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Changes in diffusion parameters, energy-related metabolites and glutamate in the rat cortex after transient hypoxia/ischemia

Aleš Homola [a,b], Norbert Zoremba [c], Karel Šlais [b,d], Ralf Kuhlen [c], Eva Syková [a,b,*]

[a] *Department of Neuroscience and Centre for Cell Therapy and Tissue Repair, 2nd Medical Faculty, Prague, Czech Republic*
[b] *Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic*
[c] *Department of Intensive Care Medicine, University Hospital RWTH Aachen, Aachen, Germany*
[d] *Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

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Abstract

It has been shown that global anoxia leads to dramatic changes in the diffusion properties of the extracellular space (ECS). In this study, we investigated how changes in ECS volume and geometry in the rat somatosensory cortex during and after transient hypoxia/ischemia correlate with extracellular concentrations of energy-related metabolites and glutamate. Adult male Wistar rats ($n = 12$) were anesthetized and subjected to hypoxia/ischemia for 30 min (ventilation with 10% oxygen and unilateral carotid artery occlusion). The ECS diffusion parameters, volume fraction and tortuosity, were determined from concentration–time profiles of tetramethylammonium applied by iontophoresis. Concentrations of lactate, glucose, pyruvate and glutamate in the extracellular fluid (ECF) were monitored by microdialysis ($n = 9$). During hypoxia/ischemia, the ECS volume fraction decreased from initial values of 0.19 ± 0.03 (mean ± S.E.M.) to 0.07 ± 0.01 and tortuosity increased from 1.57 ± 0.01 to 1.88 ± 0.03. During reperfusion the volume fraction returned to control values within 20 min and then increased to 0.23 ± 0.01, while tortuosity only returned to original values (1.53 ± 0.06). The concentrations of lactate and glutamate, and the lactate/pyruvate ratio, substantially increased during hypoxia/ischemia, followed by continuous recovery during reperfusion. The glucose concentration decreased rapidly during hypoxia/ischemia with a subsequent return to control values within 20 min of reperfusion. We conclude that transient hypoxia/ischemia causes similar changes in ECS diffusion parameters as does global anoxia and that the time course of the reduction in ECS volume fraction correlates with the increase of extracellular concentration of glutamate. The decrease in the ECS volume fraction can therefore contribute to an increased accumulation of toxic metabolites, which may aggravate functional deficits and lead to damage of the central nervous system (CNS).

Keywords: Hypoxia; Extracellular space; Microdialysis; Ischemia; Diffusion

The diffusion of neuroactive substances through the extracellular space of the CNS is the underlying mechanism of extrasynaptic (volume) transmission, which is an important mode of communication between nerve cells [1]. Diffusion in the ECS obeys Fick's law but is constrained by two factors: extracellular volume fraction α, which is the ratio of the ECS volume to total tissue volume and tortuosity λ, a parameter describing the impact of tissue geometry on diffusion compared to a free diffusion medium. Tortuosity is defined as $\lambda = (D/\mathrm{ADC})^{1/2}$, where ADC is the apparent diffusion coefficient in the brain and D is the free diffusion coefficient. The absolute values of the ECS difusion parameters can be determined by the real-time iontophoretic method using tetramethylammonium (TMA^+)-selective microelectrodes [15,16].

It has been shown that many pathological states result in changes in extracellular space volume and geometry, significantly affecting signal transmission [22,23]. Among those of major clinical relevance and experimental interest are conditions leading to brain hypoxia or ischemia. Acute hypoxia or ischemia, and also some other acute neurological disorders that involve cell membrane depolarization (cortical spreading depression, status epilepticus and hypoglycaemia), cause excessive transmembrane ionic shifts that are accompanied by the movement of water from the extracellular to the intracellular compartment (cytotoxic edema). Rapid cellular swelling inevitably results in a shrinkage of the ECS, the impaired diffusion of substances

* Corresponding author at: Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. Tel.: +420 2 41062230; fax: +420 2 41062783.
E-mail address: sykova@biomed.cas.cz (E. Syková).

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through the ECS and the greater accumulation of toxic metabolites. In turn, these consequences can contribute to functional deficits and CNS damage. Experimentally, ischemia-evoked changes in the ECS diffusion parameters in the brain cortex in vivo have, so far, been studied only in a model of global anoxia induced by cardiac arrest. These studies have revealed a dramatic decrease in ECS volume fraction and an increase in tortuosity, occurring only a few minutes after the interruption of the blood supply to the brain [25,27]. In the present study, we have examined the ECS diffusion parameters in the somatosensory cortex of adult rats during transient hypoxia combined with unilateral common carotid artery occlusion and also during subsequent reperfusion. The obtained data were correlated with changes in the energy-related metabolites lactate and glucose, the lactate/pyruvate-ratio and glutamate, monitored by intracerebral microdialysis.

Adult male Wistar rats (300–350 g) were anesthetized by an intraperitoneal injection of urethane (1.5 g/kg, Sigma-Aldrich Chemie GmbH, Seelze, Germany). The animals were intubated and connected to a ventilator (CIV 101, Columbus Instruments, Columbus, OH, USA), relaxed with pancuroniumbromide (0.6 mg/kg, Pavulon, Organon, Netherlands), and ventilated with air. The body temperature was maintained at 36–37 °C by a heating pad. The somatosensory cortex of the rat was partially exposed by a burr hole 2–3 mm caudal from the bregma and 2–3 mm lateral from the midline. A transient hypoxia/ischemia of 30 min duration was induced by reducing the inspiratory oxygen content to 10% (in nitrogen) and unilateral clamping of the common carotid artery. Following the hypoxic period, the animals were again ventilated with air ($pO_2 = 21\%$). The control animals were sham-operated and ventilated with air throughout the experiment. In order to measure in the ipsilateral somatosensory cortex, diffusion and microdialysis measurements were not performed simultaneously.

All efforts were made to minimize animal suffering and to reduce the number of animals used. The experiments were carried out in accordance with the European Communities Council Directive of 24.November 1986 (86/609/EEC) and approved by the local Institutional Animal Ethics Committee.

The ECS diffusion parameters were studied by the real-time iontophoretic method described in detail previously [13]. Briefly, an extracellular marker that is restricted to the extracellular compartment, such as tetramethylammonium ions (TMA^+, MW = 74.1 Da) to which cell membranes are relatively impermeable, is released into the extracellular space by iontophoresis and its local concentration measured with a TMA^+-selective microelectrode (TMA^+-ISM) located about 100–200 μm from the release site. The concentration of TMA^+ in the ECS is inversely proportional to the ECS volume. Double-barrelled TMA^+-ISMs were prepared by a procedure described in detail previously [21]. The tip of the ion-sensitive barrel was filled with a liquid ion exchanger (Corning 477317); the rest of the barrel was backfilled with 150 mM TMA^+ chloride. The reference barrel contained 150 mM NaCl. The shank of the iontophoretic pipette was bent so that it could be aligned parallel to that of the ion-selective microelectrode and was backfilled with 150 mM TMA^+ chloride. An electrode array was made by gluing a TMA^+-ISM to an iontophoretic micropipette with a tip separation of 100–200 μm. The iontophoresis parameters were +20 nA bias current (continuously applied current to maintain a constant electrode transport number), and a +180 nA current step of 24 s duration, to generate the diffusion curve. The TMA^+ diffusion curves were generated at regular intervals of 5 min. Before tissue measurements, diffusion curves were first recorded in 0.3% agar (Sigma-Aldrich, Steinheim, Germany) dissolved in a solution containing 150 mM NaCl, 3 mM KCl, and 1 mM TMACl. The diffusion curves were analysed to obtain the electrode transport number (n) and the free TMA^+ diffusion coefficient (D) by curve-fitting according to a modified diffusion equation using the VOLTORO program [15]. Diffusion curves were then recorded in the somatosensory cortex at a depth of 1200–1500 μm. Knowing n and D, the values of extracellular volume fraction α and tortuosity λ could be obtained from the diffusion curves.

The technique of microdialysis is based on sampling fluid via a double-lumen probe with an integrated semipermeable membrane in which the equilibration of substances in the extracellular space and perfusion fluid takes place by diffusion according to the concentration gradient. We used a double-lumen microdialysis probe with a membrane length of 2 mm, an outer diameter of 0.5 mm and a cut-off at 20,000 Da (CMA 12, 2 mm membrane length, CMA Microdialysis, Sweden). The inserted microdialysis catheter was connected by low-volume fluorinated ethylene propylene (FEP)-tubing (1.2 μl/10 cm) to a precision infusion pump (CMA 102, CMA Microdialysis, Sweden) in order to maintain a constant dialysate flow. The microdialysis catheter was continuously perfused with a dialysate containing 147 mmol/l NaCl, 2.7 mmol/l KCl, 1.2 mmol/l $CaCl_2$ and 0.85 mmol/l $MgCl_2$ (Perfusion fluid CNS, CMA Microdialysis, Sweden) at a flow rate of 2 μl/min. After a stabilisation period of 60 min following insertion into the brain, microdialysate samples were collected in 10 min intervals and immediately frozen at −40 °C until analysed. Thawed and centrifuged dialysate samples were analysed enzymatically with a CMA 600 Microdialysis Analyser (CMA/Microdialysis, Sweden) for lactate, pyruvate, glucose and glutamate concentrations.

The exchange of substances across the microdialysis membrane is limited by the total area of the membrane, the perfusion flow rate, the characteristics of the diffusing substance and the diffusion constant in the tissue surrounding the probe [24]. The recovery rate expresses the relation between the concentration of the substance in the microdialysis probe effluent and the concentration in the medium [14]. Before and at the end of the experiments, the recovery rates for each probe were determined by continuing the perfusion at the same settings in a calibration solution containing known concentrations of the different analytes. The calibration solution contained 2.50 mmol/l lactate, 250 μmol/l pyruvate, 5.55 mmol/l glucose, 250 mmol/l glycerol and 25 μmol/l glutamate (Calibrator, CMA Microdialysis, Sweden). The concentrations in the calibration solution were compared with the concentrations of the in vitro microdialysis samples to determine the relative recovery for each substance. The measured experimental values were weighted by the relative recovery to estimate the in vivo extracellular concentration of the substances in the immediate vicinity of the probes. In