Apoptosis is a form of programmed cell death that occurs in neurons during development of the nervous system and may also be a prominent form of neuronal death in chronic neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases. Recent findings also implicate apoptosis in neuronal degeneration after ischemic brain injury in animal models of stroke. Activation of both apoptotic and antiapoptotic signaling cascades occurs in neurons in animal and cell culture models of stroke. Apoptotic cascades involve: increased levels of intracellular oxyradicals and calcium; induction of expression of proteins such as Par-4 (prostate apoptosis response-4), which act by promoting mitochondrial dysfunction and suppressing antiapoptotic mechanisms; mitochondrial membrane depolarization, calcium uptake, and release of factors (e.g., cytochrome c) that ultimately induce nuclear DNA condensation and fragmentation; activation of cysteine proteases of the caspase family; activation of transcription factors such as AP-1 that may induce expression of “killer genes.” Antiapoptotic signaling pathways are activated by neurotrophic factors, certain cytokines, and increases in oxidative and metabolic stress. Such protective pathways include: activation of the transcription factors (e.g., nuclear factor-κB, NF-κB) that induce expression of stress proteins, antioxidant enzymes, and calcium-regulating proteins; phosphorylation-mediated modulation of ion channels and membrane transporters; cytoskeletal alterations that modulate calcium homeostasis; and modulation of proteins that stabilize mitochondrial function (e.g., Bcl-2). Intervention studies in experimental stroke models have identified a battery of approaches of potential benefit in reducing neuronal death in stroke patients, including administration of antioxidants, calcium-stabilizing agents, caspase inhibitors, and agents that activate NF-κB. Interestingly, recent studies suggest novel dietary approaches (e.g., food restriction and supplementation with antioxidants) that may reduce brain damage following stroke.

Key words Calcium · Free radicals · Ischemia · Neurotrophic factor · NF-κB · Par-4

Introduction

Stroke is a major cause of disability and death worldwide. Brain damage following stroke results from a reduced blood supply to brain cells which drastically reduces their access to oxygen and glucose. Studies performed during the past 20 years have identified several key biochemical and cellular events that lead to ischemic neuronal degeneration (see Mattson and Mark 1996; Dirnagl et al. 1999, for reviews). Cellular ATP levels plummet rapidly following the onset of ischemia, which impairs the ability of membrane ion-motive ATPases to remove Na⁺ and Ca²⁺ from the cell. This results in membrane depolarization, which promotes activation of synaptic glutamate receptors. Excessive accumulation of extracellular glutamate further activates glutamate receptors, resulting in massive calcium influx through N-methyl-d-aspartate (NMDA) receptors and voltage-dependent calcium channels. Mitochondrial dysfunction occurs as the result of energy failure and disruption of cellular calcium homeostasis. Increased production of free radicals results from mitochondrial dysfunction, calcium overload, and activation of enzymes such as cyclooxygenase and nitric oxide synthase. Free radicals damage cellular proteins, DNA, and membrane lipids. A particularly important aspect of oxidative stress in neurons is membrane lipid peroxidation, which results in the generation of toxic aldehydes such as 4-hydroxynonenal that...
impair the function of membrane ion-motive ATPases and glucose and glutamate transporters, thereby amplifying disruption of cellular calcium homeostasis (Mattson 1998). In addition to documenting that such alterations occur in various cell culture and animal models, intervention studies have demonstrated the efficacy of glutamate receptor antagonists, calcium-stabilizing agents, and antioxidants in reducing ischemic damage to neurons (Dinarel et al. 1999).

A variety of valuable animal and cell culture models that mimic, in part, the pathogenic environment of neurons in the brains of stroke victims have been developed. Two widely employed models of ischemic brain injury are the transient global forebrain ischemia model, in which the entire blood supply to the brain is transiently interrupted, and the focal cerebral ischemia model, in which the middle cerebral artery is occluded, resulting in damage to cerebral cortex and striatum in that hemisphere (see Ginsberg and Busto 1989; Mahair-Macrae 1992 for reviews). The focal model can involve either permanent or transient occlusion of the middle cerebral artery, with transient occlusion being generally accepted as the model that most closely duplicates stroke in human patients. In vitro models employ either dissociated cell cultures of hippocampal and cortical neurons, or hippocampal slices. The in vitro preparations can be subjected to glucose deprivation, hypoxia, excitatory amino acids, and oxidative insults (e.g., $\text{Fe}^{2+}$ and nitric oxide) to mimic specific aspects of the environment neurons encounter following stroke in vivo.

Neurons possess a variety of relatively unique features that must be kept in mind when studying mechanisms of ischemic brain injury. Neurons are, by definition, postmitotic and therefore largely irreplaceable once they die (but see Svendsen and Smith 1999 for recent evidence of the presence of neuronal precursor cells in the adult brain). Neurons also communicate with each other at highly specialized structures called synapses, in which a neurotransmitter released from the presynaptic cell activates receptors on the surface of the postsynaptic cell. Synapses are often located at a relatively large distance from the cell body, which is of considerable interest, since emerging data suggest synapses may be sites where neuronal apoptosis is often initiated (Mattson et al. 1998; Duan et al. 1999a). Finally, the glial cells that interact with neurons play important roles in modifying neuronal vulnerability to apoptosis. For example, astrocytes produce several different antiapoptotic growth factors (see below, Antiapoptotic signal transduction pathways that limit ischemic neuronal injury), while microglia (macrophages of the nervous system) produce neurotoxic substances such as nitric oxide and excitotoxins.

**Morphological and biochemical features of neuronal apoptosis**

Details of the morphological and biochemical characteristics of apoptosis in nonneuronal cells are presented in other articles in this issue. For the most part, similar changes occur in neurons undergoing apoptosis. Thus, apoptotic neurons exhibit cell body shrinkage, formation of cell surface “blebs”, and nuclear chromatin condensation and DNA fragmentation (Figs. 1, 2). The plasma membrane and organelles such as the mitochondria and endoplasmic reticulum (ER) remain intact during the cell death process. More unique to neuronal apoptosis is fragmentation of neurites (dendrites and axons), which occurs very early in the cell death process. Neurites are also damaged in neurons undergoing necrosis, but in the latter case ballooning and rapid disintegration of the neurites occurs, whereas in apoptotic neurons the fragmentation occurs with little swelling and over a longer time course. Apoptotic neurons are recognized by microglia and are engulfed; this clearance of apoptotic cells occurs without adversely affecting neighboring cells and without causing inflammation. Neurons dying by apoptosis are therefore often observed in isolation, with adjacent cells being unaffected. In many neuronal cell culture systems, microglia are not present and, in the latter settings, apoptotic cells will eventually undergo a secondary necrosis in which their plasma membranes and organelle membranes are disrupted. It is therefore important to recognize the existence of such secondary necrosis when studying neuronal apoptosis in culture systems.

Biochemical changes that may distinguish apoptosis and necrosis in neurons are beginning to be identified. Neurons undergoing apoptosis exhibit: rapid increases in prostate apoptosis response-4 (Par-4) protein levels (Guo et al. 1998; Chan et al. 1999; Duan et al. 1999a, 1999b); translocation of one or more members of the Bcl-2 protein family to mitochondrial membranes (Putcha et al. 1999); mitochondrial membrane depolarization and re-