V. CEÑA, M. FERNÁNDEZ, C. GONZÁLEZ-GARCÍA AND J. JORDÁN

Regional Center of Biomedical Investigations, University of Castilla La Mancha, Albacete, Spain

34. STROKE AND ISCHEMIC INSULTS

Summary. Brain ischemia is a common disease that is responsible for about 120 deaths per 100,000 habitants in Western countries. When cerebral blood flow decreases, there is an area, named penumbra, surrounding the core of ischemia, where neurons are perfused through collateral vessels. In this area, several molecular mechanisms involving excitatory amino acid release, increases in intracellular calcium, mitochondrial dysfunction, free radical production, release of mitochondrial proteins, proteases (mainly caspases) activation and neuronal death. These mechanisms activated in the penumbra area are responsible for the final fate of neurons and the degree of neurological damage that is produced in the patient.

1. INTRODUCTION

Stroke is the third leading cause of death in western countries being mortality rate about 120 per 100,000 persons per year. The probability of suffering a stroke is higher than 95% when cerebral blood flow decreases to about 25% of control levels. Under these conditions, mitochondrial function fails and ATP concentration in the neurons drops within minutes after cessation of blood supply. At the core of the infarct, this causes osmotic cell lysis and necrotic death. Surrounding this necrotic core is a region, called penumbra, where perfusion from collateral vessels is kept between 25-50% of control values. This would allow enough cerebral blood flow to maintain mitochondrial ATP production. However, mitochondrial function gradually fails over the ensuing days resulting in secondary cell death occurring mainly via apoptosis. Regardless of whether neuronal death is acute or secondary, ischemia is accompanied by increased efflux of excitatory amino acids, bioenergetical failure causing massive cell depolarisation, with efflux of K⁺ and uptake of Na⁺, Cl⁻, and Ca²⁺ (for a review, see Kristian and Siesjo, 1997), disruption of energy production, increased free radical generation, intracellular Ca²⁺ dyshomeostasis and induction of the apoptotic cascade.

2. EXCITATORY AMINO ACID RELEASE

Excitatory amino acids, glutamate and aspartate, are endogenous compounds acting as neurotransmitters in brain, through the activation of three types of ionotrop
receptors named after the initially described agonist activating them: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainic acid receptors. NMDA receptor-associated channels are permeable to Na\(^+\), K\(^+\) and Ca\(^{2+}\) in a voltage-dependent manner, whereas AMPA and kainic acid receptors are linked to Na\(^+\) permeable channels (Lerma, 2003). In addition, glutamate might also activate metabotropic receptors that induce G protein-mediated changes in second messengers. During brain ischemia there is a marked release of glutamate from the brain that can be monitored in plasma and cerebrospinal fluid from patients suffering ischemic stroke. Consistently with these clinical findings, pre-treatment of rats with the NMDA receptor antagonist, MK-801, decreases by 30 % infarct size following middle cerebral arterial occlusion showing that excessive stimulation of these receptors take place during cerebral ischemia, leading to neuronal degeneration.

Following glutamate release, there is a marked stimulation of glutamate receptors leading to neuronal degeneration that shows two components: an acute Na\(^+\)/Cl\(^-\)-dependent neuronal swelling and delayed Ca\(^{2+}\)-mediated cell death where a massive and prolonged Ca\(^{2+}\) influx to the cytoplasm and reactive oxygen production has been observed.

3. CALCIUM

Calcium ions play a key role as regulators of numerous cellular functions. Therefore, cells tightly control free intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\); see Berridge et al., 2003). Since the extracellular calcium concentration ([Ca\(^{2+}\)]\(_o\)) is several orders of magnitude higher (1 mM) than the intracellular one (about 100 nM), even a small increase in the permeability of cell membranes to Ca\(^{2+}\) ions lead to a significant rise in [Ca\(^{2+}\)]\(_i\). Calcium can enter cells via voltage- and agonist-operated Ca\(^{2+}\) channels and can be released from intracellular stores, i.e. the endoplasmic reticulum or the so called calciosomes. The mitochondria also represent a potential Ca\(^{2+}\) source; however, during resting physiological conditions mitochondrial Ca\(^{2+}\) content is low (about 200 nM).

During the last two decades it has become widely accepted that neuronal damage following brain ischemia is due to a perturbation of cellular Ca\(^{2+}\) metabolism. Brain ischemia, which compromises the cellular bioenergetic status, leads to cell depolarisation and to a rise in [Ca\(^{2+}\)]\(_i\). When ischemia ends, by reperfusion, the bioenergetic potential usually recovers and ion gradients are restored. However, neuronal damage can be observed following hours or days of reperfusion.

A marked and prolonged increase in [Ca\(^{2+}\)]\(_i\) is harmful to cells because it leads to activation of Ca\(^{2+}\)-dependent enzymes. Calcium might activate phopholipase A\(_2\) increasing arachidonic acid production that is metabolised by lipoxygenases or cyclooxygenases producing reactive oxygen species. On the other hand, endonucleases (enzymes that degrade DNA) play an important role in apoptosis generation and might also be activated by Ca\(^{2+}\) in absence of caspase-3. Also phosphatases, like calcineurin, or proteases, like calpain, might be activated by Ca\(^{2+}\).