NEW MAGNETIC RESONANCE SPECTROSCOPY BIOMARKER FOR MONITORING NEURODEGENERATIVE DISEASES: ANIMAL MODELS

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Creatine kinase (CK) plays a central role in energy transfer in cells with high-energy demands, and the enzyme is rather susceptible to oxidative inactivation. The aim of the present study was to investigate whether the rate constant of forward CK reaction (k_{for}) is a suitable indicator of alterations in cerebral energy metabolism. We monitored k_{for} in the rat brain non-invasively by *in vivo* phosphorus (³¹P) magnetic resonance spectroscopy (MRS). To alter energy metabolism, we applied following experimental models: Huntington's disease, diabetes mellitus, chronic alcohol intoxication and chronic cerebral hypoperfusion (vascular dementia model). Results of our ³¹P MRS experiment confirm importance of creatine kinase/phosphocreatinine (CK/PCr) system in the regulation of brain energy metabolism *in vivo* because a kinetic parameter k_{for} was significantly changed in all above animal models that simulate neurodegenerative diseases or commonly during oxidative stress. Using this method we distinguished vascular dementia (VD) and Huntington disease (HD), because in VD model a kinetic parameter k_{for} decreased and in the case HD increased. Considering the importance of CK for the maintenance of energy homeostasis in the brain, it is conceivable that an alteration of this enzyme activity in the brain may be one of the mechanisms by which various neurodegenerative diseases might be monitored just by means saturation transfer method ³¹P MRS.

INTRODUCTION

Metabolism of adenosine triphosphate (ATP) is key process for cell live and involves ATP synthesis and its utilization for supporting brain function and activation. The ATP metabolism is tightly coupled to the phosphocreatinine (PCr) metabolism *via* the enzyme system of creatine kinase. CK plays a central role in energy transfer in cells with high and fluctuating energy requirements and it is very susceptible to oxidative stress^{1–4}. It would be valuable to establish a noninvasive approach for studying this chemical process in the animal (human) brain *in vivo*. Rate constants of CK reaction can be investigated by magnetization transfer *in vivo* ³¹P MRS experiment^{5,6}. By means of this technique we studied reaction kinetics of reversible exchange of the phosphate group in the reaction catalyzed by CK

in the adult and aged rat brains in various *chronic models* of neurodegenerative diseases^{7–11}.

The forward rate constant of CK, k_{for} (PCr → ATP) was studied in adult and aged rat brains in following models of diseases: i) Huntington's disease (HD), ii) diabetes mellitus (DM), iii) alcohol intoxication and iv) vascular form dementia (VD) of Alzheimer's disease modeled by chronic cerebral hypoperfusion. The aim of these experiments was to demonstrate that in *chronic* pathological states the pseudo first-order rate constant k_{for} of the CK forward reaction is a better indicator of changes in the brain energy metabolism than the conventional steady-state *in vivo* ³¹P MRS.

MATERIAL AND METHODS

Animal models

Wistar rats with the age 3–10 (adult) and 14–24 (aged) month were used in ³¹P MRS experiments.

Huntington's disease (HD) can be simulated in an animal model with systemic administration of 3-nitropropionic acid (3-NP). Male Wistar rats with the age 20–24 months were injected i.p. with 3-NP (10 mg/kg/very

12 h) for 11 days to develop chronic model of HD (ref. ²). Group QE+HD received coenzyme Q₁₀ (CoQ₁₀) and vitamin E (250 mg CoQ₁₀ + 530 mg vit.E/kg/day, i.g.) during 10 days before application of 3-NP (ref.¹⁰).

Diabetes mellitus (DM) was induced in 11 months old male Wistar rats by single dose of streptozotocin (STZ) (55 mg/kg, i.v.). The animals were kept without insulin for 3 months. Group DM+Q received CoQ₁₀ (20 mg/kg/day, i.p.) during 3 months, starting with the onset of DM (ref.¹¹).

Chronic alcohol intoxication was modeled by administration of 30% ethanol (3 ml/day, intragastrically) to 3 months old female rats for the period of 3 weeks. Control group (C) received water as a vehicle^{7,8}.

Vascular dementia (VD) model was prepared by a minimally invasive surgical technique of 3-vascular occlusion by brachiocephalic trunk, which eliminated simultaneously right common carotid artery and right vertebral artery^{9,12}. 6–10 months old (adult) and 14–17 months old (aged) male Wistar rats were used in this experiment. Saturation transfer *in vivo* ³¹P MRS were performed two and ten weeks after vascular occlusion^{8,9}.

In vivo ³¹P magnetic resonance spectroscopy

In vivo ³¹P MRS experiments were performed at 4.7 T on SISCO 200/300 imaging spectrometer equipped with horizontal bore magnet for measurements on animals. ³¹P MR spectra were collected using 16 mm surface coil. The static magnetic field was shimmed using the proton signal of water that showed a typical line width of 20–35 Hz. Relative concentrations of phosphate metabolites were determined from integrals of their signals in ³¹P MR spectra using program MESTRE-C 1.5.1. Time-dependent ³¹P MRS saturation transfer was applied to determine the pseudo-first order rate constant of forward CK reaction (k_{for}) as described previously^{6,9,13,14}. Time dependent saturation transfer allows one to measure simultaneously two parameters, T_1 and k_{for} . Saturation of the γ -ATP resonance for increasing time periods induces an exponential decay of the PCr resonance to a new steady state (Fig. 1).

The saturation was accomplished by the DANTE pulse sequence. The k_{for} was calculated according to the equation^{9,13,14}

$$k_{\text{for}} = [1 - M_{(\text{PCr})}^* / M_{0(\text{PCr})}] / T_{1\text{app}(\text{PCr})} \quad (2)$$

where $M_{0(\text{PCr})}$ and $M_{(\text{PCr})}^*$ are the PCr signal intensities in the reference spectrum and in the spectrum after 10-second irradiation of the γ -ATP resonance, respectively. $T_{1\text{app}(\text{PCr})}$ is the apparent longitudinal relaxation time of the PCr signal in the presence of the γ -ATP saturation. To check the validity of the results, the $T_{1\text{PCr}}$ values were calculated using the following equation^{9,13,14}

$$T_{1\text{PCr}} = T_{1\text{sPCr}} [M_{\text{PCr}}^0 / M_{\text{PCr}}^\infty] \quad (3)$$

Calculated values k_{for} and $T_{1\text{PCr}}$ were expressed as means $\pm$ standard deviations. The Student's *t* tests were performed to compare differences between groups.

Fig. 1. Time dependent ³¹P NMR saturation transfer experiments. Series of saturation experiments with variable time of irradiation (0.3–10 s) of γ ATP signal. The length of the saturation period is given below each spectrum. In the control experiment, the saturation was off-resonance. The saturated γ ATP signal is labelled with asterisk.

RESULTS AND DISCUSSION

Our findings suggest that the CK reaction could play a key role in energetic system of adult and aged brains in above models of neurodegenerative diseases. While the analysis of steady-state ³¹P MR spectra revealed significant alteration in brain PCr/ β ATP ratio only in diabetic group, we found significant changes of brain k_{for} in all chronic models of neurodegenerative diseases. Compared to controls k_{for} increased in HD and DM models and decreased in chronic alcohol intoxication and VD models (Table 1).

From kinetic ³¹P MRS spectra we found in case of HD small (+13.4 %), but statistically significant an increase in k_{for} (Table 1). Exact mechanism responsible for the increase of the pseudo-first-order rate constant for the CK reaction k_{for} is presently not clear. However, *in vivo* ³¹P MRS transfer measurements revealed a correlation between CK flux and brain 2-deoxyglucose uptake^{5,6}. Our result of increased activity of CK corresponds well with reported increased 2-deoxyglucose uptake in basal ganglia of HD patients^{1,5}.

Similar increase of k_{for} (+22.9 %) we obtained in the model of diabetes mellitus in which also increased brain PCr/ β ATP ratio was found (Table 1). In both above mentioned models the function of respiratory chain in brain mitochondria was diminished¹¹. We suppose that decreased brain mitochondrial ATP production could be partly compensated in a certain stage of these pathological states by increased activity of CK system. The fact that the increase of k_{for} in these models of neurodegenerative diseases could be prevented by application of CoQ₁₀ provides evidence that k_{for} reflecting activity of CK system is sensitive marker of changes in brain energy metabolism^{10,11}.

Table 1. Parameters determined by ^{31}P MRS methods in the rat brain in models of neurodegenerative diseases

Model of neurodegenerative disease	n	k_{for} (s^{-1})	$T_1(\text{PCr})$ (s)	PCr/ β ATP
1. Huntington's disease				
Control aged rats (20–24 months)	6	0.304 ± 0.012	3.079 ± 0.076	2.032 ± 0.128
Huntington disease (HD) model	5	$0.350 \pm 0.015^*$	2.561 ± 0.119	2.164 ± 0.145
CoQ10+E + HD model	4	0.313 ± 0.012	3.006 ± 0.109	2.212 ± 0.117
2. Diabetes mellitus				
Control aged rats (11 months)	5	0.314 ± 0.007	2.702 ± 0.229	2.033 ± 0.23
Diabetes mellitus	4	$0.386 \pm 0.004^{***}$	3.105 ± 0.127	$2.706 \pm 0.11^{**}$
Diabetes mellitus + CoQ10	4	$0.310 \pm 0.013 \# \#$	3.028 ± 0.123	2.465 ± 0.132
3. Chronic alcohol intoxication				
C (female)	5	0.377 ± 0.02	3.233 ± 0.179	2.641 ± 0.092
Alcohol	4	$0.307 \pm 0.01^*$	3.268 ± 0.089	2.553 ± 0.042
4. VD model of Alzheimer disease				
<i>Adult rats</i>				
Control adult rats (6–10 months)	10	0.345 ± 0.025	3.521 ± 0.256	–
Vascular dementia model 2 weeks	10	0.284 ± 0.041	3.227 ± 0.239	–
Vascular dementia model 10 weeks	6	$0.262 \pm 0.042^*$	3.032 ± 0.411	–
<i>Aged rats</i>				
Control aged rats (14–17 months)	8	0.301 ± 0.013^a	3.421 ± 0.353	–
Vascular dementia model 2 weeks	6	$0.213 \pm 0.042^{**}$	3.501 ± 0.416	–
Vascular dementia model 10 weeks	6	$0.201 \pm 0.033^{**}$	3.610 ± 0.142	–

n – number of animals per group; k_{for} – the pseudo-first order rate constant of forward CK reaction determined in brain *in vivo* by ^{31}P MRS saturation transfer; $T_1(\text{PCr})$ – longitudinal relaxation time of phosphocreatine in absent γ -ATP saturation, a control parameter; PCr/ β ATP ratio calculated from *in vivo* ^{31}P MR spectra of the rat brain. C – control rats; $^*p < 0.05$, $^{**}p < 0.01$ vs. control group, $^{##}p < 0.001$ vs. DM group, $^ap < 0.05$ vs. control adult rats

Long-term consumption of alcohol can lead to brain atrophy and neurological dysfunction. Ethanol and its metabolite acetaldehyde are directly neurotoxic and its acute and chronic intoxication with ethanol induces oxidative stress in CNS and peripheral nerves⁷. The conventional ^{31}P MRS revealed statistically significant decrease of intracellular pH in the brain of rats treated by ethanol, but it did not show any significant variation in the content of high-energy phosphates after 14 weeks of drinking the high ethanol doses⁷. However, measurement of fluxes in the direction from PCr to ATP CK reaction showed significant decrease of the forward CK rate constant in alcoholic rat brain^{2,8} (Table 1).

The study of cerebral hypoperfusion in rats is great importance for a better understanding of cerebrovascular dynamics in various neurodegenerative diseases including Alzheimer's disease (AD). The characteristic pathology of AD involves microvascular degeneration and

chronic cerebrovascular insufficiency¹². Two factors must be present before cognitive dysfunction and neurodegeneration is expressed in AD brain: advanced aging and presence of a condition that lowers cerebral perfusion¹². Compared to the control group of healthy aged rats the hypoperfused rats showed statistically significant decrease in k_{for} both 2 and 10 weeks after occlusion (Table 1). It should be noted here that adult and aged rats kept for 10 weeks under conditions of severe hypoperfusion, showed no statistically significant changes in conventional *in vivo* ^{31}P MRS spectrum. Dysfunction of the CK system under AD conditions has also recently been reported^{4,15}. The causes of decreased levels of cytosolic brain CK isoform (BB-CK) in postmortem brain in mental pathology are subject of discussions¹⁵. As it was recently demonstrated, BB-CK energy transfer in the brain is important for habituation and spatial learning behaviour¹⁶. Dynamic study of ^{31}P MR spectroscopy revealed strong reduction of PCr

=>ATP phosphorus flux in mice with ablation of cytosolic BB-CK, while ATP and PCr levels were unaffected in these mice¹⁶. Thus, the rate constant of CK, k_{for} measured in the brain reflects changes of BB-CK activity^{8,9,16}.

We confirmed that a metabolic capacity of the creatine kinase reaction in the brain *in vivo* was significantly changed in various animal models simulating neurodegenerative diseases, such as AD, HD or generally during oxidative stress.

We have concluded that the investigation of the kinetic parameters using *in vivo* ³¹P MRS magnetization transfer method contributed to a better understanding of the underlying processes in various neurological disorders. Using this method we distinguished AD and HD, because k_{for} decreased in AD model while it increased in HD. This technique can be used as a noninvasive *in vivo* biomarker for age-related neurodegenerative diseases as it can reveal energy metabolism impairment of brain tissue, which is not yet detectable by conventional MRS methods.

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REFERENCES

1. Beal M F. (1998) Mitochondrial dysfunction in neurodegenerative diseases. *Biochim Biophys Acta* 1366, 211–23.
2. Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah Daouk R, Beal MF. (1998) Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* 18, 156–63.
3. McCord JM, Russel VJ. Inactivation of creatine phosphokinase by superoxide during reperfusion injury. In: Simic KA, Word JF, Von Sonntag C, editors. *Oxygen Radicals in Biology and Medicine*. New York: Plenum Press, 1988. p 869–73.
4. Yatin S M, Askenov M, Butterfield DA. (1999) The antioxidant vitamin E modulates amyloid β -peptide-induced creatine kinase activity inhibition and increased protein oxidation: Implications for the free radical hypothesis of Alzheimer's disease. *Neurochem Res* 24, 427–35.
5. Corbett RJT, Liptaj AR. (1994) Age-related changes in swine brain creatine kinase catalyzed 31P exchange measured *in vivo* using 31P NMR magnetization transfer. *J Cereb Blood Flow Metab* 14, 1070–7.
6. Sauter A, Rudin M. (1993) Determination of creatine kinase kinetic parameters in rat brain by NMR magnetization transfer. *J Biol Chem* 268, 13166–71.
7. Braunová Z, Kašparová S, Mlynárik V, Mierisová Š, Liptaj T, Tkáč I, Gvozdjaková A. (2000) Metabolic changes in rat brain after prolonged ethanol consumption measured by 1H and 31P MRS experiments. *Cell Mol Neurobiol* 20, 703–15.
8. Kašparová S, Dobrota D, Mlynárik V, Pham TN, Liptaj T, Horecký J, Braunová Z, Gvozdjaková A. (2000) A Study of creatine kinase reaction in rat brain under chronic pathological conditions – chronic ischemia and ethanol intoxication. *Brain Res Bull* 53, 431–5.
9. Kašparová S, Brezová V, Valko M, Horecký J, Mlynárik V, Liptaj T, Vančová O, Uličná O, Dobrota D. (2005) Study of the oxidative stress in a rat model of chronic brain hypoperfusion. *Neurochem Inter* 46, 601–11.
10. Sumbalová Z, Kašparová S, Bystrický P, Kucharská J, Mlynárik V, Gvozdjaková A. (2002) Effect of coenzyme Q10 and vitamin E on brain and skeletal muscle energy metabolism in animal model of Huntington's disease. In: *Third Conference of the International Coenzyme Q10 Association*. London. p. 193–5.
11. Sumbalová Z, Kucharská J, Kašparová S, Mlynárik V, Bystrický P, Uličná O, Vančová O, Singh RB, Gvozdjaková A. (2005) Brain energy metabolism in experimental chronic diabetes: long-term effect of coenzyme Q10 and ω -3 fatty acids. *Biologia, Bratislava, Suppl* 11, p. ///.
12. de la Torre JC, Fortin T. (1994) A chronic physiological model of dementia. *Behav Brain Res* 63, 35–40.
13. Clark JF, Harris GI, Dillon PF. (1991) Multisite saturation transfer using DANTE and continuous wave. *Magn Reson Med* 17, 274–8.
14. Mlynárik V, Kašparová S, Liptaj T, Dobrota D, Horecký J, Belan V. (1998) Creatine kinase reaction rates in rat brain during chronic ischemia. *Magnetic resonance materials in physics, biology, and medicine, MAGMA* 7, 62–165.
15. Burbaeva GSh, Savushkina OK, Dmitriev AD. (1999) Brain isoforms of creatine kinase in health and mental diseases Alzheimer's disease and schizophrenia. *Vestn Ross Akad Med Nauk* (1), 20–24.
16. Jost CR, Van der Zee CEEM, in't Zandt H J A, Oerlemans F, Verheij M, Streijger F, Fransen J, Heerschap A, Cools AR, Wieringa B. (2002). Creatine kinase B-driven energy transfer in the brain is important for habituation, and spatial learning behaviour, mossy fibre field size and determination of seizure susceptibility. *Europ J Neurosci* 15, 1692–1706.