Content

Abstract

The extracellular space (ECS) of the brain represents the microenvironment of nerve cells and enables the diffusion of neuroactive substances among neurons, axons and glia. Changes in the ECS diffusion parameters during ischemia are well known, but information about changes in ECS diffusion and energy-related metabolite concentrations in the postischemic and posthypoxic periods is insufficient.

Postischemic and posthypoxic diffusion changes were studied in the rat somatosensory cortex in different experimental hypoxia/ischemia models. Transient global hypoxia of 30 minutes duration was induced in adult male Wistar-rats by reducing inspired oxygen to 6% O_2 in nitrogen. Transient ischemia was induced by unilateral common carotid artery clamping for 30 minutes or by bilateral clamping for 10 or 15 minutes and concomitant ventilation with 6% O_2 in nitrogen. In a model of elevated oxygen consumption, seizure activity was evoked by an injection of pilocarpine. ECS volume fraction (α) and tortuosity (λ) were determined by the real-time iontophoretic method. Intracerebral microdialysis was utilized to monitor changes in the energy-related metabolites lactate, pyruvate, glucose and glutamate. Diffusion-weighted magnetic resonance imaging (DW-MRI) was used to determine the apparent diffusion coefficient of water (ADC_w) in the tissue.

Substantial changes in the extracellular diffusion properties were found in all experimental settings. During hypoxia, α decreased from 0.18 ± 0.01 during the first 20-25 minutes, followed by a further drop to 0.14 ± 0.01 after 25 minutes. Within 10 minutes of reoxygenation, α returned to control values, then increased to 0.20 ± 0.01 and remained at this level. Unilateral carotid artery occlusion led to a decrease in α from 0.19 ± 0.03 to 0.07 ± 0.01 and an increase in λ from 1.57 ± 0.01 to 1.88 ± 0.03. During reperfusion, α returned to control values within 20 minutes and then increased to 0.23 ± 0.01, while λ only returned to control values. During bilateral carotid artery occlusion, α decreased to 0.07 ± 0.01 in both groups, while λ increased to 1.80 ± 0.02. In the group of 10 minutes ischemia, normal values of α and λ were registered within 5-10 minutes of reperfusion. In the group of 15 minutes ischemia, α increased within 40-50 minutes of reperfusion to 0.29 ± 0.03 and remained at this level. λ increased to 1.81 ± 0.02 during ischemia, recovered within 5-10 minutes of reperfusion and increased to 1.62 ± 0.01 at the end of the experiment. ADC_w increased within 60 minutes after ischemia to 665 ± 15 $\mu m^2 s^{-1}$ and stayed at this level during the measurement period of 90 minutes. Seizure activity caused a decrease in α to 0.13 ± 0.01 after 100 minutes, followed by a complete recovery. Disturbances in extracellular metabolite and substrate levels were also found. Hxypoxia caused an increase in lactate concentration and the lactate/pyruvate ratio to 2.65 ± 0.24 mmol/l and 76.03 ± 13.04, respectively and a decrease in glucose levels to 1.18 ± 0.16 mmol/l after the induction of hypoxia. During recovery the metabolite and substrate concentrations returned to control levels. Glutamate levels increased 20-30 minutes after the onset of hypoxia to 22.39 ± 5.85 µmol/l and returned to prehypoxic values within 30-40 minutes of reoxygenation. Similar time courses for lactate, the lactate/pyruvate ratio and glucose were found during unilateral carotic occlusion. Seizure activity caused a rise in lactate, the lactate/pyruvate ratio and glutamate levels, reaching 2.92 ± 0.60 mmol/l, 84.80 ± 11.72 and 22.39 ± 5.85 µmol/l respectively at the maximum activity level, followed by recovery to starting values. Glucose initially increased to 3.49 ± 0.24 mmol/l, then decreased during the seizure period to 1.25 ± 0.40 mmol/l.

The observed substantial changes in the extracellular diffusion parameters and ADC_w affect the diffusion of ions, neurotransmitters, metabolic substances and drugs used in the treatment of nervous system diseases. Additionally, extracellular decreases in substrates and increases in metabolite levels during and after ischemic/hypoxic events affect the function of neuronal structures and the ionic balance of the microenvironment. In conclusion, the observed changes may aggravate functional deficits and lead to damage of the central nervous system.