Intracellular pH after Perinatal Cerebral Hypoxia-Ischaemia Estimated from Nucleotide Triphosphate, Phosphoethanolamine, and Inorganic Phosphate Chemical Shifts

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Introduction. Shortly after birth asphyxia, phosphorus (^{31}P) magnetic resonance spectroscopy shows apparently normal cerebral energetics. Phosphocreatine (PCr) and nucleotide triphosphate (NTP, mainly adenosine triphosphate (ATP)) later decline and inorganic phosphate (Pi) increases - the lower PCr and NTP the worse the outcome (1). These changes ("secondary energy failure" (SEF)) were replicated in the piglet: transient hypoxia-ischaemia (HI) caused intracellular acidosis; after HI, cerebral energy metabolism and intracellular pH (pH_i) normalised temporarily followed by SEF unaccompanied by overt acidosis (2). The Pi chemical shift (δ_{Pi}) is often used to estimate pH_i (3). However, this resonance includes extracellular and cytosolic Pi (4). Phosphoethanolamine (PEt) and γ- and β-ATP chemical shifts also depend on pH (5,6) and, contrastingly, are intracellular probes. Pi and PEt may exist in both viable and dead cells; however, ATP only exists in viable cells. This study aimed to compare pH_i - derived using Pi, PEt, and ATP - according to SEF severity in a larger cohort of piglets.

Methods. Experiments followed UK guidelines. Thirty-three healthy piglets (aged <24 hr) were anesthetised, and physiologically monitored and maintained (2): 27 had ~ 1 hr transient HI (inspired oxygen fraction reduced to ~0.12 during reversible bilateral carotid-artery occlusion); six were controls. A 25-mm diameter ^{31}P-tuned surface coil was used at 7 Tesla with single-pulse acquisition (10 s repetition time). Spectra were analysed by AMARES (7,8). Since reduced NTP predicts adversity (1), the minimum NTP/exchangeable phosphate pool (EPP = Pi + PCr + (2γ + β)-NTP) from 6 hr after HI (NTP_{min}) was used to define injury groups: mild ($0.210 > NTP_{min} > 0.165$, n = 8); moderate ($0.164 > NTP_{min} > 0.120$, n = 9); and severe ($0.119 > NTP_{min} > 0.075$, n = 10). pH_i calculations used: Pi pH = $6.77 + \log_{10}((\delta_{Pi} - 3.29)/(5.68 - \delta_{Pi}))$ (3); PEt pH = $5.625 + \log_{10}((\delta_{PEt} - 3.190)/(6.946 - \delta_{PEt}))$ (5); and MAGPAC estimated ATP pH (6). Statistical analysis used 2-tailed t-tests with unequal variances.

Results. For clarity results are only presented for controls and severe injury - mild and moderate injury results were intermediate. Compared to controls, severe-injury PEt pH_i (see figures; mean $\pm$ SEM) was mildly acid 13-33 hr after HI (all $p < .05$) with most significance 17-21 ($p < .01$), 21-25 ($p < .001$), and 25-29 hr ($p < .002$). ATP pH_i was alkaline at 1-5 hr ($p < .004$) and acid from 21-33 hr (21-25 hr $p < .01$; 25-33 hr $p < .05$) and at 41-45 hr ($p < .05$). ATP pH_i errors increased at 40-50 hr due to low ATP signal. Pi pH was acid at 13-25 hr and alkaline after 37 hr (all $p < .05$).

Discussion and Conclusions. Significant, but subtly different, pH_i measure changes were observed during SEF: in particular, there was a previously unobserved mild acidosis. The Pi pH alkalosis after 37 hr agrees with neonatal encephalopathy observations (9) and may be due to up-regulation of the sodium proton exchanger or eventual cytosolic equilibration with extracellular pH. ATP pH_i probing only viable cells, showed no such alkalosis. Precise pH_i measurement during SEF may elucidate mechanisms of post-HI cell death.

References. **1.** Azzopardi D et al (1989) Pediatr Res 25:445-51. **2.** Lorek A et al (1994) Pediatr Res 36:699-706. **3.** Petroff OAC, Prichard JW (1985) Neurology 35:781-788. **4.** Robbins RC et al (1990) J Thorac Cardiovasc Surg 99:878-884. **5.** Corbett RJT et al (1987) Neurology 37:1771-1779. **6.** Williams GD et al (1993) Analyt Biochem 214:458-467. **7.** Vanhamme L et al (1997) J Magn Reson 129:35-43. **8** Naressi A, C. MAGMA, 2001 (in press) **9.** Robertson NJ et al (2002) Ann Neurol 52:732-742.