calcium and nitric oxide can also activate apoptotic cascades involving Par-4 induction, caspase activation and mitochondrial dysfunction (13, 47). These observations suggest a role for apoptotic signaling in synaptic plasticity. We have recently obtained data suggesting that caspases may play roles in synaptic plasticity (12). Two caspase substrates, actin and spectrin (45, 61, 94) may modulate synaptic plasticity (28, 95). Subunits of the AMPA type of glutamate receptor are proteolytically degraded in cultured hippocampal neurons following exposure to apoptotic insults such as trophic factor withdrawal, staurosporine and amyloid β-peptide (12). The caspase inhibitor zVAD-fmk prevents degradation of the AMPA receptor subunits indicating that caspases mediate the proteolysis of the receptor subunits. Calcium imaging studies showed that a functional consequence of caspase-mediated cleavage of AMPA receptor subunits is a decreased calcium response to glutamate (Figure 3). The data suggest that caspase-mediated cleavage of AMPA receptor subunits may prevent excitotoxic necrosis, and thereby steer the cells to apoptosis. It remains to be established whether caspase-mediated cleavage of AMPA receptor subnits occurs during, and/or is involved in, synaptic plasticity. However, caspases can be activated in response to physiological stimuli including membrane depolarization and glutamate receptor activation (7, 79). It is likely that future investigations will reveal important roles for caspases in regulating synaptic plasticity.

A mechanism whereby cytoskeletal changes associated with apoptosis may modulate synaptic plasticity was suggested by recent studies showing that changes in actin polymerization can affect NMDA-induced currents and voltage-dependent calcium currents, and calcium responses to glutamate, in cultured hippocampal neurons (24, 28). Specifically, actin depolymerization results in enhanced rundown of NMDA and calcium currents, whereas actin stabilization enhances currents. The calcium-activated actin-severing protein gelsolin appears to serve as an important transducer of elevations of intracellular calcium levels into changes in NMDA and calcium currents (28). As actin depolymerization is associated with membrane blebbing in cells undergoing apoptosis, it may be the case that actin depolymerization-mediated decreases in calcium influx may serve to drive neurons down an apoptotic cell death pathway (as opposed to a necrotic pathway) (Figure 4).

Emerging data suggest that endoplasmic reticulum (ER) plays important roles in both synaptic plasticity and apoptosis. Studies of the mechanisms underlying long-term depression of synaptic transmission at climbing fiber — Purkinje cell synpases revealed that calcium release from IP3-sensitive ER stores is necessary for

induction of LTD (23, 93). ER is present in dendritic spines, where it may modulate intracellular calcium levels locally. Studies of the pathogenic mechanism of mutations in presenilin-1 that cause early-onset inherited Alzheimer's disease have revealed a surprising role for altered ER calcium regulation in this disorder (see 81 for review). Expression of presenilin-1 mutations in cultured neural cells and knockin mice results in increased vulnerabiilty of the cells to apoptosis and excitotoxicity (34-36, 38, 39). Neurons expressing presenilin-1 mutations exhibit enhanced calcium release from ER stores following stimulation with muscarinic agonists, glutamate and caffeine (34, 35). The enhanced calcium release mediates the endangering action of the presenilin-1 mutations because treatment of cells with agents that suppress calcium release from ER (e.g., dantrolene and xestospongin) abolish the death-enhancing effect of the mutations (34, 35; and unpublished data). Recent studies of cortical synaptosomes from wild-type and presenilin-1 mutant transgenic mice indicate that aberrant ER calcium homeostasis, and its degenerative consequences, can occur locally in synaptic terminals from mutant mice (7; Figure 5). Enhanced calcium release from ER may also account for the reported enhancement of LTP in hippocampal slices from transgenic mice expressing a presenilin-1 mutation (88).

Degeneration of synapses likely precedes neuronal cell death in many different neurodegenerative disorders including Alzheimer's disease, stroke, epileptic seizures, traumatic brain injury, Parkinson's disease and Huntington's disease. We have identified synaptic signaling mechanisms that either promote or prevent synaptic degeneration in experimental models of such neurodegenerative disorders. One example involves studies of the the cell biology of the β-amyloid precursor protein (APP) and the involvement of aberrant APP metabolism in the pathogenesis of Alzheimer's disease (see 76 for review). APP is an integral membrane protein with one membrane-spanning domain; amyloid β-peptide is a 40-42 amino acid peptide within APP that is partially embedded in the membrane at the cell surface. APP is axonally transported, and localizes to presynaptic terminals wherein it can be proteolytically processed in at least two different ways. One processing mechanism involves activity-dependent cleavage of APP in the middle of the amyloid β-peptide sequence resulting in release of a large extracellular portion of APP called sAPPα into the synaptic cleft. A second pathway involves cleavage of APP at the N-terminus of the amyloid β-peptide sequence, which leaves a C-terminal

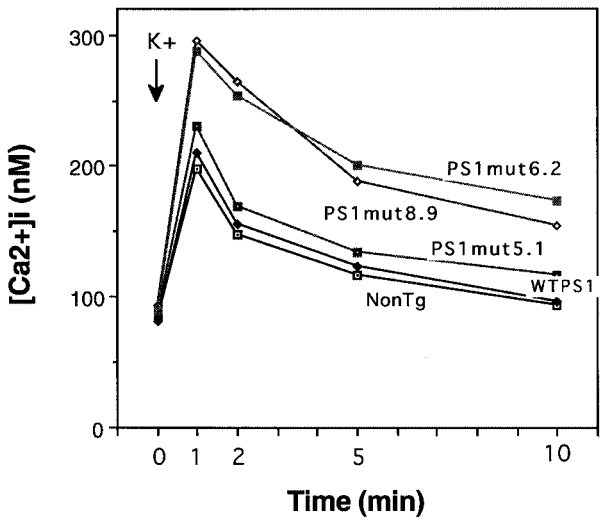

Figure 5. Synaptic calcium homeostasis is altered in transgenic mice expressing presenilin-1 mutations. Intracellular calcium levels were measured in cortical synaptosomes from the indicated lines of mice prior to exposure to (time 0) and at the indicated time points following exposure to 50 mM KCl (K^+). PS1Mut6.2 and PS1Mut8.9, transgenic mice overexpressing mutant presenilin-1 at a high level; PS1Mut5.1, transgenic mice overexpressing mutant presenilin-1 at a low level; WTPS1, transgenic mice overexpressing wild-type presenilin-1 at a high level; NonTg, nontransgenic mice. Modified from reference 7.

fragment containing intact amyloid β-peptide which is then endocytosed and further processed such that intact amyloid β-peptide is produced. In Alzheimer's disease APP processing is altered such that levels of amyloid β-peptide are increased and levels of sAPPα are decreased.

Amyloid β-peptide can directly impair synaptic function and induce degeneration of synapses. Exposure of cortical synaptosomes to amyloid β-peptide results in membrane lipid peroxidation and impairment of membrane ion-motive ATPases, and glucose and glutamate transporters (46, 57). Amyloid β-peptide also induces Par-4 production, caspase activation and mitochondrial dysfunction in synaptosomes and in dendrites of cultured hippocampal neurons (20, 80). These findings suggest that synaptic degenerative changes are likely to be early and pivotal events in the neurodegenerative process in AD. Other neurodegenerative disorders may involve a similar degenerative cascade initiated by disorder-specific factors other than amyloid β-peptide.

sAPPα plays an important role in regulating developmental and synaptic plasticity. sAPPα is released from hippocampal slices in response to electrical stimulation (85). Exposure of hippocampal slices to recombinant sAPPα results in a shift in the frequency-depend-

ence for induction of long-term depression and an increase in the amplitude of LTP (40). Whole-cell perforated patch clamp analyses in cultured primary hippocampal neurons have shown that sAPPα activates high-conductance potassium channels resulting in membrane hyperpolarization (25, 26). Activation of potassium channels may mediate rapid local protective effects of sAPPα in synaptic terminals (25, 84). sAPPα promotes neurite outgrowth and cell survival in cultured embryonic hippocampal neurons, and can protect neurons against excitotoxicity and apoptosis by a signaling mechanism involving cyclic GMP production. The sAPPα signaling pathway also activates NF-κB which may induce production of neuroprotective proteins (5). We recently found that sAPPα can enhance glucose and glutamate transport in synaptosomes (84). Pretreatment of synaptosomes with sAPPα increased their resistance to amyloid β-peptide and Fe^{2+}-induced impairment of glucose and glutamate transport. Cyclic GMP mediates sAPPα-induced enhancement of glucose and glutamate transport. These findings demonstrate that a trophic factor released in an activity-dependent manner can act locally on synaptic terminals to enhance the function of two membrane transporters that play important roles in protecting neurons against excitotoxicity and apoptosis. We have found that at least two other neurotrophic factors, ADNF (activity-dependent neurotrophic factor) and bFGF (basic fibroblast growth factor) can also activate signaling pathways within synaptic terminals that directly protect those compartments (37).

An animal model of PD involves administration of the toxin MPTP which induces mitochondrial oxidative stress in synaptic terminals of dopaminergic neurons in the substantia nigra resulting in their degeneration (41). We found that there is a rapid increase in levels of Par-4 in substantia nigra dopaminergic neurons and in their axon terminals in the striatum following MPTP administration to monkeys and mice (21). The increase in Par-4 levels precedes loss of tyrosine hydroxylase immunoreactivity and cell death. Moreover, treatment of cultured human dopaminergic cells with the complex I inhibitor rotenone and iron (two insults relevant to the pathogenesis of PD) induces Par-4 production. Dopaminergic cells pretreated with Par-4 antisense DNA were resistant to cell death induced by rotenone and iron, indicating a key role for Par-4 in the cell death process.

Well-known stress-responsive proteins such as HSP-70 and GRP-78 are present in synaptic terminals wherein they may act locally to protect synapses against various insults. Cell culture studies have provided direct evidence that increased levels of HSP-70 and GRP-78 can protect neurons against excitotoxic, apoptotic and oxidative insults (54, 96). Administration of 2-deoxyglucose (2DG, a non-metabolizable analog of glucose that induces a mild metabolic stress) to rats and mice in vivo increases levels of HSP-70 and GRP-78 in hippocampus, cortex and striatum (19, 97). We have recently found that levels of HSP-70 and GRP-78 are also increased in synaptosomes prepared from cortex of 2DG-treated rats compared to control rats (Z. Guo and M. P. Mattson, unpublished data). Synaptosomes from the 2DG-treated rats exhibited increased resistance to impairment of membrane transporter function and mitochondrial dysfunction following exposure to oxidative insults.

Another example of the mounting evidence supporting a primary role for aberrant synaptic signaling in neurodegenerative disorders comes from studies of the pathogenic action of mutations in the presenilin-1 gene which cause early-onset autosomal dominant AD. Presenilin-1 mutations increase neuronal vulnerability to apoptosis (33, 34, 39) and excitotoxicity (38). When mutant presenilin-1 is expressed in cultured PC12 cells or primary hippocampal neurons, endoplasmic reticulum calcium homeostasis is pertubed such that more calcium is released when the cells are stimulated with muscarinic cholinergic agonists or glutamate (33, 34, 38). The endangering actions of presenilin-1 mutations result from perturbed calcium regulation in that agents that block calcium release from ER or buffer cytoplasmic calcium counteract the cell death-promoting action of the presenilin-1 mutations (34). Synaptic calcium handling is perturbed in neurons from presenilin-1 mutant transgenic mice in a manner that promotes mitochondrial dysfunction (7). These findings suggest that a primary consequence of presenilin-1 mutations is to disrupt synaptic calcium homeostasis.

Future Directions

The findings described above suggest that apoptotic cascades function in a continuum in which low levels of activation play roles in adaptive responses to physiological activity and subtoxic levels of stress, whereas higher levels of activation mediate synaptic degeneration and cell death. Much further work will be required to better understand the molecular and cellular mechanisms that regulate pro- and anti-apoptotic responses in synapses. Examples of specific areas for further studies include: (1) identification of synaptic protein substrates for caspases, calpains and other proteases, and determination of how cleavage of the substrates modulates

synaptic plasticity and degeneration. (2) elucidation of the mechanisms that regulate protein synthesis locally in synaptic and dendritic terminals. Intuition, and the recent findings showing that Par-4 can be induced at the translational level in synaptic terminals (20) suggests that this is a very important control point for regulation of synaptic homeostasis. (3) defining the roles of the endoplasmic reticulum and mitochondria in synaptic function and neuronal degeneration. The critical roles of these organelles in regulating calcium homeostasis, stress responses and energy metabolism make it imperitive that we understand their involvement in synaptic physiology and pathology. (4) dissecting pathways of communication from the synapse to the nucleus and back. Synaptic signaling clearly has profound effects on gene expression, and the specific molecular cascades subserving this important aspect of synaptic function merits intense investigation. (5) elucidating mechanisms whereby oxyradicals are generated within, and removed from, synaptic terminals. Given the metabolic and signaling demands of synapses, a better understanding of local oxyradical metabolism is needed.

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