Water Exchange Kinetics in the Isolated Heart Correlate with Na^+/K^+ ATPase Activity: Potentially High Saptiotemporal Resolution *in vivo* MR Access to Cellular Metabolic Actiivity

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Introduction Intra- and extracellular water molecules undergo equilibrium exchange *via* mechanisms that include passive diffusion across the plasma membrane and movement through membrane proteins. In yeast cell suspensions steady-state water exchange across the plasma membrane correlates with H^+-ATPase activity [1]. Since the cells were at volume steady-state, this means that water cycles across the yeast membrane in response to metabolic transport activity. The H^+-ATPase, a P-type ATPase, creates the primary ion gradient (H^+) used as an energy source for secondary transport. In animal cells, the related P-type ATPase is the Na^+/K^+ ATPase (NKA). The hypothesis is tested that water cycles across the plasma membranes in concert with NKA activity.

Methods Longitudinal ^1H_2O MR relaxography (MRR) with an extracellular relaxation agent, $GdDTPA^{2-}$ (RR_e) was used to distinguish intra- and extracellular ^1H_2O signals by creating different relaxation time constant (T_1) values. Transmembrane water exchange kinetics was quantified to determine the mean intracellular water life time (τ_i) and water mole fractions (p_i and p_e) using two-site-exchange (2SX) analysis[1]. ^1H_2O T_1 values were measured using an IR pulse sequence (9.4T Varian Inova). ***Isolated Heart:*** The hearts of male Sprague-Dawley rats were isolated and perfused with Krebs Henseleit (KH) buffer at 80 mmHg constant pressure; 37°C[2]. KH RR_e concentration [RR_e] = 10 mM, KH [Na^+] = 143 mM; total Ca^{2+} was adjusted so free KH [Ca^{2+}] = 1.25 mM. Results are presented as mean (± SD) values.

Results τ_i^{-1} is the equilibrium water efflux pseudo-first order rate constant. To test whether water cycling (τ_i^{-1}) is sensitive to NKA activity, hearts were perfused with KH with varying extracellular K^+ concentration ([K_e^+]). In cardiac membrane vesicles Han [3] reported the relationship between [K_e^+] and NKA activity: at 0 mM [K_e^+], it is effectively zero; at 6 mM [K_e^+] it is ~60 % of maximal; and, at 20 mM [K_e^+] it is ~90% of maximal. In the heart, a positive, saturating correlation between τ^{-1} and [K_e^+] (**Figure 1A**) was found. Since [K_e^+] alters NKA activity, this suggests that τ^{-1} (water exchange) correlates with NKA activity. The K_m for the process was < 1 mM, closer to NKA pump current than ATPase activity [3]. Because $\tau_i^{-1} = P_w$ (A/V), the large τ_i^{-1} changes and small volume changes (**Fig 1B**) indicates that P_w (the water permeability) is changing with [K_e^+] or NKA activity.

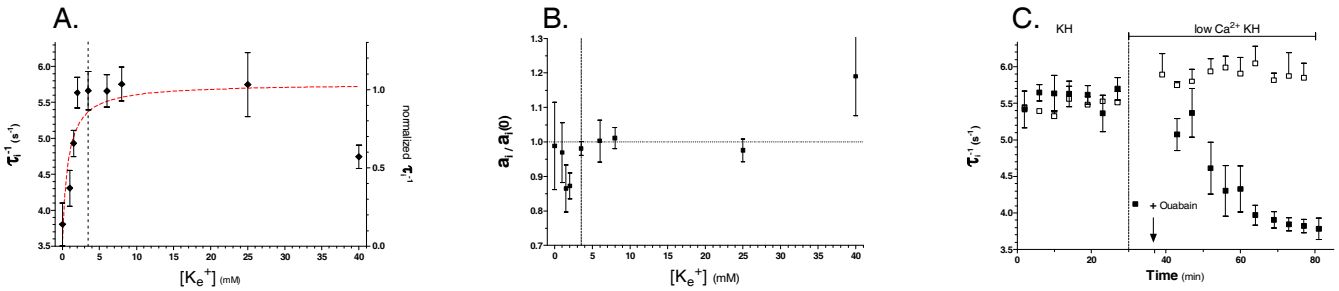

Figure 1, panel A displays the [K_e^+]-dependence of the τ_i^{-1}(s^{-1}), on left y axis, of isolated KH perfused rat hearts and on the right y axis, the normalized $\tau_i^{-1} = ((\tau_i^{-1} - \min \tau_i^{-1})/(\max \tau_i^{-1} - \min \tau_i^{-1}))$. Fitting normalized τ_i^{-1} with a Michaelis-Menten function (red dashed line) returned K_m = 0.74 ± 0.27 mM. **Panel B** shows the [K_e^+] dependence of total intracellular water volume (a_i) / a_i at time 0 ($a_i(0)$). Mean $a_i/a_i(0)$ values fluctuate no more than 15% below [K_e^+] = 25 mM, while τ_i^{-1} values vary from 5.75 s^{-1} (maximum) to 3.5 s^{-1} (minimum) a change of 40%. The vertical dotted line indicates [K_e^+] = 3.5 mM. A total of 11 hearts are included; each heart was studied at 4 different [K_e^+], beginning with 3.5 mM. **Panel C** shows the time dependence of the τ_i^{-1} (s^{-1}) of hearts perfused initially with KH ([K_e^+] was 3.5 mM); at 30 min KH [Ca^{2+}] was reduced to 0.25 mM; at 37 min ouabain was added to (■, n=5) the low Ca^{2+} KH. The [Ca^{2+}] was reduced in low Ca^{2+} KH to prevent contracture during the extended perfusion with ouabain, [K_e^+] was maintained at 3.5 mM.

Addition of a NKA inhibitor, ouabain, to the KH perfusate (**Fig 1C**) progressively reduced τ_i^{-1} over 40 min to values similar to those observed at [K_e^+] = 0 mM. These results show that steady-state water flux is high during baseline perfusion conditions in the isolated heart and correlates with NKA activity. Since NKA is found in most animal cells, ^1H_2O T_1 MRR/RR_e measured water exchange may serve as a biomarker for metabolic transport activity. This biomarker would benefit from the high SNR and spatial resolution of ^1H MRI, thus allowing high resolution functional imaging. Existing shutter-speed DCE-MRI studies have reported anatomically accurate parametric $\boldsymbol{\tau_i}$ maps of human osteosarcoma/skeletal muscle [4] and malignant breast tumors[4, 5]. The parametric τ_i maps are derived from the shutter-speed analysis but their meaning was unknown. Other patho-physiological states, e.g., ischemia, heart failure and acute renal failure may have altered $\boldsymbol{\tau_i^{-1}}$. For example, acute renal failure involves loss of renal transport activity and likely, water fluxes. Potentially the method will define the severity of metabolic damage, which will likely correlate with prognosis.

References: **1).** Zhang, Y., et al., Biophys J, 2011. 10**1**(11): p. 2833-2842; **2).** Zhang, L., H. He, and J.A. Balschi, Am J Physiol Heart Circ Physiol., 2007. 293(1): p. H457-66. **3).** Han, F., et al., Am J Physiol Cell Physiol, 2009. 297(3): p. C699-705. **4**). Yankeelov, T.E., et al., NMR Biomed, 2005. 18(3): p. 173-85. **5**). Li, X., et al., Magn Reson Med, 2005. **53**(3): p. 724-9.