Single-Voxel ^{1}H MRS of the Rat Brain: Effect of Anesthesia

I. Iltis[1], D. M. Koski[1], K. Ugurbil[1], and P-G. Henry[1]

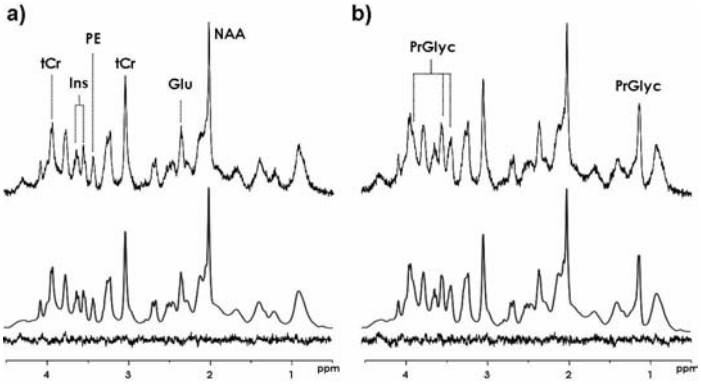
[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States

Objective. Pentobarbital is widely used as an anesthetic in animal studies. Deep pentobarbital anesthesia induces a decrease in glucose utilization (1,2). However, the effect of this deep anesthesia on brain metabolite concentrations as assessed by ^{1}H NMR spectroscopy ("neurochemical profile"), compared to an analgesic or a light anesthetic condition, is unknown. One reason for this is that the interpretation of ^{1}H NMR spectra acquired under pentobarbital is difficult, due to the solvent (propylene glycol) in which the active pentobarbital molecule is prepared. The strong contribution of propylene glycol signal to the ^{1}H spectrum has to be taken into account in spectral processing (3). Here, we included the spectrum of the injected solution for anesthesia in the LC Model basis set. Neurochemical profiles of the rat brain *in vivo* were acquired over time, while the animals were maintained under a light morphine + pancuronium anesthesia during the first hour, followed by pentobarbital anesthesia during two hours, allowing the assessment of the effect of a deep vs. a light anesthesia condition on the brain in the same animals.

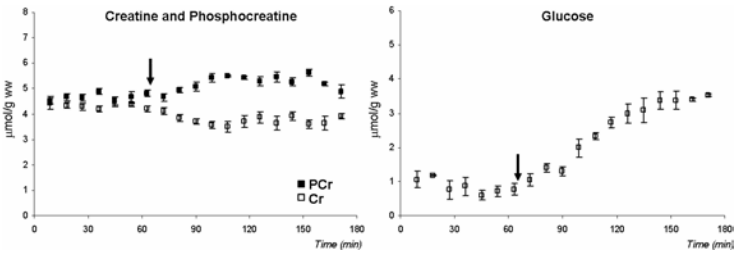
Methods. Six male Sprague-Dawley rats were anesthetized with isoflurane (1.8 %) in O_2 / N_2O (0.3 / 0.7 L.h^{-1}) for surgical procedure. Animals were intubated and ventilated. Two arterial (for blood sampling and blood pressure monitoring), one venous (for pentobarbital infusion) and one intraperitoneal (for morphine / pancuronium infusion) lines were placed in each rat. A morphine / pancuronium bolus was given at the end of the surgery, isoflurane was stopped, and a continuous infusion of morphine / pancuronium (rate, 25 / 2 ml.kg^{-1}.h^{-1}) was started for about 1 hour, then switched to pentobarbital infusion (rate, 80 mg.kg^{-1}.h^{-1}) for 2 hours. Experiments were conducted at 9.4 T. For spectra acquisition, a STEAM sequence with short echo time (TE = 2 ms, TR = 4 s) was used (4). Spectra were acquired in a 75 µl containing cortical and hippocampal structures. Absolute concentrations for metabolites were assessed with LC Model using water as an internal reference. A spectrum of the diluted solution used for anesthesia was acquired at 37 °C and included in the basis set for LC Model. Concentration time courses were obtained for 8 to 10 metabolites with a temporal resolution of 9 minutes.

Results and discussion. The inclusion of a spectrum of the anesthetic solution (pentobarbital in propylene glycol) in the LC Model basis set allowed a good fit of the in vivo spectra from the rat brain (figure 1). Propylene glycol has resonances close to macromolecules, *myo*-inositol (Ins) and phosphorylethanolamine (PE). Appearance of propylene glycol signal in brain spectra during pentobarbital infusion did not affect the quantitation of these metabolites: Cramer-Rao Lower Bounds (CRLB) obtained by LC Model were anyway comparable in spectra acquired during morphine + pancuronium and during pentobarbital anesthesia, and no change was observed in the concentration of Ins and PE after switching anesthetics. Compared to morphine / pancuronium, pentobarbital caused an increase in phosphocreatine (+ 15 %, $p < 0.0001$, $F = 8.929$) and a decrease in creatine (- 16 %, $p < 0.0001$, $F = 8.856$), suggesting a decreased energy metabolism (figure 2). The pool of glucose also showed a 4-fold increase ($p < 0.0001$, $F = 23.724$), in agreement with a decrease in glucose utilization under pentobarbital, as previously described (2).

Using single voxel ^{1}H MRS in combination with an appropriate LC Model basis set, we detected subtle changes in the brain energy metabolism under deep pentobarbital vs. a light anesthesia condition.



◄ *Figure 1. Spectra acquired under morphine (a) and one hour after starting pentobarbital anesthesia (b). TE = 2 ms, TR = 4 s, number of scans = 128. The in vivo spectra (top), the fitted spectra (middle), and the residual (bottom) are displayed. The peaks of propylene glycol are clearly visible. Including propylene glycol into the basis set for LC Model allowed proper fitting as indicated by flat residuals. tCr, creatine + phosphocreatine; Ins, myo-inositol; PE, phosphorylethanolamine; Glu, glutamate; NAA, N-acetylaspartate; PrGlyc, propylene glycol.*



►*Figure 2. Time courses of the concentration of creatine (left, open squares), phosphocreatine (left, closed squares) and glucose (right, open squares). Means ± SEM. The arrow indicates the time of the switch from morphine / pancuronium to pentobarbital anesthesia.*

1. Choi IY, et al. J Cereb Blood Flow Metab 2002;22(11):1343-1351. **2.** Crane PD, et al. Stroke 1978;9(1):12-18. **3.** Oakden WK, et al. J Comput Assist Tomogr 2005;29(1):136-139. **4.** Pfeuffer J, et al. J Magn Reson 1999;141(1):104-120.

Supported by NIH BTRRP41RR008079, R01NS38672, the MIND Institute and the KECK Foundation.