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Impact of Ischemia on Cellular Metabolism

Maximilien Gourdin and Philippe Dubois

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1. Introduction

As in all aerobic eukaryotic cells, oxygen is essential for homeostasis in human cells. The interruption of blood flow to tissues results in an arrested oxygen supply and disrupts the biochemical reactions that ensure the smooth functioning, integrity and survival of the cells. The limited oxygen reserves that are dissolved in the interstitial fluid and are bound to hemoglobin, myoglobin and neuroglobin do not maintain efficient, long-term metabolism.[1,2] Lack of oxygen affects all functions within the cell. Table 1 summarizes the main cellular consequences of ischemia.

- (1) cellular acidosis;
- (2) loss of sarcoplasmic membrane potential;
- (3) cellular swelling;
- (4) cytoskeleton disorganization;
- (5) reduction of adenosine-5'-triphosphate (ATP) and phosphocreatine is more than reduction in the energy substrates;
- (6) reduction of glutathione, of α -tocopherol;
- (7) increasing expression of leukocyte adhesion molecules;
- (8) secretion of cytokines/chemokines
 - Tumor Necrosis Factor (TNF- α)
 - Interleukins (IL-) -1, 6, 8

Table 1. Major cellular consequences of ischemia

2. Adenosine triphosphate depletion

Eukaryotic cells contain mitochondria, organelles whose main function is to produce adenosine triphosphate (ATP). ATP is an essential energy substrate, as its hydrolysis provides energy for many metabolic and biochemical reactions involved in development, adaptation and cell survival. ATP production in an aerobic cell is particularly effective when the degradation of key nutrients such as glucose and fatty acids is coupled to a supramolecular complex located in the inner membrane of mitochondria to drive oxidative phosphorylation. Oxidative phosphorylation is mediated by an electron transport chain that consists of four protein complexes and establishes a transmembrane electrochemical gradient by supporting the accumulation of protons in the intermembrane space of the mitochondria. This gradient is used as an energy source by ATP synthase during the synthesis of an ATP molecule from a molecule of adenosine diphosphate (ADP) and an inorganic phosphate (Figure 1). Without oxygen, oxidative phosphorylation stops: the proton gradient between the intermembrane space and the inner mitochondria is abolished, and ATP synthesis is interrupted. The ensuing rapid fall in intracellular ATP induces a cascade of events leading to reversible cell damage. However, over time, the damage increases and gradually becomes irreversible, which may lead to cell death and destruction of the parenchymal tissue.

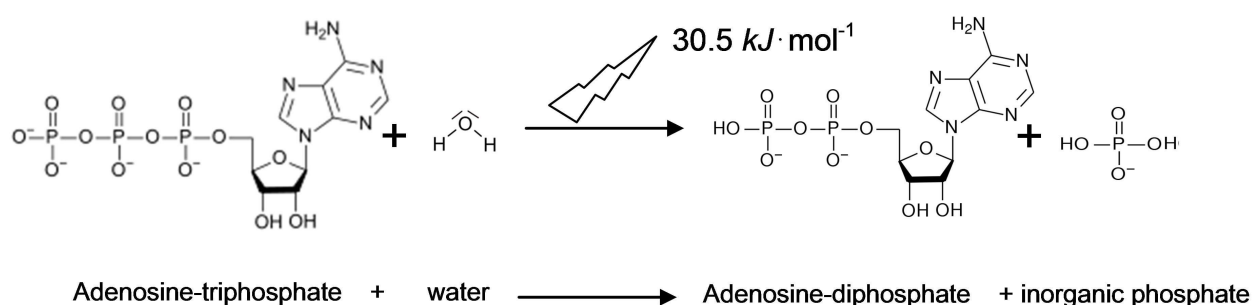


Figure 1. Hydrolysis of Adenosine-triphosphate provides energy (30.5 kJ per mole) for biochemical reactions

When devoid of ATP, the cell derives its energy from the pyrophosphate bonds of ADP as they are degraded to adenosine monophosphate (AMP) and then to adenosine. Adenosine diffuses freely out of the cell, dramatically reducing the intracellular pool of adenine nucleotides, the precursors for ATP.

3. Changes in metabolism (Figure 2)

In the presence of oxygen, human cells respire and derive their energy from the complete degradation of food (fats, carbohydrates and amino acids) by specific oxidative processes that fuel oxidative phosphorylation. A lack of oxygen completely changes these metabolic pathways, disrupting glycolysis and inhibiting the degradation pathways of lipids (beta-oxidation), amino acids and oxidative phosphorylation.

3.1. Glucose metabolism

During ischemia, the cell will change not only its glucose supply routes but also its glycolysis pathways and transition from aerobic glycolysis to anaerobic glycolysis. When this happens, the available cytosolic glucose is metabolized by anaerobic glycolysis and becomes the main source of ATP. The efficiency of this process is much lower than that of aerobic glycolysis coupled to oxidative phosphorylation; the anaerobic degradation of one molecule of glucose produces 2 ATP molecules compared to the 36 ATP molecules that are produced under aerobic conditions. Consumption quickly exceeds production, and the intracellular concentration of ATP decreases. For example, in the heart, the degree of glycolysis inhibition is directly proportional to the severity of coronary flow restriction.[3]-[5]

3.1.1. Glucose supply

With the complete interruption of or decrease in blood flow, the extracellular concentration of glucose drops very quickly. First, the cell optimizes the uptake of glucose from the interstitial space by improving glucose transmembrane transport by increasing the sarcolemmal expression of the high-affinity glucose transporters GLUT-1 and GLUT-4. [6]-[8] This protective mechanism temporarily compensates for the decrease in extracellular glucose concentration. Next, the cell uses its intracellular glucose stores of glycogen. [9] The decrease in intracellular ATP and glucose-6-phosphate, the rising lactate/pyruvate ratio and the increase in intracellular AMP and the inorganic phosphate concentration activate a phosphorylase kinase, which catalyses the conversion of glycogen phosphorylase b to its active form, glycogen phosphorylase a. This cascade reaction leads to an intense and rapid consumption of glycogen. [10]-[14]

3.1.2. Glycolysis pathways

The inhibition of oxidative phosphorylation caused by lack of oxygen does not allow the pyruvate produced by glycolysis to be degraded. Under aerobic conditions, pyruvate is transported into the mitochondria and feeds into the Krebs cycle, which provides the nicotinamide adenine dinucleotide (NADH, H⁺) and flavine adenine dinucleotide (FADH₂) cofactors for oxidative phosphorylation, significantly increasing the yield of glycolysis.

Ischemia modulates the activity of the following two key enzymes of anaerobic glycolysis: phosphofructo-1-kinase (PF1K) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Following the onset of ischemia, or during moderate ischemia, the activation of glycogenolysis accelerates glycolysis.[15]-[17] The decrease in both intracellular ATP and creatine phosphate, along with increases in the intracellular concentrations of AMP, inorganic phosphate and fructose-1,6-bisphosphate, intensify the activity of PF1K and GAPDH. [17]-[20]

During prolonged or sustained ischemia, the low intracellular glucose concentration, the disappearance of glycogen and severe intracellular acidosis eventually inhibit PF1K. Furthermore, high concentrations of lactate and protons in ischemic tissues also inhibit GAPDH. [21],[22]

Moreover, the lactate/pyruvate ratio, intracellular acidosis and the absence of regenerated essential cofactors, such as NADH, H^+ , affect the catalytic activity of the other enzymes involved in the initial step of glycolysis and prevent the optimal performance of anaerobic glycolysis. [23]

3.2. Lipid metabolism (Figure 2)

The importance of oxygen in functional oxidative phosphorylation leads to a significant reduction in ATP production from the beta-oxidation of fatty acids that is proportional to the degree of ischemia. In mild to moderate ischemia, the rate of fatty acid oxidation decreases but still fuels oxidative phosphorylation. [4],[24] In more severe ischemia, the lack of the cofactors NADH, H^+ and FAD^+ , which are normally regenerated through oxidative phosphorylation, completely inhibits acyl-CoenzymeA (acyl-CoA) dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase, which are key beta-oxidation enzymes.[4],[25] The cytosolic concentrations of fatty acids, acyl-CoA and acylcarnitine rise gradually. [26]-[28] The accumulation of these amphiphilic compounds in ischemic tissues has major functional implications. They dissolve readily in cell membranes and affect the functional properties of membrane proteins. Decreased activity of Na^+/K^+ -ATPase and the sarcoplasmic and endoplasmic reticulum Ca^{2+} -ATPase pumps, as well as the activation of ATP-dependent potassium channels, reduces the inwardly rectifying potassium current and prolongs the opening of Na^+ channels, delaying their inactivation.[29]-[31] The accumulation of amphiphilic compounds produces a time-dependent reversible reduction in gap-junction conductance. [31]

3.3. Metabolite detoxification pathways

Reducing the intracellular concentration of ATP inhibits the hexose phosphate cycle. This metabolic pathway regenerates glutathione, ascorbic acid and tocopherol, which are involved in the detoxification of metabolites from the cytosol and the sarcoplasmic membrane.

4. Intracellular acidosis

Intracellular acidosis is a cardinal feature of cellular ischemia. The increased production of protons due to metabolic modifications very quickly saturates the buffering capacity of the cell. Intracellular acidosis interferes directly and indirectly with the optimal functioning of the cell by increasing intracellular Na^+ through the activation of Na^+/H^+ exchangers and by Ca^{2+} activation of Na^+/Ca^{2+} exchangers, increasing the production of free radicals; changing the affinity of different proteins, such as enzymes and troponin C, to Ca^{2+} ; modifying tertiary protein structures; inhibiting enzymes; and disrupting the function of sarcoplasmic pumps and carriers.[29]

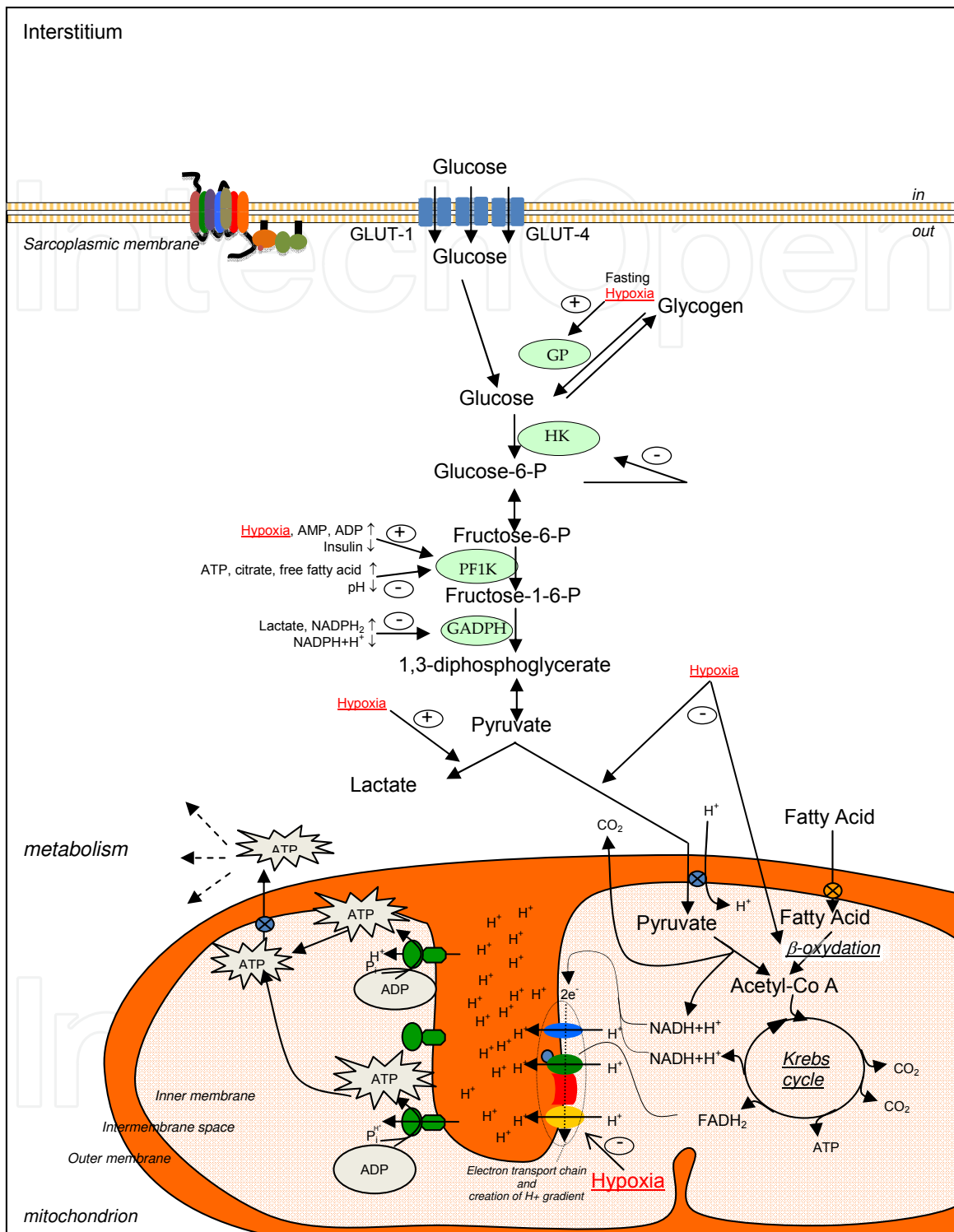


Figure 2. This figure shows schematically oxidative metabolism, ATP production and the consequences of oxygen deprivation. GLUT-1 and GLUT-4: glucose transporters; GP: Glycogene phosphorylase; HK: Hexokinase; PFK1: Phospho-fructo-1-kinase; GADPH: glyceraldehyde-3-phosphate dehydrogenase; NADH, H⁺: nicotinamide adenine dinucleotide; FADH₂: flavine adenin dinucleotide; P: phosphate; AMP, adenosine monophosphate; adenosine diphosphate; ADP: adenosine diphosphate ATP: adenosine triphosphate; CO₂: carbon dioxide; O₂ Oxygen; -: inhibition; +: activation; H⁺: proton; e⁻: electron.

The main source of protons during ischemia comes from the production of lactate from pyruvate by lactate dehydrogenase. The accumulation of extracellular lactate greatly reduces the effectiveness of the lactate/proton cotransporter, preventing the removal of protons. Additionally, the residual metabolic activity also contributes to acidosis, as the hydrolysis of an ATP molecule releases a proton.

5. Changes in the ionic cellular equilibrium (Figure 3)

Ischemia induces a profound disturbance of the ionic homeostasis of a cell. The two major changes are the loss of ionic transmembrane gradients, which causes membrane depolarization, and increased intracellular sodium ($[Na^+]_i$), which is responsible for inducing a rise in the intracellular calcium ($[Ca^{2+}]_i$) levels, leading to cellular edema.

Cellular depolarization occurs very rapidly after the onset of ischemia, and these mechanisms are not fully understood. However, it is recognized that both the inhibition of the Na^+/K^+ -ATPase and the opening of ATP-dependent K^+ channels play a crucial role. Cellular depolarization is characterized by a negative outgoing current and a decrease in the extracellular concentrations of Na^+ , Cl^- and Ca^{2+} , as well as an increase in the extracellular concentration of K^+ . Progressive depolarization of the cell also promotes prolonged activation of voltage-dependent sodium channels. [29]

The accumulation of sodium in the cytosol is multifactorial. Acidosis stimulates Na^+/H^+ exchangers to purge cellular H^+ , which results in increased intracellular Na^+ . [32]-[34] This net movement of Na^+ is accompanied by osmotic water movement. Moreover, inhibition of the Na^+/K^+ -ATPase due to a lack of ATP prevents the removal of excess intracellular Na^+ . The high intracellular concentration of Na^+ affects the function of other membrane transporters, such as the Na^+/Ca^{2+} antiporter, an accelerator. This allows the extrusion of sodium from the cell at the expense of an intracellular accumulation of Ca^{2+} . The massive entry of calcium into the cell disrupts the mechanisms that regulate its intracellular concentration and induces the release of calcium from the intracellular endoplasmic reticulum stores. [35] The lack of ATP prevents calcium excretion into the interstitium and its sequestration in the endoplasmic reticulum. The accumulation of cytosolic calcium induces degradation of membrane phospholipids and cytoskeletal proteins, alters the both the calcium affinity and the efficiency of proteins involved in contractility, activates nitric oxide synthase (NOS) and proteases such as calpains and caspases, promotes the production of free radicals and alters the tertiary structure of enzymes such as xanthine dehydrogenase, which is converted to xanthine oxidase. [36]-[38]

6. Mitochondria

The mitochondrion plays a central role in ischemic injury. Not only is it the site of critical biochemical reactions in the cell, such as oxidative phosphorylation, beta-oxidation and the

citric acid cycle, but it also occupies a unique position in the cellular balance between life and death. Inhibition of the mitochondrial respiratory chain as a result of oxygen deprivation is the cornerstone of metabolic disturbances.

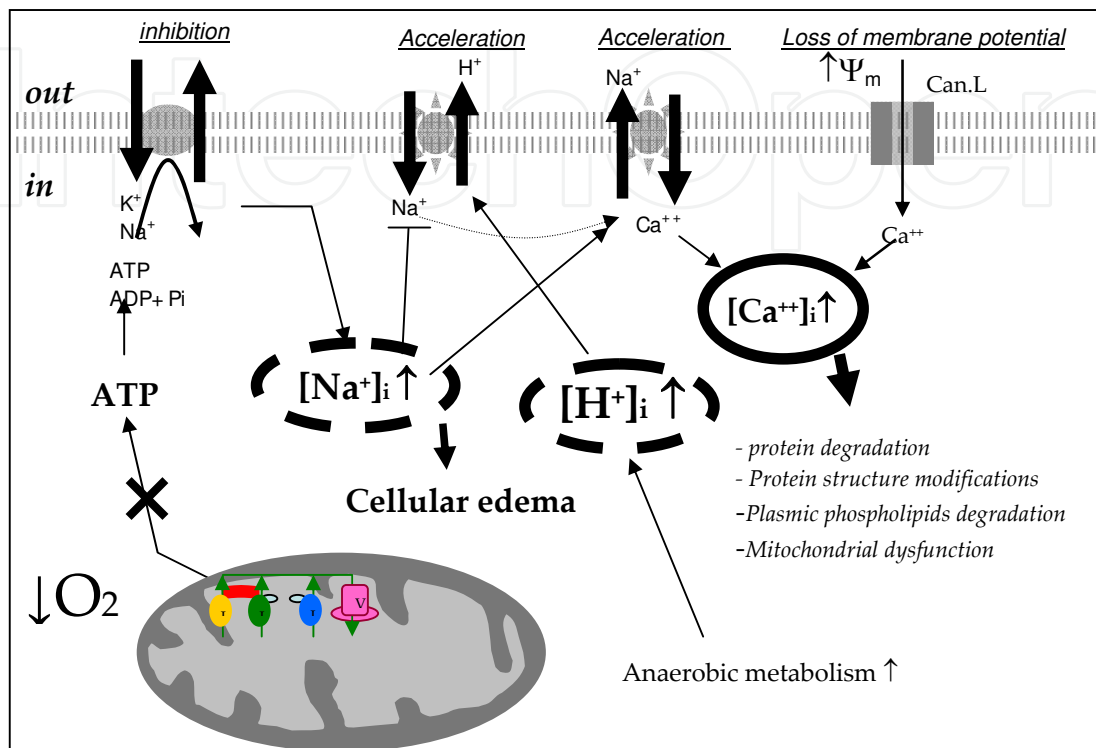


Figure 3. This figure summarizes the ionic perturbations in an ischemic cell.

6.1. Disturbance of ATP synthesis.

Without the respiratory chain oxidation-reduction reactions, proton accumulation in the mitochondrial intermembrane space is interrupted, disrupting the electrochemical gradient that allows ATP synthase to synthesize ATP. During ischemia, the proton-translocating F₀F₁-ATP synthase, which normally produces ATP, becomes an F₀F₁-ATPase and consumes ATP in order to pump protons from the matrix to the intermembrane space and maintain the mitochondrial membrane potential.[39],[40] The mitochondria therefore become a site of ATP consumption produced by anaerobic glycolysis.

6.2. An increase in free radical production

Free radical oxygen species (ROS) are highly reactive chemical compounds because they have unpaired electrons in their electron cloud. ROS are capable of oxidizing cellular constituents such as proteins, deoxyribonucleic acid (DNA), membrane phospholipids and other adjacent biological structures. In addition to their role in ischemia, ROS are constitutively generated during metabolic processes and have an important role in cell signaling. Mitochondrial respiration constitutively produces a small amount of ROS, primarily the superox-

ide anion $O_2^{\bullet-}$ at complexes I and III of the electron transport chain. The anion is rapidly converted to hydrogen peroxide (H_2O_2) by metallo-enzymes and superoxide dismutase (SOD). [41]-[43] Cellular stress, particularly oxidative stress, dramatically increases mitochondrial ROS production by disrupting and later inhibiting oxidative phosphorylation. Moreover, the rise in mitochondrial calcium increases ROS production and greatly decreases the antioxidant capacity of mitochondria by decreasing the glutathione peroxidase concentration and SOD activity.

6.3. Intramitochondrial calcium overload

The mitochondrial calcium concentration is in equilibrium between its cytosolic concentration and the proton gradient on either side of the inner membrane of mitochondria. The loss of this gradient due to the inhibition of the respiratory chain, as well as the elevated cytosolic calcium that results from ischemia, allows for the accumulation of calcium in the mitochondria and promotes mitochondrial swelling and the opening of the permeability transition pore.

6.4. Opening of the mitochondrial permeability transition pore

Ischemic disturbances within mitochondria, such as calcium overload, loss of membrane potential, oxidative stress, mass production of free radicals, low NADPH/NADP⁺ and reduced glutathione to oxidized glutathione ratios (GSH/GSSG), low intra-mitochondrial concentration of ATP or high inorganic phosphate, will promote opening of the permeability transition pore (mPTP) upon reperfusion, a major player in I/R injury-mediated cell lethality.[42], [44] mPTP is a nonspecific channel, and its opening suddenly increases the permeability of the inner mitochondrial membrane to both water and various molecules of high molecular weight (> 1,500 kDa). The opening of mPTPs abolishes the mitochondrial membrane potential and uncouples oxidative phosphorylation, which empties the mitochondria of its matrix and induces apoptosis by releasing the intra-mitochondrial proteins cytochrome c, endonuclease G, Smac/Diablo and apoptosis-inducing factor into the cytosol. [44]^{1-52]}

7. Structural and functional modifications

The cytoskeleton, the internal structural organization of a cell, is composed of a highly regulated complex network of organized structural proteins, including actin, microtubules and lamins. The cytoskeleton performs multiple functions. It maintains internal cellular compartmentalization and mediates the transmission of mechanical forces within the cell to adjacent cells and the extracellular matrix, the distribution of organelles, the movement of molecules or components and the docking of proteins such as membrane receptors or ion channels. Ischemia deconstructs the cytoskeleton. [53]-[56] The high intracellular concentrations of Ca^{2+} that are associated with ischemia activate multiple phosphorylases and proteases that disassemble and degrade the cytoskeleton, thereby eliminating the functions that rely on its integrity, such as phagocytosis, exocytosis, myofilament contraction, intercellular

communication and cell anchorage. Destruction of the internal architecture worsens I/R injuries and leads to apoptosis. [53],[56],[57] During ischemia, all elements of the cytoskeleton are affected, but with different kinetics.[54],[55] Moreover, the accumulation of osmotically active particles, including lactate, sodium, inorganic phosphate and creatine, induces cellular oedema.[38]

Regulatory cellular mechanisms provide intracellular homeostasis that enables optimal enzyme function in a relatively narrow range of environmental conditions. The conditions created by ischemia, such as acidosis and calcium overload, modify or inhibit the activity of many enzymes due to changes in the pH and tertiary structures, affecting cellular metabolism. For example, ischemia induces the conversion of xanthine dehydrogenase to xanthine oxidase.[36]-[38] These two enzymes catalyze the same reactions, converting hypoxanthine to xanthine and xanthine to uric acid. The first reaction uses NAD⁺ as a cofactor, whereas the second uses oxygen and produces O₂[•], a free radical.

8. Protein synthesis and sarcoplasmic protein expression in an ischemic cell

Protein synthesis is a complex process that requires continuous and adequate energy intake, strict control of ionic homeostasis of the cell and the smooth functioning of many other proteins. Ischemia disrupts these necessary conditions and therefore profoundly affects protein synthesis beyond acute injury. However, the transcription of several genes is initiated at the onset of ischemia, and the mechanisms underlying this phenomenon are not fully understood. Nevertheless, it appears that the mass production of free radicals, the high concentration of calcium, acidosis and the activation of the family of mitogen-activated protein kinases (MAP kinases) play an important role. Nuclear factor heat shock transcription factor-1 (HSF-1) activates the expression of heat shock proteins (HSPs), a family of chaperone proteins, and inhibits the expression of other proteins. HSPs are synthesized in different situations of stress, including hyperthermia, ischemia, hypoxia and mechanical stress, and are intended to prevent the structural modifications of key metabolic and cytoskeletal enzymes and inhibit the activity of caspases. [58]-[60]

The low oxygen partial pressure during ischemia activates other nuclear factors, such as hypoxia-inducible factor-1alpha (HIF-1 α). HIF-1 α stimulates the transcription of many genes involved in cellular defense, such as those encoding NOS and GLUT-1, and other enzymes involved in glucose metabolism.[61]

In addition, ischemia activates innate immunity by stimulating sarcoplasmic receptors, such as the Toll-like receptors (TLR) TLR-2 and TLR-6, the synthesis and sarcoplasmic expression of which are increased. Receptor stimulation supports the synthesis of chemokines and cytokines and contributes to I/R injury.[61]-[66]

At the onset of ischemia, many substances are secreted by the cell. For example, ischemic cardiomyocytes secrete bradykinin, norepinephrine, angiotensin, adenosine, acetylcholine

and opioids.[67]-[69] In addition, ischemia stimulates the expression of adhesion molecules, such as P-selectins, L-selectins, intercellular adhesion molecule-1 (ICAM-1) and platelet-endothelial cell adhesion molecules (PECAM), on the surface of endothelial cells, leukocytes and other ischemic cells. [62],[63],[70],[71] Furthermore, many cytokines, such as tumor necrosis factor- α , interleukin (IL)-1, IL-6 and IL-8, and vasoactive agents, such as endothelins and thromboxane A₂, are secreted by cells in response to ischemia. [62],[70],[72] Cytokines and chemokines, the production of which dramatically increases during reperfusion, initiate the local inflammatory response and prepare for the recruitment of inflammatory cells into the injured area, respectively.

Author details

Maximilien Gourdin* and Philippe Dubois

*Address all correspondence to: maximilien.gourdin@uclouvain.be

Université de Louvain (UCL), University Hospital CHU UCL Mont-Godinne – Dinant, Yvoir, Belgium

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