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Effects of K_{ATP} channel openers diazoxide and pinacidil in coronary-perfused atria and ventricles from failing and non-failing human hearts

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Abstract

INTRODUCTION—This study compared the effects of ATP-regulated potassium channel (K_{ATP}) openers, diazoxide and pinacidil, on diseased and normal human atria and ventricles.

METHODS—We optically mapped the endocardium of coronary-perfused right (n=11) or left (n=2) posterior atrial-ventricular free wall preparations from human hearts with congestive heart failure (CHF, n=8) and non-failing human hearts without (NF, n=3) or with (INF, n=2) infarction. We also analyzed the mRNA expression of the K_{ATP} targets $K_{ir}6.1$, $K_{ir}6.2$, SUR1, and SUR2 in the left atria and ventricles of NF (n=8) and CHF (n=4) hearts.

RESULTS—In both CHF and INF hearts, diazoxide significantly decreased action potential durations (APDs) in atria (by $-21 \pm 3\%$ and $-27 \pm 13\%$, $p < 0.01$) and ventricles (by $-28 \pm 7\%$ and $-28 \pm 4\%$, $p < 0.01$). Diazoxide did not change APD ($0 \pm 5\%$) in NF atria. Pinacidil significantly decreased APDs in both atria (-46 to -80% , $p < 0.01$) and ventricles (-65 to -93% , $p < 0.01$) in all hearts studied. The effect of pinacidil on APD was significantly higher than that of diazoxide in both atria and ventricles of all groups ($p < 0.05$). During pinacidil perfusion, burst pacing induced flutter/fibrillation in all atrial and ventricular preparations with dominant frequencies of 14.4 ± 6.1 Hz and 17.5 ± 5.1 Hz, respectively. Glibenclamide ($10 \mu\text{M}$) terminated these arrhythmias and restored APDs to control values. Relative mRNA expression levels of K_{ATP} targets were correlated to functional observations.

CONCLUSION—Remodeling in response to CHF and/or previous infarct potentiated diazoxide-induced APD shortening. The activation of atrial and ventricular K_{ATP} channels enhances arrhythmogenicity, suggesting that such activation may contribute to reentrant arrhythmias in ischemic hearts.

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Keywords

human heart; IKATP; ischemic heart disease; heart failure; arrhythmias; optical mapping

INTRODUCTION

ATP-sensitive potassium (K_{ATP}) channels are prominent in cardiac sarcolemmal membranes, and can have marked effects on cardiac repolarization and contraction during physiological and pathophysiological conditions [1–3]. Activation of K_{ATP} channels plays an important cardio-protective role during myocardial ischemia and hypoxia [4–6]. However, activation of K_{ATP} channels during myocardial ischemia leads to enhanced potassium efflux, reduction in action potential duration (APD) and reduced refractoriness, promoting ventricular fibrillation (VF) [2,7]. It has also been shown that K_{ATP} channel openers such as pinacidil promote the induction of re-entrant arrhythmias such as VT/VF [8]. Conversely, the K_{ATP} inhibitor glibenclamide can prevent or terminate VT/VF by preventing ischemia-induced anatomically heterogeneous reduction in APD and the resulting dispersion of the refractory period [9,10].

Sarcolemmal K_{ATP} channels are composed of a pore-forming subunit ($K_{ir}6.1$ or $K_{ir}6.2$) and a sulfonylurea receptor (SUR1, SUR2A, or SUR2B) [11,12]. The SUR subunit determines the specificity and selectivity of K_{ATP} agonists and antagonists [13]. In mouse isolated cells [14] and whole hearts, [15] it has been shown that atrial K_{ATP} is only sensitive to the KCO diazoxide (specific to SUR1>SUR2A) and not to pinacidil (SUR2A>SUR1), whereas ventricular K_{ATP} has the opposite sensitivity to these K_{ATP} channel openers. The few published studies on human heart K_{ATP} channels focused on isolated human single cells [16–20] or fibers [21] and there is little information on chamber-specificity of KCO action.

Recently, we have established the necessary infrastructure to investigate atrial and ventricular tissues from explanted human hearts using optical mapping techniques [22,23]. In the present study, we further used this opportunity to study intact human cardiac tissue in order to address several important questions regarding K_{ATP} openers:

1. Do the human atria and ventricles exhibit chamber specificity with respect to KCOs diazoxide and pinacidil, as described in the mouse [15]?
2. Does cardiac remodeling during human heart failure alter atrial or ventricular sensitivity to KCOs?
3. How do KCOs affect arrhythmogenicity in human atria and ventricles?

The present study was designed to evaluate the effects of two KCOs, diazoxide and pinacidil, in coronary-perfused human atria and ventricles from patients exhibiting late-stage congestive heart failure (CHF) as well as rejected non-failing donor hearts with (INF) and without (NF) a history of infarct. Atrial and ventricular electrical activity was simultaneously monitored to investigate the effects of diazoxide and pinacidil on APDs, refractory periods, and arrhythmia inducibility. Relative mRNA expression levels of K_{ATP} channel subunits were measured to examine chamber specificity of K_{ATP} channels.

MATERIALS AND METHODS

Patient groups

CHF hearts with different types of cardiomyopathy were obtained during transplantation at Barnes-Jewish Hospital, Washington University in Saint Louis, MO. For comparison, we used non-failing donor hearts, which were rejected for transplantation for various reasons,

including age, early stage hypertrophy, and coronary disease. Donor hearts were provided by the Mid-America Transplant Services (Saint Louis, MO). This study protocol was approved by the Washington University Institutional Review Board.

Explanted hearts were cardioplegically arrested and cooled to 4–7°C in the operating room following crossclamping of the aorta. The arrested heart was maintained at 4–7°C to preserve tissue during the 15–20 minute delivery from the operating room to the research laboratory. We used two separate groups of hearts for optical mapping experiments (n=13, Table 1) and for qPCR analysis (n=12, Table 2). Only two hearts were used for both optical mapping and qPCR as indicated in Table 1a. After harvesting, the hearts were immediately perfused through the aorta with a cardioplegic solution (in mmol/L: NaCl 110, CaCl₂ 1.2, KCl 16, MgCl₂ 16, NaHCO₃ 10; pH=7.65±0.05; 4°C). Cardioplegic perfusion removed all the blood and protected the hearts from ischemia during the subsequent period of tissue isolation. Tissue samples for qPCR were immediately harvested and stored in RNAlater (Sigma-Aldrich, St. Louis, MO) at –80°C.

Experimental preparation

After tissue collection, we performed functional experiments with coronary-perfused preparations (n=13, Table 1). Figure 1 shows an explanted heart, experimental design, and two types of experimental preparations used in this study: the right (n=11) and left (n=2) coronary-perfused atrial and ventricular free walls. The right (n=11) or left (n=2) coronary arteries were cannulated before the experimental preparations were isolated from the rest of the heart (Figure 1C and D). The final right atrial-ventricular (RA-RV) preparation included the RA free wall with part of the RA appendage, tricuspid valve ring, and RV free wall (Figure 1C). Coronary perfusion of the left atria was not possible in most cases due to steps involved with the clinical surgical procedure (Figure 1A). We could successfully map only 2 left atrial-ventricular (LA-LV) cardiac preparations from failing hearts, which included the LA free wall attached to part of the appendages, mitral valve ring, and LV free wall (Figure 1D). None of the atrial-ventricular preparations included sino-atrial or atrio-ventricular nodal tissues. Major arterial leaks in the atrial-ventricular preparations were ligated with silk suture. The quality of perfusion was verified by the injection of methylene blue dye (Sigma-Aldrich, St. Louis, MO). Poorly perfused tissue was trimmed from the preparations. Isolated tissues were mounted in a warm tissue chamber with the endocardial surface facing the optical apparatus (Figure 1B). Preparations were perfused with oxygenated Tyrode solution composed of (in mmol/L): 128.2 NaCl, 4.7 KCl, 1.19 NaH₂PO₄, 1.05 MgCl₂, 1.3 CaCl₂, 20.0 NaHCO₃, and 11.1 glucose, and gassed with 95% O₂-5% CO₂; pH=7.35±0.05; 37°C; coronary arterial pressure of 50–60 mmHg. The preparation was fully immersed in the perfusion efflux, ensuring appropriate superfusion.

Imaging system

After 20–30 minutes of washout and gradual warming to 37°C to ensure tissue recovery and stabilization after cold cardioplegia, the atrial-ventricular preparations were stained with di-4-ANEPPS (Invitrogen, Carlsband, CA). The preparations were immobilized with 10–15 μM Blebbistatin (Tocris Bioscience, Ellisville, MO), which blocks cardiac contraction without affecting electrical activity, including ECG parameters, atrial and ventricular effective refractory periods, and atrial and ventricular activation patterns for many mammalian species including human [22–25].

The preparations were simultaneously paced at the atrial and ventricular endocardium using two bipolar electrodes with 10 ms pulses at 2× diastolic current thresholds at a CL ranging from 1,000 ms to the atrial or ventricular functional refractory periods, respectively. Two Ag/AgCl electrodes were immersed in the superfusion solution to document the pseudo-

ECG. Imaging was simultaneously conducted with two 100×100 pixel MiCAM Ultima-L CMOS cameras (SciMedia, USA Ltd., CA) from atrial and ventricular endocardial fields of view ranging from 30×30 to 40×40 mm², sampled at 1000 frames/sec (Figure 1B). The fluorescent signals were amplified, digitized, and visualized during the experiment using specialized software (SciMedia, USA Ltd., CA).

Data processing

A custom-made Matlab-based computer program was used to analyze APs offline [25,26]. First, the signals were filtered using a low-pass Butterworth filter at 64 Hz. Activation maps were constructed from activation times, which were determined from the $dV/dt_{\max}$ in each channel. Finally, AP duration was calculated as the time difference between activation time ($dV/dt_{\max}$) and 80% of repolarization (APD80). We also used Fourier transform analyses to produce the Dominant Frequency (DF) maps, similar to our recent study [27].

Experimental protocol

Following isolation and cannulation, motion suppression, and dye staining, preparations were equilibrated for 5–10 min before imaging. Control measurements during simultaneous atrial and ventricular pacing were then made. Preparations were paced at a constant basic cycle length (BCL), which was shortened from 1,000 to 500 ms in steps of 100 ms (40–60 sec time interval between steps), and in steps of 10–20 ms from 450 ms until the functional refractory period was reached. This restitution pacing protocol was repeated after drug application.

To assess the role of different types of sulfonylurea receptors, we applied two K_{ATP} agonists: diazoxide (10–300 μ M), and pinacidil (1–100 μ M) [13]. Due to the limited time that the physiological state of the heart could be maintained during an optical mapping experiment, only the highest diazoxide (300 μ M) and pinacidil (100 μ M) concentrations were used in 9 out of 13 experiments [15]. Ultimately, since the efficacy of these drugs on their target channels depends on metabolic state, we cannot rule out an indirect effect (e.g. through mitochondrial succinate dehydrogenase), but as we have shown in animal experiments the effects of both diazoxide and pinacidil at these concentrations are blockable by glibenclamide, and absent in $K_{ir6.2}/-$ or $SUR1/-$ animals [14,15], the actions are certainly through surface membrane channels.

Diazoxide was delivered through both the perfusion and superfusion of solutions applied for 10–15 min to reach steady-state. After measuring the dynamic restitution, diazoxide was washed out from the atrial-ventricular preparations for 30 min before the delivery of pinacidil. After washout, additional staining, as well as additional injection of blebbistatin, was administered if needed. The restitution pacing protocol was repeated after 10 minutes of constant pinacidil perfusion. Lastly, the K_{ATP} channel blocker, glibenclamide (10 μ M, $n=10$), was added to reverse the effects of pinacidil and terminate arrhythmias.

RNA Extraction and Quantitative PCR

We used tissue from 3 additional CHF hearts and 7 additional NF hearts for quantitative PCR (Table 2). Total RNA was extracted from tissue of the LA (NF $n=7$, CHF $n=4$) and LV (NF $n=8$, CHF $n=4$) using the RNEasy Fibrous Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The yield was measured using a Nanodrop 1000 (Thermo Scientific), converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA), and probed using a custom-designed low-density gene array (Applied Biosystems, Foster City, CA) and the ABI PRISM 7900HT Sequence Detection System. Data were collected and analyzed by the Applied Biosystems SDS 2.1

software using the threshold cycle (Ct) relative quantification method with GAPDH as an endogenous control.

Statistical analysis

Values are expressed as means $\pm$ SD. Hypothesis testing was carried out using paired and unpaired student t-test and chi-squared analysis with Yates correction. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Action potential duration in NF, CHF and INF human atrium and ventricle

In non-failing control ventricular endocardium (INF and NF), the APD80 was 394 ± 53 ms ($n=5$), and the CHF ventricular endocardial APD80 was not statistically different (411 ± 58 ms, $n=6$), at a baseline cycle length of 1,000 ms. However, in the non-failing control atrial endocardium, APD80 was 288 ± 29 ms ($n=5$), and the CHF atrial endocardial APD80 was significantly prolonged (331 ± 33 ms, $n=6$, $p=0.02$).

Given the possibility that infarction may alter KCO sensitivity of K_{ATP} channels and that the K_{ATP} channel subunit expression has been reported to be altered in animal models of infarction [28], the non-failing hearts were subdivided into groups with (INF, $n=2$) and without (NF, $n=3$) a history of infarct. Figures 2 and 3 illustrate the effects of potassium channel openers and blockers on the APD for the CHF and NF preparation groups. All data were obtained during simultaneous mapping of both RA and RV endocardium (CL=1000 ms), and representative examples of superimposed OAPs in control and after application of pinacidil are shown in Figures 2B and 3B. Figure 4 summarizes the effects of these drugs in all eight groups: CHF-RA and CHF-RV ($n=6$); CHF-LA and CHF-LV ($n=2$); NF-RA and NF-RV ($n=3$); INF-RA and INF-RV ($n=2$).

Diazoxide (300 μ M) did not affect APD in NF-RA, and only slightly decreased APD in NF-RV, whereas pinacidil (100 μ M) shortened APD in both NF-RA and NF-RV (Figures 2 and 4). These findings are significant in demonstrating a marked difference in chamber-specificity of K_{ATP} opener action different from that seen in the rodent heart [15, 29], and suggest a different subunit composition in humans. INF hearts also exhibit no obvious chamber selectivity for KCOs - both diazoxide and pinacidil significantly shortened APD80 in RA and RV (Figure 4).

In contrast to non-failing hearts, diazoxide (300 μ M) significantly decreased APD in both atria and ventricles of failing hearts (Figures 3 and 4), and pinacidil (100 μ M) had an even more profound effect in both CHF-RA and HF-RV than in non-failing hearts (Figures 2–4).

Conduction velocity measurements were complicated by the complex anatomical architecture of the RA and RV trabeculated endocardium, but neither diazoxide nor pinacidil had a significant effect on conduction in either failing or non-failing atrial-ventricular preparations (see activation maps in Figures 3 and 4).

In most hearts, a 10–20 minute treatment with glibenclamide (10 μ M) fully reversed pinacidil-induced shortening of atrial and ventricular APD to control values, and glibenclamide (10 μ M) actually prolonged APD to significantly longer values than control in non-failing RA (288 ± 17 ms, $n=6$ vs 385 ± 29 ms, $n=5$, $p=0.017$) (Figure 4).

Arrhythmia incidence during control and Diazoxide and Pinacidil

There is much evidence in animal models that pharmacological activation of K_{ATP} channels predisposes ventricles to reentrant arrhythmias [30,31]. As shown in Figure 5, K_{ATP} -induced

atrial and ventricular APD shortening led to both pacing-induced and spontaneous AFL/AF and VT/VF. Tables 3 and 4 show data from pacing-induced and spontaneous arrhythmia incidences in control and during K_{ATP} channel treatment for all hearts. In control, atrial pacing at rates up to the functional refractory period (FRP) failed to produce any arrhythmias. However, fast ventricular pacing rates did induce sustained VF in one CHF heart and in one NF heart. Diazoxide significantly decreased both atrial and ventricular FRPs in CHF hearts and thus potentiated induction of AFL/AF and VT/VF in two atrial and three ventricular preparations, respectively. Washout from diazoxide terminated arrhythmias in all preparations. Pinacidil significantly shortened FRPs in all studied hearts and thus provoked pacing-induced reentrant arrhythmias. Moreover, spontaneous VFs were observed during the first 2–4 minutes of pinacidil perfusion in three CHF ventricular preparations (Table 4).

Figure 5A shows dominant frequency (DF) maps of different sustained atrial and ventricular arrhythmia episodes recorded during pinacidil perfusion in CHF heart #3 and NF heart #1. Figures 5B–C summarize the occurrences of arrhythmias and their corresponding DF values for CHF and NF right hearts.

It is clear that the principal mechanism of all recorded episodes of AF and AFL was a leading reentry circuit anchored to the pectinate muscles (outlined in Figure 5A) or scar. Similar mechanisms underlay VF, with single or multiple reentry circuits anchored to the myocardial trabecular network. However, due to the more complex intramural fiber architecture in ventricles compared to atria, optical mapping of the full VF reentry circuits was often challenging. The anatomical structure of both atrial pectinate muscles and ventricular trabeculae divided arrhythmia activity into regions with distinct DFs. AF and VF DFs were not significantly different between groups (Figure 5C).

Administration of glibenclamide (10 μ M) terminated pinacidil-induced AF and VF more rapidly in the ventricles than the atria (5.1 ± 2.6 versus 9.8 ± 4.5 minutes, ($p=0.02$)).

Molecular-level expression of K_{ATP} subunits in NF and CHF hearts

Figure 6 shows the relative mRNA expression levels of K_{ATP} channel subunits ($K_{ir}6.1$, $K_{ir}6.2$, SUR1, and SUR2) in the LA and LV of NF (58 ± 6 years old) and CHF (56 ± 7 years old) hearts. In choosing primers, we did not distinguish between SUR2A and 2B. All targets were expressed in both chambers with no obvious chamber specificity of either the channels or their subunits. In comparing message-level expression with functional observations resulting from the presence of diazoxide and pinacidil, we observed the following: (1) no significantly increased expression level of SUR1 in the CHF hearts (0.66 ± 0.33 (CHF-LA) vs 0.83 ± 0.42 (NF-LA), $p=0.51$; 0.59 ± 0.29 (CHF-LV) vs 0.76 ± 0.36 (NF-LV), $p=0.45$), even though diazoxide significantly shortened APDs in the CHF hearts in both the atria and ventricles.; (2) a statistically higher level of SUR2 expression in the ventricles of NF hearts (0.73 ± 0.23 (NF-LA) vs 1.28 ± 0.37 (NF-LV), $p=0.005$), consistent with our functional observation that pinacidil tends to have a greater effect on APD shortening in the ventricles as compared to atria; (3) significantly higher (0.73 ± 0.35 (overall SUR1 expression) vs 1.08 ± 0.37 (overall SUR2 expression), $p=0.002$) overall expression of SUR2 than SUR1 across all chambers, consistent with our observation that overall pinacidil has a greater effect on APDs than diazoxide; and (4) heterogeneous expression of $K_{ir}6.1$ and $K_{ir}6.2$ in both the LA and LV. Since we were unable to obtain working antibodies for immunohistochemistry, we are unable to confirm these findings at the protein level.

DISCUSSION

Validation of the experimental human coronary-perfused preparations

Our mapping study is the first to use optical AP recordings from coronary-perfused human hearts in different disease states. This study showed that simultaneous atrial and ventricular endocardial mapping can be successfully performed for at least 6 hours after the first dye staining without significant changes in the electrophysiological parameters. It is important therefore to note that atrial APD values observed during this study are comparable with previous observations made in isolated human single cells [32] or superfused slices [21,32–34]. Our observation that atrial APD and FRP were significantly longer in CHF hearts as compared to NF hearts is also consistent with previous animal [35] and clinical studies [36], which reported prolongation of atrial APD and refractoriness during congestive HF. There were no significant APD differences in the endocardial RV between CHF and NF groups. This observation was consistent with our previous study from LV transmural wedge preparations, in which we observed that CHF promotes APD prolongation in sub-epicardial, but not sub-endocardial layers [22].

Chamber specificity of K_{ATP} channel structure and pharmacology

Different species have different sensitivities to SUR1 and SUR2A agonists in the atria and ventricles [37]. Although Poitry and colleagues have performed several comparative studies of K_{ATP} activation in neonatal rat atrial and ventricular myocytes [29], relatively few studies have examined differential activation of atrial and ventricular K_{ATP} channels. Those studies have revealed a differential diazoxide sensitivity in atria as compared to ventricles, consistent with our own recent demonstration in isolated mouse myocytes and intact hearts that the atria and ventricle express only SUR1- and SUR2A- dependent K_{ATP} channels, respectively [14,15]: atrial K_{ATP} channels in mice are activated by the SUR1-specific opener diazoxide, but not by the SUR2-specific pinacidil, whereas ventricular K_{ATP} channels are activated by pinacidil, but not diazoxide. Thus, rodent atria and ventricles have different responses to K_{ATP} channel openers. Rabbit hearts share similar chamber-specific responses as murine hearts to K_{ATP} channel openers [37]. In contrast, guinea pig atrial and ventricular myocytes have similar sensitivity to pinacidil [37]. Canine hearts are also likely to exhibit mixed SUR1 and SUR2A expressions in the atria and ventricles: the direct effects of diazoxide were demonstrated in canine Purkinje fibers [38] and of pinacidil on atrial function in the coronary-perfused canine heart [39].

To our knowledge, only one study demonstrates the effect of a KCO SR 44866 (bimakalim, a SUR2A agonist similar to pinacidil and cromakalim) on both human right atrial and ventricular APD [21]. Gautier et al investigated the effects of K_{ATP} channel openers on the APDs of human superfused atrial and ventricular endocardial tissue slices obtained during open heart surgery for mitral valve replacement [21]. They found that bimakalin significantly shortened both atrial and ventricular APDs (up to 91% and 78% from control), which were fully restored by glibenclamide. Our observations, combined with this published data, lead us to conclude that human cardiac chambers do not have a distinct molecular differential pharmacology of atrial versus ventricular K_{ATP} channels in contrast to rodents. It is important to note that diazoxide and pinacidil can have additional effects on mitochondrial function at high concentrations [40] and might thereby nonspecifically cause K_{ATP} activation. In the mouse heart, lack of K_{ATP} channels in SUR1^{-/-} animals provides the definitive evidence for SUR1 role in atrial sarcolemmal K_{ATP} . In the case of human hearts, we cannot obtain such definitive evidence, and it remains a potential caveat that diazoxide might activate channels indirectly via inhibition of mitochondrial metabolism. However, even at 300 μ M diazoxide failed to activate ventricular K_{ATP} in mouse [15]. Importantly, our mRNA expression profiles also indicate that K_{ATP} channels and subunits are

heterogeneously expressed in the human heart. There may be an uneven distribution between chambers, and it also appears that ischemia and failure can change the expression of SUR1 [41] and, probably, SUR2A, as well as their sensitivity to K_{ATP} channel openers in the human heart.

The role of the metabolic state on KCO-activated K_{ATP} channel activity

Previous studies in isolated cells showed that rat and mouse atrial K_{ATP} channels activate more readily compared to ventricular K_{ATP} channels [14,29]. Poitry et al observed an increase in K_{ATP} channel sensitivity to diazoxide within the atrium versus ventricle (3:1 to $\geq 24:1$) during ischemic-like conditions in neonatal rat myocytes [29]. These findings suggest a higher atrial K_{ATP} channel sensitivity to ischemia [29] and are consistent with SUR1-based channels being more sensitive to metabolic activation than SUR2A-based channels [42]. They lead to the expectation that atrial K_{ATP} channels may activate more readily than ventricular channels during metabolic inhibition, particularly in animals exhibiting an atrial chamber-specificity for the SUR1 isoforms. Interestingly, the different etiology of the CHF and infarcted hearts is likely to produce different levels and durations of metabolic stress, which may be one explanation for the observed differences in effects of diazoxide between NF and both INF and CHF hearts (Figure 2). Koumi et al [20] demonstrated that K_{ATP} channels in atrial myocytes from patients with CHF have characteristics substantially similar to those in donors, but that the channels are less sensitive to ATP inhibition by cyanide in CHF than in donors.

Tavares et al [28] demonstrated that expression of $K_{ir6.1}$ and all SUR regulatory subunits was increased up to 3-fold in cardiomyocytes located in the infarct border zone of rat hearts, 20 weeks after coronary occlusion. They also measured the responses of K_{ATP} currents to diazoxide in these cells, whereas the response to diazoxide was undetectable in control ventricular cardiomyocytes. Thus, the higher sensitivity of the human CHF and INF hearts to diazoxide observed in our study may be attributable to three likely mechanisms: 1) failure and/or ischemia enhanced sensitivity of the SUR K_{ATP} channels [29], 2) overexpression [28] and/or 3) plasticity of SUR subunits [43]. Although our mRNA expression data has revealed interesting observations (Figure 6), our efforts to explore protein expression of SUR isoforms with commercial and custom antibodies for Western blot analysis in these human tissues have thus far proven unsuccessful. Our mRNA data show that $K_{ir6.2}$ expression is decreased in CHF atria as well as in CHF ventricles as compared to non-failing hearts. Since we are unable to obtain working antibodies for immunohistochemistry, we are unable to confirm these human findings at the protein level. The naïve assumption is that protein levels will also be expected to decrease, and such a finding could indeed reduce the efficacy of channel openers, if the net channel density is reduced. However, as we have previously demonstrated [44, 45], expressed K_{ATP} protein levels and functional channel density do not necessarily correlate, and so the lack of correlation between reduced K_{ir6} transcript levels (Figure 6) and KCO efficacy (Figure 4) in CHF may not be unexpected, and the import is unclear. Further studies will be required to determine specifically how expression of channel regulatory subunits, and channel sensitivity and specificity to KCOs is affected in human atria and ventricles during different metabolic states (ischemia, hypoxia, hypothermia, etc.) and different HF etiologies.

K_{ATP} channels and arrhythmias

Activation of K_{ATP} channels shortens ventricular APD and refractory period, which may promote reentrant arrhythmias such as VT/VF [30,31,46,47]. It remains an open question whether or not K_{ATP} openers can be proarrhythmic in the diseased human heart and thus affect the risk of sudden death in patients [48–50]. The K_{ATP} blocker, glibenclamide, prevented VF in isolated ischemic rat hearts [46] and significantly reduced incidences of VF

induced by the combination of acute ischemia during exercise in dogs with previously healed myocardial infarctions [51]. Glibenclamide also reduced the number and severity of arrhythmias during transient ischemia in diabetic patients with coronary artery disease [52] and significantly reduced the incidence of VF in non-insulin-dependent diabetic patients with acute myocardial infarction [53]. However, glibenclamide can also increase the dispersion of APD in ventricles, which may be a factor underlying increased risk of arrhythmias and sudden cardiac death in patients with diabetes [52]. Our study demonstrates that activation of K_{ATP} channels by both diazoxide and pinacidil can lead to atrial and ventricular reentrant arrhythmias (Figure 5), which can be terminated by glibenclamide. Clearly, activation of K_{ATP} channels could be one mechanism of ischemia-induced AF and VF, especially in diseased, metabolically-challenged hearts.

Cole, et al demonstrated that glibenclamide enhances, whereas pinacidil reduces, myocardial damage caused by ischemia/reperfusion [5]. These results are consistent with the many observations that activation of K_{ATP} channels during ischemia is an important adaptive mechanism for protecting the myocardium when blood flow to the tissue is compromised [5,54]. Thus, the effects of KCOs may mimic the mechanisms counteracting the harmful effects of ischemia, but the drugs can also be arrhythmogenic. The delicate balance between prevention of ischemia-induced arrhythmia by K_{ATP} blockers and prevention of myocardial damage induced by K_{ATP} openers should be considered during patient treatment by K_{ATP} active drugs.

Study Limitations

1. While the study of human hearts provides a unique opportunity to explore direct, clinical, electrophysiological effects of drugs such as diazoxide and pinacidil, the tradeoff is the limited number of explanted human hearts available. Due to limited access to functional human hearts, all of the CHF hearts with different cardiomyopathy were grouped together for the functional data analysis. Non-failing donor hearts without infarct should not be considered healthy human hearts. However, none of these donors had a history of CHF, and thus represent the best available controls for this functional study.
2. Coronary-perfused explanted hearts are not physiologically normal and are denervated. To stop muscle contraction, we used blebbistatin, which has been successfully applied in similar studies of rat and rabbit [24], canine [25], and human heart models [22,23]. However, the application of blebbistatin may help preserve ATP that may otherwise be depleted due to mechanical contraction, and thus enhance the metabolic state. Under such circumstances, metabolism-associated remodeling of K_{ATP} channels may be less evident.
3. Glibenclamide induced a prolongation of APD in NF RA but not in NF RV. This might suggest basal K_{ATP} activation occurring in the RA, but we cannot rule out the possibility that pinacidil and/or glibenclamide also affects I_{to} or I_{K1} currents [37].
4. In the normal canine heart, it has been shown that the epicardial myocardium can respond differently to K_{ATP} openers as compared to the endocardium [31]. However, we were unable to make any similar direct comparisons in the present study since the marked fibrosis and adiposity of the human epicardium obstructed optical access to it and restricted our optical mapping to the endocardium.

CONCLUSION

High-resolution optical mapping shows for the first time that remodeling due to CHF and/or infarct caused increased APD sensitivity to K_{ATP} channel openers in both human atria and ventricles. Activation of K_{ATP} channels in diseased human atria and ventricles lead to reentrant arrhythmias, such as AFL/AF and VT/VF.

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Non-standard Abbreviations and Acronyms

AFL/AF	atrial flutter and fibrillation
AP	action potential
APD	action potential duration
BCL	basic cycle length
K_{ATP}	ATP-sensitive potassium channel
CHF	heart with congestive heart failure
DF	dominant frequency
NF	heart without CHF
INF	heart without CHF but with history of infarction
RA	right atrium
RV	right ventricle
LA	left atrium
LV	left ventricle
SUR	sulfonylurea receptor
VT/VF	ventricular tachycardia and fibrillation
KCO	K_{ATP} channel opener

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Highlights

- We investigated ATP-regulated potassium channels in diseased and normal human heart
- Pinacidil decreased APD in atria and ventricles in all physiological states
- Diazoxide decreased APD in both chambers and all states, except normal human atria
- Heart failure and/or previous infarct potentiated diazoxide-induced APD shortening
- Activation of human K_{ATP} channels enhances arrhythmogenicity

A – Cross-sectional view of an explanted human heart. CS – coronary sinus; TV and MT – tricuspid and mitral valves; RAA – right atrial appendage; RAFW – right atrial free wall.

B - Experimental setup with the two CMOS cameras used simultaneously. Experimental setup and image of Left Atrial (LA) and Left Ventricular (LV) coronary perfused preparation (heart #2) with optical field of views (OFV).

C - Right heart preparation. The atria and ventricular OFVs contained the right atrial free wall (RAFW) and the right ventricular free wall (RVFW).

D – Left heart preparation. The atria and ventricular OFVs contained the left atrial free wall and appendage (LAFW and LAA) and the left ventricular free wall (LVFW).

Figure 2. Endocardial optical mapping of the human right atrial-ventricular preparation from the non-failing heart #1

A – Right atrial-ventricular preparation image of non-failing heart #1 with RA and RV OFVs.

B – Representative OAPs in the RA and RV.

C – Activation and APD80% maps of the RA during Control, diazoxide (300 μ M), and pinacidil (100 μ M). The color map scale is different between groups.

D – Activation and APD80% maps of the RV during Control, diazoxide (300 μ M), and pinacidil (100 μ M). The color map scale is different between groups.

Figure 3. Endocardial optical mapping of the human right atrial-ventricular preparation from failing heart #3

A – Right atrial-ventricular preparation image of failing heart #3 with RA and RV OFVs.

B –Representative OAPs in the RA and RV. Diazoxide (300 μ M) (green) and pinacidil (100 μ M) (pink) decreased APD compared to Control (blue).

C – Activation and APD80% maps of the RA during Control, diazoxide (300 μ M), and pinacidil (100 μ M). The color map scale is different between groups.

D - Activation and APD80% maps of the RV during Control, diazoxide (300 μ M), and pinacidil (100 μ M). The color map scale is different between groups.

Figure 4. Effect of K_{ATP} channel openers on the APD in isolated human hearts

A and **B** - Summary of the effects of diazoxide (300 μ M), pinacidil (100 μ M), and glibenclamide (10 μ M) in Failing (CHF), nonfailing with an infarct history (INF), and nonfailing with no infarct history (NF) atrial (Panel A) and ventricular (Panel B) tissues. APD80% values in Panel A and B below the threshold values indicated by the red dotted lines resulted in atrial flutter/fibrillation (AFL/AF) and ventricular tachycardia/fibrillation (VT/VF), respectively.

C - Action potential changes in percentages for CHF and NF hearts without significant infarct history.

Figure 5. KATP openers induced reentrant arrhythmias

A – Dominant Frequency maps and OAPs of pinacidil-induced reentrant arrhythmias. The OAPs in each example were selected from the site denoted by the white asterisk in the corresponding dominant frequency maps. The dotted arrows show the leading reentrant circuit in each K_{ATP} -induced reentrant arrhythmia example. The top two subpanels show sustained AF and VF simultaneously induced by fast pacing in the RA-RV preparation from the CHF heart#3. The bottom two subpanels show sustained AFL and VF simultaneously induced by fast pacing in the RA-RV preparation from the NF heart #1.

B – Arrhythmia Incidence. The incidences of arrhythmias were recorded as percentages of the total number of preparations as shown in the bar graph for Control (blue), diazoxide (green), and pinacidil (pink) groups. Pinacidil was statistically higher than the control group ($p < 0.05$).

C – Dominant Frequency. Dominant frequencies (F) of pacing-induced reentrant arrhythmias were measured and are displayed as a bar graph for the Control ($n=1$), diazoxide ($n=1$), and pinacidil ($n=6$) groups.

Figure 6.
mRNA expression of K_{ATP} subunits in the failing and nonfailing human heart. * - p<0.05

Table 1
Functional experiments heart history

Patient histories for both failing and non-failing hearts including sex, age, diagnosis.

Patient number	Preparation	Sex	Age	Diagnosis
Failing hearts				
1	RA/RV	M	59	Ischemic CM
2	RA/RV	M	52	Nonischemic CM
3	RA/RV	F	35	Restrictive CM
4	RA/RV	M	44	Ischemic CM
5	RA/RV	M	61	Ischemic CM
6	RA/RV	M	50	Hypertrophy CM
7	LA/LV	M	21	Idiopathic CM
8	LA/LV	F	18	Postpartum CM
Average			46 ± 5	
Non-Failing hearts				
1	RA/RV	M	19	Brain Death from accident
2	RA/RV	F	50	Stroke, Hypokinesia
3	RA/RV	M	54	Stroke, Coronary disease
4*	RA/RV	M	56	Stroke, Septal infarct
5*	RA/RV	F	57	Stroke, Anterior infarct
Average			47 ± 16	

CM- cardiomyopathy,

* non-failing hearts #4 and #5 with infarct history (INF group).

Table 2
mRNA experiments heart history

Patient histories for both failing and non-failing hearts including sex, age, diagnosis

Patient number	Preparation	Sex	Age	Diagnosis
Failing hearts				
1	LA/LV	M	50	Ischemic CM
2(5)	LA/LV	M	61	Ischemic CM
3	LA/LV	M	63	Ischemic CM
4	LA/LV	M	50	Ischemic CM
Average			56 ± 7	
Non-Failing hearts				
1	LA/LV	M	68	Stroke
2	LA/LV	M	55	Stroke
3(2)	LA/LV	M	50	Stroke, Hypokinesia
4	LA/LV	M	59	Brain Death from accident
5	LA/LV	F	59	Anoxic brain injury
6	LA/LV	F	55	Stroke
7	LA/LV	F	55	Anoxic brain injury
8	LA/LV	F	66	Brain death
Average			58 ± 6	

CM- cardiomyopathy,

Failing heart #2 is a heart #5 from the Table 1.

Non-failing heart #3 is a heart #2 from the Table 1.

Table 3

Incidence of Atrial Arrhythmias

Analysis of atrial arrhythmias in failing and non-failing RA and LA preparations including functional refractory period (FRP), occurrences of AFL/AF, and dominant frequency (DF) in AFL/AF. The control preparations had no occurrences of AFL/AF.

Heart number	Tissue	Control	Diazoxide		Pinacidil	
		FRP, ms	AFL/AF	FRP, ms	AFL/AF	DF, Hz
Failing hearts						
1	RA	280	no	240	no	80 AFLs 5.0
2	RA	330	no	300	no	100 AFLs 10.3
3	RA	270	no	150	no	75 AFs 16.3
4	RA	210	no	150	no	100 AFs 17.2
5	RA	300	no	300	no	150 AFL/AFs 6.8
6	RA	200	no	140	AFLs	150 AFs 14.4
7	LA	180	no	300	no	100 AFs 20.5
8	LA	190	no	125	AFns	11.5 AFs 23.0
Average						
		245 ± 20	0 out 8	213 ± 28	2 out 8	9.2 ± 2.3 126 ± 20 8 out 8 14.2 ± 2.3
Non-Failing hearts						
		FRP, ms	AFL/AF	FRP, ms	AFL/AF	DF, Hz
1	RA	170	no	150	no	80 AFLs 9.3
2	RA	150	no	150	no	125 AFL/AFs 7.3
3	RA	175	no	175	no	125 AFL/AFs 22
4*	RA	250	no	190	no	80 AFL/AFs 19.7
5*	RA	180	no	110	no	100 AFL/AFs 14.8
Average						
		185 ± 17	0 out 5	155 ± 14	0 out 5	102 ± 10 5 out 5 14.7 ± 2.9

AF/AFLs- sustained arrhythmia; AFns- nonsustained AF;

* non-failing hearts #4 and #5 with infarct history (INF group).

Incidence of Ventricular Arrhythmias

Table 4

Analysis of ventricular arrhythmias in failing and non-failing RV and LV preparations included functional refractory period (FRP), occurrences of VF/VT, and dominant frequency (DF) in VF/VT.

Heart number	Tissue	Control	Diazoxide			Pinacidil		
Failing hearts		FRP, ms	VF/VT	DF, Hz	FRP, ms	VF/VT	DF, Hz	DF, Hz
1	RV	300	VFs	5.9	240	no	350	VFs 19
2	RV	350	no		300	no	spont	VFs 9.7
3	RV	300	no		150	VFns	100	VFs 20.5
4	RV	300	no		150	VFs	spont	VFs 13.9
5	RV	300	no		300	no	150	no
6	RV	300	no		300	no	110	VFs 15
7	LV	210	no		125	VFns	spont.	VFs 27.0
8	LV	280	no		300	no	150	VFs 18.3
Average		293 ± 14	1 out 8		233 ± 28	3 out 8	210 ± 46	7 out 8 17.6 ± 2.1
Non-Failing hearts		FRP, ms	VF/VT	DF, Hz	FRP, ms	VF/VT	DF, Hz	DF, Hz
1	RV	200	no		150	no	80	VFns 14
2	RV	150	no		150	no	125	VFs 21.5
3	RV	175	VFs	10.2	175	no	125	VTs 11.7
4*	RV	250	no		180	no	spont	VTs 24.1
5*	RV	350	no		190	no	100	VTs 15.8
Average		225 ± 35	1 out 5		169 ± 7	0 out 5	108 ± 10	5 out 5 17.4 ± 2.3

VF/VTs- sustained arrhythmia; VFns- nonsustained VF