In Vivo Brain ^{1}H-MRS of Sodium Pentobarbital: A Potential Index Reflecting Anesthesia Depth and States of Brain Energy Metabolism and Activity

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Introduction

How to evaluate and monitor the depth of anesthesia is crucial for medical surgery and diagnoses. Sodium pentobarbital (PB) is commonly used in clinic and medical researches for the subject anesthesia. the purpose of this study is to find a bio-marker to determine the anesthesia depth and cerebral energy requirement at the various brain activities when subject is anesthetized by sodium pentobarbital with different dose. Therefore, the cerebral metabolic rate of ATP (CMR_{ATP}), a fundamental energy fuel, and concentration of sodium pentobarbital in the rat brain were measured by the ^{31}P and ^{1}H MRS, respectively. The brain activities were monitored and quantified by EEG entropy index. Finally, the measured concentration of brain pentobarbital was used to correlate with basal brain activity and CMR_{ATP} in the physiological range studied.

Method and Materials

Animal Preparations: Male Sprague-Dawley rats (210-350 g) were divided into three groups (each group contained 6-12 rats) for ^{1}H MRS, ^{31}P MRS and EEG measurements at three anesthesia conditions. They were first anaesthetized by inhalation of 2% (vol-vol) isoflurane (*Iso*) in nitrous oxide/oxygen (3:2), then switch to sodium pentobarbital with two different doses (*Low-Pen*: 30mg/kg bolus with 30mg/kg/h infusion rate; and *High-Pen*: after *Low-Pen,* infusion rate increased to 70mg/kg/h) to control brain basal activity. *In vivo* MRS experiments and EEG recordings were performed when the animal physiologic conditions approached steady states.

MR Measurements: MR experiments were carried out at a 9.4 T/31 cm horizontal magnet. The localized spectra (4×4×4 mm^3) were acquired by the point-resolved spectroscopy (PRESS) approach with 64 scans as well as TR/TE=3000/13 ms. CMR_{ATP} was determined by the ^{31}P saturation transfer approach, which was achieved by means of frequency-selective saturation of γ-ATP [1,2] to determine unidirectional ATP synthesis flux from inorganic phosphate (P_i) to ATP.

EEG Measurements: Two electrodes were sued for recording EEG signals. One was put on the nose of rat serving as a reference and the tip of the another electrode was inserted into the cortex through a small hole on the skull (3 mm deep, 3 mm from bregma, 3 mm lateral midline). A Shannon spectral entropy method [3] was applied to analyze EEG data and quantify the brain activity at varied brain states.

Results and Discussions

Figure 1 presents the ^{1}H MRS spectra from one rat brain acquired under three anesthesia states. With the increased dose of sodium pentobarbital which was illustrated by the elevated PB peak at 1.15ppm, the brain activity was suppressed and characterized by the neuron burst suppression, and then eventually approached to EEG silence at high dose[4]. The characteristic EEG patterns at these three anesthesia conditions are demonstrated in Figure 2: (a) burst activity; (b) burst suppression and (c) iso-electric activity. The brain activity was quantified by the EEG entropy index, which has a strong correlation with the concentration of sodium pentobarbital in the brain measured and quantified by the peak area ratio between the resonance peak at 1.15ppm and creatine (Cr) peak at 3.01ppm (see Figure 3). On the other hand, cerebral energy metabolism is tightly liked to the brain activity. ATP is the fundamental energy fuel to be directly used in the live organs. The major utilization of ATP is to restore the sodium and potassium gradients across the cellular membrane, therefore keeping sustained electrophysiological activity and cell signaling. The increased dose of sodium pentobarbital suppressed brain activity and therefore resulted to deduce cerebral energy requirements. We have proved that ATP synthesis rate equals to its utilization rate at the steady state physiology condition to maintain stable ATP concentration [1]. So the measured CMR_{ATP} should reflect the energy requirements at various brain activity states. CMR_{ATP} goes down when brain activity decreases as indicated by EEG and it decreased ~50% at the iso-electric state (see Figure 3). In contrast, the peak area ratio between the 1.15 ppm peak and Cr peak increased from zero at Iso condition to 0.53 at iso-electric state. Overall, the measured peak area ratio is tightly correlated to both basal brain activity and the cerebral ATP metabolic rate as illustrated by Figure 3. Therefore, the measured peak area ratio by in vivo ^{1}H MRS should provide a good index to noninvasively quantify anesthesia depth and the varied states of both cerebral metabolic activity and neuronal activity.

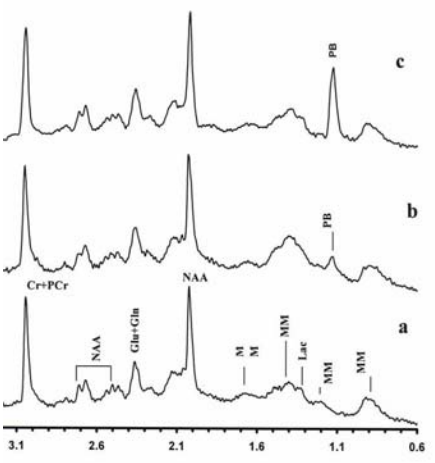

Figure 1. ^{1}H-MRS from a representative rat brain under three anesthesia states, respectively. (a). isoflurane. (b). low dose sodium pentobarbital (Low-Pen). (c). high dose sodium pentobarbital (High-Pen). The 1.15 ppm peak assigned as PB is related to the dose of sodium pentobarbital.

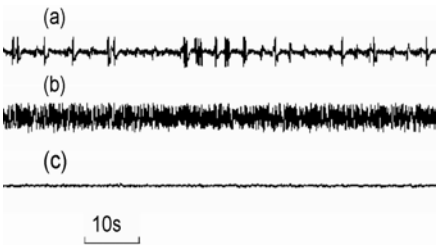

Figure 2. Characteristic changes in EEG activity produced by anesthetic agents of a representative rat. (a). isoflurane. (b). Low-Pen. (c). High-Pen.

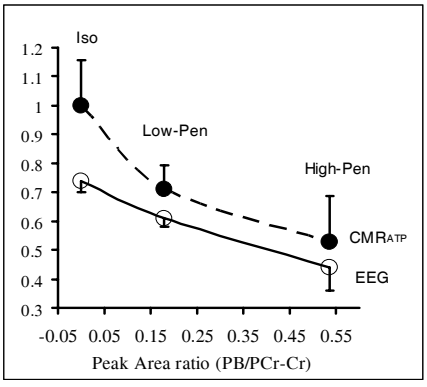

Figure 3. Correlations of brain EEG entropy index and cerebral metabolic rate of ATP (CMR_{ATP}) with peak area ratios between PB at 1.15ppm and Cr at 3.01ppm. The vertical axe indicates the EEG entropy index or relative CMR_{ATP}.

Reference

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Acknowledgements

NIH RO1 grants: NS41262, P41 RR08079; the W.M. Keck Foundation and the MIND institute.