Combining 31P-MRS with near-infrared spectroscopy to measure the concurrent changes in energy metabolites and cerebral oxygen consumption following hypoxia ischemia

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Introduction:

Hypoxia ischemia (HI) is a major cause of perinatal brain injury. Studies of brain energy metabolism in newborn piglets by ^{31}P MRS have demonstrated that the energy metabolites recover after experimentally induced HI, but a second decrease is observed 1-3 days later due to delayed energy failure (1). This delay potentially offers a therapeutic window when neuroprotection could prevent brain injury. However, a reliable method for detecting the early onset of injury has yet to be found. Our group recently developed a near-infrared spectroscopy (NIRS) method for measuring the cerebral metabolic rate of oxygen consumption ($CMRO_2$) and used this method to detect early reductions in $CMRO_2$ following HI in newborn piglets (2,3). In this study, NIRS and ^{31}P-MRS were combined to determine the temporal relationship between $CMRO_2$ and energy metabolites following HI.

Methods:

HI was induced by clamping the carotids and reducing the inspired oxygen level to 8% for 30 min. Experiments were conducted at 3T on 5 HI and 6 control piglets (< 36 hours old). ^{31}P MRS and NIRS data were collected before, during, and for six hours following insult.

The ^{31}P-MRS spectra were collected with a 25mm diameter surface coil placed on top of a piglet's head. The NIRS data were acquired from the same region by placing two fiber-optic cables, 30mm apart, on either side of the ^{31}P coil. ^{31}P spectra were acquired with 90° adiabatic RF excitation, TR = 10s and 120 averages. Spectra were fit in the time domain using a non-linear fitting algorithm developed on site (4). The concentration of nucleotide triphosphate [NTP] was determined from the β NTP peak and was expressed as a fraction of the exchangeable phosphate pool [EPP], quantified by [Pi] + [PCr] + [(γ + α + β)NTP] (1).

NIRS determinations of $CMRO_2$ were based on the Fick principle, i.e., $CMRO_2$ = CBF*(arterial – venous O_2 difference). Cerebral blood flow (CBF) was measured using indocyanine green as an intravascular NIRS contrast agent and the oxygen content in blood was determined from NIRS measurements of deoxy-hemoglobin (3,5,6).

A two-way mixed ANOVA was performed for each of the four parameters: PCr/Pi; NTP/EPP; CBF; $CMRO_2$. The within subjects factor was time and the between subjects factor was group (injury, control). A one-way ANOVA was performed for each time point for parameters that showed a significant time-by-group interaction in the two-way ANOVA.

Results:

The two-way ANOVA showed a significant time-by-group interaction [$F(6,54) = 25.8$, $p<0.001$] for the PCr/Pi ratio. PCr/Pi was significantly decreased in the HI group compared to controls up to 90 minutes post insult with no differences at later time points (figure 1). The NTP/EPP ANOVA also demonstrated a significant time-by-group interaction [$F(6,54) = 4.9$, $p<0.05$], with a slower recovery to control levels (figure 2) as compared to PCr/Pi. The $CMRO_2$ two-way ANOVA also showed a significant time-by-group interaction [$F(6,48) = 3.3$, $p<0.05$]. $CMRO_2$ was significantly decreased compared to controls for all points 150 minutes after injury (figure 3). CBF was significantly elevated for HI compared to control at the first post-insult point, but no differences were observed for any later time points.

Discussion:

As with our previous study, $CMRO_2$ was reduced following HI (2) and remained low for the duration of the study, even though CBF returned to normal levels by 90 min post injury. One explanation is that the hypoxic ischemic insult caused immediate cell death, leading to decreased $CMRO_2$. However, the ^{31}P-MRS data do not support this interpretation as the high-energy phosphates returned to control levels by ~ 2h following the insult, similar to previous studies (1). These results suggest reduced $CMRO_2$ may be an early indicator of brain injury. We plan to extend these experiments to 24 hrs to determine if early changes in $CMRO_2$ reflect the severity of delayed energy failure measured by ^{31}P-MRS. Since NIRS measurements of $CMRO_2$ can be performed at the bedside in less than two minutes, this technique could prove to be extremely useful for early diagnosis of HI.

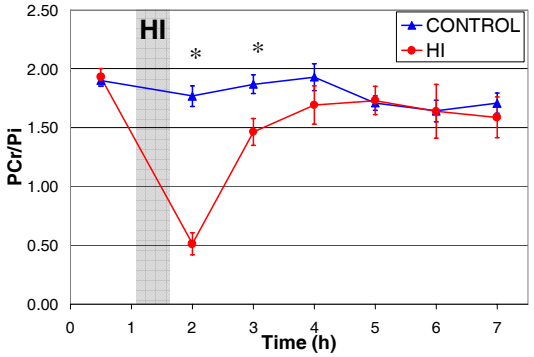

Figure 1. Ratio of phosphocreatine [PCr] to inorganic phosphate [Pi] in control and HI piglets. Error bars are SE. * p<0.05 between groups.

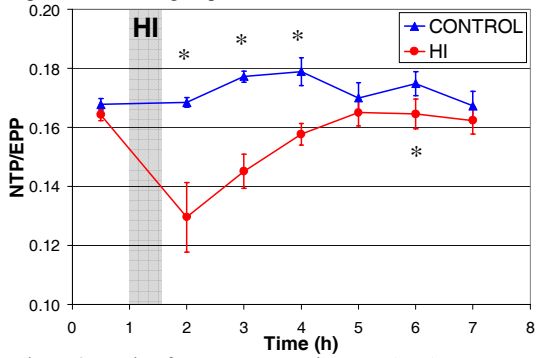

Figure 2. Ratio of [NTP] to [EPP] in control and HI piglets. Error bars are SE. * p<0.05 between groups. *p<0.05 between groups

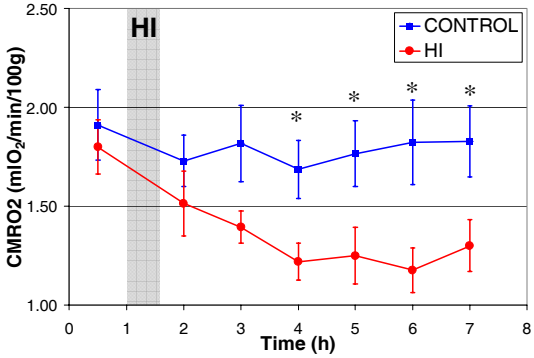

Figure 3. Cerebral metabolic rate of O_2 ($CMRO_2$) in control and HI piglets. Error bars are SE. * p<0.05 between groups.

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