Characterization of brain alkalosis during secondary energy failure in the piglet model: a shift in Pi concentration between two main intracellular pH compartments.

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Introduction: Brain intracellular pH (pH_i) has profound effects on cellular processes and cell viability. Brain alkalosis has been observed during the subacute phase following hypoxia-ischaemia (HI) in experimental models, adult stroke and in infants with neonatal encephalopathy [1]. In such infants the extent of the brain alkalosis was related to the severity of brain injury on conventional magnetic resonance (MR) imaging, neurodevelopmental outcome at one year and brain lactate/creatine, suggesting that brain pH_i may be a marker or mechanism of brain injury. Experimental data suggest that brain alkalosis after HI is deleterious to cell survival and that a delay in the rapid return of pH_i to normal or prevention of the alkaline overshoot are neuroprotective [2]. Phosphorus-31 (^{31}P) MRS provides a non-invasive measurement of brain pH_i from the chemical shift of inorganic phosphate (Pi) relative to phosphocreatine (PCr); at high field experimental models suggest brain pH_i heterogeneity due to more than one visible component of the Pi peak. This heterogeneity has been observed in both normoxic brain [3] and following HI [4]. Precise information on the pattern and timing of pH_i shifts in particular brain compartments following HI may be useful in targeting future neuroprotective strategies.

Aim: To assess brain pH_i changes and pH_i heterogeneity using high field ^{31}P MRS before, during and after an acute HI cerebral insult in an experimental model of the developing brain.

Methods: Twenty-seven newborn anaesthetised Large White piglets were studied before, during, and for 48 hours after an acute HI cerebral insult; six control animals were also studied. The insult was produced by reversibly occluding the carotid arteries and reducing fractional inspired oxygen to 12-16% for ~1 hour resulting in decreased [PCr] and adenosine tri-phosphate [ATP]. ^{31}P spectra were acquired continuously with a 7T Bruker Biospec with a 25mm diameter surface coil centred on the intact scalp over the parietal lobes. A DANTE pulse train reduced signal from bone and phospholipid prior to a non-selective excitation pulse; repetition time 10s; 192 summed transients at baseline, 64 during the insult and recovery, and 384 thereafter. Data were analysed using AMARES [5], a non-linear least square-fitting algorithm operating in the time domain, as implemented in the jMRUI package [6]. Prior knowledge of the approximate chemical shifts, linewidths, amplitudes and relative phases of the expected peaks in the spectrum was used by the fitting routine and was defined for three potential Pi peaks based on observations of the Pi lineshape. Soft constraints were used to limit the line-width of these peaks to between 10 and 50 Hz compared to a typical PCr line-width of 15-20 Hz. Estimates of chemical shift and amplitude using this methodology were found to be robust to variations in line-width and added noise in simulated ^{31}P spectra. Brain pH_i values were estimated from the chemical shifts of the Pi components relative to PCr with an appropriate Henderson-Hasselbach titration curve [7].

Results: Representative spectra demonstrating two main Pi components (Pi1 and Pi2) corresponding to two pH_i values (pH_i1 and pH_i2) at baseline and 42-48 hours following HI are shown in **Figure 1**. Piglets that did not survive to the final timepoint and insulted piglets that did not develop secondary energy failure (defined as a PCr/Pi_{total} outside 95% confidence interval for the control group at 42-48 hours) were excluded from further analysis. The analysis was performed on twenty-one insulted animals and five controls. Mean pH_i1 and pH_i2 for each group at baseline, post insult (4-10 hours) and at 42-48 hours are shown in **Figure 2**. At 42-48 hours the control group showed no change in pH_i1 or pH_i2 whereas the insulted group had slightly decreased pH_1 and pH_2 ($p<0.001$, $p < 0.05$) compared to baseline. Mean Pi_{total}/epp, Pi1/epp and Pi2/epp for each group at the respective timepoints are shown in **Figure 3.** Total Pi is denoted as Pi_{total} and concentrations are presented as a ratio to the energetic phosphate pool (epp) comprising of [PCr + Pi_{total} + ATP]. At 42-48 hours, the control group showed a slightly increased Pi_{total}/epp ($p < 0.05$) compared to baseline. The insulted group showed significantly increased Pi_{total}/epp, Pi1/epp and Pi2/epp characteristic of secondary energy failure. The amplitude of the more alkaline component, Pi1, was approximately double the amplitude of Pi2 at 42-48 hours and this difference was highly significant ($p < 0.001$).

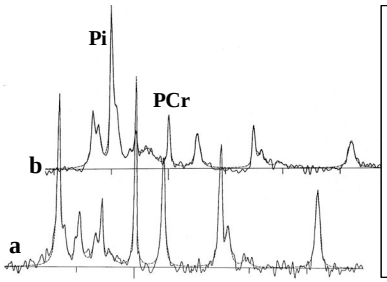


Figure 1: Spectra at (a) baseline; (b) 42-48 hrs showing 2 Pi components

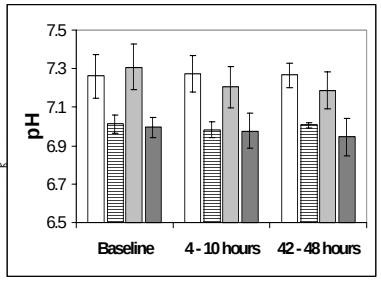


Figure 2: Brain pH_i values 1 and 2 for control and insulted groups

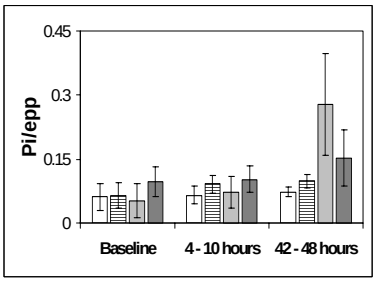
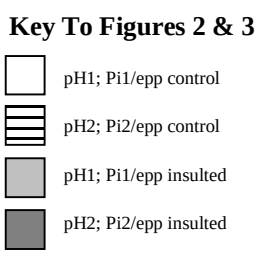


Figure 3: Pi/epp in each Pi component for control and insulted groups



Discussion: High field ^{31}P MRS demonstrated two main pH_i compartments in normoxic brain and in the acute and subacute phase following HI corresponding to pH_i values of around 7.3 and 7.0. At 42-48 hours following HI, Pi_{total} increased dramatically in keeping with secondary energy failure. In contrast to the assumption that there is an alkaline shift in pH_i during secondary energy failure, pH_i values 1 and 2 were slightly more acidic than baseline. However the proportion of Pi in pH_i1 (the more alkaline component) increased significantly more than pH_i2. At lower field strengths with poorer signal to noise ratio, this shift in relative Pi concentration between compartments might give the impression that the overall brain pH_i has become alkaline. These data suggest that during secondary energy failure there is a shift in the relative concentration of brain tissue with an alkaline pH_i. This may reflect the number of cells, neurons in particular, committed to programmed cell death. Alkalinization has been described as a critical trigger in the apoptotic process activating pro-apoptotic proteins such as Bax and transforming the mitochondrial permeability transition pore into a high conductance state. Here, the alkalosis may serve as an effector pathway mediating postischemic neuronal cell loss.

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