Title: $^{31}$PHOSPHATE NUCLEAR MAGNETIC RESONANCE SPECTROMETRY DURING CEREBRAL HYPOXIA

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Introduction. Traditionally, various techniques have been used to study the brain metabolic state. Usually, this is accomplished by rapid cooling of tissue in order to maintain metabolism at the time of sampling. Although advances have been made in this technique which allow the brain to be frozen very rapidly, the major shortcoming is that the collection of tissue is a destructive process and continuous monitoring of the brain energy state is impossible. Development of nuclear magnetic resonance (NMR) spectroscopy techniques generated speculation that this method may permit the continuous, noninvasive monitoring of cerebral metabolism. We wish to demonstrate that by incorporating a flat surface coil in a high resolution NMR spectrometer,[1] it is possible to measure phosphate metabolism in vivo in a non-destructive manner. In the present study, the value of this technique will be demonstrated by measuring the changes in adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate ($P_i$) and intracellular pH during hypoxia with relative ischemia.

Methods. Concentrations of PCr, $P_i$, and ATP were measured directly from the respective peaks in NMR spectra, whereas pH was calculated from the separation between $P_i$ and PCr peaks (this separation is a function of $H^+$ concentration).[2] A deconvolution technique was used to eliminate skeletal tissue contamination. Sixteen male, 300 gram Sprague-Dawley rats were anesthetized with halothane, tracheotomized, and bilateral carotid artery ligation performed. Halothane was discontinued immediately after tracheotomy, curare was administered, and the animals were ventilated with 79% $N_2O$ and 21% $O_2$ to maintain $pCO_2$ 35-45 torr. A 1.5 cm radiofrequency coil was then attached to the dorsal surface of the head and the animal was placed in the bore tube of the superconducting magnet where temperature was maintained at 37°C ±0.5. While inside the magnet, the first ten minutes served as a control period. Following this, a mixture containing either 3, 5, 10, or 15 percent $O_2$, 79% $N_2O$ (balance $N_2$) was delivered for ten minutes, and then for the remaining 30 minutes of the experiment, the previous mixture of 21% $O_2$ and 79% $N_2O$ was administered. Data was accumulated continuously throughout the 50 minutes. Each 3.4 minutes, 64 scans were accumulated to produce a spectrum of the phosphate metabolites.

Results. A typical NMR spectrum under well oxygenated conditions is shown in Figure 1a. PCr, ATP (all three phosphate positions: α, β, γ), and $P_i$ can be seen. Figure 1b shows the changes in these parameters in the brain of a rat that died following inspiration of 3% $O_2$. Figure 2 shows the changes in the PCr/$P_i$ ratio and pH following the different degrees of graded hypoxia. Decremental changes of inspired oxygen reveal that at 10% inspired oxygen and below, there is a 50-70% decrease in PCr/$P_i$. During the readministration of 21% $O_2$, the changes in PCr/$P_i$ and pH are as seen in fig. 2. ATP levels were not significantly affected by the lower inspired $O_2$ levels.

Discussion. Our findings show the cerebral metabolic consequences of hypoxia with relative ischemia in vivo using $^{31}$P NMR with a surface coil. CPK enzymatic activity is pH dependent; the decrease in pH and PCr with maintenance of the ATP level during hypoxia can be explained by increased CPK activity. Our calculation of normal rat intracellular brain pH of 7.09 and pH during ischemic hypoxia of 6.4 compare to the values obtained by Siesjo[3] of 7.05 and 6.0-6.5 respectively. Of interest is our observation of similar metabolic depression during hypoxia of 3, 5, and 10% $O_2$, but not at 15% $O_2$. However, during return to normoxia, the changes in metabolism appear related to given levels of graded hypoxia. Further evaluation is necessary to determine the sensitivity of NMR during the rapid metabolic changes produced by severe hypoxia and/or ischemia. We conclude that NMR is an excellent method to noninvasively investigate cerebral metabolism during hypoxia and/or ischemia.

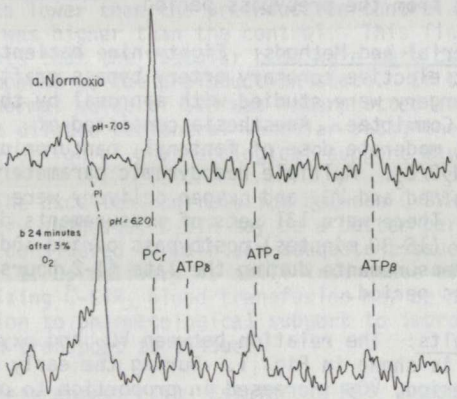


Fig. 1 $^{31}$P NMR spectra

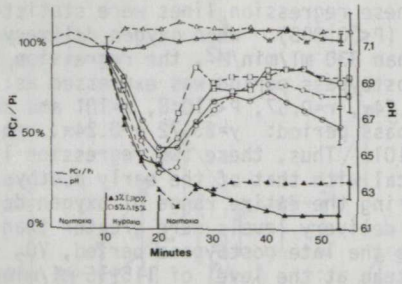


Fig. 2 PCr/$P_i$ and pH response to 10 min hypoxia

References.

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