

Hypoxic-ischemic brain injury: Pathophysiology, neuropathology and mechanisms

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Abstract. Hypoxic-ischemic brain injury is a well known consequence of cardiac arrest. Variable injuries can occur with purely hypoxic or histotoxic insults such as asphyxiation and carbon monoxide poisoning. The injury may happen at the time of the insult, but there may also be continued damage after circulation and oxygenation are reestablished. The nature and extent of the damage appears to depend on the severity, time course and duration of the oxygen deprivation and lack of blood supply, as well as on the underlying mechanism. This review describes the pathophysiological and molecular basis of hypoxic ischemic brain injury, and differentiates between the mechanisms of injury by cardiac arrest, pure respiratory arrest, and arrest secondary to cytotoxicity (e.g. carbon monoxide poisoning).

1. Introduction

Due to its high metabolic demand, the brain is very susceptible to damage from deprivation of blood supply. Animal studies have shown that after onset of ischemia, the concentrations of brain glucose, glycogen, adenosine triphosphate (ATP) and phosphocreatine fall immediately and are nearly completely depleted within 10–12 minutes of ischemia [70]. Depending on the animal model and conditions, neuronal damage and irreparable brain damage have been demonstrated within several minutes after the onset of global ischemia [49, 66], while other models have shown longer (as long as one hour) tolerance of circulatory arrest, with subsequent functional recovery [32,35]. Damage can also occur in a delayed fashion [54]. Primary respiratory arrest often leads to brain dysfunction that tends to be transient, resulting in less severe and permanent damage than that caused by a primary ischemic insult. This is particularly true if the insult is purely hypoxic with-

out circulatory arrest. Carbon monoxide poisoning can either result in a pattern of brain injury observed in isolated hypoxia, or, if the degree of toxicity leads to systemic hypotension, be comparable to an ischemic-anoxic injury.

The pathogenesis of brain injury is somewhat different in each of the etiologies, and the prognosis for neurological recovery depends on the underlying mechanism. Understanding these mechanisms help the clinician to understand the potential for recovery.

2. Pathophysiology of brain injury in cardiac arrest

The pathophysiology of ischemic brain injury has been explored with the help of different animal models. The starting point and the duration of the perfusion deficit can be defined accurately by induction of cardiac arrest or occlusion of the major arteries and ceasing the circulation to the brain. In humans, unless in situations of witnessed cardiac arrest, the exact downtime is rarely known, and reported periods of ischemia and anoxia are often vague. In general, the extent of tissue affected by ischemia, the severity of neurological findings and

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the rapidity of recovery are directly related to the length of the circulatory arrest. After 15 minutes of global ischemia with cardiac arrest, up to 95% of brain tissue is damaged [2].

Ischemic cell death is characterized by a delay between the insult and the manifestation of major cell damage. This delay varies and is dependent on the type and duration of the insult, the affected brain region and the cell type. Cell damage can appear in as little as a few hours, and as much as four days later. Based on the duration of the ischemia, the onset will be earlier or later, with longer durations leading to a shorter delay in manifestation [46]. After the onset of circulatory arrest, there is reflexive vascular dilatation if cerebrovascular autoregulation is appropriate. With ongoing arrest, stasis of the blood provokes a fall in viscosity which lowers resistance in low resistance regions (capillaries, veins) to buffer the pressure of adjacent higher resistance regions [20].

3. Biochemical changes

The microscopic mechanisms for tissue injury during the interruption of circulation are manifold. Early changes in global ischemia consist of anoxic depolarizations, which lead to changes in the intra- and extracellular electrolyte composition, and a fall in ATP. Anoxic depolarization is one of the main events observed in an anoxic-ischemic insult in vivo [71]. Between 60 and 180 seconds after the onset of global ischemia, a large negative direct-current shift occurs in both neuropil and cell body regions of the ischemic tissue. Due to impaired cell membrane function, extracellular sodium, chloride and calcium decrease, and potassium leaks into the extracellular space [71]. The abrupt fall in the extracellular calcium concentration at the time of anoxic depolarization and the calcium influx into the cell lead to an approximate 25% increase in total cell calcium [38]. The calcium entry likely takes place via NMDA receptors [25]. This increase in intracellular calcium activates calcium-dependent processes, such as the calpain system, which is involved in remodeling of cytoskeletal and membrane structures, signal transduction pathways, and apoptosis [26]. Calpain plays an important role in global ischemic damage [5], as calpain inhibitors appear to reduce cell death effectively [45]. The sodium-induced damage seems to be a combination of increasing cytosolic calcium, depleting ATP, and glutamate release. Drugs that block sodium fluxes and prevent increases in intracellular sodi-

um during anoxia or ischemia are protective in global ischemia [47].

High energy phosphates (i.e. ATP) drop to their lowest values within one to two minutes [39], and lactic acid and hydrogen ions (H^+) are released into the local environment. Acidosis abounds on a local level: the extracellular space begins to become acidic within 20 seconds of the ischemic or anoxic insult [67], and the intracellular pH follows and reaches a minimal value after two minutes [68]. A pH of 6.1 to 6.5 can be survivable for neurons for a period of ten to twelve minutes [36]. Greater and longer lasting acidosis further worsens cell function and edema. Hyperglycemia may worsen acidosis caused by increased amounts of lactic acid. Chronic hyperglycemia is associated with increased ischemic damage and mortality [36].

In addition, glutamate, an excitotoxic neurotransmitter, is released, and a number of destructive enzymes, including lipases, proteases and nucleases, are activated, which break down neuronal tissue. Free radical formation is also increased during the early period after global brain ischemia [55]. Furthermore, nitric oxide production is elevated, and peroxynitrite, the major mediator of nitric oxide toxicity, is produced after global ischemia [19]. By eliminating nitric oxide in neuronal nitric oxide synthase knock out mice, cell loss was reduced from 85% to 32% in the pyramidal cell layer in one animal study [51]. Table 1 summarizes the biochemical mechanisms leading to cell damage in a hypoxic-ischemic event.

4. Functional changes of the cell in hypoxic-ischemic insult

Mitochondrial damage occurs early after the intracellular calcium accumulation [62]. Transient mitochondrial swelling can be observed, along with disaggregation of polyribosomes, and abnormal Golgi complexes [54]. The mitochondrial dysfunction causes a further lack of ATP repletion, overproduction of free radicals, and increasing inability of the cell to buffer the calcium loads. The cytoskeleton is damaged and the cells cannot maintain their cell structure, which is an important factor in the process toward cell death. Protein synthesis is profoundly depressed by ischemia; this persists for variable periods of time, from one to 24 hours, depending on the model and the time to reperfusion [14,21]. Protein synthesis recovers completely or near completely over 12–48 hours in the regions in which cells are preserved enough to recover, but there

Table 1
Biochemical mechanisms in hypoxic-ischemic brain injury

Mechanism	Immediate effect	Result
Anoxic depolarization	↓ extracellular sodium ↓ extracellular calcium ↑ intracellular calcium	Activation of calcium-dependent processes (calpain system)
ATP depletion	Local acidosis	Generalized acidosis, cell damage, edema
Glutamate release	Excitotoxic activation of enzymes	Neuronal tissue breakdown
Free radical formation, Nitric oxide production		Cell break down

Table 2
Functional cell changes in hypoxic-ischemic injury

Underlying functional problem	Result
Mitochondrial damage	Lack of energy repletion
Cytoskeletal damage	Inability to maintain cell structure
Glutamate receptor activation	Immediate early gene upregulation Heat shock protein production

is no recovery to normal function in dying cells [69]. The increased amount of cytosolic calcium, as well as NMDA receptor activation are etiological factors in the inhibition of neuronal protein synthesis [61].

Excitotoxic activation of glutamate receptors plays a major role in the induction of heat shock proteins and in triggering the rapid transcription of a large number of immediate early genes within the first 30 minutes after the onset of global ischemia or hypoxia-ischemia [41]. There is a regional difference in the upregulation of proteins, as immediate early genes have been found to be induced in neurons and non-nerve cells in damaged regions in the setting of a moderate hypoxic-ischemic event, whereas in a severe event, they would be induced in non-damaged regions as well [8,17]. The Bcl-2 group of proteins was found to be selectively upregulated in nonvulnerable regions of the hippocampus [30], and appears to function as protective against free radical-mediated cell death [37]. Table 2 provides an overview over the functional aspects of cell damage after a hypoxic-ischemic insult.

5. Reperfusion after circulatory arrest

With the return of spontaneous circulation, there is return of oxygen and metabolite supply, and metabolic activity is resumed. Cell death does not occur as long as reperfusion is established within a certain period of time, the “revival period” [33]. However, the process of cell damage is not necessarily over once circulation is resumed. Despite the re-establishment of an adequate systemic circulation, the cerebral circulation may remain significantly impaired. After the return

of spontaneous circulation following a cardiac arrest, a rapidly occurring, short-lived period of hyperemia is soon replaced by a more protracted period of global and multifocal hypoperfusion (the “no-reflow phenomenon”) [2]. During the post-ischemic hyperemia period, which lasts for about 15 minutes, blood flow is two to three times higher than normal levels [16]. The blood flow then becomes markedly reduced, to 30 to 50% of the normal flow in the period of hypoperfusion, which lasts between 6 and 24 hours [16,44]. The cause of such circulatory impairment after a limited time of ischemia seems to be a combination of a change in the state of blood in the cerebral vasculature. The viscosity of blood is directly related to the concentrations of plasma proteins and of the formed blood elements. Due to the shift of inorganic electrolytes and water from the plasma into the surrounding parenchyma during the ischemic insult, these concentrations are increased [2].

While vascular spasm has been reported to occur during asphyxia, it preferentially affects the larger size vessels [48]. Much of the impaired blood flow in the brain occurs on the microcirculatory level. Studies have shown that stasis of blood flow for 15 minutes results in failure of reflow on the microvascular level, which involves up to 50% or more of the total brain mass [23]. The capillary lumen is reduced by swelling of the surrounding cells and the endothelium [2], and capillary flow is impeded by red blood cell sludging and disseminated intravascular coagulation [23]. It may also be impaired by the increased adhesion of leukocytes to the vascular endothelium. The resulting mismatch between blood flow and oxygen requirements of the tissue may provoke secondary hypoxia [33]. It has also been noted that the blood brain barrier becomes several fold

more permeable to normally poorly permeant small solutes (e.g. sucrose) 6 hours after global ischemia, but then returns to normal by 24 hours [58]; it is not clear whether the increased transport across the blood brain barrier contributes to further damage or serves as part of the repair mechanisms.

Once cerebral circulation is effectively reinstated, another phase of injury, the so-called “reperfusion injury”, may occur. Free radical formation and nitric oxide toxicity are more pronounced in the early reperfusion period [19,55], and further glutamate release as well as a renewed calcium shifts occur [10]. With blood inflow, there is an influx of polymorphonuclear leukocytes into the ischemic area. The polymorphonuclear cells promote peroxidative changes contributing to the reperfusion injury [9]. Furthermore, the reconstitution of blood flow can result in edema and microhemorrhages due to the dysfunctional and damaged capillaries, which increases brain swelling even further. Thus, even after resumption of systemic circulation, damaging processes may be ongoing.

6. Neuronal cell death

Pathological studies have brought evidence for different types of neuronal cell death after an ischemic insult [46]. Ischemic cell damage can culminate in primary necrotic cell death, if the revival time of brain is exceeded by the ischemic event; this is the most prevalent type [34]. The second important mechanism of cell death after hypoxic-ischemic insults is apoptosis. Another form of cell death in ischemia is free radical-induced damage and autophagocytosis [34]. The different types of cell damage can be seen at the same time in the same cell populations [18].

In necrotic cell death, visible change may be minimal in the initial hours following an ischemic event, consisting mostly of “cloudy swelling” of the nuclear region of neurons and loss of a basophilic appearance to the nucleus [1,49]. This change can be induced by NMDA agonists [57]. Calcium also has been found to be a major effector of necrotic cell death during ischemia [43]. After 8–12 hours, the classic “red neurons” begin to appear. These represent shrunken cells, with pyknotic nuclei containing coarse nuclear chromatin creating significant darkening and shrinkage of the nucleus [1,49]. The cytoplasm becomes progressively eosinophilic, visible on light microscopy as early as 30 minutes to 1 hour after an insult [15].

Table 3
Neuropathological features of cell death

Mechanism of cell death	Features
Necrosis	Loss of basophilic nucleus Pyknotic, dark nuclei Eosinophilic cytoplasm
Apoptosis	Apoptotic bodies Nucleosomal segments Cytochrome c release
Autophagocytosis	Condensed cytoplasm Large vacuoles Clumped nucleus

Apoptosis can be induced by a large number of different stimuli, such as nitric oxide production, free radical formation, reduced mitochondrial function, altered membrane potential and increased intracellular calcium. Many of these stimuli prevail during ischemia. Apoptosis in itself may further compromise the energy state of the ischemic tissue, both by consuming energy for the completion of the genomic program and by impairing the generation of ATP [34]. Morphologic features of apoptosis include formation of chromatin masses within the nucleus and subsequent formation of apoptotic bodies [3]. Biochemical changes such as breakdown of double stranded DNA into nucleosomal segments [7], activation of caspases, inhibition of proteases [52], and release of cytochrome c are strong criteria for apoptosis.

The extent of apoptosis as a mechanism of cell death after an ischemic insult is not entirely clear. Some studies have brought strong evidence for the contribution of apoptotic processes to cell death: caspase activity is increased [12], various apoptosis-promoting genes are up-regulated during the maturation of neuronal cell death [22], and TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling staining of DNA fragments) positive neurons showing condensed chromatin and apoptotic bodies are detected [9]. However, other studies found the final morphology not consistent with classic apoptosis, due to lack of spherical nuclear chromatin clumping and apoptotic bodies, and eosinophilic staining instead in most cells pointing towards necrotic cell death, and thus did not detect unequivocal signs of apoptosis [34]. Morphologic characteristics of autophagocytosis include condensation of the cytoplasm which contains many large vacuoles, and an irregularly clumped nucleus [13].

The different neuropathological features are summarized in Table 3.

7. Areas of brain damage

With ischemic and hypoxic insults, specific brain regions and distinct neuronal populations appear to be more commonly affected. A higher metabolic rate and demand of oxygen and nutrients of the neurons in those locations, or their location in vascular border zones (resulting in less tolerance of relative ischemia) makes these regions more vulnerable [11]. The knowledge of these typically affected brain regions is important, as the neurological symptoms and clinical syndromes in cardiac arrest survivors can often be explained by these specifically affected areas of brain injury.

The CA1 pyramidal neurons of the hippocampus have been found to be the most vulnerable and commonly damaged with prolonged ischemia, with resulting impairment in memory [54]. Gait disturbance from truncal ataxia can often be explained by cell injury to the Purkinje cells of the cerebellum. Apart from those cell populations, the pyramidal neurons in layers 3, 5 and 6 of the neocortex, the reticular neurons of the thalamus, and the medium-sized neurons of the striatum are preferentially affected [40,56,60]. There is a correlation between the length of circulatory arrest and the dissemination of the damage. The gradient of injury seems to diminish towards the medulla oblongata [49], and a precise topical relation between the neuronal stress response and neuronal damage after brief cardiac arrest has been shown in animal studies [9].

Even in short periods of ischemia, mechanisms of delayed neuronal death are triggered in the hippocampal CA1 neurons [65]. The vulnerability in that area also is greatest in magnitude and rapidity of neuronal loss [64]. While the neurons in the CA1 sector of the hippocampus, and also in the dorsolateral part of the striate nucleus, are often irreversibly damaged by short periods of ischemia, glial cells and vascular cells survive [60]. On a molecular level, the selective neuronal vulnerability following cerebral ischemia has been attributed to characteristic patterns of immediate early gene and heat shock protein expression [9]. The neurons in the vulnerable areas do not necessarily die during the brief period of ischemia, but rather within a few days of reperfusion [54]. Particularly for the CA1 sector neurons, delayed cell degeneration has been shown, which corresponds to a prolonged expression of heat shock proteins and early immediate genes [9].

In addition to these specific areas of vulnerability, the vascular border zone areas are the first to suffer a reduction in blood flow due to their distance from the main arterial supply. With more prolonged periods

of ischemia, these arterial borderzone regions can be appreciated macroscopically as wedge-shaped lesions with its base at the pial surface and its apex towards the lateral ventricle [1]; this pattern can be observed on neuroimaging as well [31]. The three border zone areas are comprised of the anterior border zone between anterior (ACA) and middle cerebral artery (MCA), the posterior border zone between MCA and posterior cerebral artery, and the internal border zone between the superficial branches of the MCA and the deep branches of the MCA or ACA. Apart from lying in the internal border zone, the vasculature for the deeper cerebral white matter contains widespread linear arterioles with few anastomoses. This anatomy adds to the vulnerability of the central white matter to hypoxic ischemic injury [24].

8. Pathophysiology of brain injury in hypoxic arrest

After any event of resuscitation, encephalopathy is often referred to as “hypoxic-ischemic encephalopathy.” However, a purely hypoxic event is not the same as a cardiovascular collapse. There are differences in pathophysiology and pattern of brain damage, degree of injury and prognosis. Pure hypoxic injury does not commonly lead to severe brain injury, even in the setting of prolonged and/or extreme hypoxia, as long as the systemic circulation is adequately preserved. Hypoxia seems to induce functional changes of the neuron, without necessarily causing cell death. Thus, patients may be poorly responsive and even comatose after a pure hypoxic event, but may have a much better chance for survival with good neurological recovery than those with cardiac arrest.

Hypoxic events are typically caused by airway obstruction (e.g. by swelling, as in epiglottitis or anaphylaxis), airway trauma, or intoxication (with drugs or toxins). Epidemiologically, these patients are on average younger, and there is less preexisting atherosclerotic vascular disease, which has implications on the cerebrovascular autoregulatory function [29]. Hypoxia causes elevation of the partial pressure of carbon dioxide and thus a decrease in pH. Cerebral autoregulation leads to cerebrovascular dilation, and the cerebral blood flow (CBF) increases. In the setting of a preserved systemic circulation, nutrient and glucose supply to the brain continues, and toxic metabolites are washed from the local environment – this is very different from circulatory arrest in which the nutritional supply ceases and

toxic metabolites accumulate. Anoxia is well known to cause glial cell swelling and thus overall brain swelling. The swelling of astrocytic processes is most striking in hypercapnic hypoxia with severe acidosis, and is located particularly around the capillaries. Hypercapnia alone causes less extensive damage, and hypoxia alone causes only mild astrocytic swelling [4].

There is no increased expression of heat shock proteins in pure hypoxia [6,53]. Hypoxia seems to affect neuronal processes on the level of the synapse. A selective GABA-ergic deficit has been described; this could account for the frequent occurrence of myoclonus and seizures after hypoxic insults. In histological studies of animals exposed to pure hypoxia without hypotension, there were no areas of brain necrosis, while there invariably was ischemic brain injury in combined hypoxic-ischemic insults. The neurophysiologic abnormality in the event of hypoxia, however, was manifested by EEG abnormalities of slowing and increased amplitude [50]. In a series of 3 patients with prolonged hypoxia ($pO_2 < 45$ mm Hg) without systemic hypotension for 1–8 days, who ultimately died of sudden cardiac arrest, autopsy did not show changes suggestive of ischemic damage [63]. In another series of 22 comatose patients after prolonged pure hypoxia ($pO_2 < 20$ mm Hg) without hypotension, 13 of the 22 patients experienced recovery to the pre-morbid state [28]. The time course for recovery from a purely hypoxic event is about two weeks, which parallels that for synaptic regeneration.

Another clinical manifestation that has been described for hypoxic brain damage is the so-called delayed post-anoxic encephalopathy (also known as delayed post-hypoxic leukoencephalopathy). The anoxic insult causing delayed postanoxic encephalopathy is usually prolonged and severe [56]. Tissue damage preferentially involves the subcortical and deep white matter [24]. Clinically, the patients are initially cognitively intact for an interval of two to ten days, and then become abruptly irritable, apathetic, and confused, and display poor motor control. Neuropathology reveals extensive cerebral hemispheric demyelination as the major abnormality, whereas there are no significant vascular abnormalities and no striking cerebral edema. The pathogenesis of delayed demyelination is not entirely clear. The distribution of cerebral edema occurring in anoxia is similar to the distribution of delayed demyelination. However, the edema is maximal by day 2–4, and the encephalopathy typically occurs later [56]. Cases of delayed hypoxic demyelination have been reported in which arylsulfatase A activity was reduced to as little as 50% of normal [27]. Other mechanisms which possibly

contribute include effects of a hypersensitivity reaction, vascular damage, and direct carbon monoxide toxicity (in cases of carbon monoxide-induced hypoxia) [56].

9. Pathophysiology of brain injury in carbon monoxide intoxication

Carbon monoxide (CO) blocks oxygen binding to hemoglobin, and carboxyhemoglobin is formed. The ensuing hypoxia leads to cerebral vasodilatation that is greater than that seen with pure hypoxic hypoxia. The hypoxic state also triggers release of nitric acid from platelets and endothelial cells, which leads to formation of the free radical peroxynitrate. This causes mitochondrial dysfunction with a marked decrease in cytochrome oxidase, capillary leakage and apoptotic cell death. With longer durations of exposure, oxidative stress becomes amplified by leukocyte sequestration and xanthine oxidase activity with subsequent lipid peroxidation [42]. In addition, reoxygenation leads to production of partially reduced oxygen species, which in turn provokes oxidization of essential proteins and nucleic acids, and thus can result in reperfusion injury [59].

In the heart, CO binds to intracellular myoglobin so that oxygen supply to the mitochondria is impaired. This may cause myocardial ischemia and thus lead to further brain injury through systemic hypotension. Because of their high metabolic rates, the heart and brain are the organs most susceptible to CO toxicity. The degree of injury directly correlates with the severity and duration of the CO exposure.

The neuropathology of CO toxicity in the acute stage shows petechial hemorrhages of the white matter, particularly the corpus callosum. Subsequently, there is multifocal necrosis. The brain areas affected are the ones with a high metabolic rate and oxygen demand, and include the basal ganglia, particularly the globus pallidus, substantia nigra (pars reticularis), and hippocampus. Laminar necrosis of the cortex and Purkinje cell loss is often seen in addition to the white matter damage [59]. Clinically, patients frequently present with Parkinsonism and cognitive dysfunction due to these preferentially affected areas. Table 4 provides an overview over mechanisms in pure hypoxia and carbon monoxide intoxication.

10. Conclusion

In survivors of cardiac arrest or hypoxic insults, neurological injury is a common problem. The mecha-

Table 4
Mechanisms of brain injury in hypoxia and carbon monoxide intoxication

Mechanism	Immediate effect	Result
Hypoxia	Cerebrovascular dilatation Hypersensitivity reaction, vascular damage ↓ arylsulfatase A activity	Brain edema Delayed postanoxic encephalopathy
Carbon monoxide intoxication	Carboxyhemoglobin → pronounced vasodilatation Nitric acid release Free radical formation Myocardial ischemia	Brain edema Multifocal necrosis Apoptotic cell death Systemic hypotension

nisms of injury, as well as the prognosis, are not the same for different types of insult. Prolonged periods of hypoperfusion and ischemia are associated with worse outcomes, while isolated respiratory arrest with pure hypoxia often carries a better prognosis for recovery.

Neuronal injury can occur: 1) at the time of the initial event through local biochemical changes and functional compromise; 2) in the reperfusion period due the action of free radical generation and ongoing toxic damage; 3) due to impaired cerebral blood flow after the return of spontaneous circulation because of microcirculatory impairment; and 4) as delayed damage often in the setting of hypoxia in the form of demyelination. For the clinician assessing a patient after a hypoxic ischemic insult, knowing these processes will help to understand the overall clinical picture and provide some guidance in regard to prognosis in this situation which often is devastating and uncertain for families. In order to improve neurologic recovery and prevent poor outcome, potential therapeutic options are explored by multiple methods targeting the different levels of the process of neuronal damage and ischemic cell death.

References

- [1] J.H. Adams et al., The effects of systemic hypotension upon the human brain. Clinical and neuropathological observations in 11 cases, *Brain* **89**(2) (1966), 235–268.
- [2] A. Ames, 3rd et al., Cerebral ischemia. II. The no-reflow phenomenon, *Am J Pathol* **52**(2) (1968), 437–453.
- [3] M.J. Arends and A.H. Wyllie, Apoptosis: mechanisms and roles in pathology, *Int Rev Exp Pathol* **32** (1991), 223–254.
- [4] L. Bakay and J.C. Lee, The effect of acute hypoxia and hypercapnia on the ultrastructure of the central nervous system, *Brain* **91**(4) (1968), 697–706.
- [5] R.T. Bartus et al., Time-related neuronal changes following middle cerebral artery occlusion: implications for therapeutic intervention and the role of calpain, *J Cereb Blood Flow Metab* **15**(6) (1995), 969–979.
- [6] M. Bergeron, D.M. Ferriero and F.R. Sharp, Developmental expression of heme oxygenase-1 (HSP32) in rat brain: an immunocytochemical study, *Brain Res Dev Brain Res* **105**(2) (1998), 181–194.
- [7] E. Bonfoco et al., Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures, *Proc Natl Acad Sci USA* **92**(16) (1995), 7162–7166.
- [8] B.W. Bottiger et al., Global cerebral ischemia due to cardiocirculatory arrest in mice causes neuronal degeneration and early induction of transcription factor genes in the hippocampus, *Brain Res Mol Brain Res* **65**(2) (1999), 135–142.
- [9] B.W. Bottiger et al., Neuronal stress response and neuronal cell damage after cardiocirculatory arrest in rats, *J Cereb Blood Flow Metab* **18**(10) (1998), 1077–1087.
- [10] L. Caplan, Reperfusion of ischemic brain: why and why not, in: *Thrombolytic Therapy in Acute Ischemic Stroke*, G.J. W.d.Z. Hacke and M. Hirschberg, eds, Springer: Berlin, 1991, pp. 36–45.
- [11] J. Cervos-Navarro and N.H. Diemer, Selective vulnerability in brain hypoxia, *Crit Rev Neurobiol* **6**(3) (1991), 149–182.
- [12] Y. Cheng et al., Caspase inhibitor affords neuroprotection with delayed administration in a rat model of neonatal hypoxic-ischemic brain injury, *J Clin Invest* **101**(9) (1998), 1992–1999.
- [13] P.G. Clarke, Developmental cell death: morphological diversity and multiple mechanisms, *Anat Embryol (Berl)* **181**(3) (1990), 195–213.
- [14] H.K. Cooper et al., The effect of ischaemia and recirculation on protein synthesis in the rat brain, *J Neurochem* **28**(5) (1977), 929–934.
- [15] B.J. Crain et al., Selective neuronal death after transient fore-brain ischemia in the Mongolian gerbil: a silver impregnation study, *Neuroscience* **27**(2) (1988), 387–402.
- [16] R.C. Crumrine and J.C. LaManna, Regional cerebral metabolites, blood flow, plasma volume, and mean transit time in total cerebral ischemia in the rat, *J Cereb Blood Flow Metab* **11**(2) (1991), 272–282.
- [17] M. Dragunow et al., Immediate-early gene protein expression in neurons undergoing delayed death, but not necrosis, following hypoxic-ischaemic injury to the young rat brain, *Brain Res Mol Brain Res* **25**(1–2) (1994), 19–33.
- [18] A.D. Edwards et al., Specific inhibition of apoptosis after cerebral hypoxia-ischaemia by moderate post-insult hypothermia, *Biochem Biophys Res Commun* **217**(3) (1995), 1193–1199.
- [19] M. Endoh, K. Maiese and J. Wagner, Expression of the inducible form of nitric oxide synthase by reactive astrocytes after transient global ischemia, *Brain Res* **651**(1–2) (1994), 92–100.
- [20] E.G. Fischer and A. Ames, 3d, Studies on mechanisms of impairment of cerebral circulation following ischemia: effect of hemodilution and perfusion pressure, *Stroke* **3**(5) (1972), 538–542.

- [21] S. Furuta et al., Recovery of protein synthesis in tolerance-induced hippocampal CA1 neurons after transient forebrain ischemia, *Acta Neuropathol* **86**(4) (1993), 329–336.
- [22] F. Gillardon et al., Expression of cell death-associated phospho-c-Jun and p53-activated gene 608 in hippocampal CA1 neurons following global ischemia, *Brain Res Mol Brain Res* **73**(1–2) (1999), 138–143.
- [23] M.D. Ginsberg and R.E. Myers, The topography of impaired microvascular perfusion in the primate brain following total circulatory arrest, *Neurology* **22**(10) (1972), 998–1011.
- [24] M.D. Ginsberg, E.T. Hedley-Whyte and E.P. Richardson, Jr., Hypoxic-ischemic leukoencephalopathy in man, *Arch Neurol* **33**(1) (1976), 5–14.
- [25] M.P. Goldberg and D.W. Choi, Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury, *J Neurosci* **13**(8) (1993), 3510–3524.
- [26] D.E. Goll et al., The Calpain System, *Physiol Rev* **83**(3) (2003), 731–801.
- [27] J.A. Gottfried et al., Delayed posthypoxic demyelination. Association with arylsulfatase A deficiency and lactic acidosis on proton MR spectroscopy, *Neurology* **49**(5) (1997), 1400–1404.
- [28] F.D. Gray, Jr. and G.J. Horner, Survival following extreme hypoxemia, *Jama* **211**(11) (1970), 1815–1817.
- [29] D.M. Greer, Mechanisms of injury in hypoxic-ischemic encephalopathy: implications to therapy, *Semin Neurol* **26**(4) (2006), 373–379.
- [30] A. Hara et al., Immunohistochemical detection of Bax and Bcl-2 proteins in gerbil hippocampus following transient forebrain ischemia, *Brain Res* **711**(1–2) (1996), 249–253.
- [31] J. Hendrikse et al., Cerebral border zones between distal end branches of intracranial arteries: MR imaging, *Radiology* **246**(2) (2008), 572–580.
- [32] K.A. Hossmann and V. Zimmermann, Resuscitation of the monkey brain after 1 h complete ischemia. I. Physiological and morphological observations, *Brain Res* **81**(1) (1974), 59–74.
- [33] K.A. Hossmann, Reperfusion of the brain after global ischemia: hemodynamic disturbances, *Shock* **8**(2) (1997), 95–101. discussion 102–103.
- [34] K.A. Hossmann et al., Electron microscopic investigation of rat brain after brief cardiac arrest, *Acta Neuropathol* **101**(2) (2001), 101–113.
- [35] K.A. Hossmann, R. Schmidt-Kastner and B. Grosse Ophoff, Recovery of integrative central nervous function after one hour global cerebro-circulatory arrest in normothermic cat, *J Neurol Sci* **77**(2–3) (1987), 305–320.
- [36] J.M. Hoxworth et al., Cerebral metabolic profile, selective neuron loss, and survival of acute and chronic hyperglycemic rats following cardiac arrest and resuscitation, *Brain Res* **821**(2) (1999), 467–479.
- [37] D.J. Kane et al., Expression of bcl-2 inhibits necrotic neural cell death, *J Neurosci Res* **40**(2) (1995), 269–275.
- [38] I.S. Kass and P. Lipton, Calcium and long-term transmission damage following anoxia in dentate gyrus and CA1 regions of the rat hippocampal slice, *J Physiol* **378** (1986), 313–334.
- [39] K. Katsura et al., Coupling among energy failure, loss of ion homeostasis, and phospholipase A2 and C activation during ischemia, *J Neurochem* **61**(5) (1993), 1677–1684.
- [40] K. Kawai et al., Global cerebral ischemia associated with cardiac arrest in the rat: I. Dynamics of early neuronal changes, *J Cereb Blood Flow Metab* **12**(2) (1992), 238–249.
- [41] M. Kiessling et al., Differential transcription and translation of immediate early genes in the gerbil hippocampus after transient global ischemia, *J Cereb Blood Flow Metab* **13**(6) (1993), 914–924.
- [42] R.C. Koehler and R.J. Traystman, Cerebrovascular effects of carbon monoxide, *Antioxid Redox Signal* **4**(2) (2002), 279–290.
- [43] T. Kristian and B.K. Siesjo, Calcium-related damage in ischemia, *Life Sci* **59**(5–6) (1996), 357–367.
- [44] J.C. LaManna, R.C. Crumrine and D.L. Jackson, No correlation between cerebral blood flow and neurologic recovery after reversible total cerebral ischemia in the dog, *Exp Neurol* **101**(2) (1988), 234–247.
- [45] K.S. Lee et al., Inhibition of proteolysis protects hippocampal neurons from ischemia, *Proc Natl Acad Sci USA* **88**(16) (1991), 7233–7237.
- [46] P. Lipton, Ischemic cell death in brain neurons, *Physiol Rev* **79**(4) (1999), 1431–1568.
- [47] P.G. Lysko et al., Neuroprotective effects of tetrodotoxin as a Na⁺ channel modulator and glutamate release inhibitor in cultured rat cerebellar neurons and in gerbil global brain ischemia, *Stroke* **25**(12) (1994), 2476–2482.
- [48] G.I. McHedlishvili et al., Reaction of different parts of the cerebral vascular system in asphyxia, *Exp Neurol* **18**(2) (1967), 239–252.
- [49] J.R. Miller and R.E. Myers, Neuropathology of systemic circulatory arrest in adult monkeys, *Neurology* **22**(9) (1972), 888–904.
- [50] O. Miyamoto and R.N. Auer, Hypoxia, hyperoxia, ischemia, and brain necrosis, *Neurology* **54**(2) (2000), 362–371.
- [51] N. Panahian et al., Attenuated hippocampal damage after global cerebral ischemia in mice mutant in neuronal nitric oxide synthase, *Neuroscience* **72**(2) (1996), 343–354.
- [52] T. Patel, G.J. Gores and S.H. Kaufmann, The role of proteases during apoptosis, *Faseb J* **10**(5) (1996), 587–597.
- [53] P. Pearigen, R. Gwinn and R.P. Simon, The effects in vivo of hypoxia on brain injury, *Brain Res* **725**(2) (1996), 184–191.
- [54] C.K. Petito et al., Delayed hippocampal damage in humans following cardiorespiratory arrest, *Neurology* **37**(8) (1987), 1281–1286.
- [55] C.A. Piantadosi and J. Zhang, Mitochondrial generation of reactive oxygen species after brain ischemia in the rat, *Stroke* **27**(2) (1996), 327–331; discussion 332.
- [56] F. Plum, J.B. Posner and R.F. Hain, Delayed neurological deterioration after anoxia, *Arch Intern Med* **110** (1962), 18–25.
- [57] C. Portera-Cailliau, D.L. Price and L.J. Martin, Non-NMDA and NMDA receptor-mediated excitotoxic neuronal deaths in adult brain are morphologically distinct: further evidence for an apoptosis-necrosis continuum, *J Comp Neurol* **378**(1) (1997), 88–104.
- [58] E. Preston and D.O. Foster, Evidence for pore-like opening of the blood-brain barrier following forebrain ischemia in rats, *Brain Res* **761**(1) (1997), 4–10.
- [59] L.D. Prockop and R.I. Chichkova, Carbon monoxide intoxication: an updated review, *J Neurol Sci* **262**(1–2) (2007), 122–130.
- [60] W.A. Pulsinelli, J.B. Brierley and F. Plum, Temporal profile of neuronal damage in a model of transient forebrain ischemia, *Ann Neurol* **11**(5) (1982), 491–498.
- [61] K.M. Raley-Susman and P. Lipton, In vitro ischemia and protein synthesis in the rat hippocampal slice: the role of calcium and NMDA receptor activation, *Brain Res* **515**(1–2) (1990), 27–38.
- [62] V.J. Reempts, M. Haseldonckx and M. van den Ven, Morphology and ultrastructural calcium distribution in the rat hippocampus after severe transient ischemia, *Elsevier* (1984),

- 113–118.
- [63] M. Rie and P.G. Bernad, Prolonged hypoxia in man without circulatory compromise fail to demonstrate cerebral pathology, *Neurology* **30**(443) (1980), Q4Q5.
- [64] M. Sadowski et al., Pattern of neuronal loss in the rat hippocampus following experimental cardiac arrest-induced ischemia, *J Neurol Sci* **168**(1) (1999), 13–20.
- [65] R. Schmidt-Kastner and T.F. Freund, Selective vulnerability of the hippocampus in brain ischemia, *Neuroscience* **40**(3) (1991), 599–636.
- [66] B.K. Siesjo, Brain energy metabolism and catecholaminergic activity in hypoxia, hypercapnia and ischemia, *J Neural Transm Suppl* (14) (1978), 17–22.
- [67] M.L. Smith, R. von Hanwehr and B.K. Siesjo, Changes in extra- and intracellular pH in the brain during and following ischemia in hyperglycemic and in moderately hypoglycemic rats, *J Cereb Blood Flow Metab* **6**(5) (1986), 574–583.
- [68] S. Sunagawa et al., Hydrogen ion compartmentation during and following cerebral ischemia evaluated by ³¹P NMR spectroscopy, *Brain Res* **641**(2) (1994), 328–332.
- [69] R. Thilmann et al., Persistent inhibition of protein synthesis precedes delayed neuronal death in postischemic gerbil hippocampus, *Acta Neuropathol* **71**(1–2) (1986), 88–93.
- [70] S.R.T. Wagner and W.L. Lanier, Metabolism of glucose, glycogen, and high-energy phosphates during complete cerebral ischemia. A comparison of normoglycemic, chronically hyperglycemic diabetic, and acutely hyperglycemic nondiabetic rats, *Anesthesiology* **81**(6) (1994), 1516–1526.
- [71] Y. Xie et al., Ion channel involvement in anoxic depolarization induced by cardiac arrest in rat brain, *J Cereb Blood Flow Metab* **15**(4) (1995), 587–594.