Role of $K_{ATP}$ Channels in the Maintenance of Ventricular Fibrillation in Cardiomyopathic Human Hearts


**Rationale:** Ventricular fibrillation (VF) leads to global ischemia. The modulation of ischemia-dependent pathways may alter the electrophysiological evolution of VF.

**Objective:** We addressed the hypotheses that there is regional disease-related expression of $K_{ATP}$ channels in human cardiomyopathic hearts and that $K_{ATP}$ channel blockade promotes spontaneous VF termination by attenuating spatiotemporal dispersion of refractoriness.

**Methods and Results:** In a human Langendorff model, electric mapping of 6 control and 9 treatment (10 µmol/L glibenclamide) isolated cardiomyopathic hearts was performed. Spontaneous defibrillation was studied and mean VF cycle length was compared regionally at VF onset and after 180 seconds between control and treatment groups. $K_{ATP}$ subunit gene expression was compared between LV endocardium versus epicardium in myopathic hearts. Spontaneous VF termination occurred in 1 of 6 control hearts and 7 of 8 glibenclamide-treated hearts ($P=0.026$). After 180 seconds of ischemia, a transmural dispersion in VF cycle length was observed between epicardium and endocardium ($P=0.001$), which was attenuated by glibenclamide. There was greater gene expression of all $K_{ATP}$ subunit on the endocardium compared with the epicardium ($P<0.02$). In an ischemic rat heart model, transmural dispersion of refractoriness ($\Delta ERPE_{\text{Transmural}}=\Delta ERPE_{\text{Epicardium}}-\Delta ERPE_{\text{Endocardium}}$) was verified with pacing protocols. $\Delta ERPE_{\text{Transmural}}$ in control was $5 \pm 2$ ms and increased to $36 \pm 5$ ms with ischemia. This effect was greater attenuated by glibenclamide ($\Delta ERPE_{\text{Transmural}}$ for glibenclamide+ischemia=$4.9 \pm 4$ ms, $P=0.019$ versus control ischemia).

**Conclusions:** $K_{ATP}$ channel subunit gene expression is heterogeneously altered in the cardiomyopathic human heart. Blockade of $K_{ATP}$ channels promotes spontaneous defibrillation in cardiomyopathic human hearts by attenuating the ischemia-dependent spatiotemporal heterogeneity of refractoriness during early VF. (Circ Res. 2011;109:1309-1318.)

**Key Words:** ventricular fibrillation ■ arrhythmia ■ potassium channels ■ glibenclamide

Ventricular fibrillation (VF), once established, rarely self-terminates. The factors that tend to maintain reentry in fibrillating myocardium include increases in spatial dispersion of refractoriness and time-dependent alteration of refractory periods, which may occur in a spatially heterogeneous manner.1–4 VF leads to global ischemia and resultant activation of cardiac $K_{ATP}$ channels, which shorten action potential duration and refractoriness.5 If the expression and/or function of $K_{ATP}$ channels is heterogeneous, VF-induced ischemic $K_{ATP}$ channel activation may also result in increasing spatiotemporal dispersion of refractoriness, providing conditions that are conducive for sustaining VF after its initiation. Thus, $K_{ATP}$ channel blockade may prevent shortening of refractoriness and cause attenuation of spatiotemporal dispersion of refractoriness during early VF, thereby forestalling VF perpetuation. In some conditions, such as ischemic cardiomyopathy, it has been suggested that the expression of $K_{ATP}$ subunits is altered in infarct border zone.6 We have previously presented preliminary data suggesting that there is altered expression of $K_{ATP}$ channel subunits in human cardiomyopathic hearts compared with normal hearts.7 It is not known if there is spatial heterogeneity and/or altered expression of $K_{ATP}$ channels in human cardiomyopathic hearts and if modulation of these channels in cardiomyopathic states alters the maintenance of VF.

We tested the hypotheses that (1) there is differential regional/disease-related expression of $K_{ATP}$ channels in human cardiomyopathic hearts, causing spatiotemporal dispersion of refractoriness, and that (2) blockade of $K_{ATP}$ channels...
by glibenclamide promotes spontaneous VF termination by attenuating this spatiotemporal dispersion.

**Methods**

All procedures conformed to the Helsinki Declaration of the World Medical Association. Only essential information is presented in this article in summary form. Please see the Online Data Supplement at http://circres.ahajournals.org for complete methodological details.

**Human Langendorff Model**

After informed consent, hearts were explanted from 15 patients with dilated cardiomyopathy who underwent cardiac transplantation and were Langendorff-perfused.

**Electric Mapping**

Electric mapping of the Langendorff-perfused hearts was performed with an epicardial sock covering the entire epicardial surface and a left ventricular (LV) endocardial balloon (Figure 1B and 1C). VF was recorded in ischemic condition for 20 seconds at the onset and after 180 seconds, in control and treatment groups (10 μmol/L glibenclamide) (Figure 1A). We compared spontaneous defibrillation and effective refractory period (ERP) dispersion between control and treatment groups.

**Spontaneous Defibrillation**

The abrupt termination of VF without electric defibrillation during an episode of induced VF (lasting spontaneously >30 seconds) was considered to represent spontaneous defibrillation.

**Measurement of ERP During VF**

Since the traditional method of ERP measurement cannot be used at multiple sites during VF because of the dynamic nature of VF,8 we used average local fibrillation intervals as a surrogate for ERP.9–14 To further validate the mechanisms found in human hearts, we studied the consequences of ischemia on Langendorff-perfused rat hearts with direct ERP and action potential measurements (see below).

**Spatial Dispersion of Refractoriness**

Dispersion of refractoriness was calculated between LV epicardium and endocardium (LV transmural dispersion), LV and right ventricular (RV) (interventricular dispersion), anterior and posterior LV (anterio-posterior dispersion) and apex and base (apico-basal dispersion).

**Non-standard Abbreviations and Acronyms**

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<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>APD</td>
<td>action potential duration</td>
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<td>CL</td>
<td>cycle length</td>
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<td>effective refractory period</td>
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<td>K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>ATP-sensitive potassium channels</td>
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**Figure 1.** A, Protocol used to compare the number of hearts with spontaneous terminations before and after the administration of glibenclamide. Top panel of A (subpanel A) shows the treatment group in which a VF episode was followed by the administration of glibenclamide. Glibenclamide was allowed to circulate in the Langendorff system for 5 minutes, after which VF with ischemia was reinitiated and any spontaneous terminations documented. In the control group, the initial episode of VF was followed by a rest period of 5 minutes, after which the VF with ischemia was reinitiated and any spontaneous terminations of VF documented. This allowed us to compare spontaneous terminations in the same time frame in which glibenclamide was administered, to exclude time after VF and/or ischemia as a possible contributing factor to these spontaneous terminations. B, Epicardial sock; C, endocardial balloon in LV cavity, each having 112 unipolar/bipolar electrodes, arranged in 14 columns and 8 rows and mounted on an extensible mesh.
Human Heart Samples
Human heart samples were dissected from normal and cardiomyopathic hearts for RNA and protein expression.

RNA Preparation and Quantitative PCR
RNA was isolated from endocardial and epicardial samples for quantitative PCR. β-Actin served as the control.

Langendorff-Perfused Rat Hearts
This protocol was approved by University Health Network Ethics Board. Male Sprague-Dawley rats were anesthetized. Hearts were rapidly isolated and Langendorff-perfused.

Electric Recording
The heart was placed on a plastic base in which 4 contact electrodes were inserted (Figure 2A). Two electrodes were used to record LV epicardial signals and 2 were used for pacing from LV epicardium. For endocardial mapping and pacing, 4 electrodes were inserted into the LV cavity, with 2 electrodes used for endocardial recording and 2 for endocardial pacing (Figure 2B).

ERP Dispersion Determination Using S₁-S₂ Protocol
Figure 2C shows the experimental protocol. Hearts were paced at 150 ms (S₁) at twice diastolic threshold, and an extrastimulus (S₂) was delivered, starting from S₁-S₂ = 140 ms, decreasing until capture was lost. ERP dispersion and arrhythmogenesis was measured in control group (no treatment) and after glibenclamide, pinacidil, and lidocaine.

Floating Microelectrode Recording
Recordings were obtained from epicardium and endocardium under control conditions and during ischemia with and without drug treatment.

Statistical Analysis
Data were analyzed using SAS 9.0. Values are shown as mean±SEM. The Fisher exact test and ANOVA were used, and a probability value <0.05 was considered statistically significant.
Results

Spontaneous Defibrillation

Fifteen human hearts (6 control and 9 treatment) were studied. Spontaneous VF termination occurred in only 1 of 6 control hearts. In the 9 treatment hearts, spontaneous termination occurred in 2 hearts before glibenclamide administration. Figure 3A shows an example of unipolar electrograms during VF and ischemia. After treatment with glibenclamide (n=9), VF could not be induced in 1 heart and terminated spontaneously in 7 of the remaining 8 hearts (P=0.026 versus control). Figure 3B shows an example of spontaneous termination of VF after the administration of glibenclamide.

$K_{\text{ATP}}$ Channel Blockade and Spatiotemporal Dispersion of Refractoriness During Fibrillation in Human Langendorff Model

Transmural Dispersion

Initially, mean VF cycle length (CL) was compared, averaging VF at onset and 180 seconds, between epicardium and endocardium in the control and glibenclamide treatment groups. Mean VFCL was 248±8 ms and 289±27 ms in control and 397±28 ms and 408±27 ms in the treatment group (P=0.001 versus control), in LV endocardium and epicardium, respectively. The VFCL was then compared between epicardium and endocardium in onset and early VF (180 seconds). At the onset of VF, the mean VFCL in LV endocardium and epicardium was 230±13 ms and 242±25 ms in the control group and 343±29 ms and 353±32 ms in the treatment group (Figure 4A). The difference between control group and the treatment group was statistically significant (P=0.017), whereas the difference between epicardium and endocardium was not significant. After 180 seconds of VF, as ischemia progressed, a gradient in VFCL developed in the control group, with a mean VFCL of 279±17 ms and 337±36 ms in LV endocardium and epicardium, respectively (P=0.01). In the treatment group, the difference was attenuated, with a mean VFCL of 454±34 ms and 463±27 ms, respectively, in endocardium and epicardium (P=0.6), suggesting that $K_{\text{ATP}}$ channels play a key role in establishing this gradient. Figure 3C illustrates refractoriness for the LV endocardium and epicardium during the VF sequences in 1 heart.

Interventricular Dispersion

Mean VFCL was 297±29 ms and 274±22 ms in the control group and 408±28 ms and 496±24 ms in the treatment group for LV epicardium and RV epicardium, respectively. The difference was statistically significant between control and treatment (P<0.001) but not significant between LV and RV epicardium (P=0.69). VFCL was then compared in onset and early VF (180 seconds). At the onset of VF, mean VFCL in LV and RV epicardium was 248±28 ms and 237±16 ms in control group and 353±3 ms and 365±29 ms in the treatment group. The difference between control and treatment groups was statistically significant (P=0.02), whereas the difference between LV epicardium and RV epicardium was not significant (P=0.9). During early VF, a small difference of 32 ms was observed between LV and RV epicardium (LV epicardium=345±37 ms, RV epicardium=313±34 ms), but this failed to achieve statistical significance (P=0.07). In the treatment group, the difference was attenuated, with a mean VFCL of 463±27 ms and 450±26 ms in LV and RV epicardium, respectively (P=0.23).

Antero-Posterior Dispersion

At the onset of VF, the mean VFCL was 231±30 ms and 239±30 ms in the control group (P=0.9) and 345±26 ms and 350±25 ms in the treatment group (P=0.9) in anterior and posterior LV epicardium, respectively. During early VF (180 seconds), mean VFCL in the control group was 300±33 ms and 333±33 ms in anterior and posterior LV epicardium, respectively. The difference of 33 ms between anterior and posterior LV epicardium after 180 seconds of ischemia failed to achieve statistical significance (P=0.5). After the administration of glibenclamide in the treatment group, this difference was attenuated, with a mean VFCL of 453±28 ms and 455±28 ms in the anterior and posterior LV epicardium, respectively (P=0.9).
Apico-Basal Dispersion
Mean VFCL at the apex and the base of the heart was 258 ± 110 ms and 287 ± 29 ms in the control group and 373 ± 25 ms and 423 ± 25 ms in the treatment group, respectively. In both regions, the difference was statistically significant between control and treatment groups (P < 0.001), but the difference between apex and base was not statistically significant (P = 0.15). During onset VF, the mean VFCL was 217 ± 15 ms and 264 ± 23 ms in the control group (P = 0.9) and 344 ± 29 ms and 369 ± 36 ms in the treatment group (P = 0.2) at the apex and base, respectively. During early VF (180 seconds), VFCL was 306 ± 40 ms at the apex and 347 ± 31 ms at the base in the control group (P = 0.8) and 445 ± 36 ms at the apex and 471 ± 25 ms at the base in the treatment group (P = 0.9). Though a small difference in VFCL was observed between apex and base, it was consistent in the onset, and early VF and was not affected by glibenclamide.

K<sub>ATP</sub> Channel Subunit Regional mRNA Expression in Cardiomyopathic Human Hearts
We quantified the gene expression of the 4 common subunits that make up the human cardiac K<sub>ATP</sub> channels, Kir6.1, Kir6.2, SUR1, and SUR2A, in epicardium and endocardium in 6 myopathic hearts.

K<sub>ATP</sub> Subunit Expression in LV Endocardium Versus Epicardium
K<sub>ATP</sub> subunit expression was done to evaluate if the LV transmural gradient of refractoriness was related to the differential expression of K<sub>ATP</sub> subunits in epicardium versus endocardium in cardiomyopathic hearts. All expression levels are presented relative to the housekeeping gene and normalized to LV endocardium; hence the mean expression level for all K<sub>ATP</sub> subunits in LV endocardium was 100 and the standard error was zero. Mean expression levels in LV epicardium were 62 ± 10% (P = 0.02), 59 ± 13% (P = 0.03), 57 ± 11% (P = 0.016), and 72 ± 5% (P = 0.004) for Kir6.1, Kir6.2, SUR1, and SUR2A, respectively.

K<sub>ATP</sub> Channel Expression in LV Epicardium Versus RV Epicardium
We compared K<sub>ATP</sub> gene expression in LV epicardium and RV epicardium. Mean expression levels (relative to house-
keeping gene and relative to LV endocardium) were 62±10%, 59±13%, 57±11%, and 72±5% for Kir6.1, Kir6.2, SUR1, and SUR2A, respectively, in the LV epicardium, and 80±38% (P=0.68), 106±47% (P=0.44), 80±32% (P=0.60), and 128±69% (P=0.47) in the RV epicardium.

**Langendorff-Perfused Rat Model**

**ERP Dispersion During K$_{ATP}$ Modulation**

Dispersion in refractoriness (ΔERP) was defined as the difference in the refractoriness between epicardium and endocardium (Figure 2D). Twenty-eight hearts were studied with a traditional S$_1$S$_2$ protocol. Mean ΔERP was 5.2 ms and 36±5 ms during baseline (without ischemia) and ischemia (P=0.02 versus baseline), respectively. After 10 μmol/L glibenclamide (n=13), mean ΔERP was 0.1±3.0 ms and 4.9±4.0 ms during baseline (no ischemia) and ischemia with glibenclamide (P=0.9 versus glibenclamide alone), respectively. Hence, glibenclamide significantly attenuated the ΔERP during acute ischemia (P=0.019). In 7 hearts, ERP in control conditions was followed by pinacidil. Mean ΔERP dispersion in the presence of pinacidil was 2.7±2.0 ms in the baseline condition and 28±13 ms during acute ischemia. There was no statistically significant difference in ΔERP after pinacidil, whether in the baseline condition or acute ischemia.

**Arrhythmogenesis During K$_{ATP}$ Modulation**

During control conditions, cardiac arrhythmia occurred in 15 of 28 hearts in the baseline condition and 19 of 28 hearts during acute ischemia. The difference was not statistically significant (P=0.4). In 13 hearts, measurements in control conditions were followed by 10 μmol/L glibenclamide. After glibenclamide, cardiac arrhythmias occurred in 10 of 13 hearts in the baseline condition (no ischemia) and 4 of 13 hearts during acute ischemia (P=0.047). When compared with control, glibenclamide significantly suppressed the incidence of cardiac arrhythmias during acute ischemia (4/13 versus 19/28, P=0.043). In 7 hearts, measurements during control conditions were followed by 10 μmol/L pinacidil. After pinacidil, cardiac arrhythmias occurred in 5 of 7 hearts in the baseline condition (no ischemia) and 7 of 7 hearts during acute ischemia (P=0.5). When compared with control, there was no difference in the incidence of cardiac arrhythmias after pinacidil during acute ischemia (P=0.2).

**Action Potential Changes During K$_{ATP}$ Modulation**

To study the effect of K$_{ATP}$ modulation on action potential duration, membrane potentials were measured under control conditions and after K$_{ATP}$ modulation (Figure 2E). Action potential durations at 90% of repolarization (APD$_{90}$) were analyzed. In the baseline control condition (no ischemia, no drug), mean APD$_{90}$ was 56±3 and 55±2 ms in endocardium and epicardium, respectively (n=17, P=NS). Glibenclamide did not cause any significant changes in APD in the baseline condition (n=7, APD$_{90}$ epicardium: 58±5 ms, endocardium: 53±3 ms; P=NS). Pinacidil resulted in APD shortening, mainly in epicardium (n=5; mean APD$_{90}$ epicardium: 41±8 ms; pinacidil versus baseline; P=0.049).

After 3 minutes of ischemia, APD$_{90}$ was significantly shorter on epicardium compared with endocardium (epicardium: 55±6 ms; endocardium: 82±5 ms; P=0.02). After the administration of glibenclamide, the difference in APD$_{90}$ between the epicardium and endocardium was attenuated and was not statistically significant during acute ischemia (APD$_{90}$ at 3 minutes of acute ischemia; endocardium: 83±5 ms, epicardium: 83±12 ms; P=NS).

**Lidocaine Effects on ERP and APD**

In 8 hearts, control ERP dispersion measurements were followed by administration of 10 μmol/L lidocaine. ERP dispersion measurements were repeated in the presence of lidocaine under baseline conditions as well as during ischemia. The mean ERP dispersion was 24±6 ms in the baseline condition (no ischemia). During ischemia, the mean ERP dispersion was 91±21 ms. The difference of dispersion between baseline and acute ischemia after lidocaine was statically significant (P=0.006). When compared with control, lidocaine resulted in increased transmural gradient of ERP during ischemia (P=0.02). After lidocaine, arrhythmias resulted in 50% hearts (4/8), not statistically significant from control (P=0.2). Lidocaine had no significant effect on APD$_{90}$ either in epicardium or endocardium (n=5; mean APD$_{90}$ epicardium: 50±3 ms; endocardium: 57±2 ms). Similarly, lidocaine had no significant effect on APD in acute ischemia (eg, mean APD$_{90}$ after 3 minutes of ischemia: epicardium 43±13 ms, endocardium 70±32 ms; P=NS versus no-drug control).

**Discussion**

We have demonstrated that K$_{ATP}$ channel blockade during VF promotes spontaneous defibrillation of cardiomyopathic human hearts by attenuating the ischemia-dependent spatiotemporal dispersion of refractoriness during early VF. The molecular basis probably involves heterogeneous and altered expression of K$_{ATP}$ channel subunits in cardiomyopathic hearts. After 180 seconds of VF, there is heterogeneity in refractoriness which is attenuated by blockade of K$_{ATP}$ channels. Taken together, our findings indicate that glibenclamide promotes spontaneous defibrillation of VF in the setting of global ischemia by the attenuation of spatiotemporal heterogeneity and increasing refractoriness during VF in cardiomyopathic hearts.

**Spontaneous Defibrillation**

Spontaneous defibrillation (termination of VF without high-voltage shock) is an uncommon event that has occasionally been reported in certain animal species and in humans, but rarely in a cardiomyopathic state. The development of therapeutic strategies that would promote spontaneous defibrillation has significant clinical implications for prevention of sudden cardiac death in cardiomyopathic patients at the highest risk of VF. It is well described that shortening of the APD plays an important role in maintaining cardiac arrhythmias. There are various reports that glibenclamide can either prevent or partially reverse the APD shortening in hypoxia and ischemia in both isolated pig myocardium and in situ guinea pig myocardium. Our observations in the current study suggest that during VF, glibenclamide prevents APD shortening caused by acute hypoxia, ischemia, and K$_{ATP}$
activation, leading to attenuation of spatiotemporal dispersion of refractoriness, thus causing spontaneous termination. A gradient in local activation rate/refractoriness existed between the LV endocardium and epicardium, probably due to differential $K_{ATP}$ channel gene expression. Glibenclamide, through $K_{ATP}$ channel blockade, resulted in a loss of this gradient. These observations suggest that spatiotemporal action potential characteristics within the LV contribute to the maintenance of VF in cardiomyopathic human hearts and are in part controlled by regionally differential consequences of $K_{ATP}$ activation, the effects of which are attenuated by $K_{ATP}$ channel blockade.

A dominant-frequency gradient has been reported during VF in various animal models and in human models. We hypothesized that differential function of $K_{ATP}$ channels might contribute to this gradient. The fact that blockade of $K_{ATP}$ channels by glibenclamide abolished this gradient supports this hypothesis. We further tested the hypothesis by studying the differential expression of $K_{ATP}$ channel subunits in LV epicardium and endocardium in cardiomyopathic hearts. The mRNA expression levels of $K_{ATP}$ channel subunits were significantly higher in LV endocardium compared with LV epicardium. Miyoshi et al have reported that blockade of cardiac $K_{ATP}$ channels by glibenclamide in dogs suppresses the extracellular $K^+$ rise in epicardium, whereas the extracellular $K^+$ level in endocardium remains unaffected. We attribute this difference to species specific differential functioning of $K_{ATP}$ channels in the heart. It should also be noted that the hearts that we studied were cardiomyopathic hearts explanted from patients undergoing cardiac transplantation.

$K_{ATP}$ Modulation in Experimental VF

In an in vivo ischemic porcine model, the $K_{ATP}$ channel opener pinacidil significantly shortened the ERP from 162±16 to 130±28 ms and increased the peak frequency of the LV power spectrum during VF from 9.3±0.6 to 10.5±1.0 Hz. Our data are consistent with the concept proposed regarding $K_{ATP}$ modulation during VF and also with the observed relation between refractory period and cycle length. Because measuring dynamic ERP changes during VF in human hearts was impossible, we used frequency indices as indicators of ERP change and conducted confirmatory experiments in an animal model to measure dispersion of refractoriness (ΔERP) with traditional $S_1-S_2$ methods. In the absence of ischemia, there was no difference in ΔERP between control and glibenclamide groups (5±2 versus 0.1±3.0 ms), but during ischemia there was a large, statistically significant difference (36±5 ms versus 4.9±4.0 ms). These results implicate $K_{ATP}$ activation and consequent increased ERP heterogeneity in ischemic conditions that could potentially serve as a substrate for sustaining VF. The fact that ventricular arrhythmias were ameliorated by glibenclamide supports this concept.

$K_{ATP}$ Subunit Expression

In rodent ventricular cardiomyocytes, Kir6.2/SUR2 make up the dominant $K_{ATP}$ channel expression. There is, however, heterogeneity within rodent hearts, with atrial cardiomyocytes expressing Kir6.2/SUR1. Although the classic understanding is that the $K_{ATP}$ channel in cardiomyocytes is composed mainly of Kir6.2 and SUR2A, more recent findings suggest that Kir6.1 and SUR1 subunits may also play significant roles. Our study shows that in cardiomyopathic human left ventricles, gene expression of Kir6.1, Kir6.2, SUR1, and SUR2A is heterogeneous with greater preponderance on the endocardium. Kir6 subunit upregulation may contribute to the substantial time-dependent increases in ERP-heterogeneity during VF in cardiomyopathic hearts. Tavares et al showed similar Kir6.1-upregulation in a rodent cardiomyopathic model. Hence, the relative expression of $K_{ATP}$ channels may be different not only in different tissues but also in different species and in different disease states. Establishing effective clinical treatments with $K_{ATP}$ blockade requires further evaluation of the mechanism and physiological significance of differential $K_{ATP}$ subunit expression/function with heart disease. The present study provides a basis for such further work by identifying changes in $K_{ATP}$ subunit gene expression in cardiomyopathic human hearts.

A difference in refractoriness was observed between cardiomyopathic LV epicardium and endocardium that was attenuated by glibenclamide. In our data set, it was accompanied by increased gene expression of all $K_{ATP}$ subunits in LV endocardium compared with epicardium. There are other potential mechanisms of transmural $K_{ATP}$ function gradients in addition to transmural protein expression gradients in underlying subunits. There could be another, unidentified subunit associated with Kir6/SUR subunits that importantly modifies its function and shows differential endocardial/epicardial expression. Alternatively, differences in myocardial energetics and oxygen demand could produce discrepant $K_{ATP}$ activation in LV endocardium versus epicardium. Lee et al have reported that glibenclamide inhibits Na$^+$-K$^+$ pump and Ca$^{2+}$ channels in guinea pig hearts. These effects were observed at much higher concentrations (≥100 μmol/L) than used in our study. Still, the possibility of involvement of off-target actions on other ion channels cannot be ruled out. The differential activation rate seen in our study could have another potential explanation. Conduction block of wave fronts should be considered as an explanation in addition to local refractoriness changes. Morley et al previously showed in a single-cell preparation that metabolic inhibition has a significant influence on intercellular membrane resistance and may promote conduction block. However, in our rat heart study, we paced locally in a tissue preparation and were able to establish that indeed refractoriness was locally altered.

Potential Application

The only effective therapeutic strategy by which VF can be terminated once it has occurred is electric defibrillation. For a defibrillation shock to succeed, it must extinguish existing VF activations throughout a critical mass of myocardium, as well as not initiate new fibrillatory wave fronts that propagate into regions containing excitable gaps. Therefore, the present clinical strategy involves the use of implantable defibrillator devices in cardiomyopathic patients. Various chronic pharmacological strategies have been tested for the prevention or termination of VF with
limited success, primarily due to proarrhythmic effects.36–40 The strategy that we tested presents the intriguing possibility of extinguishing VF activations throughout the myocardium by prolonging refractoriness and not initiating new fibrillatory wave fronts due to voltage gradients set up by high-voltage shocks. In addition, by virtue of having therapeutic electrophysiologic properties mainly during ischemia, this strategy has the potential of maximal effect during the global ischemia that accompanies VF. Recently, a therapeutic strategy using spatially focalized intervention to modulate Na+ channel expression in the border zone of infarcted hearts has been proposed.41 Our findings suggest a role for modulation of global ischemia-dependent pathways in a nonelectrical focal temporal defibrillation strategy by blockade of ischemia-activated KATP channels in diseased human hearts.

KATP channel blockade may have a potential role in both the primary and secondary prevention of VF arrest. We used 10 μmol/L glibenclamide, which is a higher concentration than used in reperfusion injury studies. If used clinically, this could cause hypoglycemia in normoglycemic patients. However, the use of cardioselective KATP channel blockers such as HMR188342–44 could obviate this problem in developing this treatment paradigm. In addition, cardiac KATP channel blockers could prove to be useful adjuncts when defibrillation fails or proves difficult after cardiac surgery or for resistant VF in the cardiopulmonary resuscitation setting.

Limitations
This study was performed in a Langendorff-perfused human heart model. It is not possible with this model to study the effect of interplay of different neural and autonomic mechanisms on VF that can occur in vivo; hence, care should be maintained when extrapolating the results of this study to the clinical setting. We studied the effect of KATP channel modulation in acute ischemia in isolated cardiomyopathic human hearts. Because the relative mRNA expression of KATP subunits is also different in normal and cardiomyopathic hearts, the effect of modulation of KATP channels in these scenarios needs further investigation.

It has been reported that blockade of KATP channels might counteract ischemic preconditioning and may aggravate acute ischemic injury. The emphasis of our study was on the role of KATP channel blockade in VF during acute ischemia, and we did not study the effect of KATP channel modulation on ischemic injury and preconditioning. It may, however, be possible to retain an antiarhythmic effect of KATP channel blockade without adverse consequences for cardioprotection by selectively targeting sarcolemmal KATP channels, which mediate action potential changes while leaving intact mitochondrial KATP channels, which are central to preconditioning.

In our human Langendorff model, we used VFCL as a surrogate for ERP to obtain an ERP index simultaneously in multiple cardiac regions and to study the rapidly developing temporal evolution of refractoriness. Though VFCL may overestimate absolute refractoriness, previous studies suggest that there is considerable agreement between pacing-based determination of refractoriness and CL in fibrillation.45,46 Moreover, we confirmed our principal findings with direct ERP and APD measurements in an isolated rat heart model.

In rats, transmural dispersion of ERP and APD increased with ischemia in a glibenclamide-suppressible way, as in humans; however, we observed greater ischemic ERP shortening in epicardium versus endocardium in rats, different from humans. This may be due to a species difference, because in 2 human hearts in which we were able to study monophasic action potentials during ischemia, endocardial APD decreased more than epicardial with ischemia, and the difference was attenuated with 10 μmol/L glibenclamide (Online Data Supplement), fully consistent with our VFCL results.

Western blots for protein expression of KATP channel subunits were attempted. Antibodies available for KATP subunits were imperfect. We were unable to obtain data for SUR2A, and the staining characteristics of the available SUR1 and SUR2B antibodies were suboptimal. In addition, molecular masses of bands detected by the SUR antibodies in human tissue were different from those in heterologous systems. Finally, GAPDH signals in our SUR2B control samples were degraded, and we therefore had to use Ponceau staining as a loading control. We have provided complete original blots in the Online Data Supplement (Online Figures I through Figure IV), and our results must be interpreted in the light of these limitations.

Conclusions
In this study, we have demonstrated that in cardiomyopathic human hearts, heterogeneous regulation of KATP subunit gene expression is associated with increased spatiotemporal dispersion of refractoriness during VF. Glibenclamide promotes spontaneous VF termination by attenuating spatiotemporal dispersion of refractoriness that develops with ischemia as a consequence of VF. These findings suggest a role for modulation of global ischemia-dependent pathways in nonelectrical defibrillation strategies for VF.

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Disclosures
None.

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Novelty and Significance

What Is Known?

- Ventricular fibrillation (VF) is the most common cause of sudden cardiac death.
- High-voltage electric shock is the most common procedure to terminate VF.

What New Information Does This Article Contribute?

- During VF in myopathic human hearts, there is spatio-temporal heterogeneity in refractoriness across the left ventricular myocardium.
- Blockade of $K_{ATP}$ channels by glibenclamide attenuates spatio-temporal heterogeneity in refractoriness, causing spontaneous termination of VF.

VF is the most common cause of sudden cardiac death. The only effective therapeutic strategy by which VF can be terminated once it has occurred is electric defibrillation. In this study, we demonstrate for the first time that in cardiomyopathic hearts, gene expression of $K_{ATP}$ channel subunits is altered. This is accompanied by difference in refractoriness in myopathic hearts between left ventricular epicardium and endocardium. Blocking $K_{ATP}$ channels by glibenclamide decreases this transmural difference in refractoriness and increases spontaneous termination of VF. These findings raise the intriguing possibility of promoting spontaneous defibrillation of VF using intrinsic metabolic mechanisms. In addition, by virtue of having therapeutic electrophysiological properties mainly during ischemia, this strategy has the potential of maximal effect during the global ischemia that accompanies VF. Therefore, $K_{ATP}$ channel blockade may have a potential role in both the primary and secondary prevention of VF arrest. In addition, cardiac $K_{ATP}$ channel blockers could be useful adjuncts when defibrillation fails or proves difficult after cardiac surgery or for treating resistant VF in the setting of cardiopulmonary resuscitation.
Supplemental Material

Methods:

Human Langendorff Model

Informed consent was obtained from all patients undergoing cardiac transplantation. The hearts studied were explanted from fifteen patients with dilated cardiomyopathy who underwent cardiac transplantation. Upon removal, the hearts were immediately placed in cold Tyrode solution and were taken to the laboratory within 5 minutes. Hearts were perfused retrogradely through the aorta in Langendorff mode using modified Tyrode’s solution (composition (millimolar): 118.06 NaCl, 4.69 KCl, 3.33 CaCl₂(H₂O)₂, 1.16 MgSO₄, 24.88 NaHCO₃, 1.17 NaPO₄, and 6.056 glucose) and flushed thoroughly to remove blood particles. The Tyrode-solution was oxygenated with a pediatric oxygenator connected to a carbogen (95%-O₂/5%-CO₂) cylinder. In cases where we did not have enough aortic tissue, selective cannulation of right and left coronary arteries was performed. The height of the perfusion reservoir was adjusted to maintain a constant pressure in the range of 60-65 mmHg. The temperature was continuously monitored and maintained at 37° C. The epicardial/endocardial temperatures never varied by more than 0.25° C, even during global ischemia. The flow rate of the system was maintained between 0.9 - 1.1 ml/g/min.

Electrical Mapping

The construction and configuration of the electrical arrays has been described previously¹. Each sock and balloon consists of 112 unipolar/bipolar electrodes arranged in 14 columns and 8 rows (14x8=112), mounted on an extensible mesh. Each electrode consists of two silver beads, each acting as a pole of a bipolar electrode. The inter-electrode distance between two silver beads of a bipolar electrode is 2.1 mm (centre to centre) and the distance between two electrodes is 1 cm². The electrograms acquired were amplified, multiplexed and stored on a hard drive for retrieval and analysis.

Study Protocol (Figure 1A)

After all surgical preparation was completed; the hearts were allowed to stabilize for 5 minutes before commencing the study and inducing VF. VF was induced by either burst pacing or 9-V DC current briefly applied to the right ventricular (RV) epicardium and the perfusion was halted, thus mimicking global ischemia as seen during clinical VF. All VF episodes were allowed to continue for 180 seconds to simulate clinical cardiac arrest. VF was recorded for 20 seconds each at onset (Onset VF) and 180 seconds (Early VF), after which hearts were reperfused and later defibrillated. Six hearts were used as a control group (VF Rest VF) and 9 as a treatment group (VF Glibenclamide VF) to compare spontaneous VF termination-rates.

In the treatment group (n=9), after the initial VF episode and defibrillation, 10-μmol/L glibenclamide was added to the perfusate and the drug was allowed to circulate for 10 min. After this period, the VF was initiated again and data acquisition repeated. The control group mirrored the time period and repeat inductions of the treatment group, thus allowing for comparison of the time effect and the repeat induction effect of the study protocol.

Spontaneous Defibrillation

The abrupt termination of VF without electrical defibrillation during an episode of induced VF (lasting spontaneously >30 sec) was considered to represent spontaneous defibrillation.
Dominant Frequency Analysis

The detailed methodological steps for dominant frequency (DF) analysis have been described elsewhere\(^3\). Briefly, for each of the 112 unipolar electrograms, power spectral density (PSD) was estimated using the Welch averaged modified periodogram\(^2\). Each PSD was then scanned between 1.5 and 12 Hz and the frequency associated with the highest energy component was extracted as the DF\(^2,4\). VFCL was calculated by inverse of DF (VFCL = 1/DF).

Measurement of Effective Refractory Period (ERP) during Ventricular Fibrillation

The traditional method of the measurement of effective refractory period involves a regular pacing train and a premature extrasystole with decreasing interval. This technique is limited by the fact that ERP can only be measured during pacing. VF, unlike sinus or paced rhythms, involves continuous dynamic changes in cycle length\(^5\). Hence, the ERP measured during fixed-rate pacing is unlikely to reflect values during the dynamic changes observed during VF. Because traditional methods of ERP measurement are time-consuming, it is not possible to measure rapid changes in ERP. To overcome these problems during VF, average local fibrillation intervals have been used as a surrogate for ERP\(^6-11\). This measure correlates well with local refractoriness at the short cycle lengths seen during VF in dogs, pigs and humans\(^7,9\). VF mean cycle length (VFCL) was calculated by the inverse of DF and used as a surrogate for ERP.

Spatial Dispersion of Refractoriness

\textit{LV Transmural dispersion}: The first eighty electrodes of the epicardial sock covered the LV free wall epicardium while the eighty electrodes of the endocardial balloon covered LV endocardium. VFCL from these unipolar electrodes overlying LV epicardium and LV endocardium was averaged. Mean VFCL was compared between LV free wall epicardium and endocardium.

\textit{Interventricular dispersion}: Epicardial regions were further divided into LV epicardium and RV epicardium. VFCL from the electrodes overlying the LV and RV epicardium was averaged to get mean VFCL from the LV and RV epicardium. The central parts of the LV and RV free walls were included in the data analysis to avoid signal contamination from the neighboring region.

\textit{Antero-posterior dispersion}: LV free wall epicardium was further divided into anterior and posterior LV epicardium. Central anterior and posterior LV were defined by discarding the data from electrodes overlying the border of anterior and posterior LV. VFCL from all the electrodes overlying anterior and poster LV was averaged to get a mean VFCL.

\textit{Apico-basal dispersion}: Apex and base were defined by averaging VFCL from top two and bottom rows of epicardial sock respectively. Mean VFCL was compared between epicardial apex and base.

Human Heart Samples

Human heart samples were dissected from normal and cardiomyopathic hearts for RNA and protein expression.

Cardiomyopathic Human Heart Samples

The experimental protocol was approved by the University Health Network ethics committee, and informed consent was obtained from each patient. Cardiac tissue was dissected from eight cardiomyopathic patients (2 women, 6 men) who underwent cardiac transplantation. The mean age was 53 ± 9 years, all patients had ejection fractions <20%. Immediately after explantation, each heart was
immersed in cold Tyrode solution, transported to an adjacent room (<5 minutes away), and flushed thoroughly to remove blood particles. Samples (1 mm thick) were taken from central LV epicardium and endocardium.

**Normal Human Heart Samples**

The experimental protocol was approved by the Ethical Review Board of the Medical Center of the University of Szeged, Hungary. Eight non-diseased human hearts (2 women, 6 men) were explanted from organ donors and could not be used for transplantation for technical reasons. The mean age was 54 ± 2 years. Samples were collected from endocardial and epicardial surfaces and immediately frozen with liquid nitrogen.

**RNA preparation**

We quantified K_{ATP}-channel subunit mRNA-expression in cardiomyopathic human hearts. Total RNA from each cardiac tissue was isolated and DNase-treated with the RNeasy Fibrous Tissue Mini Kit (Qiagen). The quality of total RNA was assessed by polyacrylamide-gel microelectrophoresis (Agilent 2100 Bioanalyzer). Lack of genomic DNA contamination was verified by PCR.

**TaqMan real-time reverse transcriptase-polymerase chain reaction**

Total RNA was isolated from endocardial and epicardial samples of an independent set of diseased human hearts using the TRIzol reagent (Invitrogen), treated with DNase (MBI Fermentas), and purified using the GenElute Mammalian Total RNA Miniprep Kit (Sigma). After reverse-transcription using the RevertAid H Minus First Strand cDNA Synthesis Kit (MBI Fermentas), quantitative PCRs were performed in a PRISM 7900HT sequence detection system (Applied Biosystems Inc.) to assess the expression of KIR6.1 (TaqMan® Gene Expression Assay ID: Hs00958961_m1), KIR6.2 (Hs00265026_s1), SUR1 (Hs00165861_m1), SUR2A (Hs01072316_m1) and β actin (Hs00357333_g1). Relative gene expression of each KATP-channel subunit, normalized to β-actin expression, was calculated using the 2-ΔΔCT method. The relative RNA abundance for each gene in the LV endocardial sample from each heart was expressed as 100% for comparative purposes.

**Protein extraction and Western-blot**

Enriched membrane protein fractions were obtained as follows. Freshly isolated epicardial and endocardial tissue samples from control and cardiomyopathic hearts were fast-frozen in liquid nitrogen, pulverized and further homogenized in a TNE buffer containing: Tris 25-mmol/L, EDTA 5-mmol/L, EGTA 5-mmol/L, NaCl 150-mmol/L, NaF 20-mmol/L, Na_{2}VO_{4} 0.2-mmol/L, β-glycerophosphate 20-mmol/L, AEBSF 0.1-mmol/L, leupeptin 25-μg/mL, aprotinin 10-μg/mL, pepstatin 1-μg/mL, microcystin-LR 1-μmol/L, pH 7.34, HCl. Homogenized samples were then centrifuged at 1000×g for 10 minutes, supernatant collected and ultracentrifuged at 100,000×g for 1 hour. The latter supernatant was finally resuspended and incubated in TNE buffer containing 1% Triton-X100. The protein concentration was determined for each sample using a Bradford assay (Biorad). All steps were carried out on ice at 4-5°C. Protein samples (40 μg) were separated on 8% (w/v) poly-acrylamide SDS-PAGE and transferred electrophoretically onto PVDF membranes. The PVDF membranes were blocked in a PBS-solution containing 0.05% (v/v) Tween-20 and 5% (w/v) nonfat dried milk (NDM) and incubated overnight at 4°C with primary antibodies diluted in PBS containing 0.05% Tween-20 and 1%-NDM. After washing with PBS-Tween solution/1%-NDM, membranes were incubated with corresponding HRP-conjugated secondary antibodies. Immunoreactive bands were detected by ECL using BioMax MS films. Band
densitometry was performed using Quantity One® software (Biorad). All of the protein expression data are normalized to GAPDH for the same samples on the same gels.

**Antibodies:** Primary antibodies (diluted 1/1000 unless otherwise stated) included polyclonal rabbit anti-Kir6.1 (APC-105, Alomone Labs), Kir6.2 (APC-020), polyclonal rabbit anti-SUR1 (H-80; sc-25683, Santa Cruz Biotechnologies, Inc.), goat polyclonal antibody raised against mouse SUR-2A (M-19; sc-32462), goat polyclonal antibody raised against human SUR-2B (C-15; sc-5793). Peroxidase-conjugated AffiniPure goat anti-rabbit IgG (111-035-144), and Affinipure donkey anti-goat IgG (705-035-147) from Jackson ImmunoResearch were used as secondary antibodies (1/10000).

Specificity for the above antibodies was tested using protein lysate extracted from stably transfected HEK-293 cells over-expressing murine Kir6.1 or Kir6.2 together with SUR1, SUR2A or SUR2B. (courtesy of Dr William Coetzee and Dr Andrew Tinker, University College London, The Rayne Institute, London, UK) 13, 14.

**Monophasic action potential (MAP) recordings:** Action potentials from two cardiomyopathic human hearts were recorded using MAP catheters (EP Technology)15, from the epicardium and endocardium simultaneously on opposing surfaces. MAP catheter action potentials have been validated against microelectrode action potentials and have been shown to have a strong correlation16. Data analysis was performed using a system previously described2 and measurements were made using Matlab (Mathworks). MAP recordings were obtained in the baseline condition, and acute ischemia was induced by turning off the perfusion to the heart. MAP recordings were performed again, after 3 minutes of acute ischemia without glibenclamide and then with ischemia + 10 µM glibenclamide in the treatment condition.

**Langendorff-perfused rat hearts**

This protocol was approved by University Health Network ethics board. Male Sprague Dawley rats were anesthetized using inhaled isoflurane and hearts were exposed via mid-thoracotomy. Hearts were isolated rapidly, placed in cold Tyrode's solution and transported to our laboratory within 5 min. The aorta was cannulated and hearts were Langendorff-perfused using Tyrode's solution (mM): 118.1 NaCl, 4.7 KCl, 3.3 CaCl₂(H₂O)₂, 1.16 MgSO₄, 24.9 NaHCO₃, 1.17 NaPO₄, and 6.1 glucose). The Tyrode's solution was maintained at 37°C. The perfusion-pressure was maintained at 60-65 mm Hg. The preparation was allowed to equilibrate for 10 minutes before starting the experimental protocol.

**ERP dispersion determination using S₁-S₂ Protocol**

Figure 2C shows the experimental protocol. After the preparation equilibrated, they were paced at 150 ms (S₁) at twice diastolic threshold from either surface (epicardium or endocardium). The electrogram was recorded from the same surface that was paced. An extra-stimulus (S₂) was delivered starting from 140 ms. The S₂ interval was decreased in 10-ms increments until capture was lost. The S₂ was then increased by 10 ms and decreased in 1-ms increments until loss of capture was achieved. The last capturing S₂-interval established the effective refractory period (ERP). The pacing and mapping surface was switched and the ERP again calculated. The ERP-measurement sequence was randomized (epicardium to endocardium/endocardium to epicardium) among hearts. After the ERP was calculated in the baseline condition, low-flow ischemia was induced by decreasing perfusion-rate (cardiac effluent measured to ensure perfusion <1 ml/minute) for 5 minutes. During ischemia, S1 could not capture at 150 ms, hence ERP was calculated from epicardium and endocardium consecutively using a basic train (S1) of 250 ms and the sequence of mapped surface (epicardium or endocardium) was randomized.
**K\textsubscript{ATP}-channel modulation**

The hearts were reperfused for 5 minutes and treated with drug (glibenclamide 10 μmol/L or pinacidil 10 μmol/L). The drug was allowed to perfuse the heart for 15 minutes. The protocol for ERP dispersion measurement was repeated after the administration of the drug, in the baseline condition as well as in low flow ischemia.

**Arrhythmogenesis during K\textsubscript{ATP}-modulation**

We did not study spontaneous defibrillation in the rat model; however, we documented ventricular arrhythmias (VT beats greater than 5 and VF) that occurred during the S1-S2 protocol during the above experimental conditions.

**Na\textsuperscript{+} channel blockade:** In 5 hearts, the heart was treated with 10 μmol/L lidocaine after the ERP protocol in control conditions to test the effect of Na\textsuperscript{+}-channel blockade on ERP during baseline conditions and acute ischemia, as well as on transmural dispersion of ERP during acute ischemia.

**Floating microelectrode recording**

Glass microelectrodes were pulled from borosilicate glass pipettes (0.9-mm diameter) with a vertical puller (Model 370, Needle Pipette Puller, David Kopf Instruments, CA). The tip resistance of 3-M KCl-filled pipettes ranged between 15-30 MΩ. Pipettes were mounted on a coiled Ag-AgCl electrode tip attached to a micromanipulator. Microelectrodes were connected to a Grass amplifier, output from which was fed into a computer and analyzed with custom written software. A Ag-AgCl electrode near the microelectrode served as the reference. Blebbistatin (3 μmol/L) was administered in Tyrode's solution to minimize motion and prevent dislodging of the microelectrode. Recordings were obtained from epicardium and endocardium in control conditions and during ischemia with and without drug treatment.

**Statistical Analysis**

Data was analyzed using SAS 9.0. Values are shown as mean ± SEM. Fisher’s exact test was used to compare the number of hearts with spontaneous VF termination in the control and treatment groups. ANOVA, using Generalized Linear Model in SAS, was used to compare VFCL in multiple cardiac regions between control and treatment groups. ANOVA was followed by post-hoc Tukey adjustment when significant. A two way ANOVA was employed to examine the interaction between glibenclamide and regions to assess whether the administration of glibenclamide affected different regions differently. K\textsubscript{ATP} subunit gene and protein analysis was performed using a two way ANOVA followed by bonferroni post-hoc test if significant.

In the rat Langendorff model ERP-dispersion (ΔERP) was defined as the difference in the ERP between epicardium and endocardium. ΔERP was analyzed using ANOVA in control versus treatment conditions. If significant, ANOVA was followed by post-hoc Tukey test to account for multiple comparisons and p value was adjusted accordingly. A two way ANOVA was employed to compare the expression of K\textsubscript{ATP} subunits between groups (normal and myopathic hearts) and surface (LV endocardium and epicardium). A one way ANOVA was used to detect the differences between epicardium and endocardium within myopathic hearts. A p-value <0.05 was considered statistically significant.
Supplemental Figures:

Supplemental Figure I:

**Full blots with Kir6.1 and respective GAPDH staining.** Illustration of the full blots obtained following overnight hybridization of the membranes with the anti-Kir6.1 (APC-105) antibody (1/1000). Each lane represents an individual sample included in our study. The band selected for quantification is shown with an arrow. The respective GAPDH staining was used as loading control. Note that the membrane was previously hybridized with the anti-Kir6.2 (APC-020) and further stripped before hybridization with the anti-Kir6.1 antibody.
### Supplemental Figure II:

<table>
<thead>
<tr>
<th>Control samples</th>
<th>Cardiomyopathic samples</th>
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<tr>
<td>Endocardium</td>
<td>Endocardium</td>
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<td>Epicardium</td>
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**Full blots with Kir6.2 and respective GAPDH staining.** Illustration of the full blots obtained following overnight hybridization of the membranes with the anti-Kir6.2 (APC-020) antibody (1/1000). Each lane represents an individual sample included in our study. The band selected for quantification is shown with an arrow. The respective GAPDH staining was used as loading control.
**Supplemental Figure III:**

**Full blots with SUR1 and respective GAPDH staining.** Illustration of the full blots obtained following overnight hybridization of the membranes with the anti-SUR1 (H-80) antibody (1/200). Each lane represents an individual sample included in our study. The band selected for quantification is shown with an arrow. The respective GAPDH staining was used as loading control. Note that the membrane was previously hybridized with the anti-Kir6.2 (APC-020), anti-Kir6.1 (APC-105) and further stripped before hybridization with the anti-SUR1 (H-80) antibody. The poor quality of the blot is explained by the fact that the anti-SUR1 was raised in the goat, which often lead to high background staining.
Supplemental Figure IV:

Full blots with SUR2B and respective Ponceau staining. Illustration of the full blots obtained following overnight hybridization of the membranes with the anti-SUR2B (C-15) antibody (1/200). Each lane represents an individual sample included in our study. The band selected for quantification is shown with an arrow. The respective ponceau staining was used as loading control.
Supplemental Figure V:

**Action potential duration in cardiomyopathic human hearts:** In the baseline condition (no ischemia, no drug) the mean APD for the 2 hearts was 314.5 ms and 306.5 ms in the epicardium and endocardium respectively. After three minutes of acute ischemia, APD shortening was observed that was more pronounced in the endocardium compared to epicardium (epicardium: 266 ms; endocardium: 239.5 ms). After 10 µmol/L glibenclamide, the APD after 3 minutes of ischemia was 313 ms and 296 ms at the epicardium and the endocardium respectively. Supplemental figure V shows an illustrative example of the APDs from one heart.
References:


