Cerebral Phosphate Metabolite Profiles and their Differentiation in Human, Cat and Rat Brains: A Comparison Study of *In Vivo* ^{31}P MRS at High Fields

X-H. Zhu[1], F. Du[1], Y. Zhang[1], and W. Chen[1]

[1]Department of Radiology, University of Minnesota Medical School, Center for Magnetic Resonance Research, Minneapolis, MN, United States

INTRODUCTION *In vivo* ^{31}P MRS provides useful measures for a large number of cerebral phosphate metabolites and tissue pH. It is particularly important for noninvasively studying ATP and phosphate lipid metabolisms and bioenergetics associated with brain function and neurological diseases. In addition, the reliability of ^{31}P MRS measurement can be significantly improved at high field. Although, *in vivo* ^{31}P MRS has been applied for numerous brain studies in human and various animal models, it is still lack of systematic comparison of the cerebral phosphate metabolite profiles and brain tissue pH among healthy human and commonly used animal models in medical research. In this study, we conducted a comparative ^{31}P MRS study at high fields for determining the brain phosphate metabolite profiles in the human and two animal models of cat and rat. Significant differences in the measured metabolite concentrations and tissue pH were found among these species.

METHODS *In vivo* brain ^{31}P spectra were acquired in the human subjects (n=5) on a 7T/90cm bore Magnex magnet, and in the cats (n=10) and rats (n=16) on a 9.4T/30cm bore Magnex magnet equipped with Varian INOVA consoles. A number of ^{31}P RF surface coils were home-built and applied to the human, cat and rat brains, respectively. The coil size and the flip angle of the excitation RF pulse were carefully calibrated to ensure that the detected ^{31}P signals were mainly come from the brain tissue [1-3]. A single-pulse-acquisition sequence was applied to collect *in vivo* brain ^{31}P spectra. A relatively long repetition time (TR ≥ 12 seconds) was used to minimize the saturation effect on the metabolite concentration quantification. The saturation effect was examined by the cat study using two TR values of 12 and 16 seconds. A large number of signal averaging (NEX = 80 or 128) were used for achieving sufficient SNR. Spectral processing and quantification was carried out using the AMARES method included in the software package MRUI. A total of eleven phosphate resonance peaks were detected and analyzed. They are: phosphoethanolamine (PE); phosphocholine (PC); intracellular inorganic phosphate (Pi) and extracellular Pi (Pi^{ex}); glycerophosphoethanolamine (GPE); glycerophosphocholine (GPC); phosphocreatine (PCr); three adenosine triphosphate resonances (γ-ATP, α-ATP and β-ATP); and nicotinamide adenine dinucleotides (NAD). Majority of literature suggest a stable [ATP] = 3 mM in a number of normal brains [4]. Therefore, a constant [γ-ATP] = 3 mM was applied in our study, and other metabolite concentrations were determined by the peak integral ratio between the phosphate resonances and the γ-ATP resonance. In addition, the brain tissue pH was also calculated using the chemical shift difference between Pi and PCr peaks. Paired *t*-test was used for statistical analysis and a $p<0.05$ was considered statistically significant. The results were presented by mean±SD.

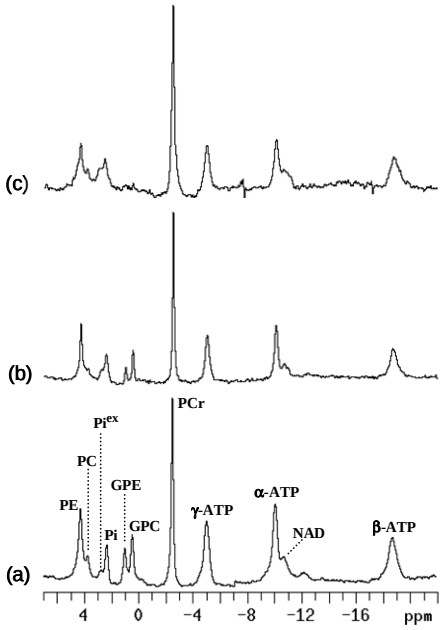

Fig. 1 *In vivo* ^{31}P brain spectrum acquired in: (a) human (TR=16s, NEX=128); cat (TR=16s, NEX=80); and (c) rat (TR=12s, NEX=128) at high fields.

RESULTS AND DISCUSSION Figure 1a shows the *in vivo* ^{31}P brain spectrum of a representative healthy human volunteer. More than eleven cerebral phosphate metabolite resonance peaks are well resolved and detected with excellent sensitivity. Figures 1b and 1c demonstrate the *in vivo* ^{31}P spectrum obtained from the cat and rat brains, respectively. Although, all resonance peaks appear in the animal brain ^{31}P spectra, there are obvious differences in the detected metabolites among human, cat and rat brains. Figure 2 summarizes the averaged results of all measured phosphate metabolite concentrations and the brain tissue pH. A number of conclusions can be drawn from these results. First, there is no significant difference in all metabolite concentrations and pH values measured with two TRs (12s vs. 16s) in the cat brain. It suggests that TR= 12s should be sufficient for achieving minimum saturation effect. Second, the detected [α-ATP] and [β-ATP] were similar with [γ-ATP] as we expected. Third, the intracellular [Pi] and total [Pi] (= [Pi]+[Pi^{ex}]) was relatively low in the human brain compared to that in the cat and rat brains. Perhaps the largest differentiation in the metabolite concentration as detected in this study is related to the two metabolites involving the phosphate lipid metabolism, i.e., GPE and GPC, which are much lower in the rat brain than that in the human and cat brains. There are also [PCr] differences among these three species. The human and cat ^{31}P spectra were from the visual cortex with minimal muscle contribution, while the rat brain [PCr] may have certain degree of muscle contamination. Therefore, caution should be taken when using the rat brain [PCr] measured in this study. Finally, there are significant differences in the brain pH showing a trend of pH (rat) > pH (cat) > pH (human) as shown in Fig. 2. The overall results indicate a closer cerebral phosphorous metabolites profiles between human and cat than that of the human and rat.

CONCLUSION Excellent *in vivo* ^{31}P NMR sensitivity and spectral resolution available at high/ultrahigh fields have benefited us significantly for improving the reliability and accuracy of the phosphorus metabolite and pH measurements. A large number of differentiations in the measured phosphate metabolites and tissue pH were detected among the brains of three different species. The results provide standard phosphate metabolite profiles of the healthy human, cat and rat brains, which should be valuable when comparing the physiology versus pathology states in a specific brain of interest. In particular, the contents of Pi, ATP and PCr are tightly coupled to the brain ATP metabolism; in other hand, the information of PE, PC, GPE and GPC is crucial for regulating the cerebral phosphate lipid metabolism. They play essential roles in brain physiology, function and dysfunction or disease.

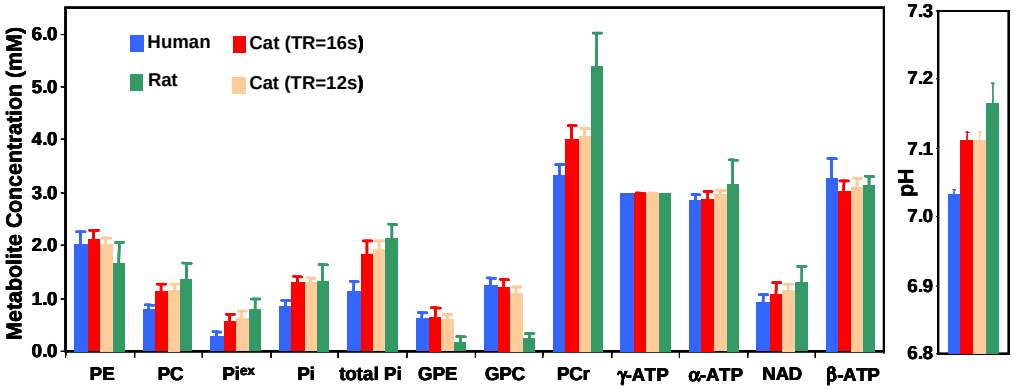

Fig. 2 Summary of cerebral phosphate metabolite concentrations and brain pH measured in the human (n=5), cat (n=10) and rat (n=16) brains.

ACKNOWLEDGEMENTS NIH grants: NS41262, EB00329, EB00513, P41 RR08079 and P30NS057091; the Keck foundation.

REFERENCES [1] Lei, et al. *MRM* 49:199-205 (2003). [2] Lei, et al. *PNAS* 100:14409-14414 (2003). [3] Du et al. *MRM* 57:103–114 (2007). [4] Siesjo, Brain Energy Metabolism, A Wiley-Interscience Publication, 1978.