Title: AN IN VIVO ^{31}P NMR STUDY OF CEREBRAL HYPOXIA AND ISCHEMIA IN ANESTHETIZED ADULT RATS

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Introduction. Efforts to protect the brain from hypoxic and ischemic injury usually include measures aimed at reducing cerebral energy metabolism. In vivo nuclear magnetic resonance (NMR) spectroscopy of ^{31}P, naturally abundant phosphorous, can be used to nondestructively measure cerebral intracellular pH and cerebral intracellular concentrations of ATP, phosphocreatine (PCr), inorganic phosphate (P_i), phosphodiesters (PD), and monophosphate sugars (MP).[1] We are exploring the use of these bioenergetic parameters as diagnostic and therapeutic indicies of brain injury and recovery, and have performed experiments with anesthetized adult rats to see if hypoxic hypoxia, ischemia during hypoxia, and global ischemia produce well defined patterns of change. Our ultimate goal is to use in vivo ^{31}P NMR spectroscopy to assess the relative benefits of different anesthetic and pharmacologic agents, to study reperfusion, and to determine irreversible damage.

Methods. Adult Sprague-Dawley rats were anesthetized with isoflurane and ventilated via an endotracheal catheter. Femoral arterial and venous catheters were placed, and the following physiological parameters were controlled: body temperature, blood pressure, serum glucose, administered anesthetic dose, arterial pH, P_aO_2 and P_aCO_2. A 14-mm, two-turn NMR detection coil, tuned for ^{31}P with a balance-matched circuit[2] was then placed over the brain, external to the animal. The prone animal was then put in a horizontal NMR spectrometer whose ^{31}P resonance frequency is 95.9 MHz. Free induction decays were collected every two seconds and spectra were generated from five minute data accumulations. The broad signal from bone was reduced by selective saturation, according to a recently developed technique.[3] Hypoxic hypoxia was induced in the first group of rats by administering nitrogen along with the inspired gases until an arterial blood gas measurement resulted in a P_aO_2 between 20 and 30 mm Hg. The blood pressure was maintained within 20% of the control value with an epinephrine infusion. Ischemia during hypoxia occurred in a second group of rats where epinephrine was not used, and the blood pressure was allowed to fall. The inspired oxygen concentration was restored to almost 100% after hypoxic periods for these first two groups. Global cerebral ischemia was induced in a third group of rats who had undergone surgical occlusion of both vertebral arteries three days before the experiment. Bilateral carotid occlusion was performed in the magnet after taking control NMR spectra, using remotely controlled suture snares around each carotid artery. After three 5-min spectral accumulations, the snares were released and cerebral blood flow was restored.

Results. Intracellular pH decreased in both hypoxia and ischemia. Initially, hypoxic hypoxia caused an increase in MP and P_i, a progressive decrease in PCr, and no change in ATP. The MP, P_i, and PCr concentrations returned to their control values when oxygen was restored (fig. 1), provided that the severity of the hypoxic insult was not so great as to make PCr disappear. Repeated insults in the same animal resulted in reproducible deterioration and recovery of the NMR spectra. Ischemia during hypoxia resulted in a rapidly progressive decrease in cerebral ATP, PCr, and MP that could not be reversed by reoxygenation and cardiovascular resuscitation. Global cerebral ischemia was characterized by immediate declines in ATP, PCr, and MP (fig. 2) that returned to control values after a brief period of reperfusion.

Discussion. Specific reversible ^{31}P NMR patterns characterize the brain during brief periods of hypoxic hypoxia and global ischemia. In vivo NMR spectroscopy can be done with an animal serving as its own control for comparisons of single spectra, and for comparisons of the time course of brain energy deterioration and recovery.

References.

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2. Murphy-Boesch J and Koretsky AP: An in vivo NMR probe circuit for improved sensitivity. J Mag Res 54:526-532, 1983.
3. Gonzalez-Mendez R, Litt L, Koretsky AP, Von Colditz J, Weiner MW, and James TL: Comparison of ^{31}P NMR spectra of in vivo rat brain using convolution difference and saturation with a surface coil. J Mag Res 57:526-533, 1984

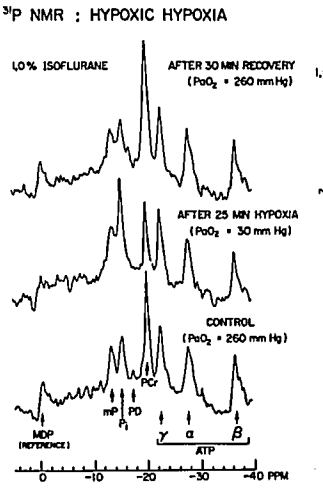

Figure 1

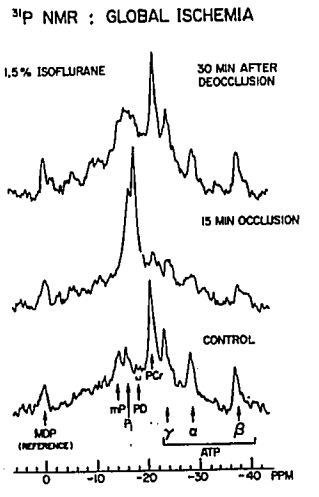

Figure 2