Quantitative 1H MRS in the rat hippocampus after global ischemia

D. Jirak[1,2], I. Vorisek[3], E. Sykova[3], M. Hajek[1,2]

[1]MR-unit, IKEM, Prague 4, Czech Republic, [2]Center for Cell Therapy and Tissue Repair, Charles University, 2nd Faculty of Medicine, Prague, Czech Republic, [3]Department of Neuroscience, Institute of Experimental Medicine, Prague, Czech Republic

Introduction

The hippocampus is one of the most vulnerable areas of the brain to ischemia in humans and animals (1). The aim of our study was to determine, using proton MR spectroscopy (^{1}H MRS), metabolic changes over the course of one month in the region of the hippocampus of the rat after ischemia.

Materials and Methods

Global brain ischemia was induced in 23 two-month old Wistar male rats. The animals were anaesthetized with pentobarbital i.p. (60mg/kg) and subjected to transient occlusion of the carotid arteries and to 6% oxygen for 15 minutes. Following ischemia, the animals underwent MRS measurement one day (4 rats), three days (7 rats), one week (5 rats) and one month (7 rats) after induction of ischemia. Ischemic rats together with 6 controls were scanned using a 4.7 T Bruker MR spectrometer with a home-made surface coil. 1H spectra were measured using a modified single voxel STEAM sequence (with selective CHESS pulses for water suppression), with short echo time (TE=3 ms) and repetition time TR=5000 ms. The volume of interest was approximately 54 mm^3 to cover both hippocampi (Figure 1). Proton spectra were evaluated using LCModel v.6 (2) to obtain absolute metabolite concentrations in laboratory units. All protocols were approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine and the experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

Results

The hippocampal proton spectra revealed peaks for the main brain metabolites, i.e. peaks assigned to N-acetyl aspartate (NAA) and N-acetyl aspartyl-glutamate (NAAG), water-soluble choline (Cho), creatine and phosphocreatine (Cr), glutamine (Gln), glutamate (Glu), inositol (Ins) and taurine (Tau). The concentrations of Cho, Cr, Ins and Tau did not change significantly during the whole experiment; however, we could observe a clear trend in the changes of the Ins concentration (Figure 2). One week after occlusion of the carotid arteries, Gln concentrations showed a significant decrease from controls. The concentration of Glu changed significantly one day after ischemia, and then gradually returned to normal. There was a marked decrease in the NAA+NAAG concentration at three days and one week after occlusion of the carotid arteries. One month after ischemia, NAA+NAAG normalized. Table 1 summarizes all concentrations of selected proton metabolites measured one day, three days, one week and one month after occlusion of the carotid arteries.

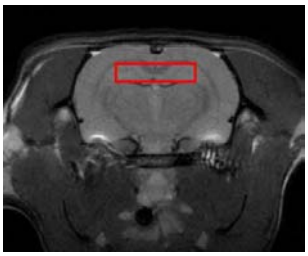

Figure 1. A typical MR image of a rat brain that served as a localizer for MR spectroscopy. The red rectangle shows the location of the hippocampus.

	Cho (mM)	Cr (mM)	Gln (mM)	Glu (mM)	Ins (mM)	Tau (mM)	NAA+ NAAG (mM)
Controls #6	1.7±0.1	8.0±0.6	2.9±0.1	6.9±0.7	2.8±0.7	5.2±0.7	6.9±0.1
1 day after ischemia #4	1.9±0.1	8.3±0.3	3.4±0.4	9.4±0.2 ▪	2.3±0.6	4.9±1.0	7.4±0.1
3 days after ischemia #7	1.7±0.1	7.6±0.5	2.8±0.1	6.8±0.9	3.3±0.1	5.4±0.5	5.7±0.1 ▪
1 week after ischemia #5	1.6±0.0	7.0±0.2	2.0±0.1 ▪	7.1±0.2	3.6±0.7	4.9±1.6	5.8±0.4 ▪
1 month after ischemia #7	1.9±0.0	7.7±0.5	2.4±0.1	7.4±0.1	3.3±0.5	4.7±0.6	6.9±0.1

Table 1. Absolute concentrations of selected metabolites in the hippocampus. Data are expressed as mean ± S.E.M. Significant differences (two-tailed t-test, $p < 0.05$) with controls are marked with a box.

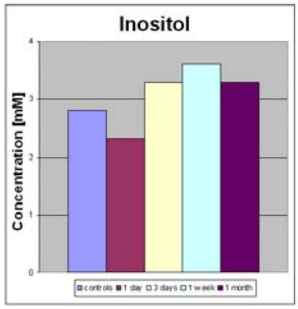

Figure 2. Hippocampal Ins concentrations of controls and following ischemia measured 1 day, 3 days, 1 week and 1 month after occlusion of the carotid arteries. The absolute concentrations are shown in Table 1.

Discussion and Conclusion

The significant increase of the excitatory neurotransmitter Glu one day after ischemia is connected with neuron activity after ischemia, and may result from reversed uptake of Glu due to a deficit in energetic processes and alteration of glial glutamate transport (3).The reduction of hippocampal NAA concentrations three days and one week after ischemia is due to neuronal death in CA1 and subsequent release and degradation of NAA in the surround tissue (4). After the decrease of the NAA+NAAG concentration, we observed its normalization one month after occlusion. This can be explained by the fact that the spectroscopic voxel included all hippocampal sub-structures, where neurogenesis is ongoing (Dentate Gyrus). Changes in Ins were not significant, but the trend indicates the maximum of its concentration at about seven days following ischemia, which corresponds to the course of astrogliosis (5).

In summary, quantitative 1H-MRS was used to determine metabolic changes in the hippocampus of rats subjected to a ischemic injury. Our results support published data which referred to a decline in NAA concentration (4) due to neuron death and an increase in Glu concentration due to release to the extracellular matrix in the brain after ischemia.

References

1) Crepel V et al. J Cell Mol Med. 2003;7(4):401-7.
2) Provencher SW. NMR Biomed. 2001;14(4):260-4.
3) Danbolt NC. Prog Neurobiol. 2001;65(1):1-105.
4) Sager TN et al. Brain Res. 2001;892(1):166-75.
5) Serkova N et al. Evaluation. Biochimica et Biophysica Acta 1996;1314:93 – 104

Acknowledgement

This study was supported by grants CEZ: MZO 00023001/983, MSMT LC554/753, AVOZ50390512, MSMT LC554 and GACR 305/03/1172.