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Review

Mitochondria, oxidative metabolism and cell death in stroke

Neil R. Sims *, Hakan Muyderman

Centre for Neuroscience and Discipline of Medical Biochemistry, Flinders Medical Science and Technology, School of Medicine, Flinders University, Adelaide, South Australia, Australia

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ABSTRACT

Stroke most commonly results from occlusion of a major artery in the brain and typically leads to the death of all cells within the affected tissue. Mitochondria are centrally involved in the development of this tissue injury due to modifications of their major role in supplying ATP and to changes in their properties that can contribute to the development of apoptotic and necrotic cell death. In animal models of stroke, the limited availability of glucose and oxygen directly impairs oxidative metabolism in severely ischemic regions of the affected tissue and leads to rapid changes in ATP and other energy-related metabolites. In the less-severely ischemic “penumbral” tissue, more moderate alterations develop in these metabolites, associated with near normal glucose use but impaired oxidative metabolism. This tissue remains potentially salvageable for at least the first few hours following stroke onset. Early restoration of blood flow can result in substantial recovery of energy-related metabolites throughout the affected tissue. However, glucose oxidation is markedly decreased due both to lower energy requirements in the post-ischemic tissue and limitations on the mitochondrial oxidation of pyruvate. A secondary deterioration of mitochondrial function subsequently develops that may contribute to progression to cell loss. Mitochondrial release of multiple apoptogenic proteins has been identified in ischemic and post-ischemic brain, mostly in neurons. Pharmacological interventions and genetic modifications in rodent models strongly implicate caspase-dependent and caspase-independent apoptosis and the mitochondrial permeability transition as important contributors to tissue damage, particularly when induced by short periods of temporary focal ischemia.

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1. Introduction

Stroke is the primary cause of adult disability in developed countries and ranks only behind cancer and cardiac disease as a cause of death [1,2]. Focal ischemia that results from occlusion of an artery in the brain (ischemic stroke) accounts for more than 80% of all strokes [1]. Unless rapidly reversed, the occlusion of a major artery usually produces tissue infarction, in which affected parts of the brain exhibit a non-selective loss of all cells including neurons, astrocytes, oligodendrocytes, microglia and endothelial cells. The size and location of these infarcts are important determinants of the long-term functional deficits resulting from ischemic stroke. Mitochondria have been implicated as central players in the development of ischemic cell death both through impairment of their normal role in generating much of the ATP for neural cell function and as key mediators in cell death pathways. This article reviews the current understanding of the mitochondrial responses to focal cerebral ischemia and the contributions of these organelles to tissue damage. Additional aspects of this topic and further discussion of some of the earlier studies can be found in previous reviews [3–6].

2. Tissue damage in response to ischemic stroke

Occlusion of a major artery within the brain produces complex cellular changes that depend in part on the severity of the ischemia that is generated and whether the occlusion is temporary or permanent. Because of the limited overlap in the perfusion territories of cerebral arteries, severe ischemia develops in the tissue immediately surrounding the occluded vessel. Blood flow usually falls to less than 20% of normal in this “core” or “focal” tissue [7–9]. The resultant disruption to delivery of glucose and oxygen leads to a greatly reduced ATP generation (see section 4.1.1). Ionic gradients across the plasma membrane quickly dissipate resulting in marked losses of intracellular potassium and large shifts of calcium into cells [2,10,11]. Because of contributions to perfusion from adjacent vessels, a lesser ischemia develops in tissue surrounding the core. This “penumbral” or “perifocal” tissue typically exhibits reductions to approximately 20–40% of normal flow [8,9,12]. Neurons in the penumbra are electrically silent for long periods, a response associated with hyperpolarization of the plasma membrane [8,12]. (Note: For simplicity, the term penumbra has been

Abbreviations: AIF, apoptosis inducing factor; AKAP121, A-kinase anchor protein 121; APAF-1, apoptotic protease activating factor 1; IAPs, inhibitor-of-apoptosis proteins; JNK, c-Jun N-terminal kinase; MCA, middle cerebral artery; Omi/HtrA2, Omi stress-regulated endoprotease/high temperature requirement protein A2; PARP, poly-ADP ribose polymerase; Smac/DIABLO, second mitochondria-derived activator of caspase/direct IAP-binding protein of low pI; t-Bid, truncated Bid

* Corresponding author. Discipline of Medical Biochemistry, School of Medicine, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia. Tel.: +61 8 8204 4242; fax: +61 8 8374 0139.
E-mail address: Neil.Sims@flinders.edu.au (N.R. Sims).

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used throughout this review to describe tissue identified as receiving moderate ischemia, even though criteria other than direct measurement of blood flow or plasma membrane potential have commonly been used.)

Following permanent arterial occlusion, infarcts initially develop in the core tissue but progress to encompass both core and penumbral regions [13,14]. The differences in the severity of the ischemia in the core and penumbra mean that different mechanisms contribute to cell death. Much of our understanding of the cellular changes induced by focal ischemia comes from animal models of stroke and from the effects of pharmacological treatments or genetic modifications on the damage that develops. Permanent or temporary occlusion of the middle cerebral artery (MCA) in rats or mice has commonly been used for these investigations. The present review focuses primarily on insights derived from such animal models into changes in energy metabolism and mitochondrial properties within the first few hours of stroke and their involvement in cell death.

Many interventions during the initial few hours following the onset of stroke in these models can reduce infarct volume, particularly in the penumbra [2,15]. Thus, irreversible damage develops relatively slowly in this region. Consistent with this conclusion, early restoration of blood flow can reduce the tissue damage and functional deficits in animal models of stroke [16,17] and following a stroke in humans [13,18]. Indeed, treatment with a thrombolytic agent to reverse arterial occlusion within the first 3 h following stroke onset provides the only approach in routine clinical use for limiting the acute effects of this disorder in humans [13,18]. Unfortunately, because of the narrow therapeutic window, only a small proportion of those affected by stroke are currently treated with thrombolysis. Spontaneous reversal of arterial occlusion occurs within the first 6 h in approximately 17% of ischemic stroke patients and in approximately 40–50% by 4 days [19]. Reperfusion beginning later than 6 h probably has limited effects on the tissue damage that develops. Thus, animal models involving permanent arterial occlusion, although less commonly investigated than temporary occlusion, are likely to be more relevant to the majority of ischemic stroke cases in humans.

Treatments targeting a diverse range of cellular properties have been found to ameliorate tissue damage and improve functional deficits in animal models of stroke [2,15]. This suggests that the mechanisms for cell loss involve interactions between multiple deleterious processes such that initial changes in one of these processes leads to more severe alterations in others. Interruption of one of these initial responses is apparently able to disrupt, or in some instances greatly delay, the spiral of increasingly abnormal changes that culminate in cell death. Intriguingly, treatments that reduce damage, including those targeting properties of specific cell populations, preserve essentially all cells in the tissue that is salvaged. A smaller but still well-demarcated infarct results. Such a uniform demise of different cell populations is not seen with many other insults and suggests a close interdependence of the different cell populations in their responses to ischemia.

Early studies demonstrated that pharmacological blockade of ionotropic glutamate receptors markedly reduced ischemic damage [2,15,20]. This effect is commonly ascribed to the involvement of an excitotoxic process in which increases in glutamate release from neurons and astrocytes induced by the ischemia cause an excessive calcium entry via these receptors and triggers other intracellular changes leading to cell death. However, alternative mechanisms have also been suggested to explain the protection by glutamate receptor antagonists, including interference with the propagation of potentially harmful spreading depression-like depolarizations that develop in the penumbral tissue during arterial occlusion [15,21]. Abnormal intracellular calcium accumulations arising from calcium entry via ion channels or transporters in addition to the ionotropic glutamate receptors have also been implicated in triggering cell death [20].

Other changes identified as important in ischemia-induced cell loss include oxidative stress, particularly involving nitric oxide and peroxynitrite, and abnormal activation of enzymes such as poly-ADP ribose polymerase (PARP) and the calpains [2,15]. Early reperfusion can limit the effects of some of these changes but also adds to the complexity of the cellular responses that develop. Oxidative stress is promoted under these conditions and inflammatory responses, arising both from resident microglia and astrocytes as well as blood-derived cells, also become important [2,15].

3. The nature of cell death in focal ischemia

Cell death resulting from cerebral ischemia was originally considered to be almost exclusively due to the process of necrosis in which catastrophic events initiated by ischemia led to cellular changes culminating in organelle swelling, disruption of the plasma membrane and release of intracellular contents. These features of cell death are usually seen in the vast majority of cells throughout the developing infarct [22]. Nonetheless, a more complex picture began to emerge in the mid-1990's with the identification of cells that exhibited features of apoptosis, including DNA fragmentation and the production of membrane enclosed apoptotic bodies [23,24]. Such changes are common features of cell death mediated by the activation of caspases either via the “intrinsic pathway” or the “extrinsic pathway” [3,25,26]. Mitochondrial changes resulting in the release of proteins are central to the intrinsic pathway (Fig. 1). These proteins lead to the activation of caspases, particularly caspase-3 in brain, which in turn induces cellular changes including internucleosomal chromatin condensation and DNA fragmentation [3,25–27]. The role of the intrinsic pathway in focal ischemia is discussed in section 5.2. The extrinsic pathway is triggered by the binding of specific ligands to plasma membrane cell death receptors. This leads to intracellular activation of caspase-8 and then of executioner caspases involved in cell death. In the extrinsic pathway, the executioner caspase activation can occur without involvement of mitochondria [3,26]. However, caspase-8 activation can also cleave Bid to produce truncated Bid (t-Bid), which initiates the release of apoptogenic proteins from mitochondria under some conditions. Caspase-independent forms of apoptosis (Fig. 1), which result from mitochondrial release of apoptosis inducing factor (AIF) and perhaps other proteins [3,25,26], have also been implicated in focal ischemic damage (see section 5.3).

Cells exhibiting features of apoptosis typically peak in number at 24 h or longer after stroke onset [23,24,28]. They are found scattered throughout the infarct following temporary or permanent occlusion but are more prominent in tissue subject to less severe ischemia within the penumbra and are more numerous in brains subjected to temporary ischemia lasting up to 2 h [23,24,28–30]. This form of cell loss shows a closer association with tissue that is potentially salvageable and has attracted particular attention as a target for neuroprotective therapies.

Attempts to characterize the mechanisms underlying ischemic cell loss have been further complicated with the recognition that necrosis also often involves specific patterns of cellular change that can develop over many hours. Furthermore, these responses can be highly regulated, or even programmed, and are potentially modifiable [26,31,32]. Alterations contributing to cerebral ischemic damage, including increased intracellular calcium and oxidative stress, have been identified as potential players in necrotic-like programmed cell death. However, the extent to which such a programmed form of necrosis might contribute to focal ischemic damage is not currently known.

4. Mitochondrial function and ATP generation during cerebral ischemia and reperfusion

Mitochondrial function in focal ischemia is altered as a direct consequence of the impaired delivery of glucose and oxygen to the

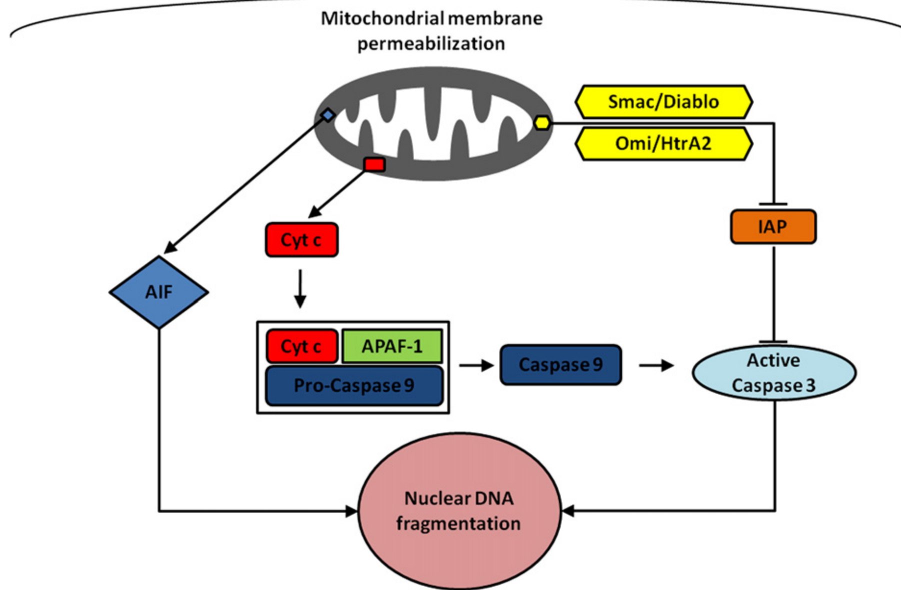

Fig. 1. Proteins released from the intermembrane space that can contribute to the development of apoptosis. The release of cytochrome *c* is a key step in the intrinsic pathway of apoptosis. This forms a complex known as an apoptosome with the proteins APAF-1 and procaspase-9 and with dATP. Apoptosome formation leads to activation of executioner caspases, particularly caspase-3, that results in internucleosomal protein degradation and other cellular changes. This process can be promoted by the release of Smac/DIABLO and Omi/HtrA2 that act to block IAPs, a family of proteins that are endogenous inhibitors of caspase-3 and other caspases. The release of AIF leads to a caspase-independent form of apoptosis. AIF interacts with other proteins to also produce DNA degradation, generating larger fragments of DNA than the caspase-dependent pathway. An interaction of AIF with cyclophilin A is essential for the development of this process, at least under some conditions. Endonuclease G, another protein released from the mitochondria, might also be involved in this process.

tissue and is further modified by changes in mitochondrial properties that develop during ischemia or following reperfusion. Limitations in the ability of cells to generate ATP can exacerbate the cellular response to other insults and can greatly influence the cell death pathways that develop. Thus, an understanding of the pattern of changes in cellular energy metabolism is essential to fully elucidate the mechanisms leading to tissue damage in ischemia. Furthermore, because the generation of ATP requires the integration of complex metabolic processes, the characterization of energy-related metabolites and of the pathways involved in their production provides a useful indicator of both the extent of preservation of essential cellular functions and the extent of cell damage or cell death in the tissue.

4.1. Energy metabolism during ischemia

Table 1 summarizes alterations in the content of energy-related metabolites and in the contributing metabolic pathways in brain tissue during ischemia and following reperfusion. An overview of the relevant metabolic processes is provided in Fig. 2, which further highlights the changes that develop in penumbral tissue during the initial 2–3 h of focal ischemia.

4.1.1. Core tissue

Within the severely ischemic core, the large reductions in blood flow lead to impaired delivery of oxygen and glucose and a mismatch between ATP use and production. Glucose and ATP content falls markedly during the first 5 min or so of arterial occlusion [33]. ATP stabilizes at values approximately 15–30% of those in non-ischemic tissue for at least the first 2 h of focal ischemia [33–36]. The initial rapid decrease in ATP content is associated with the major redistribution of ions across the plasma membrane of cells [10,11] and probably triggers this response [37]. The adenylate energy charge ([ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP]) which measures the intracellular balance between ATP, ADP and AMP is also rapidly decreased and is maintained at values of approximately 0.4–0.5 during the initial hours of focal ischemia, much lower than values in normal brain (of approximately 0.93) [33–35].

Phosphocreatine in brain tissue provides a short-term energy reserve, allowing ATP to be regenerated from ADP in a near-equilibrium reaction catalysed by creatine kinase. Phosphocreatine shows a similar pattern of change to ATP, rapidly falling to values less than 30% of normal [33–36]. Limitations in the availability of oxygen ensure that some of the glucose that does reach core tissue is metabolised via glycolysis to lactate with an associated decrease in pH. Lactate accumulates to values more than 10-fold that of non-ischemic tissue [34,36]. Reduced removal of the lactate because of the limited blood flow probably also contributes to this increase.

4.1.2. Penumbral tissue

A pattern of changes in energy metabolites similar to that in the ischemic core develops in the penumbral tissue but the alterations are

Table 1
The effects of focal ischemia for up to 2 h and of subsequent reperfusion for 1 h on the content of energy-related metabolites and pathways of energy metabolism.

	Focal ischemia		Reperfusion	
	Core	Penumbra	Core	Penumbra
Metabolites				
ATP	↓↓↓	↓↓	↓↓	↓
Adenylate energy charge	↓↓	↓	↓/N.C.	N.C.
Total adenine nucleotides	↓↓	↓↓	↓↓	↓
Phosphocreatine	↓↓↓	↓	↓	N.C.
Lactate	↑↑	↑↑	↑↑	↑/N.C.
Glucose	↓↓↓	N.C.	N.C.	N.C.
Metabolic activity				
Glucose use	↓↓↓*	N.C.	↓↓	↓↓
Oxidative metabolism	↓↓↓*	↓↓↓	↓↓	↓↓

Differences are shown compared to non-ischemic tissue. ↓: decreased to >65%; ↓↓: decreased to between 35% and 65% ; ↓↓↓: decreased to less than 35%; ↑: increased less than four-fold; ↑↑: increased greater than four-fold; N.C.: no significant change. Two symbols indicate findings that differ between published reports. *, direct evaluation of these properties in severely ischemic tissue may not give reliable information. The magnitude of these reductions is assumed from the large decrease in substrate delivery and large changes in ATP and phosphocreatine content.
Information adapted from results presented in [9,13,33–36,39–41,45,46].

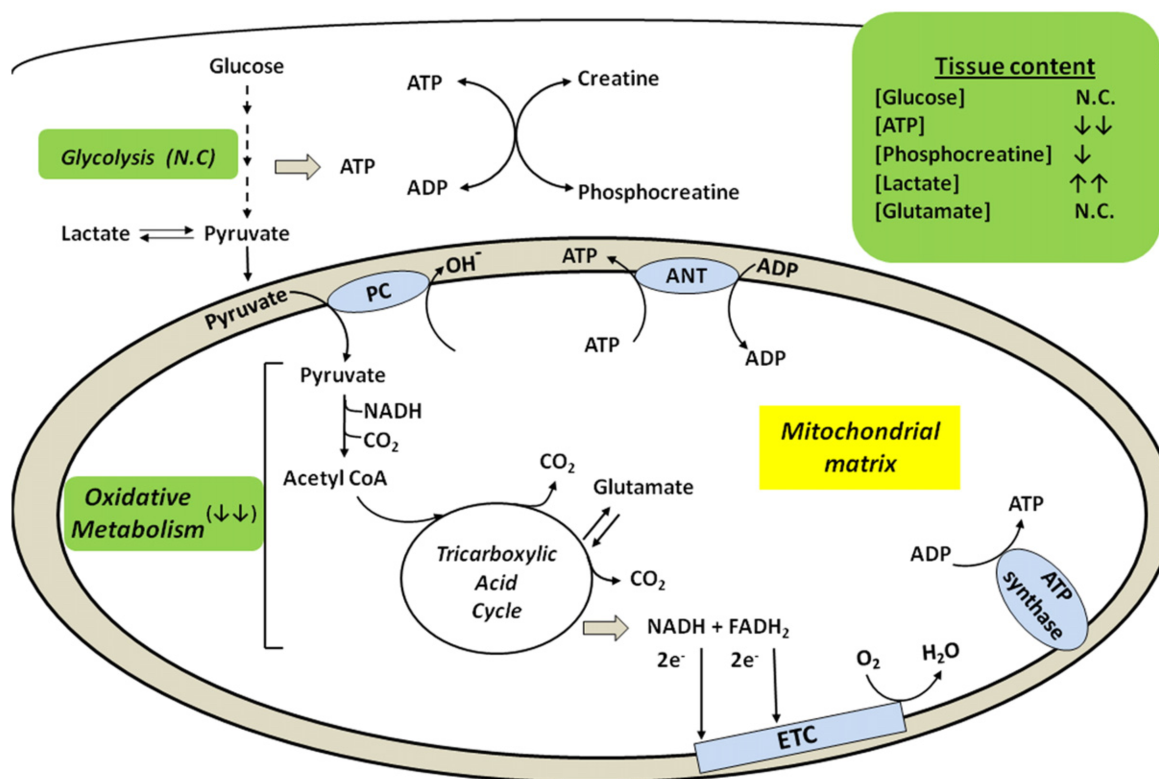

Fig. 2. Overview of the pathways of energy metabolism in cells in the brain, highlighting the major changes seen in these metabolic pathways and energy-related tissue metabolites in the penumbra within the first 2 h of stroke onset. In animal models of stroke, most cells in the penumbra remain viable during this initial period of focal ischemia and can survive if there is early restoration of blood flow or treatments that block other cellular changes critical for cell death. A single arrow indicates a moderate change and a double arrow a large change. N.C., no significant change from control values; ANT, adenine nucleotide translocase; ETC, electron transport chain; PC, pyruvate carrier. Information on metabolic status adapted from results presented in [9,13,33–36,39–41].

less severe (Table 1, Fig. 1). After 2 h of ischemia, phosphocreatine is reduced to approximately 70% of non-ischemic values and the adenylate energy charge remains above 0.8 [34]. Larger decreases are seen in ATP, with values approaching 50% of non-ischemic tissue at 2 h of ischemia [34]. Under ischemic conditions, some of the ADP generated from ATP hydrolysis is further metabolised to AMP and ATP [38]. This reaction, catalysed by adenylate kinase, normally helps to maintain ATP and meet short-term energy demands in the brain. In ischemic tissue, the AMP is further converted to inosine and hypoxanthine resulting in overall depletion of the adenine nucleotide pool [38]. The greater severity of the ATP loss compared with changes in other energy-related metabolites is to a large extent explained by this decrease in the total adenine nucleotide pool.

Surprisingly, glucose utilization in penumbral tissue as assessed from metabolism of deoxyglucose is unchanged or even increased during the first 2 h of ischemia [9,13]. An increase in glucose extraction from the blood helps to largely preserve the tissue glucose content [33,39] contributing to the maintenance of glycolytic activity. There is an increase in lactate within the penumbra to values many times higher than in normal brain but less than in the core regions [34,36]. The accumulation of lactate suggests that oxygen delivery is more severely restricted than that of glucose, resulting in impaired oxidative metabolism of pyruvate by the mitochondria. Magnetic resonance spectroscopic analysis of the products of isotopically labelled glucose provides support for this conclusion [40,41]. In particular, these studies show reductions of more than 50% in the generation of isotopically labelled glutamate from glucose. The total glutamate content in the tissue is unchanged at this time. The incorporation of label from glucose into glutamate, which occurs predominantly in neurons [41–43], requires generation of pyruvate via glycolysis, metabolism of pyruvate to α-ketoglutarate via the tricarboxylic acid cycle and subsequent conversion to glutamate (Fig. 2). Some caution is needed in interpreting these findings because of the possible confounding effects of ischemia-induced changes in the size of intermediate metabolite pools as well as any moderate changes that may exist in delivery of the labelled precursors. Nonetheless, the results strongly suggest markedly reduced oxidative metabolism of glucose in the penumbral tissue in contrast to the preservation of glycolysis.

The reduced oxidative metabolism in penumbral tissue means that there is unlikely to be reserve capacity to deal with increases in energy requirements. This conclusion is supported by comparisons of the metabolic response to spreading depression-like depolarizations that develop in the penumbra compared with KCl-induced spreading depression in normal brain [44]. Similar proportional decreases in ATP and phosphocreatine are induced by the advancing depolarization of the tissue in both situations. However, the penumbral tissue shows much larger increases in lactate and a greatly reduced ability to restore the ATP and phosphocreatine content. These changes limit the capacity of the tissue to reverse changes initiated by the redistribution of ions during the spreading depression, increasing the likelihood of deleterious consequences.

4.2. Energy metabolism following reperfusion

4.2.1. Core tissue

The greatly impaired production of ATP, the major redistribution of ions and derangement of other metabolic properties in the ischemic core are incompatible with cell survival if they are not rapidly reversed. However, the initial development of these changes within core tissue during the first 5 min or so of arterial occlusion does not immediately produce irreversible cellular deterioration. Restoration of blood flow during the first 30 to 60 min in rats and mice greatly limits the size of the infarcts that subsequently develop [16,17] and can completely block cell loss, indicating that the development of

irreversible damage in neurons and other cells in the core tissue is delayed. Even with ischemic periods up to 3 h, which will ultimately lead to infarction, there is often near-complete recovery of phosphocreatine (to more than 90% of pre-ischemic values) and of the adenylate energy charge (or other measures of adenine nucleotide balance) during the first 2 h following reperfusion [34,36,39,45,46]. The recovery of these metabolic parameters requires the presence of intact and functional cells that at least partially regain the complex metabolic activities and control processes required to meet energy demands. The concentration of ATP in core tissue recovers more slowly than phosphocreatine or the adenylate energy charge following restoration of blood flow, reaching 50–70% of control values within the first hours. This ongoing decrease in ATP is mostly due to the slow resynthesis of the adenine nucleotide pool that was depleted during ischemia, with lesser contributions from an imbalance between ATP use and production.

With further perfusion following longer ischemic periods that are sufficient to initiate infarct formation, the core tissue typically exhibits a secondary decline in energy-related metabolites that is most likely associated with final progression to the death of many cells [34,39]. Such cell loss in the core tissue is generally an inevitable consequence of ischemic periods lasting more than an hour but might not be irreversibly determined in all parts of this tissue at the onset of reperfusion. Large reductions in infarct size (exceeding 50%) have been achieved with some treatments initiated early after reperfusion following ischemic periods of at least 2 h (e.g., [47–50]). Although the penumbra is the predominant site of this protection, the magnitude of the effects suggests that parts of the core tissue are also salvaged. The initial near-complete restoration of energy metabolites on reperfusion is consistent with the possibility that cells in parts of the core can still be rescued. When temporary arterial occlusion exceeds 3 h, reperfusion results in less complete restoration of energy metabolites and a more rapid onset of secondary deterioration in the core regions, indicating that many cells are much more severely compromised at the time of reperfusion [39].

The substantial restoration of the content of energy-related metabolites is not an indication of comparable recovery of the activity of energy-generating metabolism. At 1 h of reperfusion following 2 h of ischemia in rats, glucose utilization, as assessed from deoxyglucose incorporation, is reduced to approximately 50% of normal values in tissue regions that formed the ischemic core [9]. Furthermore, the lactate content remains many times higher than in normal tissue suggesting ongoing restrictions on the oxidative metabolism of pyruvate [34,36,39,45,46]. Consistent with these results, the generation of isotopically labelled glutamate from glucose is also markedly decreased [41,46] indicating greatly reduced neuronal energy metabolism.

4.2.2. Penumbral tissue

Reperfusion in the penumbral tissue leads to complete or near-complete recovery of phosphocreatine and the adenine nucleotide balance within the first hour of reperfusion following ischemic periods lasting up to 3 h or even longer [34,39,45,46]. The restoration of ATP content is less complete, again because of slow regeneration of the depleted adenine nucleotide pool. A secondary disruption of energy metabolites associated with gross disruption of cellular function typically occurs at 6 or more hours after the initiation of reflow [39]. The timing of these changes is broadly consistent with the finding in human stroke that damage can be reduced and function improved if flow is restored within 3 h and perhaps after longer periods [8,18].

As in the core tissue, the onset of reperfusion leads to reductions (by approximately 40%) in glucose use (assessed using deoxyglucose) in the penumbra within the first hour, a finding which contrasts with the preservation of this activity during ischemia [9]. Even larger reductions in the oxidative metabolism of glucose develop in this tissue within the first hour of reperfusion, as determined from the incorporation of isotopic label from glucose into glutamate [41,46]. Lactate is substantially decreased compared with the large accumulations during ischemia. However, it usually remains significantly elevated following ischemic insults of sufficient duration to generate infarcts encompassing the former penumbral tissue [34,46] (but see also [36,41]), consistent with ongoing restrictions in pyruvate oxidation. Despite the size of the reductions in oxidative glucose metabolism, this change is not inevitably associated with the development of infarcts. Similar metabolic decreases are also seen with shorter ischemic periods that generally do not result in infarcts within the reperfused penumbral tissue [46].

As indicated previously, the isotopic labelling of glutamate from glucose primarily (but not exclusively) reflects neuronal metabolism and provides one of only a few measures available to evaluate aspects of metabolism selectively in this cell population [41–43]. A selective measure of the oxidative metabolism of astrocytes in the brain can also be obtained based on the incorporation of isotopic label from acetate to glutamine [41–43]. The selectivity results from the ability of glia but not neurons to take up acetate and convert it to acetyl CoA [51] and from the almost exclusive localization of the enzyme catalysing glutamine production (glutamine synthetase) in astrocytes [52]. The production of isotopically labelled glutamine from acetate is unchanged or even increased in the penumbra compared with normal tissue during the first hour following reperfusion [41,53]. This measure of metabolic activity continues to be nearly fully preserved for at least 4 h in reperfused penumbral tissue that is destined to become infarcted [53]. These results indicate that the majority of astrocytes remain viable in this tissue and preserve important features of oxidative metabolism for extended periods following temporary ischemia.

The finding of greatly reduced glucose conversion to glutamate but well-preserved astrocytic acetate metabolism could possibly indicate large differences in the functional preservation of astrocytes compared with neurons during early reperfusion of penumbral tissue. Alternatively, the limitations on glucose metabolism might be similar in the two cell populations but the decrease in glucose metabolism results from the effects of cellular controls or other changes decreasing activity at steps prior to acetyl CoA. If so, these restrictions on metabolism would be by-passed by the use of acetate as the metabolic precursor. The latter explanation is supported by analyses using magnetic resonance spectroscopy of the labelling patterns of individual carbons in glutamate and glutamine following injection of ^{13}C-glucose [41]. This approach allows a comparison of carbons entering the tricarboxylic acid cycle via the anaplerotic reaction catalysed by pyruvate carboxylase, which is specific to astrocytes, and those entering via pyruvate dehydrogenase, which occurs in neurons and astrocytes. The proportional contribution of these two pathways is similar in reperfused penumbral tissue and in normal tissue [41]. Thus, glucose metabolism is apparently similarly affected in both cell types following reperfusion of the penumbral tissue.

The near-complete restoration of phosphocreatine and the adenylate energy charge following reperfusion in the penumbra despite marked decreases in activity of the relevant ATP-generating metabolic pathways from glucose implies that energy-requiring functions are greatly reduced in the post-ischemic brain. This could arise partly from decreases in neuronal activity that are induced during ischemia and persist for long periods in post-ischemic brain [54,55]. A further likely major contributor to reductions in energy use in post-ischemic brain is the enzyme, AMP-activated protein kinase. This kinase is activated by many stimuli including ATP depletion and other changes produced by cerebral ischemia [56]. It induces multiple cellular alterations that reduce anabolic reactions and help to restore energy balance. The activity of AMP-activated protein kinase is increased by phosphorylation. Long-lasting increases in phosphorylated AMP-activated protein kinase are seen following reperfusion throughout the brain, including tissue from the ischemic penumbra, non-ischemic tissue and, to a lesser extent, core tissue [56,57]. Interestingly, inhibition of AMP-activated kinase initiated at the time of

occlusion or during early reperfusion reduces infarct volume whereas an activator of this enzyme causes increased damage [57,58]. Protection is also seen in genetically modified mice that do not express the α2 isoform of AMP-activated protein kinase [58]. Thus, changes leading to activation of this enzyme are deleterious in post-ischemic brain even though they can be protective following a more mild insult.

4.3. Other mitochondrial changes during ischemia and reperfusion

The capacity of mitochondria for respiratory activity has been evaluated based on oxygen utilization in preparations from brain tissue removed during focal ischemia and following reperfusion. For samples obtained during ischemia, there is a progressive deterioration of the ability of the mitochondria to increase activity of the electron transport chain in response to decreases in the proton gradient across the inner mitochondrial membrane induced by the addition of ADP or an uncoupling agent [16,59,60]. Basal respiration is largely preserved. By 2 h of MCA occlusion in rats, the ADP-stimulated or uncoupled respiration decreases by 45–60% in samples from core tissue. Reductions of 15–40% are seen in the penumbra. This reduced respiratory capacity in the penumbral mitochondria could exacerbate the effects of lower oxygen delivery and contribute to the decreases in oxidative metabolism in this region.

As is the case for energy metabolites, mitochondrial respiratory function is generally completely or near-completely restored in samples prepared from core and penumbral tissue within the first hour following reperfusion [16,59,60] but then declines at later times [59,60]. Interestingly, this secondary deterioration in mitochondrial respiration apparently develops earlier than the changes in energy-related metabolites when assessed in the same ischemic model [34,59], suggesting that the delayed alterations in mitochondrial function are an early step in the development of irreversible cell dysfunction and possibly a contributor to this process. As seen with the energy-related metabolites, there is less restoration of mitochondrial function in core tissue evaluated during early reperfusion following longer periods of ischemia [16]. Mitochondria isolated from this core tissue also exhibit a lower membrane potential when incubated under basal or ADP-stimulated conditions, providing further evidence of a decreased capacity for ATP generation in these organelles [61]. Electron micrographs of brain tissue at 2 h after reversal of a 3 h period of focal ischemia have revealed marked structural abnormalities of mitochondria in neurons [62], consistent with the functional deterioration seen under similar conditions in other studies. Interestingly, the structural alterations following reperfusion resembled those generated by much longer periods of permanent ischemia.

Although there are many potential mechanisms for disease-induced impairments of respiratory function and membrane potential in brain mitochondria [6], the molecular processes underlying the changes induced by ischemia and reperfusion are not known. Alterations in respiratory function are not explained by decreased activity of individual components of the electron transport chain as these are essentially fully preserved during ischemia and reperfusion [63]. Some subtle alterations in activities of these respiratory chain complexes in localised parts of the core tissue have been detected by immunohistochemical techniques [64]. These are most obvious at 4 h following reperfusion and may be associated with the development of early pockets of secondary deterioration in mitochondrial function.

Ischemia-induced changes in A-kinase anchor protein 121 (AKAP121) is a possible contributor to the altered mitochondrial properties. This protein was recently found to be degraded in neurons during focal cerebral ischemia [65], albeit in samples obtained at 24 h of permanent ischemia when interpretation is complicated by the advanced state of tissue damage. AKAP121 forms a complex on the outer mitochondrial membrane that is involved in inducing changes in mitochondrial functions in response to intracellular signalling pathways. Of particular interest, degradation of AKAP121 results in impaired oxidative metabolism and decreased membrane potential in the mitochondria [65]. Further studies are needed to assess the effects of shorter ischemic periods and of reperfusion on AKAP121 to evaluate its possible role in altered mitochondrial respiration.

5. Mitochondria and cell death pathways

The major deficits in mitochondrial ATP production in severely ischemic core tissue create conditions that ensure the development of necrotic death in all cells within a few hours of stroke onset. This tissue provides no prospects for promoting cell survival unless there is early reperfusion. The milder metabolic deficits in the penumbra allow longer survival of the cells and provide opportunities for protective interventions. Nonetheless, in the absence of reperfusion or other treatments, metabolic changes arising from the limited availability of substrates for oxidative metabolism and probably also from progressive deterioration of mitochondrial function again contributes to the demise of cells in this region. Other mitochondrial changes, including induction of the permeability transition and the release of proteins that trigger apoptosis, have also been implicated in tissue damage, particularly in the reperfused penumbra as discussed in the following sections.

5.1. The mitochondrial permeability transition and ischemic cell death

The mitochondrial permeability transition results from opening of a pore in the inner mitochondrial membrane that is non-selectively permeable to solutes smaller than 1.5 kDa [66–68]. Transition pore opening is usually induced by abnormal accumulations of calcium and can be promoted by multiple factors including depletion of adenine nucleotides and oxidative stress. Despite intensive research, the composition of the permeability transition pore remains incompletely understood. Three proteins have been strongly implicated: the adenine nucleotide translocase in the inner mitochondrial membrane, the voltage-dependent anion carrier in the outer membrane and cyclophilin D, a matrix protein with peptidyl-prolyl cis-trans isomerase activity [66–68]. Studies in mice that do not express cyclophilin D have confirmed a key role for this protein in development of the transition pore, although this component may be unnecessary for pore opening at very high calcium concentrations [69–71]. However, some doubts have been raised about the role of the adenine nucleotide translocase because mitochondria from mice lacking expression of the major isoforms of this protein can still develop a permeability transition [67,72].

Involvement of the permeability transition in ischemic cell death was initially suggested by the ability of cyclosporin A to produce dramatic reductions in tissue infarction (by 65–90%) when given immediately following reperfusion [49,73,74]. Cyclosporin A inhibits transition pore opening by binding to cyclophilin D. Interpretation of this result is not straightforward because cyclosporin A also has other effects that might have contributed to the observed protection including inhibition of the protein phosphatase, calcineurin. FK506, a compound that inhibits calcineurin but does not block the permeability transition, can also decrease infarct volume [49,75]. Nonetheless, impressive protection has been demonstrated when derivatives of cyclosporin A that do not inhibit calcineurin have been administered at the time of reperfusion [73,76]. In one of these studies, the inhibitor also decreased cytochrome *c* release to the cytosol but did not alter the respiratory function of mitochondria isolated from affected tissue early in the post-ischemic period [76].

The strongest evidence for a role for the mitochondrial permeability transition in ischemic damage is provided by the much smaller infarcts that develop in mice lacking the protein cyclophilin D [71]. As expected, knockout of cyclophilin D interferes with the development of the permeability transition in mitochondria. Intriguingly, cells from

these animals show reduced susceptibility to treatments including exposure to hydrogen peroxide and a calcium ionophore but not to commonly used inducers of apoptosis such as staurosporine [69–71]. This finding is consistent with the permeability transition (or at least forms of the permeability transition involving cyclophilin D) being primarily involved in necrotic cell death. Thus, the improved outcome with inhibitors acting via cyclophilin D and in the *cyclophilin D*−/− mice probably involves disruption to necrosis in the affected tissue.

The effects of deletion of cyclophilin D and of inhibitors of the permeability transition indicate a major role for this process in promoting cell death following temporary ischemia. However, the nature of this role is not yet fully understood. Halestrap [66] and Leung and Halestrap [67] have proposed a mechanism for permeability transition involvement in necrotic cell death in temporary ischemia of the heart or brain. They suggest that calcium accumulation during the ischemic period establishes conditions for pore opening but that induction is initially blocked by the lowered pH associated with lactate production. Changes initiated on reperfusion including a burst of free radical production result in opening of the pore. The inability of mitochondria to then generate ATP and the depletion of cytosolic ATP due to reversal of the ATP synthase lead to major metabolic derangements and ultimately activation of degradative enzymes leading to necrosis. At least for focal ischemia in brain, this mechanism is difficult to reconcile with the extent of recovery of energy-related metabolites during early reperfusion in core and penumbral tissue destined to become part of the infarct [34,39,45,46]. The near normal respiratory properties of mitochondria assessed in preparations from tissue early after reperfusion [16,59,60] are also at variance with induction of the permeability transition unless this was reversed during sample processing. Alternative mechanisms may be involved including a slower onset of the permeability transition that contributes to the secondary decline in energy metabolism or a possible transient induction of the permeability transition during early reperfusion that initiates changes leading to a more slowly developing programmed form of necrosis.

The evidence that the permeability transition is involved in necrosis suggests that this change might also contribute to cell loss in the penumbra in permanent ischemia but this possibility has received little attention. Protection has been achieved using cyclosporin A treatment in permanent ischemia [77,78]. However, similar protection was also provided by FK506 suggesting that induction of the permeability transition was not involved. Further investigations are needed.

5.2. Caspase-dependent apoptosis

The intrinsic pathway of apoptosis requires the release to the cytosol of specific proteins that normally reside in the intermembrane space of mitochondria [3,25–27] (Fig. 1). The best characterized of these apoptogenic mitochondrial proteins is the electron transport chain component, cytochrome *c*. Within the cytosol, cytochrome *c* forms a complex referred to as an apoptosome with procaspase-9, apoptotic protease activating factor 1 (APAF-1) and dATP. The formation of the apoptosome activates caspase-9 which then cleaves other procaspases. The activation of caspase-3 by this process is particularly important. Caspase-3 and other executioner caspases have multiple effects including proteolysis of an inhibitor of the caspase-activated DNase [3,25–27]. Degradation of the inhibitor results in translocation of caspase-activated DNase to the nucleus where it catalyses internucleosomal DNA degradation producing fragments containing multiples of 180–200 base pairs.

A release of cytochrome *c* to the cytosol following temporary ischemia has been widely observed either using immunohistochemistry on brain sections or Western blotting of tissue subfractions (see for example [79–85]). This change is usually only detected in neurons. Occasional cells exhibiting cytochrome *c* release are seen within the first hour following reperfusion. The number of affected cells typically increases substantially over the next few hours and then remains stable or increases further to 24 h. One study has reported changes restricted to penumbral tissue at 4 h following onset of reperfusion [81], but alterations more typically develop in some neurons within tissue that formed part of both the core and penumbral regions during arterial occlusion. In response to permanent ischemia, cytochrome *c* release also develops within a few hours in neurons spread throughout the ischemic tissue [30,86–88]. The active cleaved form of caspase-9 has also been detected in neurons and appears with a similar time course to that for the release of cytochrome *c* following temporary focal ischemia [79,83,87], further suggesting the development of the intrinsic pathway of apoptosis in these cells.

At least two other proteins released from the intermembrane space also promote caspase-dependent apoptosis but less directly than cytochrome *c* [3,25,26]. Both second mitochondria-derived activator of caspase/direct IAP-binding protein of low pI (Smac/DIABLO) and Omi stress-regulated endoprotease/high temperature requirement protein A2 (Omi/HtrA2) bind to and block the action of members of the family of inhibitor-of-apoptosis proteins (IAPs). IAPs inhibit caspase-3, caspase-7 and caspase-9. By blocking this effect, Smac/DIABLO and Omi/HtrA2 increase the consequences of caspase activation and promote cell death. Omi/HtrA2 but not Smac/DIABLO has a proteolytic activity that contributes to the inhibitory effects on IAPs [3,25]. Smac/DIABLO and Omi/HtrA2 increase in the cytosol of neurons following temporary focal ischemia with a time course that is similar to that of cytochrome *c* [79,80,83,89]. Interactions between Omi/HtrA2 and XIAP, the most potent member of the IAP family, have been detected by co-immunoprecipitation [89]. A delayed increase in cleaved XIAP was also seen in one study [80]. Treatment of rats with an inhibitor of Omi/HtrA2 activity shortly before induction of temporary ischemia was found to moderately reduce DNA damage in the post-ischemic tissue [80].

Taken together, these findings indicate that the intrinsic pathway of apoptosis is activated in some neurons affected by focal ischemia. The initial study describing cytochrome *c* release in focal ischemia [82] identified cells exhibiting this change that also showed features of apoptotic cell death, as indicated by positive TUNEL staining and the presence of apoptotic bodies. However, there was not a close relationship between cytosolic cytochrome *c* and this apoptotic morphology. The patterns of release of cytochrome *c* and other proteins in subsequent studies also do not suggest a close correlation with the distribution of cells exhibiting morphological features of apoptosis in the affected cells. A likely explanation for this limited correlation is that the internal pathway of apoptosis is induced in the mitochondria of the neurons showing cytoplasmic accumulation of mitochondrial proteins but that the further development of caspase-dependent apoptosis is overwhelmed by other molecular changes that result in alternative forms of cell death, at least in some of these cells. It is also likely that some of the cytochrome *c* release results from a disruption to mitochondrial structure that is unrelated to induction of caspase-dependent apoptosis.

Increases in the active cleaved form of caspase-3 are also seen following temporary ischemia with a time course and distribution similar to that for the release of proteins from the mitochondrial intermembrane space [80,81,83,90,91]. Expression is again almost exclusively detected in neurons. Mice lacking expression of caspase-3 usually die during development. However, a strain that survives to maturity develops markedly smaller infarcts than wild-type mice following 2 h of MCA occlusion [92]. The interpretation of this finding is complicated because the mice exhibit changes in brain structure, including an increased density of neurons in the cortex, arising from the lack of caspase-3 during development. Nonetheless, the results point to an important contribution of caspase-mediated cell death to ischemic damage following temporary focal ischemia. Further support for this conclusion is provided by substantial decreases in infarct

volume achieved using treatment before and after temporary ischemia with a pan-caspase inhibitor as well as an inhibitor that is relatively selective for caspase-3 [93,94]. A similar protection has also been produced by an inhibitor with selectivity for caspase-9 [95], consistent with a role for cytochrome *c* release in the development of damage.

These findings strongly suggest the activation of caspase-dependent apoptosis following temporary focal ischemia, at least in part via the intrinsic pathway. However, the relative contribution of the extrinsic and intrinsic apoptotic pathways to the caspase activation is not known. A significant role for the extrinsic pathway in inducing caspase activation is suggested by the identification of changes in upstream signalling proteins in post-ischemic brain and the development of much smaller infarcts following temporary ischemia in mice lacking components of this pathway [96,97].

Activation of caspase-3 has been less studied in permanent focal ischemia. Direct evaluation of brain tissue for caspase-3 activation during a focal ischemic insult that was restricted to the cortex identified changes only in some of the neurons in one cortical layer, although more widespread activation was seen in microglia [98]. Furthermore, treatment of rats with a caspase inhibitor prior to induction of permanent ischemia did not significantly improve infarction [99] suggesting that this form of cell death may be of little importance unless there is reperfusion.

5.3. Caspase-independent apoptosis

Mitochondrial membrane permeabilization can also lead to the release of proteins that trigger apoptosis by caspase-independent pathways [3,25,26]. The best studied mediator of this form of cell death is AIF. AIF under normal conditions is a transmembrane protein of approximately 62 kDa that resides in the inner mitochondrial membrane. Following outer membrane permeabilization, the portion of AIF (approximately 57 kDa) in the intermembrane space is released by proteolysis, via actions of calcium-activated calpains and calcium-independent cathepsins [25,26]. The AIF translocates to the nucleus where it triggers the degradation of DNA into fragments that are larger than those produced following caspase-3 activation. AIF does not have intrinsic nuclease activity. Thus, the DNA degradation requires activation of one or more other proteins. Interactions of AIF with the protein, cyclophin A in the cytosol and co-translocation of the two proteins lead to DNA degradation [3,25,26,100]. These interactions are essential for AIF-mediated apoptosis, at least under some conditions.

In focal ischemia, nuclear translocation of AIF is seen in some neurons within the first 2 h following short periods of focal ischemia (of less than 1 h) in mice and then increases greatly over the next 24 h [90,101–103]. Such changes are usually present in tissue from the core and penumbral regions [101] (but see [81]) and again are essentially restricted to neurons. By 24 h after an ischemic period of 45 min, nuclear translocation of AIF was seen in many neurons and correlated closely with overall neuronal loss [101]. Following longer periods of temporary ischemia and in permanent ischemia, nuclear translocation is apparently not initiated for several hours but again increases at later times [84,102].

The most direct evidence that AIF contributes to ischemic damage is provided by large reductions in infarct size seen following 45 min of MCA occlusion in Harlequin mice. Expression of AIF in these mice is reduced to approximately 20% [101]. Brain development in Harlequin mice is apparently normal although the decrease in AIF also alters antioxidant defences, which could potentially influence the tissue damage that develops [26]. Further support for the importance of AIF in cell death is provided by the large protective effects resulting from knockout of the PARP gene or inhibition of PARP in ischemic and post-ischemic brain. The activation of this enzyme by ischemia is believed to lead to cell death that at least in part involves AIF release and nuclear translocation (as discussed further in section 5.4.3).

Endonuclease G, another protein released from mitochondria, can interact with AIF under some conditions and can directly cause caspase-independent apoptosis [3,25,26]. In temporary or permanent ischemia, endonuclease G translocation to the nucleus follows a similar time course to AIF and is seen in neurons throughout the perfusion territory of the occluded artery [103,104]. Co-immunoprecipitation studies suggest that endonuclease G interacts with AIF in the nucleus at 24 h following temporary focal ischemia [102].

5.4. Inducers and modulators of mitochondrial involvement in cell death

5.4.1. Promoters of outer membrane permeabilization

The mechanisms for release of apoptogenic proteins from the intermembrane space to the cytosol during development of apoptosis has been intensively studied over the last decade but remains incompletely understood [3,25]. Under normal conditions, the presence of the voltage-dependent anion carrier in the outer mitochondrial membrane allows compounds with molecular weights below 1.5 kDa to readily move between the cytosol and intermembrane space. However, the release from the intermembrane space of larger molecules, including cytochrome *c* and other proteins, requires a substantial increase in permeability of the outer mitochondrial membrane. Under some conditions, this can result from formation of the mitochondrial transition pore which leads to permeabilization of the inner membrane. Subsequent swelling of the mitochondria due to water entry into the matrix following pore opening results in disruption of the outer membrane and the release of proteins from the intermembrane space [3,25]. As discussed further in section 4.1., there is considerable evidence for induction of the mitochondrial permeability transition by focal ischemia and a prominent role for this change in cell death under some conditions. However, the studies of cells from mice lacking cyclophilin D expression [69–71] suggest that this is more likely to result in necrosis than apoptosis.

An alternative process is now thought to be more commonly involved in the permeabilization of the outer mitochondrial membrane and release of apoptogenic proteins. This involves pore formation by pro-apoptotic members of the Bcl-2 family of proteins in the outer membrane [3,25]. The full details of this process are not yet elucidated but translocation of the proteins, Bim and Bax, from the cytosol to the mitochondrial membrane is commonly involved. Translocation of both proteins develops in the initial 4 h following 1 h of ischemia in rats and mice [83]. The expression of these proteins is also increased at these times [81,83]. Translocation of Bax and Bim to the mitochondria following ischemia apparently involves activation of c-Jun N-terminal kinase (JNK). Pretreatment of mice with a JNK inhibitor immediately prior to 1 h of ischemia in mice decreases movement of these proteins to the mitochondria and decreases the number of cells exhibiting features of apoptosis, predominantly in penumbral regions [83,105]. Movement of the Bcl-2 family protein, Bad, from the cytosol to the mitochondria can also contribute to mitochondrial membrane permeabilization under some circumstances. Translocation of this protein has also been detected following temporary ischemia [106] (but see also [83]).

Activation of caspase-8 by cell death receptors involved in the extrinsic apoptotic pathway can result in cleavage of another Bcl-2 family protein, Bid, and translocation of the t-Bid to the mitochondria where it promotes release of apoptogenic proteins. Cleavage of Bid develops following temporary ischemia, consistent with stimulation of this arm of the extrinsic pathway [85,107]. Furthermore, infarct volumes were found to be 67% smaller in *Bid*−/− mice compared to wild-type mice exposed to brief (30 min) focal ischemia [107] and approximately 30% smaller following more prolonged (90 min) ischemia [85]. The *Bid*−/− mice also exhibited markedly diminished and delayed cytochrome *c* release [85,107], less caspase-3 activation as well as decreases in apoptotic neurons in tissue from the penumbra [85]. Bid has also been implicated in promoting release of AIF in

neurons exposed to ischemia-like and excitotoxic insults in culture [101,108] and may therefore also contribute to caspase-independent apoptosis in ischemia.

Other members of the Bcl-2 family are anti-apoptotic either by binding pro-apoptotic Bcl-2 family proteins or via direct interactions with the mitochondria [3,25]. These proteins can provide protection against apoptotic and necrotic forms of cell death. The effects of over-expression of two of these proteins, Bcl-2 and Bcl-xL, have been investigated in permanent ischemia in mice and found to moderately reduce infarcts in some studies but not others [109–111]. Local increases in Bcl-2 expression achieved using viral vectors impeded release of cytochrome *c* and the translocation of AIF to the nucleus in neurons in the rim of infarcts produced by permanent ischemia in rats and increased the survival of these neurons [112].

5.4.2. Oxidative stress

Oxidative stress has been strongly implicated as an important factor in the development of both necrosis and apoptosis in focal ischemia, particularly when there is reperfusion [2,15]. Peroxynitrite generated from the reaction of nitric oxide and superoxide plays a prominent role. Mitochondria are a major site of production of superoxide in normal cells and probably contribute to increased oxidative stress in ischemic and post-ischemic brain. Consistent with this possibility, over-expression of Mn^{2+}-superoxide dismutase, the mitochondrial isoform of the enzyme that converts superoxide to hydrogen peroxide, results in moderate reductions in infarction in temporary ischemia [113]. Furthermore, much larger infarcts are seen in mice heterozygous for deletion of this enzyme compared with wild-type mice following both permanent and temporary ischemia [29,114].

A decrease in mitochondrial generation of free radicals has been proposed as the basis for reductions in tissue infarction in rats treated systemically with a mild dose of the mitochondrial uncoupling agent, dinitrophenol, following temporary ischemia [115]. Mitochondria isolated from the penumbra but not the core of control rats during early recirculation showed moderate increases in calcium content and an increase in production of free radicals when incubated in vitro. These changes were both substantially reduced in mitochondria from rats treated with dinitrophenol, consistent with a mitochondrial contribution to the protection [115].

The major water-soluble antioxidant, glutathione, is localized in both the cytosol and the mitochondria of cells. A selective loss of the mitochondrial pool develops during ischemic periods that are sufficient to induce infarct formation and persists during reperfusion [116]. In astrocytes in culture, losses of mitochondrial glutathione increase susceptibility to oxidative and nitrative stress but not to inducers of apoptosis [117,118]. Interestingly, the increased vulnerability in these cells apparently involves induction of the permeability transition and resembles the effects on cell viability produced by knockout of cyclophilin D [118]. The decrease in mitochondrial glutathione also seems likely to promote necrosis in ischemic and post-ischemic brain but definitive evidence is lacking.

The involvement of superoxide in the development of ischemic injury has been most extensively characterized through investigations of mice overexpressing the cytosolic enzyme, Cu^{2+} Zn^{2+}-superoxide dismutase. These animals develop smaller infarcts than wild-type mice following temporary ischemia but, interestingly, not permanent ischemia [119,120]. Following reperfusion, this enzyme leads to decreases in multiple events associated with mitochondrially mediated apoptosis including the release of cytochrome *c*, Smac/DIABLO and Omi/HtrA2 and translocation of Bad to the mitochondria [89,106,121,122].

5.4.3. PARP activation

PARP is an enzyme that is activated by DNA damage and generates poly-ADP ribose to promote repair [123]. A role for PARP activation in ischemic cell death is demonstrated by the development of much smaller infarct volumes (typically reduced by more than 50%) in response to temporary ischemia in *PARP*−/− mice [124–126] or in rats and mice treated with PARP inhibitors [47,48,125]. Decreases of infarct formation can be achieved with treatments several hours after recirculation in temporary ischemia. Markedly reduced damage is also achieved by PARP inhibition initiated within the first 2 h of permanent ischemia [48]. The effects of PARP are explained, at least partially, by the ability of poly-ADP ribose to trigger release of AIF [127]. PARP inhibition decreases AIF translocation in response to focal ischemia [101]. Furthermore, both poly-ADP ribose formation and AIF translocation induced by focal ischemia are attenuated by inhibitors of nitric oxide synthase and are also decreased in mice in which the gene for neuronal nitric oxide synthase is deleted [84]. Thus, PARP activation and subsequent AIF translocation are apparently consequences of initial induction of oxidative damage to DNA induced by peroxynitrite.

PARP activation can also initiate necrosis under some conditions [31]. Excessive activity of PARP depletes cellular NAD^+, the substrate

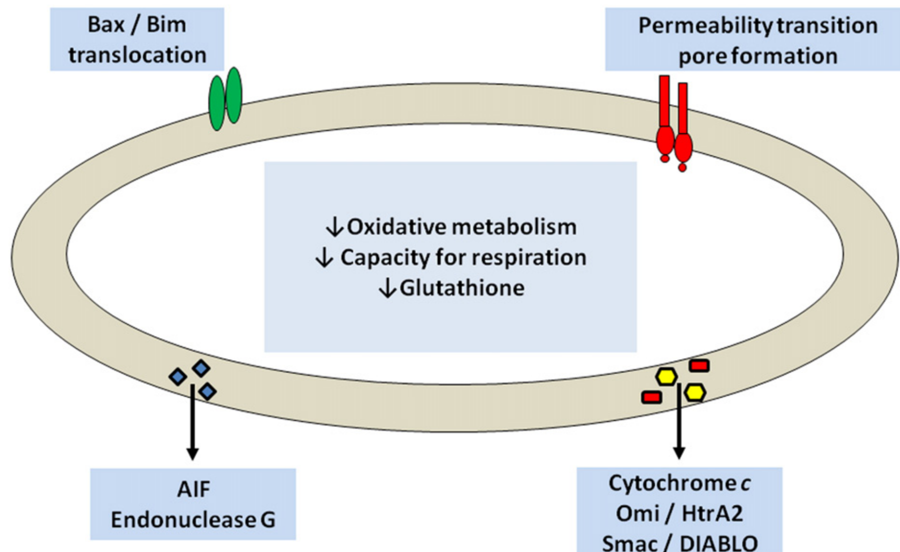

Fig. 3. Summary of major changes in mitochondrial properties that are induced by permanent or temporary focal cerebral ischemia and can contribute to cell death under some conditions.

for poly-ADP ribose formation, which then leads to utilization of ATP in an attempt to regenerate the NAD^+ [6,31]. The decrease in these key intermediates of energy generation can initiate cellular changes that result in necrotic forms of cell death. The effectiveness of PARP inhibition in limiting damage induced by both temporary and permanent ischemia could reflect the involvement of this enzyme in the development of more than one form of cell death in the ischemic and post-ischemic brain.

6. Conclusions

Cell death in stroke develops on a background of complex metabolic changes that depend on the severity and duration of the focal ischemic insult. Fig. 3 summarizes key alterations identified in mitochondrial properties that can contribute to cell death under some conditions in ischemic or post-ischemic brain. In severely ischemic core tissue, oxidative glucose metabolism is rapidly impaired by decreases in oxygen and glucose availability. These changes are not compatible with long-term cell survival. Necrosis in this tissue is a major contributor to infarct development in permanent ischemia. Of greater interest, energy metabolism in the penumbra is more moderately affected, almost certainly contributing to the ability of this tissue to be rescued by some pharmacological interventions (in animal models but not yet in humans) or by restitution of blood flow (in both animal models and humans) within the first few hours of stroke onset. In untreated permanent ischemia or with longer-term ischemia with reperfusion, impairment of mitochondrial oxidative metabolism and losses of glutathione from these organelles are likely promoters of the cell death in the penumbra that results in expansion of the infarct.

The development of the mitochondrial permeability transition and the release of mitochondrial proteins leading to caspase-dependent and caspase-independent apoptosis have all been shown to be involved in ischemic damage in animal models of stroke. A role for each of these processes in cell death, and particularly neuronal death, is best established in models of temporary ischemia, often of short duration. Thus, these responses may well contribute to the damage that develops following early restoration of blood flow in human stroke treated with thrombolysis or in the small proportion of cases with early spontaneous reperfusion. There has been much less investigation of whether these processes might also contribute significantly to the loss of the potentially salvageable cells in the penumbra under conditions of permanent or long-duration ischemia that are probably more relevant to the majority of stroke in humans. The limited evidence available suggests that neither caspase-dependent cell death nor the permeability transition is likely to have a major role. A possible broader involvement of AIF translocation is suggested by the substantial effects of inhibition of PARP in temporary and permanent ischemia, which are mediated at least in part by nuclear translocation of AIF. Further evaluation of the contribution of AIF is needed. Regardless of the ischemic conditions, there is little evidence implicating any of these three processes in the death of non-neuronal cells. The effects of ischemia on the mitochondrial properties of these non-neuronal cells and the interactions between these cells and neurons undergoing necrosis or apoptosis are currently poorly understood and also need further investigation.

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