Application of ^{87}Rb NMR spectrocopy for studies of potassium transport in intact mouse hearts

O. Jilkina[1], B. Xiang[1], B. Kuzio[1], J. Rendell[1], V. V. Kupriyanov[1,2]

[1]Institute for Biodiagnostics, National Research Council of Canada, Winnipeg, Manitoba, Canada, [2]Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada

Introduction. We have successfully used ^{87}Rb NMR spectroscopy to study regulation of potassium transport in intact rat hearts[1-4]. Rb^+ is the closest K^+ congener replacing it in all known biochemical processes. Establishing ^{87}Rb MRS of mouse hearts is presently desirable as a number of genetically modified mouse strains representing animal models of human diseases and carrying mutations in potassium channels has been developed. A mouse heart is ~10-fold smaller than a rat heart stipulating technical difficulties in cannulation and adequate perfusion of a mouse heart through several meter-long perfusion lines inside the magnet, as well as establishing satisfactory ^{87}Rb MRS parameters and kinetics of Rb^+/K^+ fluxes. Therefore, we have tested feasibility of applying ^{87}Rb MRS for studying mouse cardiac potassium transport *in situ*.

Methods. **Heart perfusion.** Male CD-1 mice of 26-30 g were anesthetized with pentobarbital (120 mg/kg). The hearts (130-190 mg) were quickly removed, arrested in ice-cold Krebs-Henseleit buffer (KHB), attached via the aorta to a cannula, and perfused in Langendorff mode with KHB containing (in mM): 25 $NaHCO_3$, 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 0.5 EDTA, 11 glucose, and 1.5 mM Na-pyruvate, aerated with a mixture of 95% O_2 and 5% CO_2. Rb-KHB has the same composition as KHB except for K^+, which was substituted with Rb^+ by 50%. Following placement of a left ventricular apical drain, a plastic balloon was inserted through the mitral valve into the left ventricular cavity and connected to a pressure transducer and to heart performance analyzers to monitor heart rate, systolic, diastolic, and perfusion pressure. The hearts were perfused at a constant flow of ~3 ml/min to provide a desired concentration of drugs during infusion.

^{87}Rb NMR spectroscopy. NMR experiments were performed using a Bruker AM-360 spectrometer with equipped Tecmag DSPect upgrade in a 10-mm Bruker broadband probe placed in a wide bore vertical 8.4-T magnet. The ^{23}Na signal (95.25 MHz) from the heart and surrounding bath was used for shimming. ^{87}Rb NMR spectra were acquired at 117.8 MHz every 1.85 min (10240 scans) using a spectral sweep width of 15 kHz, acquisition time of 8.53 ms, and a pulse duration of 40 µs (90° flip angle). Acquisition size was 128 data points. An exponential multiplier of 150 Hz was used in processing. To minimize the signal from extracardiac ^{87}Rb, a suction line was placed at the bottom of the NMR tube. A 2 µl-capillary containing 1 M RbCl and 5 M KI was used as a reference.

Results and Discussion. K^+ was partially (50%) substituted in isolated perfused mouse hearts by perfusing them with a KHB-Rb for 40-50 min. Rb^+ accumulated in the hearts, which resulted in appearance of a second ^{87}Rb peak in addition to the reference peak (Fig.1). Signal-to-noise ratio was over 20. Rb^+ accumulation was analyzed using the equation for monoexponential kinetics (Fig. 2).

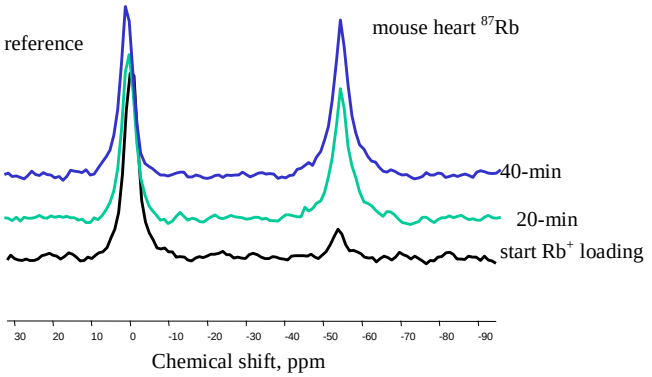

Fig. 1. Representative ^{87}Rb NMR spectra of a perfused mouse heart during Rb^+ loading.

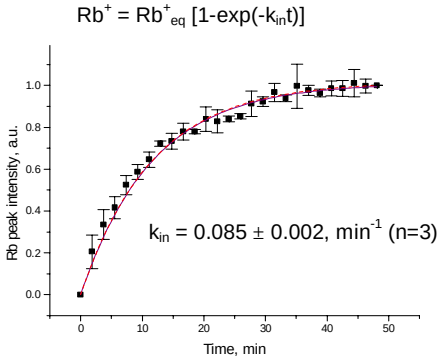

Fig.2. Kinetics of Rb^+ loading in mouse hearts, means ± SD are shown.

Rb^+ accumulation was fast, and heart's peak reached half of maximal intensity in ~12 min (Fig. 2). The amount of Rb^+ loaded into the hearts in 40-50-min was 15.7 ± 2.9 µmol/g wet weight (n = 5). Under conditions of crystalloid perfusion, 50% K^+ substitution for Rb^+, and intracellular K^+ concentration of ~ 100 mM, one can estimate intracellular volume of mouse hearts being close to 36% of total heart volume; without taking into special consideration the exact volume of cardiac mitochondria and their Rb^+ content, for which visibility factor is not known.

Rb^+ efflux was initiated by switching perfusion to Rb^+-free KHB. The initial rates of Rb^+ efflux (4 min) corresponded to the washout of extracellular Rb^+ and were discarded from the analysis. Efflux of intracellular Rb^+ followed monoexponential kinetics (Fig. 3). Under normal conditions, k_{eff} = 0.051 ± 0.005, min^{-1} (n = 4). ATP-sensitive K^+ (K_{ATP}) channels are known to be present in the cell membrane of mouse cardiomyocytes and link cardiac energetics and membrane potential, and, therefore, cardiomyocyte excitability. These channels are activated by a decrease in intracellular [ATP]/[ADP]. We have tested whether it was possible to detect activation of K_{ATP} in intact mouse hearts using ^{87}Rb MRS. Rb^+/K^+ efflux from mouse hearts was stimulated by application of a mitochondrial uncoupler, 2,4-dintrophenol (DNP). DNP decreases synthesis of cardiac ATP and therefore, should indirectly activate K_{ATP}. Indeed, 50 µM DNP activated Rb^+/K^+ efflux: $k_{eff\ DNP}$ = 0.067 ± 0.016, min^{-1} (n = 5). Similarly, the specific K_{ATP} opener, P-1075 (5 µM) stimulated Rb^+/K^+ efflux by direct activation of the channels: $k_{eff\ P\text{-}1075}$ = 0.072 ± 0.010, min^{-1} (n = 4).

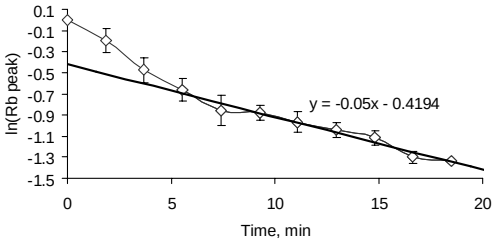

Fig. 3. Kinetics of Rb^+ efflux from mouse hearts.

Under normal conditions, the rates of Rb^+/K^+ uptake and efflux are determined mainly by the activity of Na^+/K^+ pump and K^+ voltage-gated channels that are responsible for repolarization phase of action potential. The higher heart rate of spontaneously beating mouse hearts in comparison to that of rat hearts (~ 370 vs. ~ 260 beats/min) indicates that mouse cardiac K^+ voltage-gated channels open more frequently, which is probably the factor responsible for the ~30% faster kinetics of Rb^+ uptake and efflux[1-3]. Qualitatively, the effects of DNP and P-1075 on Rb^+/K^+ efflux from mouse hearts were similar to those in rat hearts[2-4]. However, the degree of activation was less, implying that the surface density of K_{ATP} channels in mouse cardiomyocytes might be lower than that in rat cardiomyocytes or, alternatively, mouse hearts were less sensitive to DNP and P-1075. In conclusion, despite fast kinetics and the very small size of mouse hearts (~160 mg on average), ^{87}Rb MRS was demonstrated to be a suitable method for studying kinetics of Na^+/K^+ ATPase and K_{ATP} activation in intact mouse hearts, on 2-4 min time scale.

References: (1) Kupriyanov et al., Circ Res 76 (1995), 839; (2) Kupriyanov et al., NMR Biomed 11 (1998) 131; (3) Jilkina, et al., J Mol Cell Cardiol 34 (2002) 427; (4) Jilkina et al., Biochim Biophys Acta 1638 (2003) 121.