Journal of Molecular and Cellular Cardiology 86 (2015) 95–101

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Multiple quantum filtered ^{23}Na NMR in the Langendorff perfused mouse heart: Ratio of triple/double quantum filtered signals correlates with $[Na]_i$

Thomas R. Eykyn [a,b,*,1], Dunja Aksentijević [b,1], Karen L. Aughton [b], Richard Southworth [a,b], William Fuller [c], Michael J. Shattock [b]

[a] Department of Imaging Chemistry and Biology, Division of Imaging Sciences and Biomedical Engineering, King's College London, King's Health Partners, St. Thomas' Hospital, London SE1 7EH, United Kingdom
[b] The British Heart Foundation Centre of Research Excellence, The Rayne Institute, King's College London, St. Thomas' Hospital, London SE1 7EH, United Kingdom
[c] Division of Cardiovascular and Diabetes Medicine, University of Dundee, Dundee, United Kingdom

ARTICLE INFO

Article history:
Received 21 March 2015
Received in revised form 8 July 2015
Accepted 10 July 2015
Available online 18 July 2015

Keywords:
Multiple quantum filtered ^{23}Na
TQF
DQF
Langendorff perfused mouse heart
Shift reagent

ABSTRACT

We investigate the potential of multiple quantum filtered (MQF) ^{23}Na NMR to probe intracellular $[Na]_i$ in the Langendorff perfused mouse heart. In the presence of Tm(DOTP) shift reagent the triple quantum filtered (TQF) signal originated largely from the intracellular sodium pool with a 32 ± 6% contribution of the total TQF signal arising from extracellular sodium, whilst the rank 2 double-quantum filtered signal (DQF), acquired with a 54.7° flip-angle pulse, originated exclusively from the extracellular sodium pool. Given the different cellular origins of the ^{23}Na MQF signals we propose that the TQF/DQF ratio can be used as a semi-quantitative measure of $[Na]_i$ in the mouse heart. We demonstrate a good correlation of this ratio with $[Na]_i$ measured with shift reagent at baseline and under conditions of elevated $[Na]_i$. We compare the measurements of $[Na]_i$ using both shift reagent and TQF/DQF ratio in a cohort of wild type mouse hearts and in a transgenic PLM^{3SA} mouse expressing a non-phosphorylatable form of phospholemman, showing a modest but measurable elevation of baseline $[Na]_i$. MQF filtered ^{23}Na NMR is a potentially useful tool for studying normal and pathophysiological changes in $[Na]_i$, particularly in transgenic mouse models with altered Na regulation.

1. Introduction

Intracellular Na concentration $[Na]_i$ is a key modulator of cardiac cell function [1]. At steady-state $[Na]_i$ has been measured between 4–16 mM depending on species and preparation [2]. The Na electrochemical gradient across the cell membrane provides the energy for the action potential upstroke, as well as the active transport of many other ions, amino acids and substrates into the cell [2,3]. $[Na]_i$ is also critical for the control of intracellular calcium $[Ca]_i$ via the sodium–calcium exchanger (NCX), thereby determining sarcoplasmic reticulum (SR) Ca content and cardiac contractility [4]. Maintenance of the Na^+ gradient is therefore fundamentally important in normal physiology but is also critically important in cardiac hypertrophy and heart failure where the elevation of $[Na]_i$ contributes to contractile and electrical dysfunction [5–7].

Historically, many techniques have been used to measure intracellular Na but few are physiologically relevant, either measuring Na at room temperature or in quiescent preparations [1]. ^{23}Na nuclear magnetic resonance (NMR) is able to distinguish intra versus extracellular Na pools, employing paramagnetic shift reagents such as Tm(DOTP) [8] to separate the two. However, these reagents are efficient chelators of Ca and Mg leading to modified ion homeostasis and reduced cardiac contractility [9]. As a result, shift reagents exhibit significant toxicity precluding their use in vivo. In contrast, multiple quantum filtered (MQF) ^{23}Na nuclear magnetic resonance has shown great potential to probe intra and extracellular pools of Na in the *absence* of shift reagent and therefore under more physiological conditions [10,11]. Much work has been carried out developing multiple quantum filtered ^{23}Na NMR in the perfused rat heart [10,12,13], in vivo in skeletal muscle and the brain [14] and in tumours [15]. In the perfused rat heart, the ^{23}Na TQF signal has contributions from the intra and extracellular Na pools [11,12,16] and has been shown to be proportional to intracellular $[Na]_i$ measured by atomic absorption spectroscopy [13]. Methods have been developed to suppress the extracellular contribution to the TQF signal [17]. On the other hand, the ^{23}Na DQF signal was found previously to arise only from the extracellular Na pool in the rat heart suggesting

* Corresponding author at: Department of Imaging Chemistry and Biology, Division of Imaging Sciences and Biomedical Engineering, King's College London, King's Health Partners, St. Thomas' Hospital, London SE1 7EH, United Kingdom.
E-mail address: thomas.eykyn@kcl.ac.uk (T.R. Eykyn).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.yjmcc.2015.07.009

that TQF and DQF ^{23}Na NMR signals arise from different cellular compartments [18].

Whilst the isolated rat heart has historically been an important model for cardiovascular research, the ability to rapidly and efficiently manipulate the genome of the mouse has recently made this the species of choice for many physiological and biochemical studies. The application of MQF ^{23}Na NMR techniques to the isolated perfused mouse heart is much more challenging due to technical issues of the smaller heart size and, prior to this study, has not been described. The aim of the present study was therefore to revisit the utility of the multiple quantum filtered experiment and to establish and characterise the methods necessary to apply MQF ^{23}Na NMR measurements to isolated Langendorff-perfused mouse hearts in both wild-type and a transgenic PLM^{3SA} genotype displaying altered $[Na]_i$ homeostasis. The PLM^{3SA} transgenic mouse has three mutations of the PLM phosphorylation sites Ser63, 68 and 69 which have been substituted for alanine, rendering PLM unphosphorylatable, leading to altered Na/K ATPase activity and elevated intracellular Na [6].

2. Methods

2.1. Langendorff mouse heart perfusion

C57BL/6J male mice (~28 g body weight) were purchased from Harlan (Harlan, UK). PLM^{3SA} and WT mice were bred 'in house' as previously described [6]. Mice were kept in individually ventilated cages with a 12 h light–dark cycle, controlled humidity and temperature (20–22 °C), fed standard chow and water ad libitum. All experiments were approved by institutional ethical review committee and conform to the UK Home Office Guidance on the Operation of the Animals Scientific Procedures Act 1986 (HMSO).

Mice were administered terminal anaesthesia via intra-peritoneal pentobarbitone injection (~140 mg/kg body weight). Hearts were rapidly excised, cannulated and perfused in isovolumic Langendorff mode and perfused at 80 mm Hg pressure maintained by a STH peristaltic pump controller feedback system (AD Instruments, Oxford, UK), at 37 °C with Krebs–Henseleit (KH) buffer continuously gassed with 95% O_2/5% CO_2 (pH 7.4) containing (in mM): NaCl 116, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.4, $CaCl_2$ 1.4, glucose 11 [19]. Cardiac function was assessed using a fluid-filled cling-film balloon inserted into left ventricle (LV), and connected via a rigid polyethylene line to an MR-compatible pressure transducer (DTX Plus TNF-R, Becton-Dickinson) and a PowerLab system (AD Instruments, Australia). The volume in the intraventricular balloon was adjusted using a 1.0 ml syringe to achieve an initial LV diastolic pressure of ~9 mm Hg. Left ventricular developed pressure (LVDP) was calculated from the difference between systolic (SP) and diastolic pressures (DP). Functional parameters (systolic pressure, end-diastolic pressure, heart rate, LVDP, coronary flow, perfusion pressure) were monitored using LabChart software v.7 (AD Instruments, Australia) throughout the experiment [19].

The low flow rates and the long perfusion lines in NMR systems can create problems with adequate temperature control. In addition, these long perfusion lines create significant 'dead-spaces' (where temperature, gas solubility and pH may change) and introduce long delays on switching solutions. These problems were solved in the current system by the use of two parallel continuously recirculating water-jacketed perfusion lines (Fig. 1a). Control, and test solutions were gassed at 37 °C before being pumped (Gilson Minipuls 3) through two parallel lines within a water-jacketed (37 °C) umbilical line (approx 3 m in length). These two lines then enter a novel three-way pneumatically operated valve positioned directly above the cannula (Fig. 1a). Control solution was passed via the pneumatic valve to the heart whilst the test solution was recirculated back up the umbilicus to the external reservoir. When the valve is activated, the control solution is recirculated and the test solution passes to the heart with effectively no distal stagnant dead-space and a switching time of <2 s, at the level of the heart. In order to protect the magnet, coils etc. from accidental leakage, the perfusion system includes a 'flood plain' between the perfusion lines, valve etc. and the cannula (Fig. 1a). This flood plain includes a moisture sensor connected to a remote alarm. The umbilical line, transducers, valve and all fittings were manufactured exclusively of non-magnetic parts and was fed via the top of the magnet and lowered into the active region of the bore/microimaging coil. At the end of each experiment, the perfusion system was removed from the magnet and hearts were immediately snap frozen using Wollenberger tongs, pre-cooled in liquid nitrogen, and wet and dry weights were recorded.

2.2. NMR spectroscopy

All experiments were carried out on a Bruker Avance III 400 MHz wide-bore spectrometer (Bruker, Karlsruhe, Germany) equipped with triple-axis gradients, a microimaging probe and exchangeable RF coil inserts (10 mm ^{23}Na coil or ^{1}H/^{31}P dual tune coil) that enable rapid switching between different nuclei. NMR tubes (O.D. 10 mm) (Wilmad, UK) were shortened in length to just greater than the NMR active coil region in order to minimize the perfusate dead volume beneath the heart. The bore of the NMR was maintained (~313 K) by continuous delivery of warm water, from a thermostatically controlled water bath, through the imaging gradients of the MR. Temperature calibration was performed in situ using a capillary containing ethylene glycol at the position of the heart.

To analyse the relationship between $[Na]_i$ elevation and TQF and DQF signal intensities, hearts were equilibrated for 20 min, followed by 20 min treatment with: (i) standard KHB ($n = 6$) (ii) 50 μM ouabain ($n = 6$), (iii) K^+-free buffer ($n = 3$), (iv) K^+-free/Ca^{2+}-free ($n = 3$) or (v) K^+-free/Ca^{2+}-free/Mg^{2+}-free ($n = 3$) buffer. Interleaved TQF and DQF NMR acquisitions were recorded throughout the stability period and during the intracellular sodium elevation protocols. At the end of each intervention, perfusate was switched to KHB containing 5 mM $Tm(DOTP)^{5-}$ to shift the extracellular $[Na]_e$ signal and quantify $[Na]_i$. An intracellular volume of 2.5 ml/g dry weight of tissue was assumed for calculation of $[Na]_i$ [20].

In order to confirm that cardiac energetics were normal and not compromised prior to any elevation in $[Na]_i$, fully relaxed ^{31}P experiments were also performed with the same perfusion system and phosphate free Krebs–Henseleit buffer, acquired with a 60° flip angle, 256 scans, a repetition time of 3.8 s and a total experiment duration of 16 min.

2.3. Multiple quantum filtered ^{23}Na MRS

The multiple quantum filtered pulse sequence consists of [10]: $90°(\phi_1) - \tau_m/2 - 180°(\phi_2) - \tau_m/2 - \beta°(\phi_2) - \delta - \beta°(\phi_3) - FID(\phi_{rec})$. DQF experiments were acquired with a four-step phase cycle: $\phi_1 = \phi_2 = 90°, 180°, 270°, 0°$; $\phi_3 = 0°$; $\phi_{rec} = 0°, 180°, 0°, 180°$. The flip angle β was set either to the magic angle, 54.7° (rank 2 $T_{2,\pm2}$ only) or to 90° (both rank 2 $T_{2,\pm2}$ and rank 3 $T_{3,\pm2}$). TQF experiments were acquired with a six-step phase cycle: $\phi_1 = 30°, 90°, 150°, 210°, 270°, 330°$; $\phi_2 = 120°, 180°, 240°, 300°, 0°, 60°$; $\phi_3 = 0°$; $\phi_{rec} = 0°, 180°, 0°, 180°, 0°, 180°$ with the flip angle $\beta = 90°$ (rank 3 $T_{3,\pm3}$ only). The mixing time ($\tau_m = 3.6$ ms) was calibrated for the maximum TQF signal in the mouse heart and set to be the same for the DQF experiments. All MQF experiments were acquired with 192 scans, 2048 data points, sweep width of 50 ppm, an acquisition time of 200 ms, pre-scan delay of 200 ms and a total acquisition time of 1.24 min. An exponential line broadening factor of 10 Hz was applied prior to Fourier transformation and subsequent baseline correction.

2.4. Shift reagent ^{23}Na MRS

All hearts were perfused at the end of the protocol with modified KHB containing 5 mM $Tm(DOTP)^{5-}$ (thulium (III) 1,4,7,10-