Review Article

Brain protection: physiological and pharmacological considerations. Part I: The physiology of brain injury*

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Ischaemia, whether focal or global in nature, produces a sequence of intracellular events leading to increased cell permeability to water and ions including Ca^{++}. There is a loss of cellular integrity and function, with increased production of prostaglandins, free radicals, and acidosis with lactate accumulation. These events may be exacerbated by glucose administration. Pharmacological agents aimed at alleviating ischaemic injury could be directed at decreasing cerebral metabolic requirements for oxygen, improving flow to ischaemic areas, preventing Ca^{++}-induced injury, inhibition of free radical formation, lactate removal, inhibition of prostaglandin synthesis, and prevention of complement-mediated leukocyte aggregation. Part 1 of this paper describes some of the pathophysiological events leading to ischaemic brain injury. Part 2 of this paper will consider the current agents available for brain protection.

L'ischémie, qu'elle soit globale ou non, entraîne une cascade de modifications intracellulaires amenant une augmentation de la perméabilité cellulaire à l'eau et aux ions dont le Ca^{++}. Intégrité et fonction cellulaires sont compromises avec une augmentation de la production des prostaglandines, des radicaux libres et de l'acidose avec accumulation de lactate, le tout pouvant être exacerbé par la perfusion de glucose. Afin de limiter les dommages induits par l'ischémie cérébrale, les agents thérapeutiques peuvent emprunter plusieurs avenues : diminution des besoins métaboliques en oxygène, amélioration du flot sanguin dans les zones ischémiques, prévention des dommages dus au Ca^{++}, inhibition de la synthèse des radicaux libres et/ou des prostaglandines, élimination des lactates et prévention de l'agrégation leucocytaire induite par le complément. Dans un premier temps, nous décrivons la cascade pathophysiologique entraînant la lésion cérébrale ischémique. Dans la deuxième partie de notre travail, nous nous attarderons aux modes de protection cérébrale.

Key words
BRAIN: brain blood flow, ischaemia, metabolism, oxygenation;
IONS: calcium.

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Cerebral neurological injury may occur during anaesthesia and surgery, particularly cardiac surgery where extracorporeal circulation (cardiopulmonary bypass (CPB)) is employed.[1-15] During cardiac surgery, the

reported incidence of injury is extremely variable (0.3–80 per cent) but in prospective studies is in the order of 17 per cent with permanent residua occurring in five per cent.[2,6,8,9,11–15] Part of the variability in the reported incidence is reflected by differing definitions of adverse neurological outcome (e.g., subtle neurological abnormalities (39 per cent) vs stroke (five per cent)[11] and the use of retrospective vs prospective data analysis.[11,13] Neurological complications may lead to prolonged stay in the intensive care unit and are associated with increased morbidity, mortality[3,8,9,13,15] and cost.

This paper will address the mechanisms and physiology of central nervous system injury and Part II will address the pharmacology of brain protection.

Normal cellular events

The central nervous system is comprised of a vast network of neurons, supporting glial cells, and vasculature.[16] The neuron is a unique cell in that it can transmit depolarization impulses along its membrane in a nondecremental manner.[17] Energy is required to facilitate neuronal impulse transmission and for basic cellular maintenance.[18,19]

The brain is a very metabolically active organ and may utilize up to 20 per cent of the total body oxygen consumption.[19] The energy requirements of the brain can be differentiated into two components: (1) energy necessary for the preservation of cellular integrity (40 per cent); (2) energy required for the transmission of nerve impulses (60 per cent).[18] The energy needed for neuronal metabolism is obtained from the adenosine triphosphate (ATP) molecule produced through oxidation of glucose in the mitochondria via oxidative phosphorylation.[20] Difficulties in maintaining cellular integrity arise when there is a reduction in ATP production as the brain contains low concentrations of ATP and stores very little glucose in the form of glycogen.[21] Hence, the brain requires a constant delivery of metabolic substrate and tolerates ischaemia poorly.[19]

Extraction of glucose and oxygen from blood is increased as cerebral blood flow decreases.[22] Glucose, the primary metabolic substrate, is brought across the cell membrane by facilitated diffusion.[22] Transport of oxygen across the membrane is by passive diffusion.[23] The cerebral metabolic requirement for oxygen ($CMRO_2$) varies depending on the activity of the CNS.[17]

The cell membrane where depolarization takes place is composed of a lipid-bilayer framework, intercalated with a number of components including cholesterol, carbohydrates and proteins.[24] The proteins comprise such complexes as Na^+K^+ATPase pumps, ion-selective (K^+, Na^+, Ca^{++}) and voltage-sensitive channels, adenylate cyclase and cytochrome oxidases.[24,25] The ion-selective and voltage-sensitive channels are important in regulating the excitability of the neuron. Whether depolarization or hyperpolarization of the neuron occurs depends upon which ion is able to pass along its electrochemical gradient through its specific channel.[25]

Role of calcium

Calcium plays a critical role in a number of intracellular reactions including: (a) inhibition of some enzymes (e.g., by increasing production of cAMP, Ca^{++} inhibits the glycolytic enzyme hexokinase);[17] (b) activation of other enzymes (e.g., Ca^{++}-ATPase, adenylate kinase, phospholipases A and C);[26,27] (c) regulation of actin-myosin cross-bridging, and thus muscle contraction (via myosin light-chain kinase).[28]

Intracellular calcium levels are maintained at three sites: the plasma membrane, mitochondria, and endoplasmic reticulum (sarcoplasmic reticulum in muscle cells). At the plasma membrane, a Ca^{++}-Na^+ exchange system exists which is indirectly dependent on Na^+-K^+ATPase activity and there is also an ATP-dependent Ca^{++}-ATPase containing calmodulin. Both systems transport calcium out of the cell.[26] At the mitochondria, H^+ ions are exchanged for Ca^{++} ions across the inner mitochondrial membrane which operates at the expense of oxidative phosphorylation.[24,26,27,29] A Na^+-Ca^{++} exchange system is also present in the mitochondria. In this system, mitochondria may take up or release Ca^{++} in response to Na^+ shifts secondary to membrane depolarization.[26,30] In general, the presence of high intracellular Na^+ levels inhibits Ca^{++} uptake by mitochondria.[30]

The endoplasmic reticulum aids in sequestering Ca^{++} by expending ATP to exchange the H^+ for a Ca^{++} ion.[17,24,31]

In reducing intracellular Ca^{++} levels, energy must be utilized. In contrast, accumulation of intracellular Ca^{++} does not require energy.[17,24]

The ischaemic brain

When the very large number of cellular processes occurring in the neuron are considered, the importance of adequate oxygen delivery and ATP generation is obvious. When the minute to minute delivery of oxygen to the brain is impaired and the cellular demand for oxygen exceeds the supply, a series of pathogenetic cellular events occur which leads to cellular dysfunction and loss of cellular integrity (Figure). Generally, the extent of cerebral ischaemic injury, and thus the neurologic outcome, is directly related to the severity and the duration of reduced blood flow.

Ischaemia and cerebral blood flow (CBF)

Normal cerebral blood flow in humans approximates 50 $ml \cdot 100\ g^{-1} \cdot min^{-1}$.[32] At this rate, tissue requirements

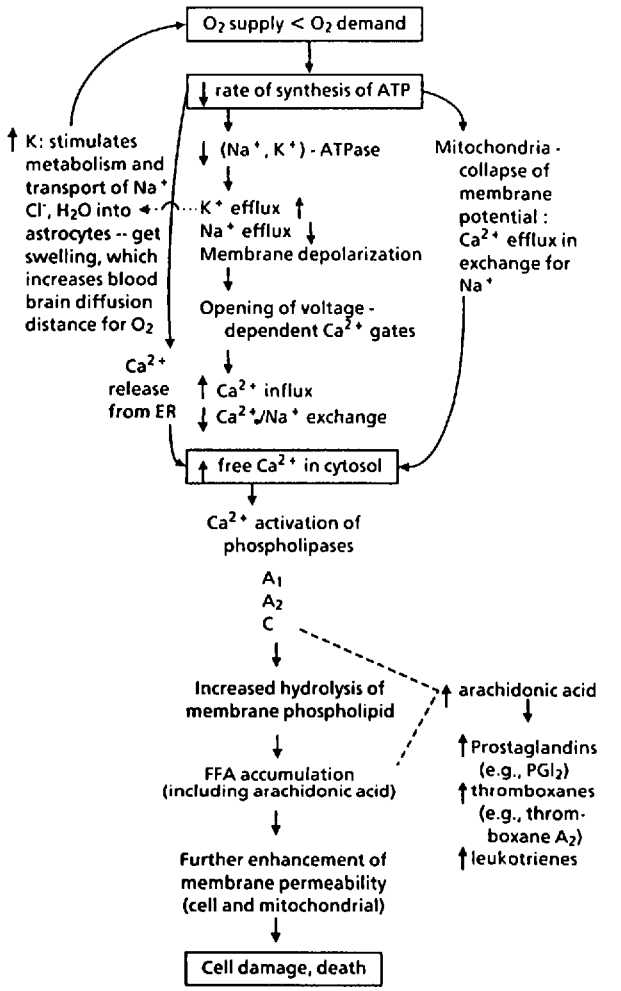

FIGURE Progression of probable metabolic events initiated in hypoxia-sensitive cells by limitation of oxygen supply. Reprinted from *Messick JM, Milde LN.* Brain Protection. Advances in Anesthesiology 1987; 4: 47–88. By permission of Mayo Foundation.

for oxygen and glucose are met and cellular integrity is preserved. However, the ischaemic thresholds of CBF below which the function of neurons cannot be maintained have been established.[24] The threshold of electrical failure is 15 to 18 ml · 100 g^{-1} · min^{-1} and is characterized by an isoelectric EEG.[21,33–35] At this flow, enough oxygen for maintenance of cell function is provided but it is insufficient to support synaptic transmission. The threshold of ionic failure is approximately 10 ml · 100 g^{-1} · min^{-1} and is presumed to be the threshold of CBF at which irreversible neurological damage occurs.[36,37]

ATP depletion

In order to provide sufficient amounts of ATP, the neuron must carry out oxidative phosphorylation, which requires the presence of oxygen. After only 20 sec of complete ischaemia, ATP is depleted to such a level that synaptic transmission is not possible and the EEG becomes isoelectric.[27] The creatine phosphate concentration approaches zero after one minute and ATP stores are depleted after five to seven minutes.[24] In the absence of oxygen, anaerobic metabolism of glycolytic substrates ensues in an effort to generate more ATP. However, the yield in this process is inefficient and during ischaemia ATP depletion is inevitable.

Ionic failure

A number of ATP-dependent mechanisms exist that maintain normal intracellular concentrations of ions. With depletion of ATP stores, these mechanisms fail and redistribution of ions results.[17,24] Perhaps the most important disruptionis that of the Na^+K^+-ATPase pump.[17] As a result of failure of this pump, active extrusion of intracellular Na^+ ceases leading to an influx of Na^+ and Cl^- in excess of any K^+ lost. There is a concomitant influx of water and neuronal swelling (cyototoxic oedema) as well as depolarization of the neuron.[24,38] Depolarization of the neuronal membrane stimulates the opening of voltage-sensitive Ca^{++} channels. A massive influx of Ca^{++} into the neuron ensues.[24] Normal mechanisms for maintaining low intracellular Ca^{++} concentrations, previously described, fail due to the lack of ATP. The end result is accumulation of intracellular Ca^{++}.

Calcium accumulation

Redistribution of Ca^{++} ions plays a central role in the pathogenesis of ischaemic cell injury.[39,40] Calcium ions activate phospholipases A and C.[27] These phospholipases hydrolyze membrane phospholipids with a resultant accumulation of free fatty acids (FFA), especially arachidonic acid.[38] The hydrolysis of membrane phospholipids disrupts a variety of membraned organelles including the mitochondria and plasma membrane.[38–40] These disruptions can cause further alterations in membrane permeability and ion distribution, including further influx of calcium.[39,40]

During incomplete ischaemia, as in reperfusion, arachidonic acid is further oxidized via cyclooxygenase and lipooxygenase pathways to prostaglandins, thromboxanes and leukotrienes,[24] collectively known as eicosanoids, all of which can contribute further to pathophysiological reactions and cell injury.[41] Free radicals are also produced during oxidation of arachidonic acid.[24,42,43] The production of these mediators is a direct result of high cytoplasmic Ca^{++}.

NMDA receptor hyperactivation

The excitatory amino acid (EAA) neurotransmitters have recently been implicated in the production of ischaemic

brain damage.[44,45] Evidence suggesting that activation of the N-methyl-D-aspartate (NMDA) receptor by EAA's is involved in cerebral ischaemic damage comes from a number of sources. This receptor type is predominantly found in those areas of the brain that are selectively vulnerable during ischaemia.[46] Within these areas, these receptors participate in synaptic transmission.[47] However, their activity is particularly prevalent during periods of neuronal hyperactivity[47] which is often the case following ischaemia.[48] The activation of these NMDA receptors induces burst firing[49] which may be responsible for instances of ischaemia-induced seizures.[50] The NMDA receptor complex is associated with an ionophore which possesses an especially high Ca^{++} conductance[51] and as such may contribute to intracellular Ca^{++} overload which, as described above, is strongly implicated as a major factor contributing to cell death during ischaemia.[52] Unlike other EAA receptors, NMDA receptors do not experience desensitization in the presence of ischaemia[53] and thus their activation can continue after ischaemia.

If activation of the NMDA-receptor is a mechanism for ischaemic damage, then blockade of these receptors via pharmacologic intervention should reduce the neuronal damage that occurs in selectively vulnerable regions of the brain. The acute changes in energy metabolism that arise during ischaemia may be attenuated by NMDA antagonists.[54] This is likely due to a reduction of Ca^{++} influx.[55]

Lactic acidosis

During ischaemia, glucose metabolism is switched from the aerobic phosphorylation pathway to the anaerobic glycolytic pathway, which results in the accumulation of cellular lactate and a decrease in pH.[27,42]

In a primate model of severe focal ischaemia, Michenfelder *et al.* noted lactate accumulation of almost four times the mean control level of 2.14 $\mu mol \cdot g^{-1}$ within 30 min of middle cerebral artery (MCA) occlusion.[56] By the end of three hours post-occlusion of the MCA, lactate levels increased to approximately 17 $mmol \cdot kg^{-1}$ of tissue.[56] Lactate levels in the range of 16–20 $mmol \cdot kg^{-1}$ are considered to be the threshold above which tissue damage occurs.[57]

It has been suggested that lactate is a major contributor to ischaemic neuronal damage when it is present in high concentrations.[57] Mechanisms by which lactate accumulation can contribute to neuronal injury are given in Table I.

Glucose potentiation of ischaemic damage

The concentration of glucose in the brain at the onset of ischaemia may influence outcome after the ischaemic event.[24,57,60]

During complete ischaemia, high levels of brain glucose allow for continued glycolysis, but only by an anaerobic pathway. This leads to a neurotoxic accumulation of lactate, increased acidosis, and consequently the potential for greater histopathological and neurological injury. This concept is supported by both primate models[61] and by retrospective studies of patients surviving cardiac arrest.[62]

In cases of severe incomplete ischaemia, glucose is continually being supplied to the neurons. However, the oxygen delivery to these tissues is not sufficient to surpass the ischaemic threshold. As a result, and in an effort to replenish the oxidizing agent NAD^+ (from NADH), this continued supply of glucose is anaerobically metabolized to lactate. As long as glucose is supplied, lactate will accumulate.[17] A high blood glucose concentration during severe incomplete ischaemia may increase the potential for a worse neurological outcome as has been shown in animals[27] and in a prospective study of human patients.[63]

Intravenous administration of glucose during or prior to an ischaemic insult may worsen the neurological outcome and perhaps should be avoided in high-risk situations[57] (e.g., cardiac surgery, carotid endarterectomy).

Free radical generation

A free radical is a chemical species with an unpaired electron.[64,65] Free radicals can be generated as a result of ischaemia and are contributing factors to neurological damage.

Ischaemia can generate free radicals in a number of ways. The most important free radical is the superoxide radical (O_2^-).[66] During ischaemia there is an increase in reducing equivalents in the form of NADH, hydrogen ion and lactate,[67] an increase in xanthine concentration,[68] and the conversion of xanthine dehydrogenase to xanthine oxidase.[69] This conversion is probably due to the ischaemic redistribution of Ca^{++} into the cytosol of cells. Calcium activates a protease that may catalyse conversion of xanthine dehydrogenase to xanthine oxidase.[66,69] It is xanthine oxidase which serves as the major source of O_2^- in the post-ischaemic tissue.[66,70]

The actual production of the O_2^- occurs during reperfusion when there is a surge of O_2 supply to the tissues.[17] In this environment, O_2 acts as an electron acceptor during

TABLE I Mechanisms of neuronal injury due to lactate accumulation during ischaemia[41,42,57–59]

Necrosis of endothelial cells with swelling and rupture of astrocytes leading to a reduction in collateral flow
Denaturation and inactivation of proteins including enzymes
Suppression of the regeneration of NAD^+ [NAD^+-NADH]
Production of oxygen-free radicals

TABLE II Cellular derangements due to the action of oxygen-free radicals[17,52,65,70,74]

Alteration of phospholipase activity with increased arachidonic acid release leading to prostaglandin formation
Increased membrane permeability
Amino acid oxidation
Protein-protein cross-linking
Protein strand scission
Increased Ca^{++} influx
Increased release of enzymes from liposomes
Mitochondrial disruption with decreased ATP production

the xanthine oxidase-catalysed conversion of xanthine to urate and, in the process, O_2 is reduced to O_2^-.[70-72]

The fate of the O_2^- radical after its production is variable.[72] The $O_2.^-$ may attack proteins and polyunsaturated phospholipids in membranes, including plasma membranes and cellular organelles, leading to disruption of these organelles (lysosomes, mitochondria, peroxisomes, etc.) [70,73,74] When one considers the functional role of these organelles in maintaining cellular integrity, it becomes apparent how disruption of these organelles leads to cellular injury.

Some examples of cellular derrangements due to free radicals are given in Table II.

Further generation of lipid peroxide radicals (ROO), lipid hydroperoxides (ROOH), the highly reactive hydroxyl radical (OH^-), and fragmentation products such as malonaldehyde ensues.[70,73-75]

There is a number of naturally occurring enzymes that will terminate the propagation of O_2^- and other radicals. Superoxide dismutase will catalyse the reaction of O_2 to hydrogen peroxide (H_2O_2).[52,76] Catalase and peroxidase are responsible for the elimination of hydrogen peroxide (H_2O_2) by converting it to water and oxygen, when present in normal intracellular concentrations.[72,73] There is no physiological defence or enzyme system that can scavenge excessive quantities of hydroxyl radicals (OH^-). Their production is normally prevented by eliminating the substrates from which they are produced, H_2O_2 and O_2^-.[72] However, under pathophysiological conditions, this radical can cause extensive cellular damage.[72]

Cardiac surgery and the cardiopulmonary bypass apparatus

During cardiac surgery, risk factors for an increased incidence of neurological injury include advanced age (>60 yr);[1,12] history of previous neurological disease;[1,11,77-82] the duration of extracorporeal circulation,[1,8,10] the level of the mean systemic pressure maintained while on cardiopulmonary bypass (<50 mmHg),[6,14] the type of surgical procedure performed (e.g., open-vs-closed chamber),[12] the development of unanticipated intraoperative events (e.g., air embolism),[1,81-84] difficult intubation,[1] the observation of a calcified and atherematous aorta at the time of application of the aortic cross-clamp,[83] the type of oxygenator used (membrane vs bubble)[84-86] the nature of the priming solution (blood vs crystalloid),[1] the use of femoral arterial cannulation,[7] and arrhythmias.[3]

The equipment used to provide extracorporeal circulation has been implicated in pathophysiological processes that may aggravate an ischaemic event. It has been demonstrated by Cavarocchi *et al.* that cardiopulmonary bypass activates complement.[86] Foreign substances and surfaces in the cardiopulmonary bypass circuit initiate the alternative pathway of the complement cascade, liberating C3a and C5a. C5a is an anaphylatoxin with chemotactic properties for leukocytes including polymorphonuclear cells (neutrophils). Complement causes aggregation of these cells in the area of complement activation. Aggregation of leukocytes by this manner has been demonstrated in the lungs,[86] but is also likely to occur to some extent in the cerebral tissue. Phagocytosing neutrophils generate superoxide and hydrogen peroxide-free radicals.

Microvascular damage and reperfusion injury

Microvascular damage is a complication of the ischaemic damage at the cellular level and is especially apparent after reperfusion. During ischaemia, the normal mechanisms that contribute to autoregulation are non-functional and cerebral blood flow (CBF) is dependent on the perfusion pressure and the degree of vessel occlusion.[17]

Upon reestablishment of cerebral circulation following complete ischaemia, there is a five- to ten-minute period of hyperperfusion which is then followed by a prolonged period of hypoperfusion.[87-89] This decrease in CBF is heterogeneous with different areas showing different degrees of hypoperfusion.[89] During this period of hypoperfusion neurons may still be capable of functional recovery (i.e., above the threshold for energy failure), but because of reduced delivery of O_2 and substrates, recovery is hampered.[17] This state of incomplete ischaemia may contribute further to neurological damage.[90]

There are several hypothesized causes of post-ischaemia hypoperfusion phenomena. Prostaglandins have been implicated. Endothelial cells can be damaged during ischaemia. This damage can disrupt the endothelial cells in such a way that there arises an imbalance in the production of the prostaglandins prostacyclin (PGI_2) and thromboxane A_2 (TBA_2).[17,27] Under normal conditions these two substances regulate platelet aggregation and thrombus formation.[27,91] However, during ischaemia free radicals are often formed in disrupted cells, including neurons and endothelial cells. These radicals can react with membrane phospholipids to produce lipid peroxides.[74] The lipid peroxides will inhibit, selectively, the

formation of PGI_2 and result in the unopposed action of TBA_2 in endothelial cells.[27] This imbalance is further enhanced as a result of the high arachidonic acid pool that is generated during ischaemia due to membrane degradation. Upon reoxygenation, oxygen-dependent enzymes responsible for the further degradation of arachidonic acid are activated and increased production of TBA_2 occurs.[17] TBA_2 causes platelets to adhere to endothelial cells,[92] thus leading to thrombus formation[93] and possibly the post-ischaemia hypoperfusion syndrome.[27,94-96]

Another possible mechanism of hypoperfusion appears to be the increase in intracellular Ca^{++} in vascular smooth muscle during reperfusion. Like other ischaemic cell membranes, the permeability of ischaemic smooth muscle cell membranes is altered leading to ionic disturbances, including increased Ca^{++} influx,[24,39] especially upon reperfusion. This Ca^{++} activates myosin light-chain kinase which catalyzes the formation of myosin, thus increasing actin-myosin interaction and contraction of this smooth muscle.[28,96] Contraction of vascular smooth muscle leads to vasoconstriction.

The deformability of erythrocytes is often lost during ischaemia which will result in an increased blood viscosity which in turn will cause a decrease in blood flow.[95]

Ischaemic cytotoxic oedema may also contribute to hypoperfusion. Initially, cytotoxic oedema contributes through swelling of perivascular glial cells that results in an impingement on potential collateral flow.[40] Iannotti *et al.* have shown an increase in local tissue pressure to occur within 10–15 minutes of MCA occlusion.[97]

Vasogenic oedema arises hours to days after vessel occlusion and is indicative of irreversible ischaemic endothelial damage.[40] With vasogenic oedema, the blood-brain-barrier (BBB) ultimately breaks down with resultant accumulation of plasma in the extracellular compartment,[98] which in turn would further impinge on surrounding vasculature.

Conclusion

With reduction in cerebral blood flow and oxygen delivery, a chain of events is initiated which, if uninterrupted, will lead to cell damage and eventually to neuronal cell death. Calcium plays an important role in sustaining ongoing tissue damage. Production of excitatory amino acids may occur which may increase neuronal firing. Lactate accumulates and free radical ions are generated leading to further neuronal damage.

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