Role of intracellular buffering power on the mitochondria-cytosol pH gradient in the rat liver perfused at 4°C.


Introduction:
Although hypothermic conditions are largely employed for organ preservation, only a few studies have been undertaken to determine the mechanisms which control the cytosolic pH (pHcyt) at 4°C in mammals. The observation of the mitochondrial Pi NMR signal, and thus the determination of the mitochondrial pH (pHmito) at 4°C, may arise from an increase in the Pi level within the matrix, in response to mitochondrial swelling induced by hypothermia as shown by electron microscopy. The aim of this work was to determine the factors (i.e., buffering power) regulating the amplitude and the pH gradient between cytosol and mitochondria (ΔpHmito-cyt) in the isolated rat liver perfused at 4°C. Liver ATP content, pH and buffering power of cytosolic and mitochondrial compartments were evaluated in situ using phosphorus-31 nuclear magnetic resonance spectroscopy.

Methods:
Liver (4-6g) from fed male Wistar rats was perfused at 4°C in situ (4mL/min.g wet weight), excised and placed in 20 mm NMR cell. Perfusion medium was a Krebs-Henseleit buffer (pH 7.4) in absence (KH) or presence of bicarbonate (KHB) gassed with 95%O2/5%CO2 or 100% O2, respectively. The protocol was as follows: (i) 30 min KH or KHB perfusion, (ii) 40 min isobutyrate (25, 50 or 100 mM in KH or KHB) perfusion, (iii) 30 min KH or KHB reperfusion. 31P NMR spectra were recorded at 161.9 MHz within 2 min on a Bruker DPX400 spectrometer. pHcyt and pHmito were determined from the chemical shift of cytosolic and mitochondrial Pi resonance, respectively, taking the glycerophosphocholine resonance as an internal reference at 0.47 ppm and methylene diphosphonic acid (MDPA) in capillary as external reference at 18.4 ppm.

Results:
No ΔpHmito-cyt was detected in the liver perfused without bicarbonate. Permeant weak acid in the perfusate (H2CO3, 25 mM or isobutyric acid, 25, 50 or 100 mM) acidified both cytosol and mitochondria, and revealed a ΔpHmito-cyt from 0.06 to 0.31 pH unit (Fig1). Nevertheless the manipulations of the ΔpHmito-cyt were more effective in bicarbonate-free conditions, due to the absence of buffering by H2CO3/HCO3-. In the absence of bicarbonate, the intracellular buffering power (Fig2) was 3-fold higher in the mitochondria (110 mmol/pH unit at pHmito 7.16) than in the cytosol (44 mmol/pH unit at pHcyt 7.30) and dependent on the matrix and cytosol pH, respectively. These buffering powers were almost double in the presence of bicarbonate. In the bicarbonate-free perfused liver, the respiratory activity was 0.08±0.02 μmol O2/min.g liver wet weight and the ATP turnover was only 40±7 nmol/min.g liver wet weight, indicating the weak activity of liver mitochondria when ΔpHmito-cyt was <0.05 pH unit. The ATP turnover was determined during a metabolic steady state using 2.5 mM KCN and 0.5 mM iodoacetate (IAA) simultaneously added to the perfusate to suppress oxidative phosphorylation and to prevent glycolysis respectively. During a 50 mM isobutyric acid load, the ATP turnover was 35±4 nmol/min.g liver wet weight whereas ΔpHmito-cyt rose to 0.26±0.02 pH unit and pHmito remained alkaline. Hence, although ΔpHmito-cyt was increased the ATP turnover remained unchanged.

Discussion and conclusion:
This work is the first evaluation of the mitochondrial buffering power in the isolated liver. The ΔpHmito-cyt observed within various acid loads reflected the differential titration of cytosol and mitochondria containing proteins and H2CO3/HCO3- buffering systems. Moreover, no direct relationship between ΔpHmito-cyt and ATP turnover could be shown.

References:

Fig1. The effect of different concentrations of isobutyric acid (from 0 to 100 mM) on pHcyt and pHmito in the absence (KH) and presence (KHB) of bicarbonate. Results are shown as means±SEM.

Fig2. Effect of compartment pH (pHcyt or pHmito) on the buffering power (β) of the cytosol and the mitochondria in the presence (KHB) and absence (KH) of bicarbonate.