Disruption of Kir6.2 gene affects potassium fluxes in perfused mouse hearts

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Introduction. ATP-sensitive potassium channels (K_{ATP}) couple metabolic status (ATP level) to changes in potassium fluxes and membrane potential-dependent functions, such as insulin secretion in the pancreas or myocyte contractility in the heart. Mice lacking pore-forming subunit Kir6.2 of K_{ATP} (Kir6.2-/-) are non-insulin dependent diabetic [1] with compromised stress reactions, while in humans, abnormal K_{ATP} have been implicated in conditions such as dilated cardiomyopathy and permanent neonatal diabetes. Even though K_{ATP} channels have been cloned and their properties studied in cell membranes, regulation of potassium fluxes in intact perfused hearts of Kir6.2-/- mice has not been investigated. Rubidium (Rb^+) is a well-known K^+ tracer substituting K^+ in all known biochemical reactions. ^{87}Rb MRS provides a unique opportunity to compare potassium fluxes under metabolic stress conditions in the hearts of Kir6.2-/- and healthy control mice.

Methods. Heart perfusion. Kir6.2-/- and control (C57Bl6) mice of 19-34 g were anesthetized with pentobarbital (120 mg/kg). The hearts (130-200 mg) were quickly removed, arrested in ice-cold Krebs-Henseleit buffer (KHB), attached via the aorta to a cannulae, and perfused in a 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 75%. 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 equipped with Tecmag DSPect upgrade in a 10-mm Bruker broadband probe placed in a wide bore vertical 8.4-T magnet. The 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 3 µl-capillary containing 1 M RbCl and 5 M KI was used as a reference.

Results and Discussion. C57Bl6 and Kir6.2-/- mouse hearts were loaded with Rb^+ by perfusion with Rb-KHB (containing 75% Rb^+ and 25% K^+) for 40 min. Rb^+ accumulation in the hearts resulted in the appearance of a second ^{87}Rb peak separated from the reference by ~50 ppm (Fig. 1). Even though the real kinetics of Rb^+ uptake and efflux can be multiexponential, we used simple monoexponential fits to analyse the data (Fig. 2, and 3). Kinetics of Rb^+ loading in K_{ATP}-deficient mouse hearts under baseline conditions was not significantly different from the kinetics in control C57Bl6 hearts (Fig.2). The Rb^+ uptake rate constants were 0.077 ± 0.006 (n=10) and 0.069 ± 0.005 (n=8) for the control and diabetic mouse hearts, respectively. Thus, deficiency in K_{ATP} channels do not affect the basal activity of Na^+/K^+ ATPase that mostly determines the rate of Rb^+ uptake in situ.

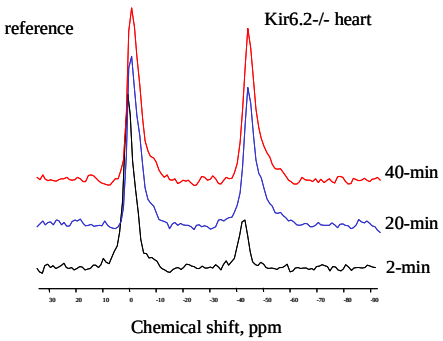

Fig. 1. Representative ^{87}Rb NMR spectra at 2, 20, and 40 min loading of a Kir6.2-/- mouse heart with Rb^+.

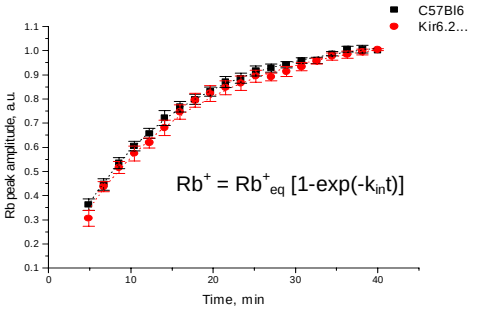

Fig. 2. Kinetics of Rb^+ loading in mouse hearts; means ± SE are shown.

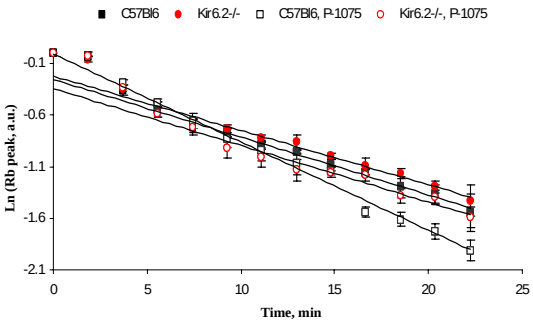

Fig. 3 Activation of Rb^+ efflux by P-1075 from C57Bl6 hearts and lack of similar activation from Kir6.2-/- hearts.

Rb^+ efflux was initiated by changing perfusion to Rb^+-free buffer. The first two spectra during Rb^+ washout reflected mostly extracardiac Rb^+ and were discarded from the analysis. The basal rate constants (k) were similar: 0.052 ± 0.06 (n=3) and 0.054 ± 0.009 (n=3) for the control and diabetic mouse hearts, respectively. A K_{ATP} opener, P-1075 binds to a SUR2A regulatory subunit of cardiac K_{ATP}. This binding induces potassium movement through the Kir6.2 subunit of K_{ATP}. Infusion of P-1075 (20 µM) activated K_{ATP} and stimulated Rb^+ efflux: k=0.086 ± 0.008 (n=3) in normal mouse hearts. P-1075 also induced a decline in contractile function of the mouse hearts to ~20% due to cardiac membrane hyperpolarization and a decrease in calcium fluxes. This activation and function changes were reversed upon infusion of a K_{ATP} inhibitor antidiabetic compound, glibenclamide (5 µM, k=0.063 ± 0.006, n=3). However, in Kir6.2-/- mice P-1075 produced no stimulation (k=0.05 ± 0.002 n=2) and no changes in cardiac function; thus confirming that knockout of Kir6.2 gene rendered K_{ATP} channels non-functional in these mice. The role of K_{ATP} channels in normal myocardium is controversial, as K_{ATP} channels are closed under the baseline conditions. However, these channels open in ischemia or during metabolic inhibition, when the level of ATP decreases. It has been shown that short activation of K_{ATP} somehow protects the myocardium against the subsequent long-term ischemia. We mimicked metabolic inhibition by infusion of a mitochondrial uncoupler, 2,4-dintrophenol (DNP, 50 µM) that stimulated Rb^+ efflux from the Rb^+-preloaded mouse hearts, similar to P-1075 [2]. However, in Kir6.2-/- knockouts, DNP produced no such stimulation (k=0.042 ± 0.01 n=2).

Interestingly, in healthy mouse hearts infusion of 50 µM DNP for 20 min induced a decline in ATP to ~40% and phosphocreatine to ~20% and, as a result, a decline in cardiac function to ~20%; however, cardiac function recovered to ~50% upon DNP withdrawal. In Kir6.2-/- mouse hearts, DNP stopped the hearts almost immediately and no recovery was observed.

Conclusions. Our ^{87}Rb MRS data indicate that a mutation in K_{ATP} channels does not affect basal potassium fluxes on an intact heart level; confirming that these channels are closed under normal conditions. However, there was a clear difference in stimulation of potassium effluxes under conditions that activate K_{ATP} (infusion of a K_{ATP} opener or metabolic inhibition). To establish further links between K_{ATP} and energetics, studies of Kir6.2 knockout on sensitivity of cardiac energetics to metabolic stress would be necessary.

References: 1. Miki et al., Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. Proc Natl Acad Sci U S A. 1998;95:10402.

2. Jilkina et al., Application of ^{87}Rb NMR spectroscopy for studies of potassium homeostasis in intact mouse hearts, Proc. Intl Soc Mag Res Med 11 (2004):1780.