Neuronal and Astrocytic Metabolism following Reperfusion after Middle Cerebral Artery Occlusion

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Introduction: Successful reperfusion in thromboembolic stroke depends on the viability of the ischemic brain cells. Ischemia induces changes in neuronal and astrocytic metabolism, and disrupts neuro-astrocytic interactions. In the intact brain astrocytes provide metabolic and trophic support to neurons, and modulates synaptic activity. Accordingly, impairment in astrocyte functions can critically influence neuronal survival. In the present study we sought to identify the metabolic events in previously ischemic, but successfully reperfused brain tissue in a rat model of middle cerebral artery occlusion (MCAO). The aim was to uncover the changes in neuronal and astrocytic metabolism brought on by reperfusion in order to provide insight into metabolic prerequisites for successful reperfusion.

Materials and Methods: Right-sided MCAO was induced with the intraluminal filament model in fasted male Wistar rats (320-340 g) under isoflurane anesthesia. A catheter was introduced into the right femoral vein, externalized at the tail radix. 1000 IE Fragmin was administered im. After surgery, the rats were placed in individual cages and allowed to recover. The rats assigned to reperfusion were re-anesthetized 115 min after MCAO, the filament retracted 120 min after MCAO, and the rats returned to their cages. There were four ^{13}C MRS groups that received an intravenous injection of 0.3 mmol/L [1-^{13}C]glucose and [1,2-^{13}C]acetate (1 mL/100g rat) in sterile water over 2 min before decapitation into liquid N_2 15 min later. The ^{13}C MRS groups were: 120 min of reperfusion after 120 min of MCAO (n=8), permanent MCAO lasting120 min (n=7) and 240 min (n=7), and sham operated rats (n=7). From the frozen brains a 5 mm coronal slice extending from chiasma opticum and caudally was cut using a brain matrix, and the upper frontoparietal cortex was sampled. The brain samples were extracted, redissolved in D_2O containing 0.15% ethyleneglycol as a chemical shift and quantification standard, and neutralized. Proton decoupled 125.5 MHz ^{13}C MR spectra were obtained on a Bruker DRX-500 spectrometer (35° pulse angel, 25 kHz spectral width, 64 K data points, acquisition time 1.3 s per scan, 2.5 s relaxation delay). Some spectra were broadband decoupled during acquisition only to avoid nOe. Amino acid concentrations were determined with HPLC. DWI was performed on a 2.35T Bruker Biospec in rats subjected to 240 min of permanent MCAO (n=8) and 120 min of reperfusion after120 min of MCAO (n=7). DWIs were acquired with a spin echo sequence (TE 32 ms, TR 1500 ms, five axial diffusion gradients, b-values between 270 and 1468 s/mm^2). Eight transaxial slices, slice thickness 1.5 mm, gap 0.3 mm, were obtained. During imaging the rats were re-anesthetized with isoflurane. Ischemic tissue volume was calculated from the ADC maps. All values are mean ± SD. Statistical analysis was performed with unpaired Student's T-test and ANOVA followed by LSD posthoc test. $p < 0.05$ was considered statistically significant.

Results: Reperfusion for 120 min after 120 of MCAO reduced the ischemic tissue volume significantly to 133 mm^3 compared to 346 mm^3 in rats subjected to 240 min permanent MCAO ($p<0.001$). The upper frontoparietal cortex (see Fig. 1) was not incorporated in the ischemic volume in the reperfused rats.

Figure 1. Example of ADC maps obtained from rats subjected to 240 min of permanent MCAO (A) or 120 min of reperfusion following 120 min of MCAO (B). Ischemic tissue is colored blue.

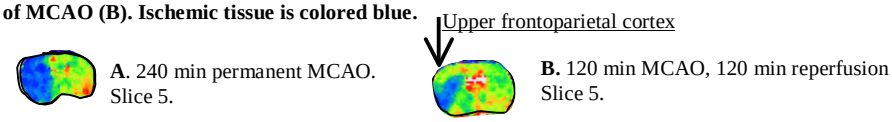
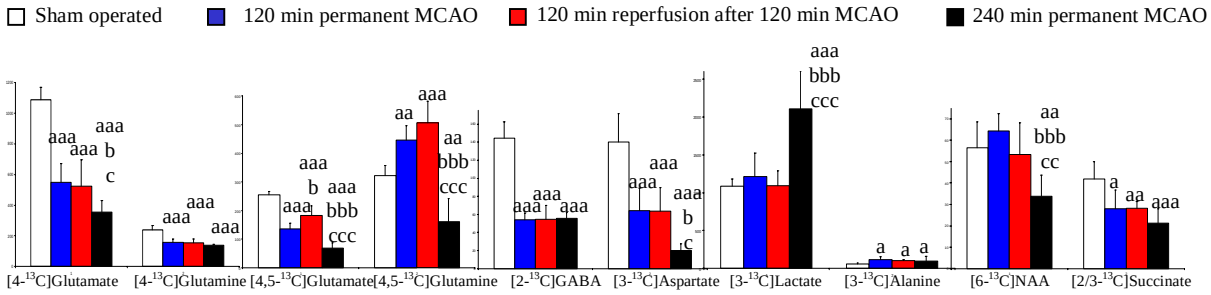


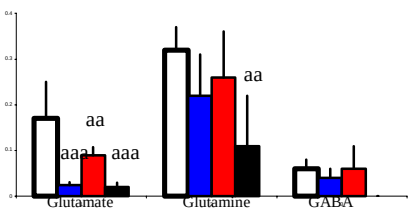
Figure 2. The total amount of ^{13}C (10^{-9} mol/g) in amino acids and other compounds from the upper frontoparietal cortex



a: Statistical different from sham operated rats, $p<0.05$, aa $p<0.001$, aaa $p<0.0001$. b: Statistical different from 120 min permanent MCAO, $p<0.05$, bbb $p<0.0001$. c: Statistical different from 120 min reperfusion following 120 min MCAO, c $p<0.05$, ccc $p<0.0001$.

^{13}C MRS revealed no significant differences in amount of label in the major isotopomeres between 120 min of permanent MCAO and 120 min of MCA followed by 120 min of reperfusion (Fig.2). At 240 min of permanent MCAO both neuronal and astrocytic metabolism was severely affected as demonstrated by the marked reduction in amount of [4-^{13}C]glutamate and [4,5-^{13}C]glutamine, and the increased lactate production. Also the amount of [6-^{13}C]NAA and [2-^{13}C]/ [3-^{13}C]succinate, which are markers of neuronal mitochondrial function and TCA cycle flux respectively, were at the same level after 120 min of reperfusion and 120 min of permanent MCAO.

Figure 3. Pyruvate carboxylase versus pyruvate dehydrogenase activity ratio in glutamate, glutamine and GABA synthesis



Pyruvate carboxylation is the major anaplerotic pathway in the brain, and is localized to astrocytes. In glutamate there was a non-significant increase in PC/PDH activity following reperfusion compared to 120 min of permanent MCAO (Fig. 3). In glutamine and GABA the PC/PDH activity ratio was not significantly reduced before 240 min of permanent MCAO.

Discussion and Conclusion: 120 min of reperfusion did not improve astrocytic and neuronal metabolism significantly although there was a normalization of the ADC maps, demonstrating that there was no intracellular edema, and that reperfusion was successful. There was a trend towards increased pyruvate carboxylase activity. Pyruvate caboxyalse activity is a prerequisite in order to restore both neuronal and astrocytic TCA cycle activity. The level of neuronal metabolism detected at 120 min of permanent MCAO and after reperfusion may represent a minimum of metabolic activity necessary for neuronal viability. At 240 min of MCAO the metabolic profile from both neurons and astrocytes were markedly reduced, and the upperfrontoparietal cortex is considered to be irreversibly damaged in this MCAO model.