

Neural Injury, Repair, and Adaptation in the GI Tract

I. New insights into neuronal injury: a cautionary tale*

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Hall, Karen E., and John W. Wiley. Neural Injury, Repair, and Adaptation in the GI Tract. I. New insights into neuronal injury: a cautionary tale. *Am. J. Physiol.* 274 (*Gastrointest. Liver Physiol.* 37): G978–G983, 1998.—Understanding of the pathophysiology of neuronal injury has advanced remarkably in the last decade. This largely reflects the burgeoning application of molecular techniques to neuronal cell biology. Although there is certainly no consensus hypothesis that explains all aspects of neuronal injury, a number of interesting observations have been published. In this brief review, we examine mechanisms that appear to contribute to the pathophysiology of neuronal injury, including altered Ca^{2+} signaling, activation of the protease cascades coupled to apoptosis, and mitochondrial deenergization associated with release of cytochrome *c*, production of free radicals, and oxidative injury. Finally, evidence for neuroprotective mechanisms that may ameliorate cell injury and/or death are reviewed. Little information has been published regarding the mechanisms that mediate injury in the enteric nervous system, necessitating a focus on models outside the gastrointestinal (GI) tract, which may provide insights into enteric nervous system injury.

calcium signaling; apoptosis; oxidative injury; free radicals; ceramide; nuclear factor- $\kappa\beta$; nerve growth factor

THE PATHOPHYSIOLOGY and treatment of neurodegeneration have become a major focus of research in the past decade. This has been driven in part by the substantial health care burden attributed to chronic neurodegenerative diseases, such as dementia and chronic neuromuscular disorders. Dysfunctional neuromuscular coordination in the gastrointestinal (GI) tract can occur with aging and systemic diseases such as diabetes mellitus. Little is known of the mechanisms responsible for neurodegeneration in the digestive tract. Much of the existing data have been generated using neuronal cell lines or primary neuronal preparations from outside the digestive tract. It is likely that similar mechanisms contribute to the pathophysiology of neuronal injury in the digestive system. In this review, we focus on those mechanisms that contribute to neuronal injury that we believe may have relevance to the GI tract.

ROLE OF ALTERED Ca^{2+} SIGNALING IN NEURAL INJURY

The Ca^{2+} hypothesis of cellular injury consists of several interrelated postulates (13). First, it proposes that cellular mechanisms that regulate the homeostasis of cytosolic free Ca^{2+} [Ca^{2+}]_i, the so-called Ca^{2+} “set-point,” play a critical role in a variety of neurodegenerative processes, and that altered [Ca^{2+}]_i might account for a number of the changes in neural function. Second, it postulates that the plasticity of neuroarchitecture is regulated by functional equilibrium between molecular mechanisms promoting growth and regeneration and processes that control regression and degeneration. Third, it proposes a systematic interaction between the magnitude of the change in cytosolic Ca^{2+} and the duration of the deregulation in Ca^{2+} homeostasis. Therefore, a small change in [Ca^{2+}]_i that is sustained over a long period can result in cellular damage similar to that produced by a large change in [Ca^{2+}]_i sustained over a short period. Fourth, it suggests that altered regulation of [Ca^{2+}]_i homeostasis may be a part of the final common pathway for the cellular changes leading to cell dysfunction and death. This hypothesis accounts for several alternative mechanisms through which the regulation of [Ca^{2+}]_i can be disrupted. These include changes in ion channel function or formation of new channels; changes in membrane structure that alter the function of transmembrane proteins; alterations in the behavior of Ca^{2+} binding proteins, extrusion pumps, and buffers; and altered Ca^{2+} sequestration. Fifth, it proposes that cell injury may not be due to a single event or insult but may be brought about by a series of different antecedent events occurring, in combination or in sequence, over a long period. Most of the events triggered by a rise in [Ca^{2+}]_i reflect the action of Ca^{2+} on enzymes, notably lipases, protein kinases (or phosphatases), proteases, and endonucleases.

In neurons, [Ca^{2+}]_i is regulated by Ca^{2+} influx through voltage-activated Ca^{2+} channels in the plasma membrane. Stimulation of receptors by excitatory amino acids, such as glutamate, has been correlated with Ca^{2+} influx and subsequent neural damage in various models of injury (20). However, a modest elevation in [Ca^{2+}]_i may be protective.

Neuronal degeneration within the central nervous system (CNS) appears to play a role in the development of Alzheimer's disease. Postmortem studies support

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activation of programmed cell death (apoptosis) in the brains of patients with Alzheimer's disease. Increased hippocampal Ca^{2+} influx via voltage-sensitive Ca^{2+} channels or by novel cation-selective channels composed of β -amyloid protein has been proposed to contribute to neuronal damage in Alzheimer's disease (16).

CONTRIBUTION OF AUTOIMMUNE IMMUNOGLOBULINS TO ALTERED NEURONAL Ca^{2+} SIGNALING

Serum factor(s) may play a role in the pathophysiology of elevated Ca^{2+} influx. Serum from type 1 diabetic patients increases Ca^{2+} influx and markers of neuronal injury in several cultured cell systems, including non-neuronal (9) and neuroblastoma cell lines (21). The serum factor (or factors) has yet to be fully characterized; however, immunoglobulins of either the IgM or IgG classes have been implicated. Several autoantigens have been proposed as possible sites for targeting of autoimmune immunoglobulins, including phospholipids, glutamic acid decarboxylase, and cell adhesion molecules (CAMs) of the immunoglobulin superfamily. CAMs (including neuronal CAM and myelin-associated glycoprotein) have immunoglobulin folds in their extracellular domains and play a role in the myelination of axons.

Autoantibodies directed against several voltage-activated Ca^{2+} channel subtypes have been described in amyotrophic lateral sclerosis (ALS) that were associated with decreased Ca^{2+} entry (23). Passive transfer of immunoglobulin-containing fractions into mice produced changes at the neuromuscular junction that were similar to changes observed in patients with ALS. ALS immunoglobulins induce Ca^{2+} -dependent apoptosis in a differentiated motoneuron hybrid cell line via a mechanism that involves oxidative injury and Ca^{2+} -dependent apoptosis (5). The relationship of decreased Ca^{2+} influx at the neuromuscular junction and promotion of Ca^{2+} -dependent apoptosis in the cell body requires further study but may involve mobilization of intracellular stores of Ca^{2+} .

Somatic muscle weakness and autonomic symptoms characterize the Lambert-Eaton myasthenic syndrome (LEMS). The somatic muscle weakness results from autoantibody (IgG class)-mediated downregulation of voltage-activated Ca^{2+} channels at motor nerve terminals and a consequent reduction in acetylcholine release. The basis for the autonomic symptoms is unknown. Using specific Ca^{2+} channel antagonists, Waterman et al. (27) demonstrated the presence of IgG autoimmune immunoglobulin directed against several different Ca^{2+} channel subtypes in LEMS serum. Ca^{2+} influx and subsequent neurotransmitter release were decreased in bladder postganglionic parasympathetic neurons and postganglionic sympathetic neurons in the vas deferens of mice injected with IgG from LEMS patients.

The marked improvement in neuropathic syndromes after intravenous immunoglobulin or plasmapheresis supports the participation of an autoimmune mechanism in the pathogenesis of some neuropathies, includ-

ing diabetic neuropathy (14). Therefore evidence exists supporting both increased and decreased autoantibody-mediated Ca^{2+} entry in neurons. The membrane epitopes involved in the binding of the autoantibodies and the linkage to increased Ca^{2+} influx have not been delineated. In contrast, the reduction in Ca^{2+} influx in LEMS and ALS appears to involve autoantibodies directed against presynaptic Ca^{2+} channels.

ROLE OF MITOCHONDRIAL DYSFUNCTION IN NEURAL INJURY

There have been significant advances recently in our understanding of the contribution of mitochondria to basic cellular function such as energy supply, Ca^{2+} homeostasis, and, more recently, programmed cell death. Neuronal death evoked by excitatory neurotransmitters such as glutamate has been associated with mitochondrial dysfunction. However, it is not clear whether this is a primary or a secondary event. Exposure to supraphysiological glutamate concentrations triggers both an early necrotic and a later apoptotic cell death pathway. Recent studies indicate that irreversible mitochondrial depolarization is associated with necrosis in cerebellar granule cells, whereas recovery of mitochondrial potential after transient glutamate-mediated depolarization is followed several hours later by apoptosis. Some studies suggest that partially energy-deficient neurons are less likely to undergo apoptosis, perhaps because initiation of apoptosis is energy dependent.

Alterations in mitochondrial membrane potential can be monitored using the voltage-sensitive dye JC-1. There is a close correlation between mitochondrial depolarization measured by this method and decreased ATP generation. In ischemic hippocampal neurons, elevation of $[\text{Ca}^{2+}]_i$ by glutamate-induced depolarization results in mitochondrial depolarization and structural damage to mitochondria (17). Mitochondria sequester large quantities of Ca^{2+} ; thus impaired mitochondrial function could elevate $[\text{Ca}^{2+}]_i$ by diffusion into the cytosol. This has been demonstrated to occur in mitochondrial encephalomyopathy, an inherited mutation resulting in neuronal injury (18). Decreased ATP generation due to mitochondrial dysfunction could also affect $[\text{Ca}^{2+}]_i$ indirectly by decreasing energy-dependent Ca^{2+} extrusion. Decreased Bcl-2 (B cell lymphoma-associated protein) concentrations in the mitochondrial membrane have been implicated in mitochondrial deenergization and subsequent neuronal damage.

ROLE OF OXIDANT PATHWAYS IN NEURAL INJURY

Oxidative stress resulting from increased formation and/or reduced scavenging of reactive oxygen species (ROS) can damage critical cell components, including intracellular lipids, proteins, and DNA (19). Mitochondrial dysfunction may play a pivotal role. Exposure to ROS such as superoxide or hydrogen peroxide (both of which can be produced in the mitochondria on depolarization) is associated with apoptosis and impaired

[Ca²⁺]_i regulation. Decreased mitochondrial Bcl-2 activity (whether secondary to decreased expression or impaired function) results in release of cytochrome *c* and subsequent activation of a variety of oxidative pathways. These include activation of the caspase (ICE/CED-3) family of proteases (24), which have been implicated in several models of apoptosis. Activation of caspases appears to be irreversibly associated with cell death.

Ischemic injury in diabetes mellitus is associated with oxidative stress (26). The mechanism(s) of excitotoxic/ischemic injury have been studied extensively in the CNS and appear to involve elevation of intracellular Ca²⁺ and generation of free radicals. Indexes of oxidative stress include an increase in neuronal lipid hydroperoxides, conjugated dienes, and lower levels of reduced glutathione. Histopathology reveals a vacuolar neuropathy involving the mitochondria. There appears to be a consistent theme that impaired mitochondrial function and generation of ROS play an important role in several models of neuronal injury. The pathways that link activation of extracellular proapoptotic ligands to mitochondrial dysfunction require clarification.

ROLE OF NITRIC OXIDE IN NEURAL INJURY

Nitric oxide (NO) has been implicated in initiation of apoptosis in a variety of neurodegenerative models. Evidence for both initiation of and protection against apoptosis has been reported. Increased endogenous NO production and exposure to peroxynitrite or NO donors result in apoptosis in a variety of neural preparations, perhaps via a pathway involving caspase activation and increased Ca²⁺ influx correlated with activation of excitatory receptors (15).

Diminished NO production may also be injurious. NO-mediated *S*-nitrosylation decreases caspase activation in nonneuronal cells (25), conferring protection from apoptosis. Decreased NO has been implicated in the pathogenesis of the ischemic injury observed in diabetes. In the diabetic rat model, treatment with aldose reductase inhibitor restores NO-mediated endothelium-dependent relaxation to within normal limits, and inhibition of NO synthase (NOS) can block the beneficial effects of an aldose reductase inhibitor on slowing of diabetic nerve conduction. The constitutive form of NOS is dependent on Ca²⁺ and calmodulin, providing a mechanism for altered Ca²⁺ homeostasis to decrease NO production.

ROLE OF MAPK, JNK, AND PI 3-KINASE IN NEURAL INJURY

Several downstream signal transduction pathways have been implicated in neural injury, including mitogen-activated protein kinase (MAPK), c-Jun NH₂-terminal protein kinase (JNK), and phosphatidylinositol 3-kinase (PI-3K) (10). Paradoxically, a large body of evidence also suggests that these same pathways may also be trophic or protective in some tissues (10). One

mechanism by which Ca²⁺ influx may regulate downstream intracellular signal transduction pathways is provided by studies documenting that increased [Ca²⁺]_i activates MAPK in cultured PC-12 neuroblastoma cells. Of interest, activation of MAPK can be dissociated from downstream induction of apoptosis, raising questions regarding the specificity of this pathway to neuronal injury vs. neuroprotection. Neuronal injury due to axotomy causes activation of constitutive JNK in sensory neurons in vivo, upregulation of *c-jun* expression and DNA binding, subsequent neuroprotection, and regeneration of axonal processes (12). However, JNK activation and subsequent *c-jun* upregulation have also been correlated with apoptosis. These apparent discrepancies may be related to differences in tissue specificity and/or experimental conditions. PI-3K activation has been implicated in growth factor-mediated survival in a variety of cell types, including primary neurons in culture (6). A link with depolarization-mediated neuroprotection via modest elevation of [Ca²⁺]_i has been proposed; however, activation of the PI-3K pathway is not essential in the promotion of depolarization-mediated neuronal survival in embryonic neurons. This may be explained by the observation that neurons become more sensitive to depolarization-mediated neuroprotection with increasing culture time, perhaps as a result of increased expression of Ca²⁺ channels or release of neurotrophins.

ROLE OF CERAMIDE/SPHINGOLIPID SIGNALING IN NEURAL INJURY

Ceramide is a sphingolipid produced by hydrolysis of membrane sphingomyelin to phosphorylcholine and ceramide and may be an important mediator of cellular growth and differentiation, as well as programmed cell death. Interest in ceramide as a second messenger signaling pathway has increased because of evidence suggesting ceramide is a signal pathway in neurotrophin-mediated neuroprotection (8). However, the role of sphingolipid signaling in neuronal injury is unclear, as both neuroprotective and proapoptotic effects of modulation of the ceramide pathway have been described in neuronal cells. In nonneuronal tumor cell lines, lymphokines such as tumor necrosis factor- α and interferon- γ increase endogenous ceramide production and induce apoptosis. A similar effect has been observed in cultured neurons, possibly due to stimulation of the p75 neurotrophin receptor as described in the following section. Conversely, elevation of ceramide by exposure of primary neurons to cell-permeable ceramide analogs attenuates the proapoptotic effect of neurotrophin withdrawal. Many aspects of this novel pathway remain to be elucidated, including the mechanism by which neurotrophin stimulation modulates sphingolipid synthesis. The downstream effects of sphingolipid signaling may be exerted via activation of second messengers such as nuclear factor- κ B (NF- κ B), which has demonstrated a similar dichotomy of both pro- (3) and antiapoptotic effects (2).

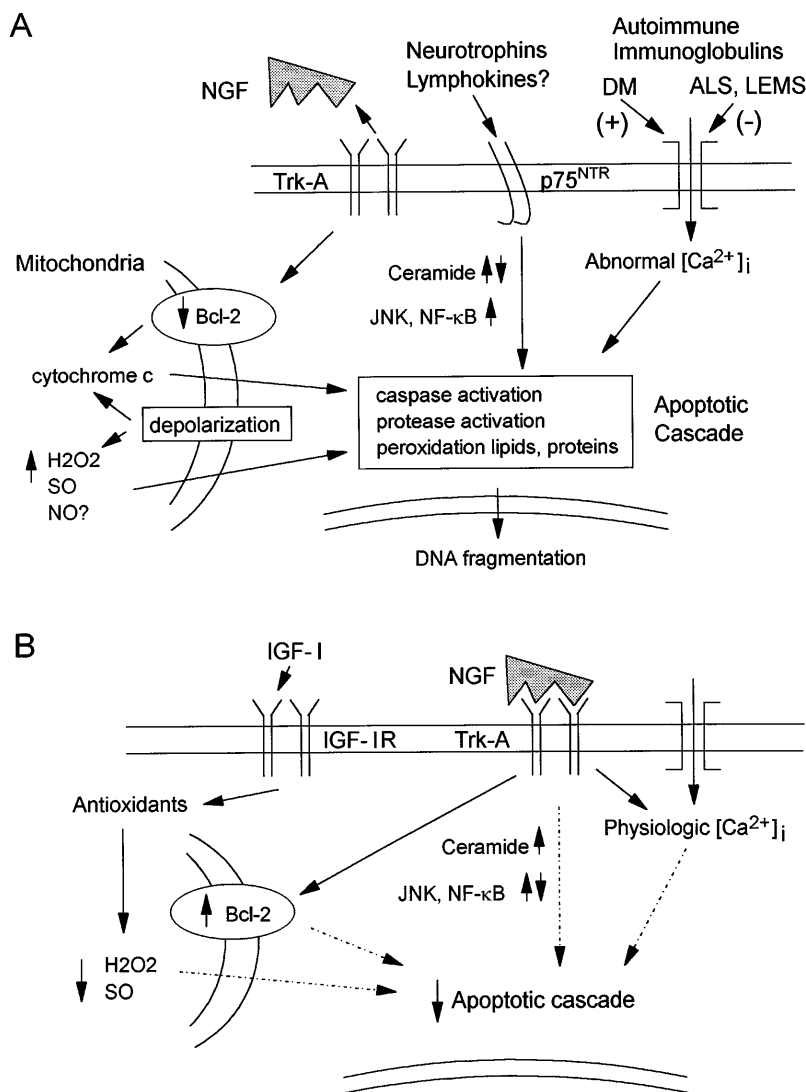


Fig. 1. Proposed models of neuronal injury (A) and neuroprotection (B). A: neuronal apoptosis can be triggered by decreased tyrosine kinase receptor A (Trk A) neurotrophin receptor stimulation due to nerve growth factor (NGF) withdrawal, neurotrophin/lymphokine(?) stimulation of p75 neurotrophin receptor (p75^{NTR}), and autoimmune immunoglobulin modulation of Ca²⁺ influx, resulting in abnormal cytosolic Ca²⁺ concentration ([Ca²⁺]_i). Abnormal [Ca²⁺]_i, decreased mitochondrial Bcl-2, release of cytochrome c, and increased production of reactive oxygen species have been linked to activation of an "apoptotic cascade." The role of other intermediate signal pathways [ceramides, c-Jun NH₂-terminal protein kinase (JNK), nuclear factor-κβ (NF-κβ)] is unresolved; however, they appear to act upstream of the apoptotic cascade. B: neurotrophins such as NGF and other extracellular ligands [insulin-like growth factor I (IGF-I)] appear to prevent activation of the apoptotic cascade by several mechanisms. These include maintenance of mitochondrial Bcl-2 function and decreased oxidative damage. Neurotrophin-mediated maintenance of [Ca²⁺]_i within the physiological range also appears to be protective. The role of intermediate pathways (ceramide, JNK, and NF-κβ) in neuroprotection is unresolved. Solid lines indicate stimulatory pathways, dashed lines indicate inhibitory pathways. ALS, amyotrophic lateral sclerosis; LEMS, Lambert-Eaton myasthenic syndrome. DM, diabetes mellitus. SO, superoxide. NO, nitric oxide.

NEUROPROTECTION: ROLE OF NEUROTROPHINS AND ANTIOXIDANTS

During embryonic development of the nervous system, approximately one-half of all the neurons that are produced die as a result of apoptosis. This process adapts the size of the neuronal population to their target site and likely contributes to target specificity. It appears that specific target-derived neurotrophic factors [nerve growth factor (NGF), brain-derived neurotrophic factor, ciliary neurotrophic factor, and insulin-like growth factor I (IGF-I)] are involved in this process. Neurotrophins bind to specific tyrosine kinase receptors (Trk) in the tumor necrosis factor receptor superfamily, and specifically to Trk A in the case of NGF. Neurotrophins also bind to the p75 neurotrophin receptor, which has a "death domain" sequence implicated in neuronal apoptosis (4). NGF is required for normal function of adult sympathetic and sensory neurons, including maintenance of axonal processes, and loss of NGF leads to neuronal dysfunction and eventually neuronal death. Administration of exogenous NGF or increased endogenous production of NGF decreases

neuronal death induced by axotomy, target removal, and even certain chemical and virological insults to these neurons. Production and release of NGF is impaired in diabetes, and restitution of adequate levels of NGF attenuates diabetic neuropathy (1).

Neurotrophins and IGF-I can protect neurons from excitotoxic/ischemic injury, possibly by preventing the excessive elevation of Ca²⁺ and attenuating the effects of free radical-induced injury such as metal-catalyzed oxidation. IGF-I immunoreactivity is present in Schwann cell cytoplasm, is increased by sciatic nerve transection, and enhances regeneration in a dose-dependent fashion in lesioned sciatic nerve (7). In streptozotocin-diabetic rats, serum and tissue IGF-I levels are diminished. Therefore, blunted local neurotrophic effects may contribute to the pathogenesis of diabetic neuropathy.

Several studies indicate a potential beneficial effect of antioxidants in decreasing neural injury (22). Antioxidants such as α-lipoic (thioctic) acid and ascorbyl γ-linolenic acid appear to have a beneficial therapeutic effect in diabetic neuropathy. Treatment with antioxi-

dants has been shown to decrease age-related protein oxidation and cognitive decline. Mitochondrial superoxide dismutase overexpression in animal and cell line models decreases peroxynitrite production and lipid peroxidation and significantly ameliorates neuronal apoptosis (11). In addition to scavenging ROS directly, some exogenous antioxidants may induce upregulation of antioxidant molecules such as glutathione.

CONCLUSIONS

Several pathways appear to play a role in the development of neural injury. These are summarized in Fig. 1A. Acute elevation of $[Ca^{2+}]_i$ due to excitatory amino acids or serum factors has been implicated in triggering pathways that culminate in cell death due to necrosis or apoptosis. The “ Ca^{2+} hypothesis” of neural damage proposes that moderate increases in free intracellular Ca^{2+} over prolonged periods may also be injurious. Impaired mitochondrial function leading to decreased ATP generation may play a pivotal role in triggering cell death in many neurodegenerative conditions. Production of oxidant species is also a common finding in neural injury, and treatment with antioxidants has proven beneficial in some models of neural damage. Reduced levels and impaired function of neurotrophins have been documented in several neurodegenerative disorders, with subsequent effects on second messenger pathways such as Ca^{2+} signaling. Restoration of neurotrophin levels may ameliorate neural injury. Figure 1B summarizes the neuroprotective pathways discussed in this review.

Many aspects of the events associated with neuronal injury require clarification. Future studies will need to address the apparent paradox of the same signal transduction pathway being both pro- and antiapoptotic. Experiments utilizing primary neurons may help to clarify which pathways are likely to be activated under pathological conditions. Our current understanding of the mechanisms underlying the control of apoptotic neuronal death is based primarily on studies utilizing immortalized cell lines. These cell lines can demonstrate significant differences compared with primary neurons in both the activity and specificity of pathways implicated in neuronal injury and death. Elucidation of the specific sequence of events (e.g., direct vs. indirect activation of apoptosis), as well as points of convergence between pathways that subserve injury or protection in neurons, has just begun. Finally, it should be appreciated that many neurodegenerative disorders exhibit a slowly progressive time course. Any hypothesis proposed to explain the pathophysiology of these disorders must take into account their chronic natural history.

We have just begun to elucidate the pathways involved in GI neurodegeneration. Several systemic disorders such as diabetes mellitus and LEMS are known to affect gut neuromuscular coordination. It is likely that one or more of the pathophysiological mechanisms described in neuronal models outside the GI tract will contribute to neuronal injury in the enteric nervous system.

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