Handbook of Clinical Neurology, Vol. 92 (3rd series)
Stroke, Part I
M. Fisher, Editor

Chapter 2

The ischemic cascade and mediators of ischemic injury

MATTHIAS ENDRES[1]*, ULRICH DIRNAGL[1], AND MICHAEL A. MOSKOWITZ[2]

[1]*Charité-Universitätsmedizin Berlin, Berlin, Germany*

[2]*Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA*

Cerebrovascular disease ranks second as the cause of death worldwide; mortality over the first year after first stroke is approximately 20%. The economic and social burdens of stroke, however, are not consequences of mortality; they are imposed by the large majority of stroke patients who survive but are physically and mentally disabled by stroke-induced brain damage (Stroke Progress Review Group, 2002).

Stroke is a heterogeneous disease and refers to an umbrella of conditions. In the Western world, ischemic stroke comprises 80–85%, while primary intracerebral hemorrhages, subarachnoidal hemorrhages, and sinus thrombosis account for the remaining 15–20%. By contrast, hemorrhagic stroke may comprise up to 50% of all strokes in Asia. Ischemic stroke is caused by a transient or permanent reduction of blood flow restricted to the territory of a cerebral artery—typically by embolic or thrombotic occlusion. In any case, ischemic stroke eventually results in the death and dysfunction of brain cells. Central to our current understanding of stroke pathophysiology is evidence derived from animal models. Mimicking the condition of ischemic stroke are models of focal cerebral ischemia. The most frequently used species are rodents such as mice and rats but also other mammals, including dogs, cats, and sheep as well as primates (Lo et al., 2003).

2.1. Temporal and spatial events after stroke: the concept of an ischemic penumbra

Not all brain cells die immediately after an ischemic stroke. Ever since Astrup first introduced the concept of an ischemic penumbra, i.e. a perilesional area that surrounds the ischemic core, it is generally accepted that cell death evolves in a temporal and spatial continuum (Astrup et al., 1981). Almost immediately after vessel occlusion, an ischemic core is defined that is destined to die irrespective of therapeutic interventions unless blood flow is rapidly restored. Surrounding this core lies the penumbra, which is functionally silent but metabolically still active and hence salvageable. Early reperfusion is the major target of most experimental interventions, to render cells in the penumbra resistant to cell death. Importantly, however, the penumbra is dynamic: indeed, over time the 'core grows at the cost of the penumbra' and previously viable brain becomes infarcted tissue (Ginsberg et al., 1999) (Fig. 2.1).

2.2. Active cell death mechanisms

Although the exact timing and cellular pathways are incompletely understood it is commonly believed that mechanisms actively promoting cell death are triggered after stroke (Lo et al., 2005). Cell death occurs by a necrotic pathway characterized by ischemic or edematous cell changes, by an apoptotic pathway with a number of morphological (e.g., apoptotic bodies, blebbing), biochemical (e.g., DNA laddering), pharmacological, and molecular characteristics (e.g., activation of caspases), or by autophagocytosis. A number of different stages of the cell death process and major pathophysiological pathways have emerged from the literature (Dirnagl et al., 1999; Lo et al., 2005). First comes an induction stage with energy failure, increase of intracellular calcium, and release of excitatory amino acids (Lipton et al., 1999). This in turn triggers the activation of downstream perpetrators of ischemic

*Correspondence to: Priv.-Doz. Dr M. Endres, Klinik und Poliklinik für Neurologie, Charité-Universitätsmedizin Berlin, Campus Mitte, Schumannstrasse. 20/21, D-10117 Berlin, Germany. E-mail: matthias.endres@charite.de, Tel: +49.30.450.560.257, Fax: +49.30.450.560.932.

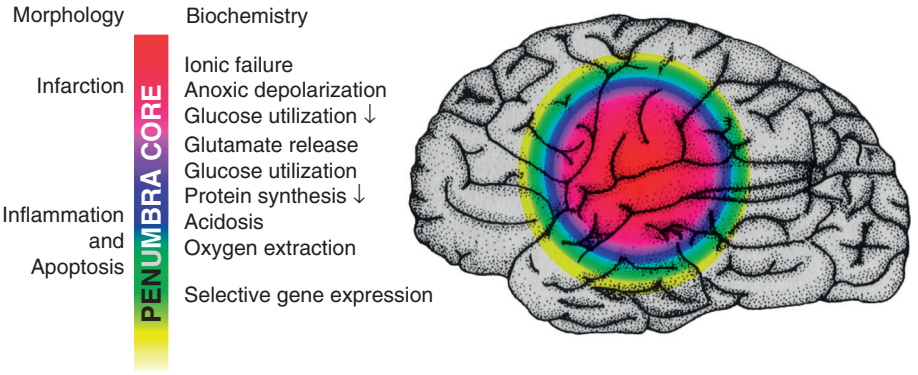

Fig. 2.1. The ischemic penumbra. A brain region of low perfusion in which cells have lost their membrane potential terminally ("core") is surrounded by an area in which intermediate perfusion prevails ("penumbra")(from Dirnagl et al., 1999).

damage, including free radical and peroxynitrite production, calpain, phospholipases, and poly-ADP-ribose polymerase activation. Concomitantly, apoptotic pathways are initiated. Waves of peri-infarct depolarization further compromise the energy balance of ischemic neurons in the penumbra. Inflammation then amplifies tissue damage. Secondary stages of cell death may involve long-term changes in macromolecules and other key metabolites. All these events are potential targets for therapeutic interventions (Fig. 2.2).

2.3. Excitotoxicity, energy failure, and ionic imbalance

Brain is one of the most metabolically active organs and depends almost exclusively upon oxidative phosphorylation for energy. It is exquisitely sensitive to disturbances in oxygen and glucose supply (Siesjo, 1978; Hansen, 1985; Erenciska and Silver, 1989). Following focal ischemia there is a profound deprivation of oxygen and glucose. Available evidence suggests that gray and white matter both suffer immediate loss

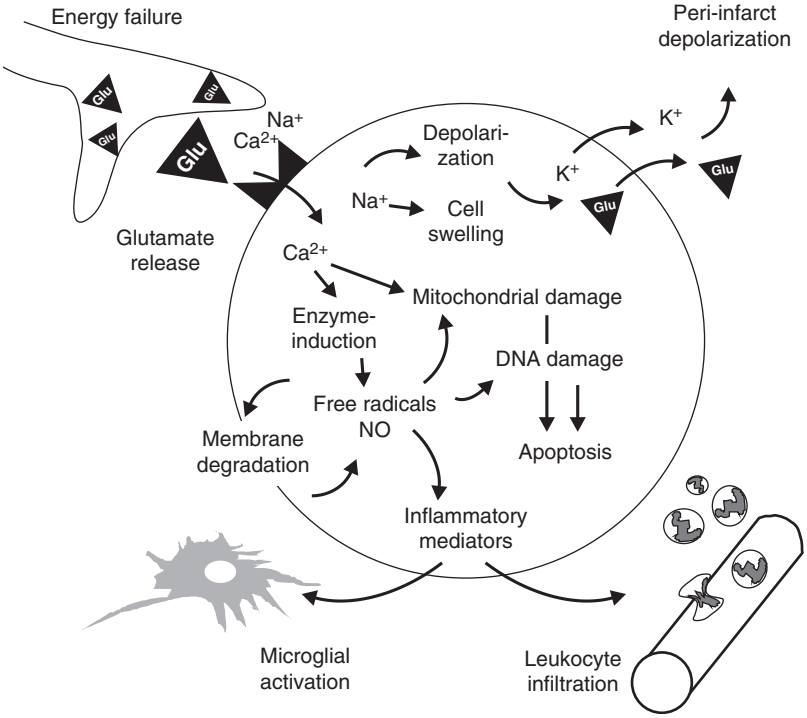

Fig. 2.2. Simplified overview of pathophysiological mechanisms in the focally ischemic brain (from Dirnagl et al., 1999).

of function with anoxia (Stys et al., 1992). Within minutes both neuronal and non-neuronal cells become depolarized (e.g., anoxic depolarization) and voltage-dependent calcium channels are activated (Martin et al., 1994; Paschen, 2000). Depolarization also induces the release of (predominantly excitatory) neurotransmitters from presynaptic terminals into the synaptic cleft (Zipfel et al., 1999). In particular, binding of glutamate to ionotropic *N*-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA) receptors promotes excessive calcium influx. In turn, increased intracellular calcium levels act as universal second and third messenger to trigger an array of downstream phospholipases and proteases that degrade membrane and proteins essential for cellular integrity (such as actin, spectrin, laminin, etc.; Chen et al., 1998; Furukawa et al., 1997; Endres et al., 1999). Calcium ions and other ions also enter mitochondria through the mitochondrial permeability transition pore, causing dysfunction and mitochondrial swelling. Moreover, sodium and chloride enter neurons via channels for monovalent ions (e.g., the AMPA receptor) passively followed by water. The ensuing intracellular ('cytotoxic') edema may negatively impact perfusion in the peri-infarct region. Imbalances of other ions are also important: for example, large amounts of zinc that are stored in vesicles of excitatory neurons become released upon depolarization and contribute to excitotoxic cell death (Frederickson, 1989; Weiss et al., 1993; Sorensen et al., 1998).

2.4. Oxidative and nitrosative stress

As a consequence of ischemia but particularly after reperfusion, reactive free oxygen species are generated. These reactive free oxygen species are also key mediators of tissue damage in other organs subjected to reperfusion injury, such as heart or kidney. Free radicals trigger a vicious cycle in the mitochondria with inhibition of electron transport mechanisms leading to excess superoxide production and also to activation of mitochondrial permeability transition (Fiskum et al., 1999; Kroemer and Reed, 2000; Chan, 2001). Additional sources of reactive free oxygen species are enzymatic processes such as the cyclooxygenase-dependent conversion of arachidonic acid to prostanoids, or the degradation of hypoxanthine. In fact, oxidative stress is closely linked to excitotoxicity, energy loss, and ionic imbalances, and all these events contribute to tissue damage. Injury induced by oxidative stress is mitigated by endogenous mechanisms involving superoxide dismutase or glutathione; however, these scavenging mechanisms are typically insufficient during an injury as robust as focal ischemia (Kondo et al., 1997; Fujimura et al., 1998, 1999). Mice overexpressing superoxide dismutase have reduced injury following focal ischemia, giving support to the notion that interventions aimed at enhancing endogenous repair mechanism may be attractive therapeutic targets for ischemic stroke (Kinouchi et al., 1991; Sheng et al., 1999).

In addition to reactive free oxygen species, nitrosative stress contributes to tissue damage. Nitric oxide (NO) itself may play both beneficial and deleterious roles during brain ischemia depending upon when and where it is produced. NO is synthesized from L-arginine by several isoforms of NO synthase (NOS; i.e. NOS I, II, and III). In situations of increased oxidative stress NO reacts with superoxide anions to generate the highly reactive and cytotoxic peroxynitrite, which may damage virtually every cellular component (Beckman and Koppenol, 1997; Iadecola, 1997). During ischemia constitutive, calcium-dependent neuronal type I NOS (nNOS) may be activated via calcium influx. Within minutes after induction of focal cerebral ischemia cortical NO levels may rise from 10 nmol/L to 2 μmol/L (Dalkara and Moskowitz, 1994). In addition, inducible (type II) NOS (iNOS), which is calcium/calmodulin-independent and is not normally present in healthy brain tissue, is induced after brain ischemia in non-neuronal cells such as microglia and leukocytes, but also astrocytes and endothelial cells. Once expressed, iNOS is constitutively active and confers high NO output, contributing to secondary late-phase tissue damage (Iadecola et al., 1997). Experiments using knockout mice have helped to clarify the respective roles of the different NOS isoforms in ischemic injury. Animals that lack expression of nNOS (so called nNOS knockout mice) have 40% smaller ischemia lesion volumes (Huang et al., 1994). Similarly, pharmacological inhibition of iNOS or deletion of the iNOS gene leads to a reduction in ischemic damage (Iadecola et al., 1997). Interestingly, iNOS is not expressed before 24 hours after ischemia onset, and iNOS inhibition reduces tissue damage even when administered as late as 1 day after the occlusion, which is consistent with the hypothesis that ischemic injury indeed evolves over several days.

The generation of nitric oxide and reactive free oxygen species is linked to DNA damage and activation of the nuclear enzyme poly-ADP-ribose polymerase (PARP-1). PARP-1 is activated by single-stranded DNA nicks (which may be generated by oxidative and nitrosative damage) and in turn consumes large amounts of β-nicotinamide adenine dinucleotide (NAD^+) to produce poly-ADP chains for post-translational modification of a number of repair enzymes including PARP-1 itself (Zhang et al., 1994). It has been assumed that the

consumption of NAD^+ and subsequent depletion of ATP results in cellular energy depletion and subsequent cell death (Zhang et al., 1994; Eliasson et al., 1997). Ischemic cell death is suppressed by inhibiting PARP-1 activity or by deleting the *PARP1* gene, indicating the potential of PARP-1 as a therapeutic target (Eliasson et al., 1997; Endres et al., 1997). Consistent with the notion that PARP-1 is a downstream effector of NO-mediated neurotoxicity are experiments to demonstrate that nNOS knockout mice have significantly reduced levels of poly-ADP-ribose formation in ischemic tissue compared to wild-type controls (Endres et al., 1998a; Dawson and Dawson, 2004).

In contrast to nNOS and iNOS, an additional isoform, type III or endothelial NOS (eNOS), plays a protective role during cerebral ischemia (Endres et al., 2004). Endothelial NO possesses vasodilatory, anti-inflammatory, antithrombotic, and antiproliferative properties. NO generated by eNOS is crucial in vascular function, maintaining cerebral blood flow and reducing cerebral infarct volume, especially in the early stages following cerebral ischemia. Animals lacking eNOS expression develop larger cerebral infarctions following focal brain ischemia (Huang et al., 1994, 1996). Several modalities that upregulate eNOS expression and/or activity have been identified, including HMG-CoA reductase inhibitors (statins), physical activity, steroid hormones, and nutrients (Endres et al., 1998b; Hafezi-Moghadam et al., 2002; Endres et al., 2004). They all increase NO bioavailability, leading to enhanced cerebral blood flow and protection from ischemic stroke.

2.5. Peri-infarct depolarizations

Anoxic depolarization is caused by the loss of energy and oxygen in neurons and non-neuronal cells and the acute release of glutamate and other excitatory amino acids. Cells in the core of the lesion remain depolarized and go on to die. In the peri-infarct zone, cells may repolarize, however at a high cost of energy expenditure. Following depolarization neurotransmitters and potassium are released into the extracellular space and then initiate repetitive waves of so-called peri-infarct depolarizations (Nedergaard and Hansen, 1993; Back et al., 1994, 1996). In animals, these peri-infarct depolarizations occur at a frequency of 1–4 events per hour for at least 6–8 hours. They typically originate in the core of the lesion and then propagate to the periphery in a wave-like fashion (Busch et al., 1996; Wolf et al., 1997). There are several lines of evidence from ischemic animal models (in mouse, rat, and cat) that the phenomenon of peri-infarct depolarizations in fact contributes to tissue damage: (1) The total number and frequency of peri-infarct depolarizations correlate with the final lesion volume and cell loss and it has been shown that each propagating depolarization contributes to the immediate growth of the lesion from the core to the periphery (Mies et al., 1993; Back et al., 1994, 1996; Hoehn-Berlage, 1995); (2) therapeutic interventions aimed at reducing peri-infarct depolarizations (such as NMDA and AMPA receptor blockade) provide tissue sparing (Iijima et al., 1992) while (3) induction of additional peri-infarct depolarizations (e.g., by administration of topical potassium chloride solution) further increases infarct volume. So far, however, convincing evidence that peri-infarct depolarizations occur after ischemic stroke in humans is lacking (Back et al., 2000).

2.6. Inflammation and immunity

Early after the onset of ischemia, the expression of pro-inflammatory genes, including those encoding NF-κB, hypoxia-inducible factor, and interferon 1β, is triggered (Pirttilä and Kauppinen, 1992; Lindsberg et al., 1996; Iadecola, 1997). A cascade of events is then initiated that includes the expression of adhesion molecules (such as intercellular and vascular adhesion molecules, ICAM and VCAM, as well as selectins), endothelial activation, pro-inflammatory and pro-thrombotic interactions between vessel wall and blood constituents promoting thrombogenesis, and microvascular plugging (Connolly et al., 1996; Stanimirovich and Satoh, 2000; Frijns and Kappelle, 2002). Of note, serological markers of vessel inflammation including C-reactive protein and sICAM have been linked to increased stroke risk (Ridker et al., 2000; Tanne et al., 2002).

Adhesion molecules expressed by the endothelium interact with receptors on neutrophils to promote their entry into brain, followed days later by macrophages and monocytes (Iadecola, 1997). Inflammatory responses, however, are mediated not only by bone marrow and blood-derived cells but also by immunocompetent cells resident in the brain such as microglia. These cells constitute up to 20% of the total brain cell number and become activated after brain insults—particularly in the penumbra (Tarozzo et al., 2002). Typically, microglial cells retract their processes and adapt an 'ameboid' morphology as they become activated. Similar to leukocytes, activated microglia produce a plethora of pro-inflammatory cytokines along with toxic metabolites (Yrjanheikki et al., 1999). Recent evidence demonstrates that bone-marrow-derived mononuclear cells that invade the brain upon ischemic insult may differentiate into microglia and become part of the surviving brain (Priller et al., 2001). Also, macroglia such as astrocytes become activated and proliferate. These cells also contribute to the

production of pro-inflammatory cytokines and also neuroprotective factors including erythropoietin, transforming growth factor (TGF)-β, and metallothionein (Letterio, 2000; Ruscher et al., 2002; Trendelenburg et al., 2002).

There is evidence to support the notion that the inflammatory reaction following ischemic stroke is both protective and destructive. In support of the latter notion, it was demonstrated that ischemic brain injury is reduced either when neutrophil invasion is blocked by systemic neutropenia or when the expression of adhesion molecules is blocked by specific antibodies or via genetic deletion (e.g., using ICAM-1 antibodies or ICAM-1-knockout mice; Connolly et al., 1996).

In addition, it has recently become clear that brain ischemia also increases susceptibility to systemic infection by brain-specific mechanisms (Meisel et al., 2005). Ischemic brain injury disturbs the homeostasis between the central nervous system and the immune system, leading to immunosuppression. In fact, in a rodent model of focal brain ischemia neuroendocrine-mediated systemic immunosuppression results in the development of spontaneous systemic bacterial infections (Prass et al., 2003).

2.7. Apoptosis-like pathways

Loss of membrane integrity, cell swelling, and organelle failure, i.e. typical characteristics of necrosis, are the most prominent features of cell death following brain ischemia (Majno and Joris, 1995). There has been vigorous scientific debate about the mechanism of ischemic cell death with respect to apoptosis versus necrosis, although most agree that necrotic cell death predominates in ischemic stroke. The term 'apoptosis' was introduced into the scientific literature by Kerr in 1972, although the typical morphological hallmarks of apoptotic cell death were described by pathologists more than 100 years ago (Kerr et al., 1972; Majno and Joris, 1995). These hallmarks include cytoplasmic shrinkage, blebbing, nuclear segmentation, chromatin condensation, and formation of apoptotic bodies. In general, the 'decision' for apoptotic versus necrotic cell death depends on the nature and intensity of the stimulus, the type of cell, and the stage reached in its developmental life-cycle (Bonfoco et al., 1995; Nicotera et al., 2000). In line with this, a number of reports have proposed that necrosis is the predominant mechanism that follows acute, permanent vascular occlusion, whereas in milder injury, cell suicide becomes unmasked and death resembles apoptosis, particularly within the ischemic penumbra (Du et al., 1996; Endres et al., 1998c).

Although bona fide morphological criteria of apoptosis are apparently lacking (van Lookeren Campagne and Gill, 1996), a number of molecular, biochemical, and pharmacological criteria to define apoptotic cell death are fulfilled in ischemic neuronal death. Both caspase-dependent and independent mechanisms have been described. Caspases are aspartate-specific cysteine proteases, constitutively expressed in brain, including neurons, and are activated by intrinsic and extrinsic stimuli (Yuan and Yanker, 2000). Extrinsic pathways include the activation of death receptors, e.g., Fas and tumor necrosis factor (TNF)-α receptors (Martin-Villaba et al., 1999). Upon activation, Fas assembles as part of a death-inducing signaling complex along with Fas-associated protein with death domain (FADD) and pro-caspase-8 (Qiu et al., 2002). Intrinsic activators comprise oxygen radical formation, DNA damage, rise in intracellular calcium, and lysosomal protease activation (Nicotera and Lipton, 1999; Budd et al., 2000; Salvesen, 2001). Once activated, caspases cleave a number of downstream substrates that include other ('executioner') caspases, DNA repair enzymes such as PARP, cytoskeletal proteins, presenilin, huntingtin, and caspase-activated deoxyribonuclease (ICAD). The most important executioner caspase in the brain is caspase-3, which is activated early after ischemia, particularly in the peri-infarct region (Namura et al., 1998; Endres et al., 1998c). Cytochrome *c* plays an important role in the activation of caspases. Once released from the mitochondria cytochrome *c* forms an 'apoptosome' complex in the presence of ATP and caspase-9. Apoptosome-formation is suppressed by Bcl-Xl (or BCL2L1) (Green and Reed, 1998) while cytosolic Bid, a pro-apoptotic Bcl-2 family member, facilitates cytochrome *c* release and apoptosome formation (Wei et al., 2001). Downstream markers of apoptosis include the appearance of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeled (TUNEL) cells and biochemical evidence of oligonucleosomal DNA fragmentation ('DNA laddering'), which has been demonstrated in stroke models in numerous reports (Linnik et al., 1993; MacManus et al., 1993; Tominaga et al., 1993; Li et al., 1995a, b; Endres et al., 1998c). Inhibition of caspase, for example using small oligopeptides that mimic the cleavage site of a caspase substrate, protects from cerebral ischemia (Hara et al., 1997; Fink et al., 1998; Endres et al., 1998c). Interestingly, following mild insults, caspase-3 activation is delayed until 9 hours, consistent with the treatment window for caspase-inhibitors, which is also extended to 9 hours after vessel occlusion (Fink et al., 1998). Moreover, animals with reduced expression of caspase-1 or -3 are protected from mild experimental ischemia (Friedlander et al., 1997; Schielke et al., 1998).

In addition, there is evidence for caspase-independent apoptotic mechanisms in cerebral ischemia. Apoptosis-inducing factor (AIF) is considered as a key-signaling molecule in this cascade (Susin et al., 1999). Apparently, PARP-1 activation may promote the mitochondrial release of AIF. AIF then re-locates to the nucleus, where it promotes chromatin condensation. Cell death by AIF is resistant to treatment with pan-caspase inhibitors but can be suppressed by neutralizing AIF before nuclear translocation, supporting the notion that AIF acts independently of caspases.

A growing body of evidence indicates that components of the cell cycle machinery become activated in neurons subjected to cell death stimuli and play specific roles in their death (Katchanov et al., 2001; Herrup and Arendt, 2002; Greene et al., 2004). In this context it has been suggested that multiple cyclin-dependent kinases (CDKs) may participate in neuronal death and two central hypotheses have been espoused. The first describes the paradoxical situation by which terminally differentiated post-mitotic neurons reactivate components of the cell cycle machinery, including CDKs, which however confers cell death instead of mitosis (Copani et al., 2002). Indeed, activation and up regulation of a number of cell cycle components has been reported in several cell death models including stroke (Timsit et al., 1999; Osuga et al., 2000; Wang et al., 2002; Qiu et al., 2002). Activation of the cell cycle machinery may be triggered by the stroke-induced downregulation of endogenously expressed CDK inhibitors of the INK and KIP family, such as p16, p19, p21, or p27 (Katchanov et al., 2001). A second hypothesis proposes that deregulated CDK 5, which is not involved in cell cycle progression but rather implicated in transcription, neuronal function, and differentiation, induces neuronal damage. Activation of CDK 5 following brain ischemia may be triggered via calcium-mediated activation of the protease calpain, which in turn converts p35 into a smaller, more stable and mislocalized p25 form. This then activates CDK 5 to induce cell death. Indeed, accumulation of p25 after transient forebrain ischemia activates CDK 5 and induces ischemic cell death (Wang et al., 2003). In fact, Park and co-workers have demonstrated that CDK 5 acts preferentially to regulate excitotoxic damage while cell-cycle-promoting CDKs such as CDK 2, 4, and 6 are involved in more delayed types of cell death (Rashidian et al., 2005). However, the downstream effectors that mediate neuron cell death in response to cell cycle activation are largely unknown. Recently, the pro-apoptotic Bcl-2 homologue of BH3 domain-only molecule, Bim, has been identified as a direct target of a neuronal E2F-dependent apoptotic pathway (Biswas et al., 2005).

2.8. Stroke-induced endogenous neuroprotection

Historically, research on the pathophysiology of stroke was biased towards investigating mechanisms of tissue damage, while endogenous protective responses to challenges like substrate deprivation and the secondary effectors of cell death (reviewed above) received little attention. Recently, however, it became clear that the final outcome of a stroke is the result not only of tissue destruction and reorganization or plasticity but also of endogenous neuroprotection.

Central to this research are models of ischemic preconditioning (Dirnagl et al., 2003). Ischemic preconditioning refers to a process of protecting cells, tissues, or whole organisms induced by one or more exposures to a subliminal noxious stimulus (trigger). Subliminal ischemia (‘ischemic tolerance’), hypoxia, reactive oxygen free radicals, inflammation, etc. can serve as tolerance-inducing stimuli. Studies in patients experiencing transient ischemic attacks indicate that ischemic tolerance does also exist in humans. A transient ischemic attack, by definition, does not lead to infarction. Subsequent to a transient ischemic attack, brain infarcts that occur are smaller compared to lesions without preceding transient ischemic attack (Weih et al., 1999; Moncayo et al., 2000). In models of cerebral ischemia preconditioning, many sensors, transducers, and effectors of stroke leading to endogenous neuroprotection have been identified (Kirino, 2002; Dirnagl et al., 2003; Kariko et al., 2004; Trendelenburg and Dirnagl, 2005). These include anti-excitotoxic, anti-inflammatory, and anti-apoptotic mechanisms. Hypoxia-inducible-factor (HIF)-1-dependent signaling may serve as a typical example of such an endogenous neuroprotective response. Focal cerebral ischemia leads to local hypoxia and consequently to the nuclear translocation and DNA-binding of HIF-1, a key sensor of hypoxia in almost all mammalian cells. HIF-1, among other proteins, including glycolytic enzymes, induces the expression of erythropoietin in astrocytes. Erythropoietin, in a paracrine fashion, binds to neuronal erythropoietin receptors. Via the activation of a protein kinase cascade, in particular phosphoinositol-3 kinase, the pro-apoptotic protein BAD becomes phosphorylated and thus inactivated (Ruscher et al., 2002). By this mechanism HIF-1-induced erythropoietin expression can salvage neurons challenged by ischemia, particularly in the penumbra. Understanding the signaling by which the brain inactivates mechanisms of cell death in focal cerebral ischemia may lead us to novel strategies for the treatment of patients with stroke. For example, erythropoietin, as well as other neuroprotective hematopoietic cytokines expressed in the brain

(such as granulocyte colony stimulating factor and granulocyte–macrophage colony stimulating factors), is presently being tested as a neuroprotectant in clinical stroke trials (Ratan et al., 2004).

2.9. The blood–brain barrier, the microcirculation, and the neurovascular unit

The newly introduced concept of the 'neurovascular unit' comprised of cerebral endothelial cells, astrocytes, and neurons, along with extracellular matrix, provides an opportunity to look beyond a single cell and focus on cell–cell interaction—particularly after ischemic stroke (Lo et al., 2003, 2005). The integrity of the blood–brain barrier depends primarily on the interaction of the extracellular matrix with endothelial cells and astrocytes. Perturbation of the extracellular matrix following ischemic stroke includes the degradation of type IV collagen, laminin, and fibronectin. A number of proteases such as cathepsins, plus heparanases, contribute to this, as do plasminogen activator and matrix metalloproteinases (MMPs). MMP-2 and -9 are induced very early after ischemia onset, which correlates with the risk for hemorrhagic transformation, edema formation, and the extent of tissue demise (Gasche et al., 1999; Heo et al., 1999; Lapchak et al., 2000). Indeed, increased MMP plasma and brain levels have been demonstrated in stroke patients (Clark et al., 1997; Montaner et al., 2001). In experimental stroke models, MMP inhibitors reduce ischemic brain damage (Rosenberg and Navratil, 1997; Asahi et al., 2001) and MMP-9 knockout mice are protected against cerebral ischemia (Asahi et al., 2001)

2.10. White matter

In contrast to research on gray matter injury, white matter injury following stroke has been largely neglected. Indeed, it may well be that the failure to develop clinically effective neuroprotective strategies for stroke in part relate to the inattention to white matter injury and the failure to ameliorate ischemic damage to white matter. Most stroke models are performed in rodents, whose brains are a little less than 10% white matter by volume (Zhang and Sejnowski, 2000) while the human brain contains more than 50% white matter by volume. Hence, protecting gray matter alone may have considerably less benefit in humans than in rodents. However, much less is understood about the cellular pathways of ischemic injury in white matter, which is composed of axons, oligodendrocytes, astrocytes, and blood vessels. Laminin, fibronectin, and chondroitin proteoglycans are matrix proteins that envelop these cells. Furthermore excitotoxic injury differs from gray matter as there are no synapses and vesicular release is minimal. Recently, a number of quantitative techniques have been developed to assess white matter injury as well as to protect white matter in ischemia (Dewar et al., 1999). Similar to gray matter, energy deprivation leads to dysfunction of axons via activation of voltage-dependent sodium and calcium channels, and consecutive activation of calcium-dependent disruptive pathways (Goldberg and Ransom, 2003). Of interest, the white matter seems particularly resistant to hypoglycemia with astrocytic intracellular glycogen stores playing a protective role (Wender et al., 2000). Indeed, the astrocyte glycogen content defines the period of hypoglycemia in which cells are resistant to injury. Particularly vulnerable to ischemic damage are oligodendrocytes, the myelin-forming cells of the central nervous system. These cells express non-NMDA glutamate receptors and can be damaged and killed by glutamate excitotoxicity via intracellular calcium overload and subsequent calcium-dependent cascades, as described above (McDonald et al., 1998). Notably, oligodendrocytic AMPA receptors lack the calcium-impermeable GluR2 subunit, which may render these cells particularly vulnerable to injury (Matute et al., 2002).

2.11. Concluding remarks

Cell death following brain ischemia is mediated by a complex interplay of a number of pathophysiologically distinct mechanisms. Both blood vessels and parenchyma have been implicated and these complex interactions define the fate of compromised tissues and cells. The past two decades have witnessed an enormous expansion in our understanding of events that determine the fate of brain cells following vessel occlusion. In fact, novel death mechanisms have been implicated to explain the diversity of cell death phenotypes resembling necrosis, apoptosis, necroptosis, and autophagy in ischemic models. Their relevance to embolic, thrombotic strokes and transient ischemic attacks in humans, however, remains to be determined. Each cell death mechanism critically relates in unique ways to energy depletion, deployment of endogenous proteases, oxygen radical generation, fluxing of cations, receptor activation, and active cell death programs that determine the fate of injured parenchymal and vascular cells. Despite this complexity, there is reason for optimism, as recent developments in molecular and cell biology, plus neuroimaging, are providing tools for better translation of discoveries from bench to bedside (Endres et al., 2007).

References

Asahi M, Wang X, Mori T, et al. (2001). Effects of matrix metalloproteinase 9 gene knockout on the proteolysis of blood–brain barrier and white matter components after cerebral ischemia. J Neurosci 21: 7724–7732.

Back T, Kohno K, Hossmann KA (1994). Cortical negative DC deflections following middle cerebral artery occlusion and KCl-induced spreading depression: effect on blood flow, tissue oxygenation, and electroencephalogram. J Cereb Blood Flow Metab 14: 12–19.

Back T, Ginsberg MD, Dietrich DW, et al. (1996). Induction of spreading depression in the ischemic hemisphere following experimental middle cerebral artery occlusion: effect on infarct morphology. J Cereb Blood Flow Metab 16: 202–213.

Back T, Hirsch JG, Szabo K (2000). Failure to demonstrate peri-infarct depolarizations by repetitive MR diffusion imaging in acute human stroke. Stroke 31: 2901–2906.

Beckman JS, Koppenol WH (1997). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271: C1424–C1437.

Biswas SC, Liu DX, Greene LA (2005). Bim is a direct target of a neuronal E2F-dependent apoptotic pathway. J Neurosci 25: 8349–8358.

Bonfoco E, Krainc D, Ankarcrona M, et al. (1995). Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc Natl Acad Sci U S A 92: 72162–72166.

Budd SL, Tenneti L, Lishnak T, et al. (2000). Mitochondrial and extramitochondrial apoptotic signalling pathways in cerebrocortical neurons. Proc Natl Acad Sci U S A 97: 6161–6666.

Busch E, Gyngell ML, Eis M, et al. (1996). Potassium-induced cortical spreading depression during focal cerebral ischemia in rats: contribution to lesion growth assessed by diffusion-weighted NMR and biochemical imaging. J Cereb Blood Flow Metab 16: 1090–1099.

Chan PH (2001). Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21: 2–14.

Chen J, Nagayama T, Jin K (1998). Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J Neurosci 18: 4914–4928.

Clark AW, Krekoski CA, Bou SS, et al. (1997). Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. Neurosci Lett 238: 53–56.

Connolly ES Jr, Winfree CJ, Springer TA, et al. (1996). Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 97: 209–216.

Dalkara T, Moskowitz MA (1994). The complex role of nitric oxide in the pathophysiology of focal cerebral ischemia. Brain Pathol 4: 49–57.

Dawson VL, Dawson T (2004). Deadly conversations: nuclear-mitochondrial cross-talk. J Bioenerg Biomembr 36: 287–294.

Dewar D, Yam P, McCulloch J (1999). Drug development for stroke: importance of protecting cerebral white matter. Eur J Pharmacol 375: 41–50.

Dirnagl U, Iadecola C, Moskowitz MA (1999). Pathobiology of ischaemic stroke. An integrated view. Trends Neurosci 22: 391–397.

Dirnagl U, Simon RP, Hallenbeck JM (2003). Ischemic tolerance and endogenous neuroprotection. Trends Neurosci 26: 248–254.

Du C, Hu R, Csernansky CA, et al. (1996). Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J Cereb Blood Flow Metab 16: 195–201.

Eliasson MJL, Sampei K, Mandir AS, et al. (1997). Poly (ADP-ribose)polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 3: 1089–1095.

Endres M, Wang Z-Q, Namura S, et al. (1997). Ischemic brain injury is mediated by the activation of poly(ADP-ribose)polymerase. J Cereb Blood Flow Metab 17: 1143–1151.

Endres M, Scott GS, Namura S, et al. (1998a). Role of perosynitrite and neuronal nitric oxide synthase in the activation of poly(ADP-ribose)synthetase, against peroxynitrite-induced glial damage and stroke development. Neurosci Lett 248: 41–44.

Endres M, Laufs U, Huang Z, et al. (1998b). Stroke protection by 3-hydroxy-3 methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 95: 8880–8885.

Endres M, Namura S, Shimizu-Sasamata M, et al. (1998c). Attenuation of delayed neuronal death after mild focal cerebral ischemia in mice by inhibitors of the caspase family. J Cereb Blood Flow Metab 18: 238–247.

Endres M, Fink KB, Zhu J (1999). Neuroprotective effects of gelsolin during murine stroke. J Clin Invest 103: 347–354.

Endres M, Laufs U, Liao JK, et al. (2004). Targeting ENOS for stroke protection. Trends Neurosci 27: 283–289.

Endres M, Engelhardt B, Koistinaho J, et al. (2008). Improving outcome after stroke: translational research after SAINT-II. Cerebrovasc Dis 25(3): 268–278.

Erenciska M, Silver IA (1989). ATP and brain function. J Cereb Blood Flow Metab 9: 2–19.

Fink K, Zhu J, Namura S, et al. (1998). Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. J Cereb Blood Flow Metab 18: 1071–1076.

Fiskum G, Murphy AN, Beal MF (1999). Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases. J Cereb Blood Flow Metab 19: 351–369.

Frederickson CJ (1989). Neurobiology of zinc and zinc-containing neurons. Int Rev Neurobiol 31: 145–238.

Friedlander RM, Gagliardini V, Hara H, et al. (1997). Expression of a dominant negative mutant of interleukin-1 beta converting enzyme in transgenic mice prevents neuronal death induced by trophic factor withdrawal and ischemic brain injury. J Exp Med 185: 933–940.

Frijns CJM, Kappelle LJ (2002). Inflammatory cell adhesion molecules in ischemic cerebrovascular disease. Stroke 33: 2115–2122.

Fujimura M, Morita-Fujimura Y, Murakami K (1998). Cytosolic redistribution of cytochrome *c* after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 18: 1239–1247.

Fujimura M, Morita-Fujimura Y, Kawase M, et al. (1999). Manganese superoxide dismutase mediates the early release of mitochondrial cytochrome C and subsequent DNA fragmentation after permanent focal cerebral ischemia in mice. J Neurosci 19: 3414–3422.

Furukawa K, Fu W, Li Y (1997). The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. J Neurosci 17: 8178–8186.

Gasche Y, Fujimura M, Morita-Fujimura Y, et al. (1999). Early appearance of activated MMP-9 after focal cerebral ischemia in mice. J Cereb Blood Flow Metab 19: 1020–1028.

Ginsberg MD, Belayev L, Zhao W, et al. (1999). The acute ischemic penumbra: topography, life span, and therapeutic response. Acta Neurochir Suppl (Wien) 73: 45–50.

Goldberg MP, Ransom BR (2003). New light on white matter. Stroke 34: 330–332.

Green DR, Reed JC (1998). Mitochondria and apoptosis. Science 281: 1309–1312.

Greene LA, Biswas SC, Liu DX (2004). Cell cycle molecules and vertebrate neuron death: E2F at the hub. Cell Death Differ 11: 49–60.

Hafezi-Moghadam A, Simoncini T, Yang Z, et al. (2002). Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. Nat Med 8: 473–479.

Hansen AJ (1985). Effect of anoxia on ion disturbances in the brain. Physiol Rev 65: 101–148.

Hara H, Friedlander RM, Gagliardini V, et al. (1997). Inhibition of interleukin 1β converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A 94: 2007–2012.

Heo JH, Lucero J, Abumiya T, et al. (1999). Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab 19: 624–633.

Herrup K, Arendt T (2002). Re-expression of cell cycle proteins induces neuronal cell death during Alzheimer's disease. J Alzheimers Dis 4: 243–247.

Hoehn-Berlage M (1995). Diffusion-weighted NMD imaging: application to experimental focal cerebral ischemia. NMR Biomed 8: 345–358.

Huang Z, Huang PL, Panahian N, et al. (1994). Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 265: 1883–1885.

Huang Z, Huang PL, Ma J, et al. (1996). Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. J Cereb Blood Flow Metab 16: 981–987.

Iadecola C (1997). Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci 20: 132–139.

Iadecola C, Zhan F, Casey R, et al. (1997). Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci 17: 9157–9164.

Iijima T, Mies G, Hossmann KA (1992). Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: effect on volume of ischemic injury. J Cereb Blood Flow Metab 12: 727–733.

Kariko K, Weissman D, Welsh FA (2004). Inhibition of toll-like receptor and cytokine signaling—a unifying theme in ischemic tolerance. J Cereb Blood Flow Metab 24: 1288–1304.

Katchanov J, Harms C, Gertz C, et al. (2001). Mild cerebral ischemia induces loss of cyclin-dependent kinase inhibitors and activation of cell cycle machinery before delayed neuronal cell death. J Neurosci 21: 5045–5053.

Kerr JF, Wyllie AH, Currie AR (1972). Apoptosis: a basic biological phenomenon with wide-ranging implication in tissue kinetics. Br J Cancer 26: 239.

Kinouchi H, et al. (1991). Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. Proc Natl Acad Sci U S A 88: 11158–11162.

Kirino T (2002). Ischemic tolerance. J Cereb Blood Flow Metab 22: 1283–1296.

Kondo T, et al. (1997). Reduction of CuZn-superoxide dismutase activity exacerbates neuronal cell injury and edema formation after transient focal cerebral ischemia. J Neurosci 17: 4180–4189.

Kroemer G, Reed JC (2000). Mitochondrial control of cell death. Nat Med 6: 513–519.

Lapchak PA, Chapman DF, Zivin JA (2000). Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. Stroke 31: 3034–3040.

Letterio JJ (2000). Murine models define the role of TGF-beta as a master regulator of immune cell function. Cytokine Growth Factor Rev 11: 81–87.

Li Y, Chopp M, Jiang N, et al. (1995a). In situ detection of DNA fragmentation after focal cerebral ischemia in mice. Mol Brain Res 28: 164–168.

Li Y, Chopp M, Jiang N, et al. (1995b). Induction of DNA fragmentation after 10 to 120 min of focal cerebral ischemia in rats. Stroke 26: 1252–1258.

Lindsberg PJ, Carpen O, Paetau A, et al. (1996). Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. Circulation 94: 939–945.

Linnik MD, Zobrist RH, Hatfield MD (1993). Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. Stroke 24: 2002–2009.

Lipton P (1999). Ischemic cell death in neurons. Physiol Rev 79: 1431–1568.

Lo EH, Dalkara T, Moskowitz MA (2003). Mechanisms, challenges, and opportunities in stroke. Nat Rev Neurosci 4: 399–415.

Lo EH, Moskowitz MA, Jacobs TP (2005). Exciting, radical, suicidal. How brain cells die after stroke? Stroke 36: 189–192.

McDonald JW, Althomsons SP, Hyra KL, et al. (1998). Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor mediated excitotoxicity. Nat Med 4: 291–297.

MacManus JP, Buchan AM, Hill IE, et al. (1993). Global ischemia can cause DNA fragmentation indicative of apoptosis in rat brain. Neurosci Lett 164: 89–92.

Majno G, Joris I (1995). Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146: 3–15.

Martin RL, Lloyd HG, Cowan AI (1994). The early events of oxygen and glucose deprivation: setting the scene for neuronal death? Trends Neurosci 17: 251–257.

Martin-Villalba A, Herr I, Jeremias I, et al. (1999). CD95 ligand (Fas-L/APO-1L) and tumor necrosis factor-related apoptosis-inducing ligand mediate ischemia-induced apoptosis in neurons. J Neurosci 19: 3809–3817.

Matute C, Alberdi E, Ibarretxe G, et al. (2002). Excitotoxicity in glial cells. Eur J Pharmacol 447: 239–246.

Meisel C, Schwab JM, Prass K, et al. (2005). Central nervous system injury-induced immune deficiency syndrome. Nat Rev Neurosci 6: 775–786.

Mies G, Iijima T, Hossmann KA (1993). Correlation between peri-infarct DC shift and ischaemic neuronal damage in rat. Neuroreport 4: 709–711.

Moncayo J, de Freitas GR, Bogousslavsky J, et al. (2000). Do transient ischemic attacks have a neuroprotective effect? Neurology 54: 2089–2094.

Montaner J, Alvarez-Sabín J, Molina C, et al. (2001). Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 32: 1759–1766.

Namura S, Zhu J, Fink K, et al. (1998). Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. J Neurosci 18: 3659–3668.

Nedergaard M, Hansen AJ (1993). Characterization of cortical depolarizations evoked in focal cerebral ischemia. J Cereb Blood Flow Metab 13: 568–574.

Nicotera P, Lipton SA (1999). Excitotoxins in neuronal apoptosis and necrosis. J Cereb Blood Flow Metab 19: 583–591.

Nicotera P, Leist M, Fava E, et al. (2000). Energy requirement for caspase activation and neuronal cell death. Brain Pathol 10: 276–282.

Osuga H, Osuga S, Wang F, et al. (2000). Cyclin-dependent kinases as a therapeutic target for stroke. Proc Natl Acad Sci U S A 97: 10254–10259.

Paschen W (2000). Role of calcium in neuronal injury. Brain Res Bull 53: 409–413.

Pirttilä TR, Kauppinen RA (1992). Recovery of intracellular pH in cortical brain slices following anoxia studied by nuclear magnetic resonance spectroscopy: role of lactate removal, extracellular sodium and sodium/hydrogen exchange. Neuroscience 47: 155–164.

Prass K, Meisel C, Hoflich C, et al. (2003). Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation—reversal by poststroke T helper cell type 1-like immunostimulation. J Exp Med 198: 725–736.

Priller J, Fluger A, Wehner T, et al. (2001). Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat Med 7: 1356–1361.

Qiu J, Whalen MJ, Lowenstein P, et al. (2002). Upregulation of the Fas receptor death-inducing signaling complex after traumatic brain injury in mice and humans. J Neurosci 22: 3504–3511.

Rashidian J, Iyirharo G, Aleyasin H, et al. (2005). Multiple cyclin-dependent kinases signals are critical mediators of ischemic/hypoxic neuronal death in vitro and in vivo. Proc Natl Acad Sci U S A 102: 14080–14085.

Ratan RR, Siddiq A, Aminova L, et al. (2004). Translation of ischemic preconditioning to the patient: prolyl hydroxylase inhibition and hypoxia inducible factor-1 as novel targets for stroke therapy. Stroke 35: 2687–2689.

Ridker PM, Hennekens CH, Buring JE, et al. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342: 836–843.

Rosenberg GA, Navratil M (1997). Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 48: 921–927.

Ruscher K, Freyer D, Karsch M, et al. (2002). Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. J Neurosci 22: 10291–10301.

Salvesen GS (2001). A lysosomal protease enters the death scene. J Clin Invest 107: 21–22.

Schielke GP, Yang GY, Shivers BD, et al. (1998). Reduced ischemic brain injury in interleukin-1 beta converting enzyme-deficient mice. J Cereb Blood Flow Metab 18: 180–185.

Sheng H, Bart RD, Oury TD, et al. (1999). Mice overexpressing extracellular superoxide dismutase have increased resistance to focal cerebral ischemia. Neuroscience 88: 185–191.

Siesjo BK (1978). Brain energy metabolism and catecholaminergic activity in hypoxia, hypercapnia and ischemia. J Neuro Trans Suppl 14: 17–22.

Sorensen JC, Mattsson B, Andreasen A, et al. (1998). Rapid disappearance of zinc positive terminals in focal brain ischemia. Brain Res 812: 265–269.

Stanimirovich D, Satoh K (2000). Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation. Brain Pathol 10: 113–126.

Stroke Progress Review Group (2002). Report of the Stroke Progress Review Group. National Institute of Neurological Disorders and Stroke, Bethesda, MD, pp. 1–116.

Stys PK, Waxman SG, Ransom BR (1992). Effects of temperature on evoked electrical activity and anoxic injury in CNS white matter. J Cereb Blood Flow Metab 12: 977–986.

Susin SA, Lorenzo HK, Zamzami N, et al. (1999). Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397: 441–446.

Tanne D, Haim M, Boyko V, et al. (2002). Soluble intercellular adhesion molecule-1 and risk of future ischemic stroke: a nested case-control study from the Bezafibrate Infarction Prevention (BIP) study cohort. Stroke 33: 2182–2186.

Tarozzo G, Campanella M, Ghiani M, et al. (2002). Expression of fractalkine and its receptor, CX3CR1, in response to ischaemia-reperfusion brain injury in the rat. Eur J Neurosci 15: 1663–1668.

Timsit S, Rivera S, Ouaghi P, et al. (1999). Increased cyclin D1 in vulnerable neurons in the hippocampus after ischaemia and epilepsy: a modulator of in vivo programmed cell death? Eur J Neurosci 11: 263–278.

Tominaga T, Kure S, Narisawa K, et al. (1993). Endonuclease activation following focal ischemic injury in the rat. Brain Res 608: 21–26.

Trendelenburg G, Dirnagl U (2005). Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. Glia 50: 307–320.

Trendelenburg G, Prass K, Priller J, et al. (2002). Serial analysis of gene expression identifies metallothionein-II as major neuroprotective gene in mouse focal cerebral ischemia. J Neurosci 22: 5879–5888.

Van Lookeren Campagne M, Gill R (1996). Ultrastructural morphological changes are not characteristic of apoptotic cell death following focal cerebral ischaemia in the rat. Neurosci Lett 213: 111–114.

Wang F, Corbett D, Osuga H, et al. (2002). Inhibition of cyclin-dependent kinases improves CA1 neuronal survival and behavioral performance after global ischemia in the rat. J Cereb Blood Flow Metab 22: 171–182.

Wang J, Liu S, Fu Y, et al. (2003). Cdk5 activation induces hippocampal CA1 cell death by directly phosphorylating NMDA receptor. Nat Neurosci 6: 1039–1047.

Wei MC, Zong WX, Cheng EH, et al. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292: 727–730.

Weih M, Kallenberg K, Bergk A, et al. (1999). Attenuated stroke severity after prodromal TIA: a role for ischemic tolerance in the brain? Stroke 30: 1851–1854.

Weiss JH, Hartley DM, Koh JY, et al. (1993). AMPA receptor activation potentiates zinc neurotoxicity. Neuron 10: 43–49.

Wender R, Brown AM, Fern R, et al. (2000). Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. J Neurosci 20: 6804–6810.

Wolf T, Lindauer U, Reuter U, et al. (1997). Noninvasive near-infrared spectroscopy monitoring of regional cerebral blood oxygenation changes during peri-infarct depolarizations in focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 17: 950–954.

Yrjanheikki J, Tikka T, Keinanan R, et al. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 96: 13496–13500.

Yuan J, Yanker BY (2000). Apoptosis in the nervous system. Nature 407: 802–809.

Zhang K, Sejnowkoski TJ (2000). A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A 97: 5621–5626.

Zhang J, Dawson VL, Dawson TM (1994). Nitric oxide activation of poly(ADP-ribose)synthetase in neurotoxicity. Science 263: 687–689.

Zipfel GJ, Lee JM, Choi DW (1999). Reducing calcium overload in ischemic brain. N Engl J Med 341: 1543–1544.