RESEARCH ARTICLE

Loss of ATP-Sensitive Potassium Channel Surface Expression in Heart Failure Underlies Dysregulation of Action Potential Duration and Myocardial Vulnerability to Injury

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Abstract

The search for new approaches to treatment and prevention of heart failure is a major challenge in medicine. The adenosine triphosphate-sensitive potassium ($K_{ATP}$) channel has been long associated with the ability to preserve myocardial function and viability under stress. High surface expression of membrane $K_{ATP}$ channels ensures a rapid energy-sparing reduction in action potential duration (APD) in response to metabolic challenges, while cellular signaling that reduces surface $K_{ATP}$ channel expression blunts APD shortening, thus sacrificing energetic efficiency in exchange for greater cellular calcium entry and increased contractile force. In healthy hearts, calcium/calmodulin-dependent protein kinase II (CaMKII) phosphorylates the Kir6.2 $K_{ATP}$ channel subunit initiating a cascade responsible for $K_{ATP}$ channel endocytosis. Here, activation of CaMKII in a transaortic banding (TAB) model of heart failure is coupled with a 35–40% reduction in surface expression of $K_{ATP}$ channels compared to hearts from sham-operated mice. Linkage between $K_{ATP}$ channel expression and CaMKII is verified in isolated cardiomyocytes in which activation of CaMKII results in downregulation of $K_{ATP}$ channel current. Accordingly, shortening of monophasic APD is slowed in response to hypoxia or heart rate acceleration in failing compared to non-failing hearts, a phenomenon previously shown to result in significant increases in oxygen consumption. Even in the absence of coronary artery disease, failing myocardium can be further injured by ischemia due to a mismatch between metabolic supply and demand. Ischemia-reperfusion injury, following ischemic preconditioning, is diminished in hearts with CaMKII inhibition compared to wild-type hearts and this advantage is largely eliminated.
when myocardial $K_{\text{ATP}}$ channel expression is absent, supporting that the myocardial protective benefit of CaMKII inhibition in heart failure may be substantially mediated by $K_{\text{ATP}}$ channels. Recognition of CaMKII-dependent downregulation of $K_{\text{ATP}}$ channel expression as a mechanism for vulnerability to injury in failing hearts points to strategies targeting this interaction for potential preventives or treatments.

Introduction

Over the past two decades, there has been considerable progress in the treatment of chronic heart failure yet, even with the best of modern therapy, heart failure is still associated with 5-year mortality rate of 45%-60% [1]. Therefore, the search for new approaches to treatment and prevention of heart failure is one of the major challenges in medicine. One possible target is the adenosine triphosphate-sensitive potassium (KATP) channel that has been long associated with the ability to preserve myocardial function and viability under various stressors [2–7]. The $K_{\text{ATP}}$ channel is one of the most abundant cardiac membrane protein complexes and has the unique ability to adjust membrane excitability in response to changes in the energetic status of the cell [4,5,8–14]. More recently, $K_{\text{ATP}}$ channels have also been shown to be critical regulators of cardiac membrane excitability in response to heart rate acceleration [15]. When activated by increased cellular metabolic demand, $K_{\text{ATP}}$ channel-dependent cellular potassium efflux shortens cardiac action potential duration (APD) [2, 3, 8, 9, 16–20]. The outward $K_{\text{ATP}}$ current also limits sodium and calcium entry into the cell and thus reduces energy requirements for ion homeostasis and contraction, as well as prolongs the diastolic interval that supports myocardial relaxation and replenishment of resources [2–6, 21–27]. In addition to effects of channel gating, the efficiency of $K_{\text{ATP}}$ channel-dependent membrane electrical responses to changes in myocardial energetics has been shown to be highly dependent on the regulation of $K_{\text{ATP}}$ channel membrane expression [17, 28–30]. Specifically, a high surface expression of membrane $K_{\text{ATP}}$ channels ensures a rapid reduction in APD in response to metabolic challenges thereby providing optimal myocardial energetics, while cellular signaling that reduces surface $K_{\text{ATP}}$ channel expression blunts APD shortening, thus sacrificing energetic efficiency in exchange for greater cellular calcium entry and increased contractile force [16, 17, 29–32].

Membrane $K_{\text{ATP}}$ channel expression in healthy hearts can be regulated by calcium/calmodulin dependent protein kinase II (CaMKII) [28, 29]. This densely expressed multifunctional kinase targets numerous proteins involved in excitation contraction coupling and excitability to support enhanced cardiac mechanical performance, while its persistent activation under pathophysiological conditions, such as heart failure, promotes cardiomyocyte death and dysfunction [33–36]. In healthy hearts, CaMKII phosphorylates the Kir6.2 pore-forming $K_{\text{ATP}}$ channel subunit that initiates a signaling cascade responsible for endocytosis of $K_{\text{ATP}}$ channels [29]. This signaling results in a rapid reduction in $K_{\text{ATP}}$ channel current capacity that quickly rebounds when CaMKII activation subsides [29]. Excessive and persistent activation of CaMKII, presumably triggered to bolster waning mechanical performance, is a common feature of various types of heart failure [33]. We hypothesize that myocardial vulnerability to injury in failing hearts may be mediated in part by a chronic suppressive effect of CaMKII activation on membrane $K_{\text{ATP}}$ channel expression.

Here, we confirm that CaMKII activation is upregulated in a murine model of non-ischemic heart failure induced by transverse aortic banding and that this is associated with a significant reduction in the membrane surface expression of $K_{\text{ATP}}$ channels, in their current capacity, and
consequently in the responsiveness of ventricular APD shortening under the metabolic stresses of heart rate acceleration and hypoxia. Such changes could aggravate depletion of cardiac energy resources thus contributing to myocardial injury, cell death and heart failure progression, and are consistent with the known beneficial effects on cardiac stress resistance that occur with CaMKII inhibition [33–35, 37–39]. Understanding the interaction between CaMKII activation and cardiac $K_{ATP}$ channel cell membrane expression could present alternative strategies to avoid or treat the excessive myocardial vulnerability to stress that characterizes and promotes heart failure.

Materials and Methods

All animal protocols conform to the Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Institutional Animal Care and Use Committee.

Heart failure model

Male and female mice aged 10–12 wks were subjected to cardiac pressure overload by transverse aortic banding (TAB) as described [40]. Briefly, mice were anesthetized with ketamine/xylazine (40/5 mg/kg, respectively) by intraperitoneal injection. The mice were intubated with a 16 gauge tube, and ventilated with a small rodent ventilator (Harvard Apparatus, Holliston, MA). A thoracotomy was created between the second and third intercostal space, and the aortic arch visualized. TAB was performed by placing a suture around the aorta and the shank of an 18-gauge needle. The needle was then removed. In sham operated animals, the aortic arch was visualized but not banded. The chest wall was closed, and the pneumothorax evacuated.

Mouse genetic models

Transgenic mice expressing a specific peptide inhibitor of CaMKII (AC3-I) under control of the cardiac specific Myh6 promoter [37] and homozygous Kir6.2-KO mice generated by targeted disruption of the kcnj11 gene [41] were compared to WT littermates. Breeding of AC3-I and Kir6.2-KO mice was performed resulting in offspring homozygous for Kir6.2-KO while expressing the AC3-I transgene (AC3-I/Kir6.2-KO).

Echocardiography of cardiac function

Two-dimensional transthoracic echocardiography was performed by the University of Iowa Cardiology Animal Phenotyping Core Laboratory using a 30 MHz linear array transducer (Vevo 2100, VisualSonics, Toronto, Canada). Midazolam (.1 mg SC) was used for conscious sedation. Parasternal long- and short-axis views were obtained to evaluate left ventricular function, using the bi-plane area-length method.

Cardiomyocyte isolation

Single ventricular cardiomyocytes were isolated as described previously [15]. Briefly, hearts were cannulated, then rapidly excised and retrogradely perfused with normal Tyrode solution for 5 min, 3 min with a “low calcium” medium (in mM): 100 NaCl, 10KCl, 1.2 KH$_2$PO$_4$, 5 MgSO$_4$, 20 glucose, 50 taurine, 10 HEPES supplemented with 0.13 CaCl$_2$, 2.1 EGTA, then 13 min with low calcium medium supplemented with 1% bovine serum albumin, 0.2 mM CaCl$_2$, collagenase (type IV, 22 units/ml, Worthington) and Protease (0.1 mg/ml, Type 16, Sigma). Left ventricles were dissected away and were cut into pieces (~3 x 3 mm). Subsequently the tissue was gently triturated with a glass pipette until dissociated cardiomyocytes were obtained.
Electrophysiology

Studies were performed using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) integrated with a Nikon TE2000-U microscope. Experiments were performed at 36–37°C using a temperature controller TC2r (Cell MicroControls, Norfolk, VA). For whole-cell recording, borosilicate glass pipettes (2–3 MΩ) were filled with internal solution (in mM): KCl 140, MgCl₂ 1, EGTA 5, ATP 5, HEPES-KOH 5, pH 7.3 with KOH. Cardiomyocytes were superfused with Tyrode solution (in mM): NaCl 136.5, KCl 5.4, CaCl₂ 1, MgCl₂ 0.53, glucose 5.5, HEPES-NaOH 5, pH 7.4. Whole-cell current traces were obtained in response to 1 s rectangular pulses from a holding potential of −50 mV to test potentials from −80 to +50 mV. For quantification, whole cell KᵥATP channel current was measured as the difference between baseline and pinacidil- and 2,4-dinitrophenol (DNP)-stimulated current recorded at same voltage potentials. For analysis, only whole cell recordings in which beginning and ending capacitance were within 10% were used. Transmembrane action potentials were recorded in the current-clamp configuration at a stimulation frequency of 1 Hz. All recordings were monitored, sampled, and analyzed using pClamp software (Molecular Devices, Sunnyvale, CA).

Isolated heart studies

Hearts were extracted from anesthetized mice and retrogradely perfused at 90 mmHg with Krebs-Henseleit buffer bubbled with 95% O₂/5% CO₂, at 37°C and pH 7.4. The atrioventricular node was mechanically ablated and hearts paced at 150 ms cycle length (Bloom Electrophysiology, Fischer Imaging Corp., Denver, CO) using a platinum bipolar pacing catheter positioned in the right ventricle (NuMed, Hopkinton, NY). For heart rate acceleration tests, the pacing cycle length was abruptly switched to 90 msec. For hypoxia tests, a separate reservoir of Krebs-Henseleit buffer was bubbled with 95%N₂/5%CO₂, at 37°C and pH 7.4. A valve followed by a small bubble chamber and an oxygen sensor immediately above the heart was used to quickly switch the perfusing solutions. Oxygen partial tension was measured in the perfusate prior to heart passage (Model 210, Instech Laboratories, Plymouth Meeting, PA). Coronary flow was measured in series with the aortic cannula (T402, Transonic Systems, Ithica, NY). A monophasic action potential (MAP) probe (EP Technologies, Sunnyvale, CA) was maintained at a single stable position on the LV epicardium, and amplified signals (IsoDam, World Precision Instruments, Sarasota, FL) were acquired at 2 kHz. MAP recordings were analyzed (Clampfit, Molecular Devices, Sunnyvale, CA) for duration at 90% repolarization (MAPD₉₀). Steady state changes in MAPD₉₀ were calculated using the duration of the MAP just prior to the acceleration of pacing rate or initiation of hypoxia as a reference. MAPs were only analyzed from tracings in which pacing capture was maintained without interruption. All calculations were manually reviewed.

Western blot

Whole cell protein extracts were used for immunoblotting with CaMKII (Pan, Cell Signaling), phospho-CaMKII (Thr-286, Cell Signaling) and oxidized CaMKII (Ox-CaMKII, gifted from the Anderson laboratory), and anti-GAPDH (Santa Cruz Biotechnologies) antibodies.

Myocardial ischemia-reperfusion injury

The myocardial ischemia-reperfusion injury model was employed as previously described [28]. Briefly, after allowing an isolated, retrogradely perfused heart to stabilize for 20 minutes, ischemia preconditioning (IP) with two 2 min cycles of global ischemia (stop flow) were followed by 5 min of reperfusion. All hearts were then subjected to 20 min of global ischemia and 45 min
reperfusion. At the end of ischemia/reperfusion experiments, hearts were removed from the Langendorff perfusion apparatus and immediately frozen at -20°C. The frozen hearts were cut from apex to base into transverse slices of approximately equal thickness (~0.8 mm). The slices were placed into a small cell culture dish and then incubated in 1% triphenyltetrazolium chloride (TTC) in phosphate buffer (Na2HPO4: 88 mM, NaH2PO4 1.8 mM, pH 7.4) at 37°C for 20 min by rocking the dish. The development of the red formazan pigment relies on the presence of lactate dehydrogenase or NADH in living tissues, while failure to stain red indicates a loss of these constituents from necrotic tissue. After staining, the TTC buffer was replaced by 10% formaldehyde. The slices were fixed for the next 4–6 h before the areas of infarct tissue were determined by ImageJ software. The risk area was the sum of total ventricular area. The infarct size was calculated and presented as percentage of risk area.

Drugs
The following drugs were purchased from Sigma: isoproterenol, pinacidil, triphenyltetrazolium chloride (TTC) and 2,4-dinitrophenol (DNP).

Statistical analysis
The data are presented as mean ± the standard error of the mean. Statistical significance is evaluated with the Student’s t-test. p<0.05 is considered statistically significant.

Results
Decreased contractile function, ventricular enlargement, cellular hypertrophy and action potential duration prolongation occur in the transverse aortic banding model of cardiomyopathy
Hearts from mice after transverse aortic banding (TAB) vs. sham operation were compared by echocardiogram (Fig 1A). Five to six weeks after operation, the ejection fraction was significantly reduced in the TAB group compared to sham controls (38±4%, n = 12 vs. 72±2%, n = 9, p < .01, Fig 1B). Heart weight from TAB operated mice was increased compared to controls (heart weight to body weight ratio of 10.1 mg/g, n = 11 vs. 5.9 mg/g, n = 9, p < .01). Isolated ventricular cardiomyocytes from hearts of TAB mice had increased capacitance, representing cell size (291±16, n = 14 vs. 158±11 pF, n = 17, p < .01), and longer action potentials than controls (8.0±1.1 vs. 3.1±2, 19.2±2.0 vs. 10.3±6, and 32.0±2.4 vs. 20.5±1.1 msec for action potential duration at 50%, 75% and 90% repolarization, respectively, n = 25 cells from 3 TAB mice and 27 cells from 4 sham mice, p < .01 for each comparison, Fig 1C and 1D). These findings indicate that TAB effectively resulted in typical findings of decreased contractile function, ventricular enlargement, cellular hypertrophy and action potential changes associated with heart failure.

CaMKII is activated in heart failure
It is well established that CaMKII is activated in failing hearts [36, 42–44]. To determine whether the TAB generated model of non-ischemic cardiomyopathy used here recapitulates these reported findings, we assayed CaMKII phosphorylated at residue 286 (P-CaMKII), oxidized CaMKII (Ox-CaMKII), and total CaMKII (T-CaMKII) by western blot in ventricular tissue (Fig 2A). This indicated that expression of P-CaMKII is increased by 32% (132.1±1.0 vs. 100.0±6.6 AU, n = 3 each, p < .05, Fig 2B, left panel) and Ox-CaMKII is increased by 34% (133.9±5.5 vs. 100.0±3.3, n = 3 each, p < .01, Fig 2B, middle panel) in hearts of TAB compared to sham mice.
K_ATP channel membrane expression and current density are decreased in failing hearts

We previously found that activated CaMKII phosphorylates the Kir6.2 subunit and promotes endocytosis of cardiac K_ATP channels in healthy hearts [29]. To determine if K_ATP channel expression is reduced in association with the persistent CaMKII activation of pressure overload-induced heart failure, we assessed the hearts of TAB and sham operated mice by

Fig 1. Morphological and electrophysiological changes in transverse aortic banding (TAB) vs. sham operated mice. A) Representative parasternal long axis echocardiographic images of the ventricles of sham and TAB operated mice. B) Summary data of left ventricular ejection fraction (**p<0.01 vs. sham). C) Representative action potentials recorded in isolated ventricular cardiomyocytes from sham and TAB mice. D) Summary data of action potential duration in isolated ventricular myocytes of sham and TAB mice (**p<0.01 vs. sham). APD50, APD75, APD90: action potential duration at 50%, 75% and 90% repolarization, respectively. E_m: membrane potential.

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whole heart biotinylation for the presence of the Kir6.2 subunit on the surface of ventricular cardiomyocytes. We find ventricular surface expression of Kir6.2 (Fig 3A) to be reduced by 37% in hearts from TAB vs. sham mice (62.8±1.4 vs. 100.0±10.3 AU, n = 3 hearts each, p < .05, Fig 3B). Accordingly, in isolated ventricular myocytes, the KATP channel current density as stimulated by application of the KATP channel opener, pinacidil, with the mitochondrial uncoupler, 2,4-dinitrophenol (DNP, Fig 3C) was reduced approximately 40% in TAB compared to sham controls (41.5±6.5 pA/pF, n = 7 cells from 3 TAB mice vs. 72.9±8.7 pA/pF, n = 11 cells from 3 sham mice, p < .01, Fig 3D). These data indicate that heart failure and CaMKII activation are associated with a significant reduction in KATP channel membrane surface expression.

CaMKII activation results in dynamic reduction of $K_{ATP}$ channel current density in heart failure

We have also previously shown that acute activation of CaMKII in isolated cardiomyocytes by the application of the beta-agonist isoproterenol does not alter $K_{ATP}$ channel gating but results in the endocytosis of $K_{ATP}$ channels causing a rapid decrease in $K_{ATP}$ channel current measured by patch clamp [29]. Here, we tested whether the same dynamic response remains operative in cardiomyocytes from failing TAB hearts. Because $K_{ATP}$ channel current density is
already reduced in hearts of TAB vs. sham mice, changes in response to isoproterenol are expressed as a % of baseline. We find that the decrease in $K_{ATP}$ channel current density elicited by isoproterenol (Fig 4A) is blunted but still present in ventricular myocytes from hearts of TAB compared to sham control mice (20.8±5.1%, $n = 6$ cells from 3 TAB mice vs. 43.0±3.8%, $n = 10$ cells from 3 sham mice, $p < .01$, Fig 4B). Thus these data indicate that the CaMKII-dependent mechanism of $K_{ATP}$ channel membrane expression regulation is pertinent to heart failure conditions.

Reduction in membrane expression of $K_{ATP}$ channels is associated with a reduced rate of APD shortening

To assess the physiologic significance of the reduced expression of $K_{ATP}$ channels in failing cardiomyocytes, left ventricular epicardial monophasic action potential duration at 90% repolarization ($\text{MAPD}_{90}$) in response to hypoxia (Fig 5A) and heart rate acceleration was measured in isolated hearts from TAB and sham operated mice. Previous data in mice without heart failure indicate that $\text{MAPD}_{90}$ shortening and the rate of shortening in response to hypoxia and heart rate acceleration are largely regulated by $K_{ATP}$ channel current [17, 30]. Here, in response to hypoxia, we find that there is a significant slowing in the rate of $\text{MAPD}_{90}$ shortening (.14±.02 vs. .35±.08 msec/sec, $p < .05$, Fig 5B right panel), also reflected in the greater half-time of maximal shortening (42.9±2.8 vs. 26.9±4.1 sec, $p < .05$, Fig 5B middle panel) for hearts from TAB.
Similarly, there is a slowing in the rate of MAPD90 shortening to abrupt heart rate acceleration (0.36±0.08 vs. 1.34±0.26 msec/sec, p < .05, Fig 5C right panel), again also reflected in a prolonged half-time of shortening (18.2±2.5 vs. 7.6±1.5 sec, p < .05, Fig 5C middle panel) in hearts from TAB (n = 12) compared to sham controls (n = 9). In response to both stressors, there is a trend toward slightly less steady state MAPD90 shortening that is not statistically significant (11.8±1.5 msec, n = 10 TAB vs. 16.7±2.6 msec, n = 9 sham for hypoxia and 11.1±1.8 msec, n = 12 TAB vs. 16.8±2.5 msec, n = 9 sham for heart rate acceleration, Fig 5B, C left panels). Thus, reduction of membrane $K_{\text{ATP}}$ channel expression results in a significant loss of ventricular APD adaptation in failing hearts.
Disruption of $K_{ATP}$ channel expression eliminates the protective effect of CaMKII inhibition on ischemia-reperfusion injury

Even in the absence of coronary artery disease, failing myocardium can be injured by ischemia due to a mismatch between metabolic supply and demand [45]. Many studies have
demonstrated that CaMKII is activated during cardiac ischemia-reperfusion and contributes significantly to the resulting injury via multiple signal cascades [46–56]. Specifically, CaMKII has been shown to be activated by acute ischemia and reperfusion in isolated rodent hearts [49–53, 55, 56]. We have also documented that CaMKII activation triggers the rapid endocytosis of Kir6.2 subunits in the myocardium while CaMKII inhibition results in greater numbers of $K_{\text{ATP}}$ channels at the myocyte surface and increased $K_{\text{ATP}}$ channel current capacity [29]. This has been confirmed in situ with measurement of ventricular myocyte surface Kir6.2 by whole heart biotinylation [29]. Opening of $K_{\text{ATP}}$ channels is a well-characterized protective mechanism against cardiac ischemia-reperfusion injury [20, 57, 58]. Previous data indicate that the increased $K_{\text{ATP}}$ channel membrane expression in mice transgenically expressing a peptide inhibitor of CaMKII (AC3-I), or increased $K_{\text{ATP}}$ channel current induced by $K_{\text{ATP}}$ channel openers, correlates with reduced myocardial infarction size following ischemia/reperfusion [28]. Similarly, cardiac CaMKII inhibition or targeted deletion of CaMKIIδ protects against adverse myocardial remodeling following myocardial infarction or TAB [37–39], presumably mediated in part by the loss of CaMKII suppression of $K_{\text{ATP}}$ channel expression. Indeed, obtaining comparable degrees of ventricular dysfunction in the presence and absence of CaMKII inhibition in order to replicate such experiments in heart failure would be difficult because CaMKII inhibition significantly affects vulnerability to myocardial dysfunction. Therefore, to assess whether the down-regulation of $K_{\text{ATP}}$ channels that we have demonstrated in the hearts of TAB mice may contribute to injury and further heart failure progression, and whether resistance to injury in the presence of CaMKII inhibition can be attributed to increased $K_{\text{ATP}}$ channel expression, we assessed ischemia-reperfusion injury in isolated non-failing hearts from WT compared to the AC3-I, Kir6.2-KO and AC3-I/Kir6.2-KO genetic mouse models (Fig 6A). As expected, the area of injury was significantly increased in hearts lacking sarcolemmal $K_{\text{ATP}}$ channels (Kir6.2-KO) compared to WT controls (61.7±1.2%, n = 5 vs. 49.4±3.5%, n = 8, p < .05, Fig 6B) and significantly reduced in hearts in which a CaMKII inhibitor is constitutively expressed (AC3-I, 40.3±2.2, n = 10, p < .05 vs WT, Fig 6B). Interestingly, the protective benefit of CaMKII inhibition was eliminated in the absence of sarcolemmal $K_{\text{ATP}}$ channels (AC3-I/Kir6.2-KO, 57.7±3.5%, n = 8, p < .05 vs WT, p = NS vs Kir6.2-KO, Fig 6B), indicating that release of $K_{\text{ATP}}$ channel expression from downregulation is a major mechanism for the myocardial protective effects of CaMKII inhibition, at least in response to ischemia-reperfusion, but possibly in response to other pathologic stressors as well.

**Discussion**

Here we examine whether chronic, persistent CaMKII activation in failing hearts could promote the heart failure phenotype of action potential prolongation and metabolic vulnerability through an effect on $K_{\text{ATP}}$ channel surface expression and current. Indeed, our data indicate a significant reduction of about 35–40% in the myocyte membrane presence of $K_{\text{ATP}}$ channels in failing compared to healthy hearts. Since a significant reservoir of sub-membrane $K_{\text{ATP}}$ channel subunits is present in cardiomyocytes [29, 31], we use corroborating methods that exclude these spare components from the quantification of membrane surface $K_{\text{ATP}}$ channel expression. Specifically, we use the technique of whole heart biotinylation immediately after heart isolation to label only surface-accessible subunits. The result from this method correlates well with the other technique employed—$K_{\text{ATP}}$ channel current density measured by whole cell patch clamp in isolated cardiomyocytes.

We have previously demonstrated that the high baseline surface expression of $K_{\text{ATP}}$ channels in healthy hearts supports a rapid change in APD in response to myocardial stress that prevents excessive oxygen consumption thereby preserving an optimal myocardial energetic
state [2]. Conversely, a significantly slowed adjustment of MAPD₉₀ in response to heart rate acceleration or hypoxia occurs with both a decrease in ventricular $K_{ATP}$ channel surface expression of 75–85% in a transgenic mouse model [17, 30] and a 30–50% reduced surface expression in sedentary vs. active mice [17], both coupled with proportional increases in oxygen consumption [17]. In the current study, we demonstrate an approximately 30–40% reduction in surface $K_{ATP}$ channels in failing compared to non-failing hearts that is also coupled to a significant slowing in MAPD₉₀ adjustment with both heart rate acceleration and hypoxia. Given the similar scale to our previous experiments with regard to $K_{ATP}$ channel surface expression deficit and slowing of MAPD₉₀ adjustment, a similar effect on oxygen consumption in the TAB mice is expected. Thus we conclude that the reduction in $K_{ATP}$ channel surface expression and sluggish membrane electrical response in the failing hearts of TAB mice is likely sufficient to contribute significantly to the sick metabolic phenotype of heart failure.

We have previously established that $K_{ATP}$ channel expression on the surface of healthy cardiomyocytes is directly affected by CaMKII activation due to phosphorylation of the Kir6.2 subunit by CaMKII that then initiates endocytosis of channel subunits [29]. Yet in heart failure,
where remodeling re-orders numerous cell processes, it is possible that the loss of surface $K_{ATP}$ channels at baseline in failing hearts is unrelated to the demonstrated CaMKII activation. However, we find that the diminished $K_{ATP}$ channel current density in the failing hearts can be further downregulated, albeit in a blunted fashion, in response to activation of CaMKII by isoproterenol. We interpret this to indicate that, despite many profound remodeling effects in heart failure, the mechanism of CaMKII-induced internalization of $K_{ATP}$ channels is still operable. Furthermore, the chronic activation of CaMKII in the failing TAB hearts and the consequent low baseline surface expression of $K_{ATP}$ channels probably preclude further vigorous $K_{ATP}$ channel downregulation since there is likely little remaining dynamic range for either CaMKII activation or $K_{ATP}$ channel internalization.

In non-failing hearts, we demonstrate that CaMKII inhibition attenuates ischemia-reperfusion injury—an effect that is eliminated by knockout of Kir6.2—corroborating a previous pharmacologic study [28] and indicating that this cardioprotective CaMKII inhibition-driven phenomenon is mediated substantially by $K_{ATP}$ channels. Since our data support that $K_{ATP}$ channel surface expression remains both chronically and acutely under the influence of CaMKII, it is reasonable to expect that a protective effect of CaMKII inhibition in failing hearts is also largely mediated by a release of $K_{ATP}$ channel expression from suppression. Further studies with induction of CaMKII inhibition after the establishment of heart failure, with and without Kir6.2-KO, may confirm these findings.

Recognition of a CaMKII-dependent downregulation of $K_{ATP}$ channel expression as a mechanism of heart failure development and progression points to strategies targeting this interaction for potential preventives or treatments. Indeed, CaMKII inhibition has been scrutinized as a promising therapeutic approach in heart failure. However, diverse tissue expression and pleiotropic vital functions of CaMKII have made it a problematic target. Meanwhile, the presently available $K_{ATP}$ channel openers do not have cardiac specificity and their therapeutic use is limited due to potent effects on vasculature tone causing hypotension [59–61]. Given these limitations, deciphering the interaction between CaMKII and $K_{ATP}$ channel surface expression could provide for development of more specific approaches to securing myocardial metabolic well-being and preventing or treating heart failure.

We recognize that the alteration in $K_{ATP}$ channel surface expression identified in this study is one element in a complex array of ion channel adaptations identified in heart failure. Indeed, heart failure has been associated with numerous modifications of ion channels, the precise profile of which can be specific to the myocardial layer, stage and etiology of heart failure [62–64]. Furthermore, interactions between ion channels contribute to the electrophysiologic phenotype of various types of heart failure [62, 63]. It is likely that $K_{ATP}$ channel surface expression in heart failure is similarly non-uniform across layers, models and stage and that the impact of their downregulation depends in part on interactions between $K_{ATP}$ channels and other ion channels. Further study will be needed to determine whether our findings here in a TAB model will translate to other etiologies and severities of heart failure.

Whether the demonstrated perturbation in $K_{ATP}$ channel surface expression, and its effect on APD shortening and myocardial energetics, is relevant to human disease also remains to be tested. One might argue that the short cycle length in rodents necessitates strong repolarizing currents to prevent action potential fusion and that rodents would thus be more dependent on $K_{ATP}$ channel opening under stressful conditions than larger mammals. However, a comparison of studies across animal models indicates that the cardioprotective effect of $K_{ATP}$ channels occurs within a broad range of ion channel profiles and associated shapes and durations of action potentials [65]. Furthermore, the current study and previous studies [17, 30] identify changes in APD related to stress as important for the $K_{ATP}$ channel effect on myocardial energetics and cardioprotection. The question of applicability of our findings to human heart...
failure is also partially addressed by a previous study indicating a defect in K\textsubscript{ATP} channel gating as a potential cause of familial cardiomyopathy [27]. While that study did not identify deficient K\textsubscript{ATP} channel expression, it reinforces the concept that insufficient K\textsubscript{ATP} channel current, which could be caused by either gating or expression defects, may predispose human myocardium to injury and mechanical failure.

**Conclusions**

Our data indicate that ventricular K\textsubscript{ATP} channel surface expression is under the control of CaMKII in failing hearts and that the chronic CaMKII activation in this disease state suppresses K\textsubscript{ATP} channel surface expression sufficiently to incur a failure of membrane electrical responsiveness associated with negative metabolic consequences. Our data support that this mechanism underlies an increased risk of myocardial injury that can translate to heart failure progression.

**Author Contributions**

Conceived and designed the experiments: LVZ DMH-Z. Performed the experiments: ZG AS ZZ SK ES AM LVZ DMH-Z. Analyzed the data: ZG AS ZZ ES LVZ DMH-Z. Contributed reagents/materials/analysis tools: MEA LVZ DMH-Z. Wrote the paper: ZG LVZ DMH-Z.

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