Contractility and ischemic response of hearts from transgenic mice with altered sarcolemmal K$_{\text{ATP}}$ channels

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Rajashree, R., J. C. Koster, K. P. Markova, C. G. Nichols, and P. A. Hofmann. Contractility and ischemic response of hearts from transgenic mice with altered sarcolemmal K$_{\text{ATP}}$ channels. Am J Physiol Heart Circ Physiol 283: H584–H590, 2002. First published April 18, 2002; 10.1152/ajpheart.00107.2002.—The functional significance of ATP-sensitive K$^+$ (K$_{\text{ATP}}$) channels is controversial. In the present study, transgenic mice expressing a mutant Kir6.2, with reduced ATP sensitivity, were used to examine the role of sarcolemmal K$_{\text{ATP}}$ in normal cardiac function and after an ischemic or metabolic challenge. We found left ventricular developed pressure (LVDP) was 15–20% higher in hearts from transgenics and ischemic or metabolic challenges. We found left ventricular developed pressure; heart rate; diastolic pressure; Kir6.2 ischemic preconditioning; metabolic inhibition; left ventricular ischemic or metabolic challenges.

Native K$_{\text{ATP}}$ channels are closed at physiological concentrations of ATP but open as ATP decreases (17). ATP responsiveness may be significant in allowing the coupling of metabolic state to membrane potential and hence myocardial excitability. Supporting the theory that K$_{\text{ATP}}$ channels are typically inactive at physiological ATP concentrations, basal myocardial contractility is unaffected by knockout of the murine Kir6.2 channel (25). However, acute opening of sarcolemmal K$_{\text{ATP}}$ channels due to metabolic inhibition shortens action potential duration and decreases left ventricular developed pressure (LVDP) (25). To gain further insight into the effect(s) of activation of K$_{\text{ATP}}$ channels on cardiac function, we generated transgenic (TG) mice expressing mutant sarcolemmal K$_{\text{ATP}}$ channels that have greatly reduced ATP sensitivity and are open under physiological conditions. The first goal of the present study was therefore to characterize the contractile function of hearts from these TG animals.

Improved myocardial postischemic recovery brought about by preischemic conditioning with brief, transient ischemia (ischemic preconditioning) may involve opening of K$_{\text{ATP}}$ channels (for reviews, see Refs. 4, 9, 18, and 20). Gross and Auchampach (8) were the first to show that K$_{\text{ATP}}$ channel blockers inhibit the protective effects of ischemic preconditioning, and preischemic treatment with K$_{\text{ATP}}$ channel openers mimics the cardioprotection afforded by ischemic preconditioning. It was initially hypothesized that during ischemic preconditioning the resultant fall in ATP would allow sarcolemmal K$_{\text{ATP}}$ channels to open, reduce action potential duration, decrease Ca$^{2+}$ influx, and protect the heart from subsequent ischemic damage due to Ca$^{2+}$ overload. However, it was subsequently determined that a decrease in action potential duration may not be necessary to observe the reduction in postischemic infarct size associated with ischemic preconditioning (27). More recent studies using K$_{\text{ATP}}$ inhibitors and activators purportedly specific for mitochondrial K$_{\text{ATP}}$ channels implicate mitochondrial rather than sarcolemmal K$_{\text{ATP}}$ channels as responsible for the cardioprotective effects of ischemic preconditioning (4, 9, 18, 20).

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20). However, the interpretation of these studies may not be straightforward because the specificity of K\(_{ATP}\) openers/blockers are condition specific. For example, diazoxide, a K\(_{ATP}\) opener considered specific for mitochondrial K\(_{ATP}\) channels, has no effect on sarcolemmal K\(_{ATP}\) channels in the absence of MgADP but activates sarcolemmal K\(_{ATP}\) at in vivo levels of MgADP (6). Thus the second goal of the present study was to determine whether hearts from TG mice with sarcolemmal K\(_{ATP}\) channels having decreased ATP sensitivity respond differently than non-TG hearts to 1) global ischemia with and without ischemic preconditioning, and 2) metabolic inhibition using cyanide and 2-deoxyglucose (2-DG) pretreatment.

For these studies we examined the contractility of hearts expressing mutant Kir6.2 (12). In excised patch-clamp experiments, sarcolemmal K\(_{ATP}\) channels from TG mice were nearly 100-fold less sensitive to inhibition by ATP than non-TG controls (12). Somewhat counterintuitively, the maximal K\(_{ATP}\) current density was also decreased fourfold in myocytes from TG mice (12). Therefore, when the intracellular [ATP] falls (e.g., during the onset of ischemia), K\(_{ATP}\) channels in TG hearts are expected to open earlier than those in non-TG controls. Similarly, TG K\(_{ATP}\) channels will stay open longer as intracellular [ATP] rises (e.g., during the onset of reperfusion), even though the maximal K\(_{ATP}\) channel conductance is lower than that in control. Thus the present study provides insight into the role the sarcolemmal K\(_{ATP}\) plays in normal and pathological myocardial function using TG mice that have a greatly decreased ATP sensitivity and a reduced number of functioning K\(_{ATP}\) channels at the sarcolemma.

**MATERIALS AND METHODS**

**TG mice.** Previously, we demonstrated COSm6 cells cotransfected with SUR2A and Kir6.2 containing an NH\(_2\)-terminal truncation of 30 amino acids (∆N) in combination with a point mutation, K185Q, have a pronounced ATP insensitivity of their K\(_{ATP}\) channels (13). Thus TG constructs were made using the cardiac specific α-myosin heavy chain promoter with Kir6.2[∆N,K185Q] and a green fluorescent protein (GFP) tag on the COOH-terminus (Kir6.2[∆N,K185Q]-GFP) (12). TG mice were identified by PCR on mouse tail DNA using GFP-specific oligonucleotide primers. Four founder mice expressing the Kir6.2[∆N,K185Q]-GFP transgene were isolated and bred to obtain N,K185Q-GFP transgenic mice. N,K185Q-GFP transgenic mice were crossed with male or female mice expressing the N,K185Q-GFP transgene to obtain N,K185Q-GFP transgenic mice. N,K185Q-GFP transgenic mice were isolated and bred to obtain N,K185Q-GFP transgenic mice.

For ischemia-reperfusion protocols, hearts were instrumented but not paced, and baseline contractility was obtained over 30 min. Hearts were then exposed to Krebs-Henseleit containing 10 nM Iso for 5 min, washed with Krebs-Henseleit solution without Iso for 25 min, and exposed to Krebs-Henseleit containing 100 nM Iso for 5 min. Average heart rate and peak increase in LVDP at each concentration of Iso were determined. Immediately after the Iso dose-response determination, carbachol was added to the Krebs-Henseleit solution at a constant pressure of 65 mmHg with a balloon inflating in the heart chamber. Fluid from the chamber was continually retained in the tubing because the tube with the transducer forms a vacuum. Pressures from the transducer were digitized using an analog-to-digital conversion board (NB-MIO-16XL-18 μs, National Instruments; Austin, TX) and stored in a Macintosh computer. Acquisition and data analysis was carried out using computer programs generated in LABVIEW software (National Instruments).

LVDP was calculated as the difference between peak systolic pressure and end-diastolic pressure (EDP). Averages of LVDP and EDP from the final 3–5 min under a given experimental condition are reported. Postischemic increases in EDP were calculated as the difference between postischemic EDP and preischemic EDP.

**Experimental protocols.** Dose-response effects of isoproterenol (Iso) and carbachol were obtained in the same set of hearts. Hearts were instrumented but not paced, and baseline contractility was obtained over 30 min. Hearts were then exposed to Krebs-Henseleit containing 10 nM Iso for 5 min, washed with Krebs-Henseleit solution without Iso for 25 min, and exposed to Krebs-Henseleit containing 100 nM Iso for 5 min. Average heart rate and peak increase in LVDP at each concentration of Iso were determined. Immediately after the Iso dose-response determination, carbachol was added to the Krebs-Henseleit solution containing 100 nM Iso. Carbachol concentration was sequentially increased every 5 min without washing in the maintained presence of 100 nM Iso. Heart rate was reported as the average heart rate over the entire period of carbachol exposure at a given concentration. After exposure to 500 nM carbachol (highest dose), hearts were exposed to a Krebs-Henseleit solution containing 100 nM Iso without carbachol for 10 min.

For ischemia-reperfusion protocols, hearts were instrumented and paced at 6 Hz for a 20-min equilibration period. After the equilibration period, either no change or an additional 10 min (no preconditioning) or four cycles of 5 min of ischemia-5 min of reperfusion were carried out (ischemic preconditioning). This was followed by a prolongation of 22 min and reperfusion for 60 min.

Effects of decreasing extracellular [Ca\(^{2+}\)] and metabolic inhibition were obtained in the same set of hearts. Hearts were instrumented, allowed to recover for 15 min, paced, and sequentially exposed to Krebs-Henseleit solution containing 1.75 mM Ca\(^{2+}\), 1.50 mM Ca\(^{2+}\), and 1.25 mM Ca\(^{2+}\). Data were averaged and reported for the final 3 min of a 10-min exposure time/Ca\(^{2+}\) concentration. Hearts were then returned to Krebs-Henseleit solution containing 1.75 mM Ca\(^{2+}\) for 10
min to reestablish baseline pressure values. Subsequently, glycolysis was reduced by perfusion with a modified Krebs-Henseleit solution containing 1 mM 2-DOG without glucose or lactate for 5 min (2). Oxidative phosphorylation was blocked by perfusion with modified Krebs-Henseleit solution containing 2 mM NaCN without glucose, lactate, or 2-DOG for 10 min (2). Hearts were then perfused for 60 min with a Krebs-Henseleit solution with glucose and lactate but without 2-DOG or CN.

**Statistics.** ANOVA and either a Fisher's least-significant-difference post hoc test or Student’s t-test were applied. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Baseline characterization of contractility.** Heart-to-body weight ratios did not change in the K$_{ATP}$ TG mice compared with gender-matched non-TG littermates (Table 1). Systolic pressure (Table 1) and LVDP (Fig. 1) were significantly higher in TG hearts than in paired non-TG hearts both in the presence and absence of external pacing. Intrinsic heart rates of the excised, Langendorff-perfused hearts were not different between hearts from K$_{ATP}$ TG mice compared with gender-matched non-TG littermates [383 ± 35 vs. 380 ± 23 beats/min, respectively (means ± SE, n = 5)].

Calcium dependence on contractility was determined in hearts from TG and paired non-TG mice (Fig. 2). Decreasing extracellular [Ca$^{2+}$] from 1.75 to 1.50 to 1.25 mM significantly decreased LVDP in hearts from non-TG mice, whereas there was no systematic decrease in LVDP in TG hearts.

**Inotropic reserve in control and K$_{ATP}$ TG hearts.** The inotropic effects of β-adrenergic and muscarinic receptor activation were determined in hearts from TG and paired non-TG mice. The β-adrenergic receptor agonist Iso increased heart rate to a similar extent in TG and non-TG hearts (Fig. 3). Concomitantly, an incremental, positive inotropic effect was observed in hearts of non-TG mice, whereas in TG hearts LVDP did not change upon exposure to 10 nM Iso (Fig. 3). Increasing the Iso concentration to 100 nM led to a similar increase in LVDP [237 ± 38% for non-TG and 183 ± 42% for TG (means ± SE, n = 5)] and heart rate. It should be noted that control hearts from TG mice demonstrated a trend toward higher LVDP compared with control non-TG mice, but this did not reach statistical significance ($P = 0.20$ for 5 hearts/group). Statistical analysis using data from all control hearts in this study.
(Fig. 1) indicates LVDP is significantly higher in hearts of TG mice.

The negative chronotropic effect of muscarinic action was determined in the presence of 100 nM Iso. No statistically significant difference existed in the carbachol concentration-heart rate relationship between hearts from TG and non-TG mice (Fig. 4). After application of the highest concentration of carbachol, hearts were washed free of carbachol, and the effect of 100 nM Iso alone was redetermined to identify the extent of rundown and receptor desensitization in the preparations. Similar heart rates were observed in hearts stimulated with isoproterenol before and after the carbachol dose-response measurements (Fig. 4).

Functional response to global ischemia and reperfusion in the presence and absence of ischemic preconditioning. Figure 5 presents the postischemic recovery of contractile function of hearts that underwent 22 min of ischemia, followed by 60 min of reperfusion with and without ischemic preconditioning. Similar postischemic recovery of LVDP and increase in EDP were obtained in nonpreconditioned hearts from both TG and non-TG mice. Postischemic recovery of LVDP and EDP improved in ischemic preconditioned hearts from non-TG mice but not in hearts from TG mice (Fig. 5).

Functional response to metabolic inhibition. Figure 6 presents the results from representative experiments to examine the response of metabolic inhibition in hearts of gender-matched TG and non-TG mice. Both hearts underwent metabolic inhibition by replacement of glucose with 2-DOG and exposure to NaCN (see MATERIALS AND METHODS). Recovery of the non-TG heart from metabolic inhibition was significantly better as determined by a higher final LVDP (Fig. 6A) and lower EDP (Fig. 6B). During metabolic inhibition the decrease in LVDP was delayed, but the development of rigor was accelerated in TG hearts (Fig. 6). Cumulative results of hearts that underwent metabolic inhibition (Fig. 7) are consistent with this individual paired observation in that an earlier onset of rigor contracture and a lower recovery of LVDP was observed in TG hearts, yet spontaneous beating ceased at a significantly later time in hearts from TG mice (Fig. 7).

DISCUSSION

$K_{ATP}$ TG hearts have increased myocardial developed pressure and are insensitive to changes in extracellular $[Ca^{2+}]$ and submaximum β-adrenergic stimulation. Knockout of Kir6.2, the pore-forming subunit of the $K_{ATP}$ channel, abolishes $K_{ATP}$ channel activity in the mouse ventricle and the effects of $K_{ATP}$ channel openers on action potential duration (25). Expression of Kir6.2[ΔN,K185Q] in the mouse heart leads to profound reduction of ATP sensitivity yet decreased $K_{ATP}$ channel density (12). Counter to predictions from previous studies, the reduction of ATP sensitivity does not lead to marked action potential shortening or excitation failure under normal conditions, and heart rate is reduced by $-15\%$ in conscious animals (12). Why the phenotype is so mild and what are the broader consequences of altered $K_{ATP}$ channel activity on cardiac function remain open questions. The present study begins to address these questions by examination of contractile function in Kir6.2[ΔN,K185Q] TG hearts.
LVDP was higher in TG hearts compared with hearts from paired non-TG animals. This increase was not due to hypertrophy. In addition, there was no decrease in LVDP of TG hearts when extracellular Ca\(^{2+}\) was decreased from 1.75 to 1.25 mM, and \(\beta\)-adrenergic responsiveness decreased in TG hearts. This decrease in sensitivity of LVDP to extracellular Ca\(^{2+}\) could be explained by either 1) an increase in myofilament Ca\(^{2+}\) sensitivity of force production, or 2) altered Ca\(^{2+}\) handling. Loss of \(\beta\)-adrenergic responsiveness in other TG mouse models has been correlated with an increased intracellular Ca\(^{2+}\) and resultant Ca\(^{2+}\)-induced inhibition of adenylate cyclase (22). Thus our data are most consistent with the hypothesis that there is an increase in intracellular [Ca\(^{2+}\)] in TG hearts that accounts for the increase in LVDP, maintained contractility at lower [Ca\(^{2+}\)], and reduced inotropic reserves as assessed by 10 nM Iso stimulation. Future cellular studies will definitively establish whether intracellular Ca\(^{2+}\) and/or myofilament Ca\(^{2+}\) sensitivity are increased in TG hearts with a high expression of Kir6.2\(\Delta\text{N,K185Q}\)-GFP.

At least two possibilities exist as to how transgene overexpression can lead to the apparent elevation in contractile state in TG hearts under control conditions. First, the altered \(\text{K}_{\text{ATP}}\) channel activity in the sarcolemma may lead to compensatory increases in Ca\(^{2+}\) current or decreases in voltage-gated K\(^{+}\) current, either of which might explain the variable prolongation of action potential duration seen in isolated TG myocytes (12). Second, \(\text{K}_{\text{ATP}}\) channels may be present in sarcoplasmic reticular membranes and affect transsarcoplasmic reticular membrane Ca\(^{2+}\) distributions. At the present time, these possibilities are speculation, but may be resolved by future studies at the cellular level.

**TG hearts and muscarinic activation.** Muscarinic modulation of heart rate is an important physiological index of heart function. The negative chronotropic effect of muscarinics is due to activation of muscarinic K\(^{+}\) (KACh) channels in the sinoatrial node and atrium. The channel is activated by G protein-coupled receptors and is thought to be a heterotetrameric complex with equal numbers of Kir3.1 and Kir3.4 subunits (5).

In the present study, hearts from TG and non-TG mice responded in a similar fashion to increasing concentrations of a muscarinic agonist. This suggests G protein-coupled \(\text{K}_{\text{ACH}}\) channels are not altered in hearts of TG mice with Kir6.2\(\Delta\text{N,K185Q}\)-GFP. In addition, it is
consistent with the idea that mutagenesis, in and of itself, does not lead to nonspecific changes in cardiac ion channels.

\( K_{ATP} \) TG hearts have differential recovery from ischemia and metabolic inhibition. Myocardial functional recovery after 22 min of global ischemia was similar in hearts from paired TG and non-TG mice. The rapid contractile failure observed in ischemia results from decreasing pH, accumulation of phosphate, and subsequent inhibition of the myofilaments (7). Consistent with ischemia-induced cardiac failure being independent of \( K_{ATP} \) channels, recovery from ischemia was similar in \( K_{ATP} \) TG and non-TG hearts. In contrast, metabolic inhibition leads to a delayed contractile failure (cessation of beating occurred at 600 s rather than 400 s), but an earlier onset of rigor contracture and a very poor functional recovery in TG compared with non-TG hearts. The contractile failure normally observed in metabolic inhibition is likely due to \( K_{ATP} \) channel activation and subsequent action potential shortening (7, 14). We previously demonstrated that at greater than 200 s into metabolic inhibition, the current density in \( K_{ATP} \) TG myocytes is fourfold less than in non-TG myocytes (12). Thus, in \( K_{ATP} \) TG hearts, a lower absolute level of \( K_{ATP} \) channel current during metabolic inhibition in combination with a higher contractile state may delay the cessation of contraction. This continued contraction during metabolic inhibition and the higher baseline inotropic state would result in increased ATP consumption, which would cause an earlier onset of rigor and concomitant worsening of contractile recovery after removal of metabolic blockers. Hence, any protective effects brought about by an initial, more rapid activation of the \( K_{ATP} \) channel in TG hearts is counteracted.

Acute and/or chronic changes in sarcolemmal \( K_{ATP} \) channels impede myocardial functional recovery from ischemic preconditioning. In hearts from TG mice, ischemic preconditioning failed to improve postsischemic myocardial functional recovery. This result seems contradictory to the observations that implicate the mitochondrial \( K_{ATP} \) channel as the primary transducer of cardioprotection afforded by preconditioning (4, 9, 18, 20) and is consistent with studies indicating a role for the sarcolemmal \( K_{ATP} \) channels in cardioprotection (11, 15, 19). However, it should be noted there may be indirect consequences of the \( K_{ATP} \) transgene expression on preconditioning. First, chronic reduction in \( K_{ATP} \) channel density may allow for an increase in cardiac electrical abnormalities during development and aging and subsequent damage to the heart. However, our observations of an increase in the baseline LVDP of TG hearts and a similar postsischemic recovery in LVDP in TG and non-TG hearts that were not preconditioned suggest the TG hearts are not failing in general. Second, experimental conditions such as duration and timing of preconditioning, heart rate, and inotropic state of the heart can have a significant impact on the benefits brought about by ischemic preconditioning. Because the inotropic state appears to be affected by the transgene, it is conceivable that the necessary conditions for effective preconditioning may be altered in TG hearts. Nevertheless, the recent demonstration that preconditioning is also abolished in hearts from Kir6.2 knockout animals (26) indicates further consideration of the role of sarcolemmal \( K_{ATP} \) channel in this phenomena is warranted.

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REFERENCES


