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

Svatava Kašparová, Zuzana Sumbalová, Jaromír Horecký, Peter Bystrický, Vladimír Mlynárik, Anna Gvozdjáková, Tibor Liptaj

a NMR Laboratory, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava
b Pharmacobiochemical Laboratory of the 3rd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
c Slovak Medical University Bratislava, Slovakia
d Metabolic Imaging, EPFL, Lausanne, Switzerland

Received: June 10, 2005; Accepted: September 25, 2005

Key words: Creatine kinase/Saturation transfer 31P NMR/Chronic cerebral hypoperfusion/Neurodegenerative diseases/Animal model

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 \textit{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 \textit{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 \textit{in vivo}. Rate constants of CK reaction can be investigated by magnetization transfer \textit{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

\[
PCr^{2-} + MgADP^-- + H^+ ⇄ MgATP^{2-} + Cr
\]

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 \textit{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/every
12 h) for 11 days to develop chronic model of HD (ref. 7). 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 vehicle7, 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 and right vertebral artery9, 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 occlusion8, 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 (kfor) as described previously6, 9, 13, 14. Time dependent saturation transfer allows one to measure simultaneously two parameters, T1 and T2. Saturation transfer in vivo 31P MRS experiments with variable time of irradiation (0.3–10 s) of γATP signal. The length of the saturation period is given below each spectrum. In the control experiment, the saturation was off-resonance. The saturated γATP 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/βATP ratio only in diabetic group, we found significant changes of brain kfor in all chronic models of neurodegenerative diseases. Compared to controls kfor increased in HD and DM models and decreased in chronic alcohol intoxication and VD models (Table 1).

From kinetic 31P MRS transfer measurements we found in case of HD small (+13.4 %), but statistically significant an increase in kfor (Table 1). Exact mechanism responsible for the increase of the pseudo-first-order rate constant for the CK reaction kfor is presently not clear. However, in vivo 31P MRS transfer measurements revealed a correlation between CK flux and brain 2-deoxyglucose uptake3, 4. Our result of increased activity of CK corresponds well with reported increased 2-deoxyglucose uptake in basal ganglia of HD patients1, 5.

Similar increase of kfor (+22.9 %) we obtained in the model of diabetes mellitus in which also increased brain PCr/βATP 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 kfor in these models of neurodegenerative diseases could be prevented by application of CoQ10 provides evidence that kfor reflecting activity of CK system is sensitive marker of changes in brain energy metabolism10, 11.
Table 1. Parameters determined by $^{31}$P MRS methods in the rat brain in models of neurodegenerative diseases

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

n – number of animals per group; $k_{fo}$ – the pseudo-first order rate constant of forward CK reaction determined in brain in vivo by $^{31}$P MRS saturation transfer; $T_1$(PCr) – longitudinal relaxation time of phosphocreatine in absent γ-ATP saturation, a control parameter; PCr/$\beta$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, a$P$ < 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 nerves. 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 doses. 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 brain2, 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 insufficiency. 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 perfusion. Compared to the control group of healthy aged rats the hypoperfused rats showed statistically significant decrease in $k_{fo}$ 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. The causes of decreased levels of cytosolic brain CK isoform (BB-CK) in postmortem brain in mental pathology are subject of discussions. As it was recently demonstrated, BB-CK energy transfer in the brain is important for habituation and spatial learning behaviour. 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 mice\textsuperscript{16}. Thus, the rate constant of CK, $k_\text{rate}$, measured in the brain reflects changes of BB-CK activity\textsuperscript{8,9,16}.

We confirmed that a metabolic capacity of the creatine kinase reaction in the brain \textit{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 \textit{in vivo} \textsuperscript{31}P 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 $k_\text{rate}$ decreased in AD model while it increased in HD. This technique can be used as a noninvasive \textit{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.

**ACKNOWLEDGEMENT**

This work was facilitated by the support of the Slovak State Program of Research and Development No. 2003SP200280203, and grants VEGA No. 1/4112/97-99, 1/7547/20, 1/0546/03.

**REFERENCES**