Activation of Glibenclamide-Sensitive ATP-Sensitive K⁺ Channels During β-Adrenergically Induced Metabolic Stress Produces a Substrate for Atrial Tachyarrhythmia

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Background—Cardiac ATP-sensitive K⁺ channels have been suggested to contribute to the adaptive physiological response to metabolic challenge after β-adrenoceptor stimulation. However, an increased atrial K⁺-conductance might be expected to be proarrhythmic. We investigated the effect of ATP-sensitive K⁺ channel blockade on the electrophysiological responses to β-adrenoceptor-induced metabolic challenge in intact atria.

Methods and Results—Atrial electrograms were recorded from the left atrial epicardial surface of Langendorff-perfused rat hearts using a 5x5 electrode array. Atrial effective refractory period and conduction velocity were measured using an S1–S2 protocol. The proportion of hearts in which atrial tachyarrhythmia was produced by burst-pacing was used as an index of atrial tachyarrhythmia-inducibility. Atrial nucleotide concentrations were measured by high performance liquid chromatography. Perfusion with ≥10⁻⁹ mol/L of the β-adrenoceptor agonist, isoproterenol (ISO), resulted in a concentration-dependent reduction of atrial effective refractory period and conduction velocity. The ISO-induced changes produced a proarrhythmic substrate such that atrial tachyarrhythmia could be induced by burst-pacing. Atrial [ATP] was significantly reduced by ISO (10⁻⁴ mol/L). Perfusion with either of the ATP-sensitive K⁺ channel blockers, glibenclamide (10⁻⁵ mol/L) or tolbutamide (10⁻³ mol/L), in the absence of ISO had no effect on basal atrial electrophysiology. On the other hand, the proarrhythmic substrate induced by 10⁻⁴ mol/L ISO was abolished by either of the sulfonylureas, which prevented induction of atrial tachyarrhythmia.

Conclusions—Atrial ATP-sensitive K⁺ channels activate in response to β-adrenergic metabolic stress in Langendorff-perfused rat hearts, resulting in a proarrhythmic substrate. (Circ Arrhythm Electrophysiol. 2012;5:1184-1192.)

Key Words: arrhythmia ■ re-entry ■ K₂,ATP channel ■ sulfonylurea

In contrast to the inward rectifier K⁺ (Kᵢ) channels that underlie the acetylcholine-dependent, G-protein-gated current (I}$/acm/ $_/GIRK$ and the background K⁺-conductance (I}$/Kᵢ/), relatively little is known concerning the role of ATP-sensitive K⁺ (K₂,ATP) channels in atrial tachyarrhythmia (AT).1–3 The availability of the K²/ATP channel current and the expression of the associated pore-forming α-subunit, K₆.2, is reduced in chronic human atrial fibrillation (AF), suggesting that K₂,ATP channels do not contribute to the electric remodeling of chronic AF.4,5 The K₂,ATP channel is formed as a hetero-multimeric complex of K₆.2 with a sulfonylurea receptor (SURx) regulatory β-subunit.6 Binding of ATP to K₆.2 results in channel inhibition whereas binding of Mg²⁺-ADP to SURx activates the channel.5 mRNA for K₆.1, K₆.2, SUR1, and SUR2A have been shown to be expressed in rat atrium.7 There is evidence from genetically modified mice of differences in the SURx subunit composition of atrial and ventricular K₂,ATP channels, with SUR2A suggested to be the predominant subunit in the ventricles whereas SUR1 is essential for the formation of atrial K₂,ATP channels.6,8 On the other hand, more recent data indicate that SUR1 may not predominate over SUR2A in the human atrium.9

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Cardiac K₂,ATP channels exist in the sarcolemma as macromolecular complexes with phosphotransfer and glycolytic enzymes that serve to fine-tune channel gating, thereby coupling cellular electric activity with metabolism.6,10 It has long been recognized that in the ventricles cardiac K₂,ATP channels open during ischemia and that their activation can be protective; the increase in K⁺ conductance during metabolic insult stabilizing...
the resting membrane potential, shortening action potential duration (APD), and thereby reducing Ca\(^{2+}\) influx leading to the conservation of energy stores. More recently, data from K\(_{ATP}\) channels in middle-aged patient with adrenergic AF has led to the suggestion of a physiological role for K\(_{ATP}\) channels in contributing to the cardiac adaptation during adrenergic stress. The identification of a loss-of-function mutation in the SUR2A isoform of the SURx in a middle-aged patient with adrenergic AF and implies a role in the adaptive response to adrenergic stimulation of the atria similar to that proposed for the ventricles. Consistent with this view, the β-adrenergic agonist, isoproterenol (ISO), was shown to induce AF in Langendorff-perfused hearts from K\(_{ATP}\) mice. However, it is well established that the opening of K\(_{ATP}\) channels in the ventricles during metabolic challenge can also be proarrhythmic, with ST-segment abnormalities of the ECG, spatially heterogeneous shortening of APD, increased dispersion of refractoriness, and the generation of regions of inexcitability and conduction block promoting re-entrant tachyarrhythmias and fibrillation. Perhaps surprisingly, given the potential involvement of K\(_{ATP}\) channels in the genesis and maintenance of arrhythmia, there is currently very little information about the role of K\(_{ATP}\) channels in the atria in comparison with the ventricles. Therefore, the aim of this study was to examine the role of K\(_{ATP}\) channels in the electrophysiological responses of the intact left atrium (LA) to metabolic challenge through β-adrenoceptor stimulation.

**Methods**

Full details of methods are available in the online-only Data Supplement.

**Animal Procedures**

All procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986). Hearts were excised from adult male rats (200–250 g) under general anesthesia (80–100 mg/kg sodium pentobarbital, IP), mounted on a whole-heart perfusion apparatus, perfused retrogradely at a rate of 8 mL.min\(^{-1}\).g\(^{-1}\) heart weight via the aorta with a Krebs' Henseleit solution, unipolar atrial electrograms and ECG were recorded and atrial effective refractory period (AERP), conduction velocity (CV) and AT-inducibility were measured, as described previously. The wavelength of excitation (WL) was calculated as AERP×CV.

**Langendorff Perfusion**

The concentration-dependence of effects of ISO on AERP, CV, and AT-inducibility were examined in 26 hearts (data shown in Figures 1 and 2). Hearts were perfused with control solution and, after an equilibration period, AERP and CV were measured at cycle lengths (CL) of 150, 100, and 75 ms. Burst-pacing was then applied to test for AF-inducibility. Each heart was then perfused with a single concentration of ISO of either 10\(^{-9}\) (n=5), 10\(^{-8}\) (n=6), 10\(^{-7}\) mol/L (n=6), or 3×10\(^{-6}\) mol/L (n=3) and, once a steady-state had been achieved, the measurements were repeated at each CL and AT-inducibility was tested again. The concentration-dependence of the effects of the sulfonylurea and glibenclamide (GLIB), in the presence of ISO, was examined in 25 hearts using a similar paradigm. In this case, after AERP, CV, and AT-inducibility measurement in control solution, hearts were perfused with 10\(^{-4}\) mol/L ISO, measurements repeated, and then, in the continued presence of ISO, the hearts were perfused with GLIB at a single concentration of either 10\(^{-7}\) (n=6), 10\(^{-6}\) (n=6), 3×10\(^{-6}\) (n=6), or 10\(^{-5}\) mol/L (n=7) and the measurements were repeated once again. The effects of 10\(^{-3}\) mol/L tolbutamide (TOLB) were examined at a single CL of 100 ms in the presence of 10\(^{-6}\) mol/L ISO in 6 hearts after a similar paradigm. AERP, CV, and AT-inducibility were not altered during time-matched to experiments involving 2 sequential changes of solution (n=8; data not shown).

**Data Analysis and Statistics**

Data are presented as mean±SEM. All parametric data were subject to D'Agostino and Pearson omnibus normality test. Data examining the effect of drug treatment on AERP and CV (ie, Figure 1A and 1B, Figure 3A and 3B and Figure 4A and 4B) were analyzed by 2-way repeated measures (RM) ANOVA comparing the data in independent variables. Probability values to the right of A and B represent the level of significance from 2-way repeated measures (RM) ANOVA comparing the data in that concentration of ISO with the corresponding control (see Methods). The concentration-dependence of the shortening of atrial effective refractory period (AERP) (D) and slowing of conduction velocity (CV) (E) expressed as percentage change relative to control (cycle length [CL]=150 ms). Curves represent fits to equation 2. The logEC\(_{50}\) for the ISO-concentration-dependently reduces atrial effective refractory period (AERP) (D) and slowing of conduction velocity (CV) (E) expressed as percentage change relative to control (cycle length [CL]=150 ms). Curves represent fits to equation 2. The logEC\(_{50}\) for the ISO-concentration-dependently reduces atrial effective refractory period (AERP) (D) and slowing of conduction velocity (CV) (E) expressed as percentage change relative to control (cycle length [CL]=150 ms). Curves represent fits to equation 2.
control data. Data examining the effects of TOLB were analyzed by 1-way RM ANOVA and Bonferroni multiple comparisons test (3 comparisons). Differences in AT-inducibility were analyzed by 1-tailed Fisher exact test (eg, Figures 2B and 5A). The relationship between WL and the CL and duration of AT was examined by Pearson and Spearman correlation analysis, respectively (Figure 2C and 2D). Data on the ATP content of left ventricular (LV) and LA tissue (Figure 6) were analyzed by 2-way ANOVA with Bonferroni multiple comparisons test (2 comparisons). Data from the recording of whole-cell KATP currents (Figure 7) were analyzed by 1-way RM ANOVA with Bonferroni multiple comparisons tests (6 comparisons). Data on the effects of ISO and GLIB on the ECG intervals (Table) were analyzed by either paired Student t test or 1-way RM ANOVA with Bonferroni multiple comparisons tests (3 comparisons). P<0.05 was considered statistically significant.

The percentage inhibition of AERP and CV was calculated using equation 1:

$$\% \text{Inhibition} = \left( \frac{\text{Control} - \text{Drug}}{\text{Control}} \right) \times 100$$ (1)

where control represents the control value of AERP or CV, drug represents the corresponding value of AERP or CV in the presence of drug. The concentration-dependence of the percentage inhibition was fitted with equation 2 by nonlinear least squares:

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{LogEC}_{50} - x)}}$$ (2)

where Bottom and Top correspond to the minimum and maximum plateaus, respectively, and X represents log_{10}[drug].

### Results

Under control conditions, AERP, CV, and WL showed a dependence on CL (Figure 1). Perfusion with the ISO at concentrations of 10^{-9} mol/L and above resulted in a significant reduction of AERP in a concentration-dependent fashion (Figure 1A). The ISO-dependent shortening of AERP was concentration dependent, with an EC_{50} of 2.71×10^{-8} mol/L at CL=150 ms (Figure 1D). CV was significantly slowed by perfusion with ISO at concentrations of ≥10^{-7} mol/L (Figure 1B) and the EC_{50} at CL=150 ms was 6.45×10^{-8} mol/L (Figure 1E). There was no significant interaction between CL and drug treatment in the effects on either AERP or CV in 2-way RM ANOVA. Moreover, there were no significant differences in the EC_{50} at different CL (online-only Data Supplement Table I). The effects of ISO on AERP and CV were reflected in a marked shortening of WL at concentrations of ≥10^{-8} mol/L (Figure 1C). Because shortening of WL might lead to a substrate for re-entry, the effect of ISO on AT-inducibility was investigated. Under control conditions it was not possible to induce AT in the Langendorff-perfused rat hearts either through

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Isoproterenol (ISO) increases atrial tachyarrhythmia (AT) inducibility. A, Example ECG (top trace) and atrial electrogram (lower 3 traces) immediately after burst-pacing during perfusion with 10^{-6} mol/L ISO. B, Incidence of AT induced by either burst-pacing or a programmed extrastimulation protocol under control conditions or during perfusion with ISO. Data from 3 hearts perfused with 3×10^{-6} mol/L ISO are not shown as one heart showed ventricular fibrillation after burst-pacing. C, Correlation of cycle lengths (CL) during AT with wavelength of excitation (WL) (r=0.7852, P<0.0001). Solid line was fitted by linear regression (dotted lines represent 95% CIs) and had slope that was significantly different from zero (P<0.0001). D, Correlation of duration of paroxysms of AT with WL (Spearman r=0.6158, P=0.0050). Note the use of the logarithmic scale for AT duration.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Glibenclamide (10^{-5} mol/L) is without effect on baseline atrial effective refractory period (AERP) (P=0.25) (A), conduction velocity (CV) (P=0.95) (B), and wavelength of excitation (WL) (C).
burst-pacing or via the S1–S2 protocol. However, AT could be induced after perfusion of hearts with ISO at concentrations of $\geq 10^{-9}$ mol/L (Figure 2). Representative examples of atrial electrogram recordings during a paroxysm of AT are shown in Figure 2A. The same data are shown on a faster time scale in online-only Data Supplement Figure I to allow closer inspection of the electrograms and of the rhythm. The effect of ISO on AT-inducibility showed concentration-dependence: whereas AT was induced in just 1 of 5 hearts perfused with $10^{-9}$ mol/L ISO, in 4 of 6 hearts perfused with $10^{-8}$ mol/L ISO, and in all hearts perfused with an ISO concentration of $\geq 10^{-7}$ mol/L (Figure 2B). Notably, WL strongly correlated with CL during paroxysms of AT (Figure 2C) and was inversely correlated with AT duration (Figure 2D). Taken together, these data demonstrate that ISO caused a concentration-dependent shortening of the WL of excitation that was associated with a substrate for tachyarrhythmia.

The sulfonylurea, GLIB, was used as a pharmacological tool to investigate the contribution of $K_{ATP}$ channels to the responses of the perfused hearts. GLIB ($10^{-5}$ mol/L) was without effect on baseline AERP, CV, or WL, indicating both that $K_{ATP}$ channels did not contribute to atrial refractoriness or excitability under control conditions in Langendorff-perfused hearts and that GLIB was without nonselective effect on other currents underlying atrial electrophysiology (Figure 3). Unsurprisingly, therefore, AT-inducibility was not increased by perfusion with the $K_{ATP}$ channel blockers (data not shown).

In addition, GLIB had no significant effect on either the RR, QT, or QT intervals during spontaneous activity at the sinus rate (Table), demonstrating the lack of contribution of $K_{ATP}$ channels to either sinus pacemaking or ventricular repolarization in the perfused hearts. Because GLIB has been suggested to inhibit G-protein–gated inward rectifier K+ channel (GIcK) and background inward rectifier channels and there is evidence that $\beta$-adrenoceptor stimulation potentiates the background K+ conductance and a constitutively active form of GIcK,17–19 the effects of GLIB on the GIcK currents in human embryonic kidney 293 cells stably transfected with either hKir2.1 or rKir3.1/3.4 were examined. GLIB ($10^{-5}$ mol/L) had no significant effect on the inward rectifier currents through either hKir2.1 (3.7±1.7% inhibition, n=5) or rKir3.1/3.4 (3.6±3.3% inhibition, n=6) channels.

In contrast to control perfusion, GLIB ($10^{-7}$ to $10^{-5}$ mol/L) inhibited the ISO ($10^{-6}$ mol/L)-induced changes in atrial electrophysiological parameters in a concentration-dependent fashion (Figure 4). In the presence of ISO, the mean AERP values were prolonged by each concentration of GLIB examined (Figure 4A). Interestingly, the slowing in CV induced by $10^{-6}$ mol/L ISO was reversed by GLIB ($\geq 10^{-6}$ mol/L) such that the CV values in the presence of $10^{-5}$ mol/L GLIB at CL of 100 ms and 150 ms were faster than the corresponding control values (Figure 4B). In consequence, the WL in the presence of

![Figure 4](http://circ.ahajournals.org/)}
10−6 mol/L ISO and 10−5 mol/L GLIB were longer than control (Figure 4C). The ISO-induced changes in AERP (Figure 4D) and CV (Figure 4E) were concentration dependently inhibited by GLIB with IC50s of 5.37×10−7 mol/L and 1.16×10−6 mol/L, respectively. There was no interaction between CL and drug treatment and there were no significant differences in the IC50s of GLIB at different CL (online-only Data Supplement II). The lengthening of WL induced by GLIB led to a reduction in AT-inducibility in the presence of ISO: Perfusion with ISO (10−6 mol/L) resulted in AT being induced in 23 out of 24 hearts (95% of hearts). Subsequent perfusion with GLIB in the continued presence of ISO reduced the ISO-induced increase in AT-inducibility in a concentration-dependent fashion over the same range of concentrations of the sulfonylurea as were effective in lengthening WL (10−7 to 10−5 mol/L) (Figure 5A). AT-inducibility was reduced to 66% (4 of 6 hearts) after perfusion with 10−7 mol/L GLIB and to 17% (1 of 6 hearts) by 10−6 mol/L GLIB (Figure 5A). AT could not be induced at all in the presence of the sulfonylurea at 3×10−6 mol/L or at 10−5 mol/L (Figure 5A). TOLB is an alternative sulfonylurea that has been shown to prevent ventricular tachyarrhythmia and to block KATP channels in human atrial myocytes at millimolar concentrations.20,21 TOLB (10−3 mol/L) was found to abolish the 10−6 mol/L ISO-dependent increase of AT-inducibility (ISO, 5/6 hearts; ISO+TOLB, 0/6 hearts; P=0.0076). AERP, measured at a CL of 100 ms, was reduced by ISO from 67.3±3.0 ms to 41.3±3.1 ms (n=6, P=0.0001). In the presence of ISO, AERP was prolonged by TOLB from 41.3±3.1 ms to 53.7±3.7 ms (n=6, P=0.0033). In separate experiments, TOLB had no effect on AERP in the absence of ISO (control, 67.8±1.9 ms; TOLB, 71.3±3.1 ms; P=0.0900).

To examine the modulation of sinus node pacemaking and ventricular repolarization by β-adrenoceptors, and the possible contribution of nodal and ventricular KATP channels therein, the effects of ISO and of GLIB on the RR and QT intervals of ECG were investigated. Perfusion with ISO (10−6 mol/L) produced a marked positive chronotropic response reflected in a significant shortening of the RR interval (Table).
Perfusion with ISO also resulted in a shortening of the uncorrected QT interval (Table). However, the rate-corrected QT interval was not affected by the β-adrenoceptor agonist (Table), indicating that the shortening in QT interval caused by ISO could be accounted for by heart rate-dependent shortening of the QT interval. Most notably, GLIB (10−5 mol/L) had no significant effect on RR interval or on the uncorrected QT and rate-corrected QT intervals, consistent with a lack of contribution of KATP channels to any sinus node activity or to ventricular repolarization in the presence of ISO. As with GLIB, TOLB also did not affect pacemaking or ventricular repolarization (online-only Data Supplement Table III). The data are consistent with a lack of effect of either sulfonylurea on sinus nodal and ventricular ion channels in the presence of ISO. Most significantly, taken together, these data indicate that β-adrenoceptor agonism resulted in the activation of atrial, but not of ventricular, KATP channels.

The effect of β-adrenoceptor stimulation on tissue ATP concentrations was investigated in LA and LV apex samples taken immediately after Langendorff perfusion with either control Krebs’ Henseleit solution or Krebs’ Henseleit solution containing 10−6 mol/L ISO. In comparison with time-matched controls, 30 minutes perfusion with ISO resulted in significant reduction of ATP in the LA (Figure 6). In contrast, ISO had no significant effect on [ATP] in LV tissue (Figure 6). These data demonstrate that perfusion with ISO resulted in severe LA metabolic stress.

The action of ISO on atrial KATP channel currents was investigated by whole-cell recording from isolated rat LA myocytes using conditions suitable for the surveying of background K+ currents (Figure 7).22 Excitation–contraction coupling was inhibited by the inclusion in the pipette of the Ca2+-chelator, BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid). After a prepulse (−40 mV), a step depolarization to +20 mV (100 ms) elicited a transient inward current that decayed to a steady-state outward current level by the end of the pulse (Figure 7B). A descending voltage-ramp from +20 mV to −120 mV (500 ms) allowed the instantaneous current–voltage relation for the background conductance to be obtained (Figure 7C). Mean outward current densities at +20 mV are shown in Figure 7D. Although the transient inward currents were increased by ISO, consistent with an increase in L-type Ca2+ current (Ica,L), β-adrenoceptor-agonism had no statistically significant effect on the background currents. However, metabolic inhibition (MI) with NaCN (10−3 mol/L) and 2-deoxy-glucose (10−3 mol/L) resulted in a marked increase in the background conductance, which became linear (Figure 7C). The mean current density at +20 mV was increased >23-fold (Figure 7D). Subsequent superfusion with the KATP channel blocker, GLIB (3×10−6 mol/L), in the continued presence of MI resulted in a marked reduction in the outward current at +20 mV. The GLIB-sensitive difference current reversed at −91.1±1.32 mV, demonstrating the involvement of K+–selective channels in this current. Thus, under these recording conditions, ISO does not activate KATP currents directly. However, substantial GLIB-sensitive currents could be activated after MI in these cells. Taken together, the data of the present study indicate that the activation of atrial KATP channels after β-adrenoceptor stimulation in Langendorff-perfused hearts involves severe metabolic stress and depletion of intracellular ATP levels rather than being through the direct modulation of KATP channel gating.

**Discussion**

This study provides evidence for the first time that metabolic stress arising from β-adrenoceptor stimulation produces a substrate for AT in the intact atria of structurally normal hearts. The ISO-induced substrate for arrhythmia could be completely reversed by superfusion with a sulfonylurea, consistent with the notion that activation of the sarcolemmal KATP channel in response to metabolic stress underlies the electrophysiological response to β-adrenergic stimulation of the LA of Langendorff-perfused hearts.

**Selectivity of Sulfonylurea Action**
The data in the present study are consistent with a selective action of sulfonylurea on sarcolemmal KATP channels activated after perfusion with ISO. The concentrations of GLIB and of TOLB effective in inhibiting the ISO-induced changes in atrial electrophysiology corresponded to those shown previously to be effective at reducing ischemia-induced shortening of the ventricular action potential and ventricular fibrillation in perfused rat hearts.20,21 For example, the ISO-induced shortening of WL and AT-inducibility were significantly reduced by 10−4 mol/L GLIB and were completely reversed by a 3-fold higher concentration of the sulfonylurea (Figures 4C and 5A). GLIB has been reported to inhibit other cardiac ion currents including, IK1, IGIRK, ICa,L, the transient outward K+ current, the ultrarapid delayed rectifier K+ current, a protein kinase A-dependent Cl− current, and the Na+ pump.17,24–26 However, in the present study when GLIB or TOLB were applied at high concentration (10−5 mol/L or 10−4 mol/L, respectively), they had no effect on baseline atrial or ventricular electrophysiological parameters. GLIB had no effect on recombinant Ks,1/3.4 and Ks,2.1 channels. Moreover, neither sulfonylurea had any effect on sinus rate or on ventricular repolarization in the presence of ISO (Table and online-only Data Supplement Table III). Although GLIB has been reported to have nanomolar affinity for cardiac SURx, the efficacy of the drug...
is limited during metabolic stress. This may account for the incomplete inhibition by 3×10⁻⁶ mol/L GLIB of the atrial Kₐtp channel currents activated by MI (Figure 7). Thus, taken together, these data demonstrate that nonspecific effects of the sulfonylureas on cardiac ion currents other than Kₐtp channels had no effect on atrial or ventricular electrophysiology at the concentrations used in this study.

Although GLIB has been shown also to inhibit mitochondrial Kₐtp channels, the effects of the sulfonylurea on AERP and CV implicate atrial sarcolemmal Kₐtp channels in the responses to ISO. For example, the reduction in AERP during perfusion with the β-adrenoceptor agonist is likely to have resulted from shortening of APD attributable to increased outward current through GLIB-sensitive sarcolemmal Kₐtp channels. Similarly, the slowing of CV is likely to be attributable to an increase in the K⁺-selective conductance of the resting sarcolemmal membrane leading to a reduction in membrane excitability: the increased membrane K⁺-conductance resulting from activation of the Kₐtp channel can be considered as an increased sink for the source current, resulting in a reduction in CV. Presumably, the increase in CV above control values at all CLs after perfusion with 10⁻⁵ mol/L GLIB in the continued presence of 10⁻⁶ mol/L ISO (Figure 4B) reflected the β-adrenergic potentiation of cardiac voltage-gated Na⁺ current (IₚNa) and IₚCaL. Similarly, the lesser potency of ISO in the slowing of CV compared with the reduction of AERP is likely to be the result of the concomitant potentiation of IₚNa and IₚCaL by β-adrenergic pathways (Figure 1B). Thus, taken together, the effects of GLIB seen here were specific to the inhibition of atrial sarcolemmal Kₐtp channels activated in the presence of ISO.

**Mechanism of Atrial Kₐtp Channel Activation**

Interestingly, Findlay et al., working with isolated multicellular rat LA and pulmonary vein sleeve preparations, have recently demonstrated a sustained hyperpolarization of the resting membrane potential in response to selective β-adrenoceptor stimulation, consistent with a β-adrenoceptor-mediated increase in K⁺-selective background conductance. Moreover, ISO has been reported to shorten APD in LA from normal mice. However, in the present study, the atrial myocyte K⁺-conductance was not increased by ISO, although the ISO-mediated increase in peak inward current indicates that β-adrenoceptor pathways were intact in these cells. On the other hand, MI resulted in a profound increase in background K⁺-conductance, which could be largely inhibited by Kₐtp channel blockade. Thus, activation of β-adrenergic-signaling pathways alone does not lead to the activation of atrial Kₐtp channels although β-adrenoceptor-activation may potentiate the effects of ATP depletion. The markedly reduced LA [ATP] after perfusion with 10⁻⁶ mol/L ISO indicates that substrate delivery in the perfused hearts was insufficient to meet the increased metabolic demand during β-adrenergic stimulation at the agonist concentration tested, resulting in metabolic stress. Thus, the present study provides evidence for the activation of atrial sarcolemmal Kₐtp channels in response to β-adrenergic metabolic stress and is consistent with the well-established opening of Kₐtp channels in the ventricles in response to hypoxia or substrate deprivation.

Conversely, the lack of effect of GLIB in the absence of ISO indicates that substrate delivery was sufficient to meet metabolic demand under control conditions. Presumably, the ISO-induced increase in metabolic demand arose from the augmented activity of myosin ATPase and membrane ion transporters (eg, sarcoplasmic reticulum Ca²⁺ and Na⁺/K⁺-ATPases) associated with the positive inotropic action of β-adrenergic stimulation. Consistent with this hypothesis, the concentration-dependence of ISO-induced changes in AERP and WL corresponded well with the positive inotropic action of the β-adrenoceptor agonist. Although the positive inotropic action of ISO in the rat atrium is reported to be entirely accounted for by β₁-adrenoceptors, a role for β₂-adrenoceptors in atrial Kₐtp channel activation cannot be entirely ruled out. Conversely, β₁-adrenoceptors are negatively inotropic in the rat heart and may have attenuated the ISO-induced metabolic-stress.

**Ventricular Kₐtp Channels Were Not Activated**

In the present study, neither GLIB nor TOLB had any effect on ventricular repolarization, indicating that ventricular Kₐtp channels were not activated during perfusion with ISO. This is contrary to the putative pivotal role for cardiac Kₐtp channels in the physiological adaptation to adrenergically mediated increase in myocardial metabolic demand. On the other hand, the data are entirely consistent with numerous studies indicating that the activation of ventricular Kₐtp-channels requires severe metabolic stress, presumably reflecting the buffering of cytosolic ATP concentration changes by phosphotransfer and glycolytic enzymes. As, unlike the LA, the reduction of [ATP] in the LV apex after perfusion with ISO was not statistically significant, presumably the β-adrenergic-increase in metabolic demand was not sufficient to result in metabolic stress in the LV of the Langendorff-perfused hearts. Thus, the data of the present study are consistent with the activation of cardiac Kₐtp channels during metabolic stress involving changes in tissue [ATP], but not through increased metabolic demand alone. The induction of metabolic stress in the LA but not the LV is likely to be attributed to differences in Q₀ and substrate delivery to the 2 tissues (see online-only Data Supplement). Although under control conditions perfusion was clearly adequate to meet the metabolic demand of both the LV and LA, the increase in metabolic demand with ISO appears to have resulted in significant metabolic stress in the LA but not the ventricle. In addition to atrial–ventricular differences in the ISO-induced reduction of [ATP], the lower sensitivity to metabolic stress of ventricular compared with atrial Kₐtp-channels, possibly attributable to differences in SURx subunit composition, may have also contributed to the atrio-selective action of β-adrenergic stimulation on atrial electrophysiology in the present study.

**Involvement of Re-Entrant Mechanisms**

β-adrenoceptor stimulation has frequently been associated with atrial-triggered activity and may involve modulation of Ca²⁺ handling. Furthermore, glycolytic inhibition has been associated with triggered activity in the LA of...
Langendorff-perfused hearts from aged rats. However, the data of the present study are entirely consistent with a re-entrant mechanism: The ISO-induced increase in AT-inducibility correlated with the reduction in WL (Figure 1E). There was also a close correlation between ISO-induced changes in WL and CL during AT (Figure 1D). Moreover, the GLIB concentration-dependent reduction in AT-inducibility appeared to correlate with the GLIB-dependent lengthening of WL, although this was not statistically significant (Figure 5B). Taken together, these observations indicate that the shortening of AERP and WL played a fundamental role in the AT-inducibility during β-adrenergic stimulation and that prolongation of AERP and WL by GLIB underlies the antiarrhythmic action of sulfonylurea. Two very recent reports are consistent with the proposal that atrial KATP channel activation produces a substrate for AT and AF: The SUR2A-selective KATP channel opener, pinacidil, shortened atrial APD and increased AT-inducibility in a coronary-perfused preparation of human right atrial and right ventricular free wall. In addition, atrial electric remodeling and increased AF-inducibility in a mouse model of salt-induced hypertension has been shown to be associated with increased GLIB-sensitive atrial K_ATP channel current and expression of SUR1. Moreover, a substrate for re-entry attributable to β-adrenergic shortening of APD has been demonstrated in a canine atrial preparation.44

In summary, the present study demonstrated that activation of atrial sarcloemmal K_ATP channels through β-adrenergic metabolic stress resulted in a substrate for AT involving shortening of the AERP and WL and provides evidence in support of the suggestion that the K_ATP channel may represent a target for antiarrhythmic therapy in AF. Further studies are warranted to establish the mechanism of K_ATP channel activation in the atrium and its contribution to a substrate for arrhythmia during increased metabolic demand with physiological oxygen tension and substrate delivery.

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Disclosures

S.C.M. Choisy, J.C. Hancock, and A.F. James were coholders of the project grant PG/09/046. J.C. Hancock and A.F. James were coholders of project grant PG/08/104. I. Khaliulin, M.S. Suleiman, and A.F. James are coholders of PG/11/079. R. Bond, J.C. Hancock and A.F. James are coholders of grant FS/10/68. S.E. Haou and J.T. Milnes are employed by Xention Ltd, which has interests in drugs targeting ion channels for the treatment of atrial fibrillation; S.E. Haou and J.T. Milnes have stock options in Xention. Xention has no specific interest in the results of this study. The other authors have no conflicts to report.

References

ATP-sensitive K⁺ (K_ATP) channels couple membrane conductance to metabolism and are highly expressed in the sarcolemma of cardiac myocytes. In principle, their activation can be both protective and proarrhythmic. It has been suggested that K_ATP channels may function to protect against arrhythmias during β-adrenergic metabolic stress. On the other hand, there is considerable evidence from ventricular preparations that K_ATP channel activation contributes to the ECG abnormalities, ventricular tachyarrhythmia and fibrillation associated with ischemia, and myocardial infarction. However, there is comparatively little information about the role of K_ATP channels in the atria. This is a proof-of-concept study, using electrophysiological recordings from Langendorff-perfused rat hearts to examine the potential role of K_ATP channels in atrial electrophysiology.

Under the conditions of the study, the β-adrenoceptor-selective agonist, isoproterenol, induced metabolic stress and produced a substrate for tachyarrhythmia that could be prevented by concomitant perfusion with a sulfonylurea K_ATP channel blocker (glibenclamide or tolbutamide). Whole-cell recordings from isolated atrial myocytes indicated that K_ATP channel currents were not activated by stimulation of isoproterenol-dependent signaling pathways alone, although metabolic inhibition elicited very large glibenclamide-sensitive K_ATP currents under the same conditions. It is concluded that atrial K_ATP channel activation during β-adrenergic metabolic stress in perfused hearts produces a substrate for tachyarrhythmia. Thus, atrial K_ATP channels may represent a target for antiarrhythmic therapy under conditions of metabolic stress. Further studies are warranted to establish whether K_ATP channels contribute to a substrate for atrial tachyarrhythmia during β-adrenergically increased metabolic demand with physiological oxygen tension and substrate delivery.
Activation of Glibenclamide-Sensitive ATP-Sensitive K⁺ Channels During β
-Adrenergically Induced Metabolic Stress Produces a Substrate for Atrial
Tachyarrhythmia

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Activation of glibenclamide-sensitive $K_{\text{ATP}}$ channels during $\beta$-adrenergically-induced metabolic stress produces a substrate for atrial tachyarrhythmia

Short title: Kim/Zhang, $\beta$-adrenergic stress, $K_{\text{ATP}}$ channels & AF

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METHODS

Electrophysiological recordings from whole hearts. Hearts were excised under general anesthesia (80-100 mg/kg sodium pentobarbital, IP), mounted on a whole heart perfusion apparatus and perfused retrogradely at a rate of 8 ml.min⁻¹.g⁻¹ heart weight via the aorta with a Krebs’ Henseleit (KH) solution (in mM; 118.5 NaCl, 25.0 NaHCO₃, 3.0 KCl, 1.2 MgSO₄.7H₂O, 1.2 KH₂PO₄, 2.5 CaCl₂, 11.1 D-glucose at 37°C) gassed with 95% O₂/5% CO₂, as described previously¹,². Left atrial unipolar electrograms were recorded using a 5×5 array of recording electrodes². The electrode array was pressed against the epicardial surface of the anterior aspect of the left atrial free wall. The interelectrode distance was 334 μm; therefore, the recording area was 1.336 mm × 1.336 mm. All electrograms were individually amplified and were acquired to a PC hard disk via a Power1401 interface using Spike 2 software (Cambridge Electronic Design, UK). Bipolar stimulation was applied via electrodes adjacent to the recording array and stimulus protocols were generated using an AMPI Master-8 programmable stimulator with Iso-Flex stimulus isolators (Intracel Ltd., UK). Atrial effective refractory period (AERP) was measured using an S₁-S₂ stimulus protocol¹,². The activation time at each electrode was taken as the time at the point of maximum negative deflection and was measured relative to the earliest fiducial point of activation²,³. The maximal vectorial conduction velocity (CV) across the array was calculated from the electrogram most distal, and with the shortest activation time, relative to the earliest fiducial point²,⁴. The wavelength of excitation (WL) was calculated as the product, AERP×CV. To examine the inducibility of atrial tachyarrhythmia (AT), each heart was subjected to three consecutive bursts of rapid pacing, each of 5 s duration at cycle lengths of, respectively, 2.5, 5 & 10 ms. The duration of the longest paroxysm of AT induced in each heart, whether by burst pacing or by the S₁-S₂ protocol, was recorded. In control experiments, AERP, CV, WL
and inducibility of AT were not altered during a protocol time-matched to experiments involving the assessment of atrial electrophysiology under control conditions followed by two sequential changes of solution (n=8; data not shown). The unipolar electrocardiogram (ECG) was recorded via electrodes placed on the epicardial surface of the apex of the left ventricle and the aortic cannula. RR and QT intervals were measured and the average of 5 consecutive ECG complexes recorded. The rate-corrected QT (QT<sub>c</sub>) interval was calculated according to Kmecova et al<sup>5</sup> using a modified Bazett’s formula normalized to the average resting RR interval in perfused rat hearts (f), according to equation S1.

Equation 1:  
\[ QTc = \frac{QT}{\sqrt{(RR/f)}} \]

**Whole-cell voltage clamp recording of inward rectifier channel currents.** HEK293 cells stably transfected with either rat K<sub>i3.1/3.4</sub> (cell line a generous gift of Professor Andrew Tinker, University College & Queen Mary’s, London) or human K<sub>i2.1</sub> inward rectifier K<sup>+</sup> channels were seeded onto sterile glass coverslips at a density to allow isolated cells to be selected for recording. The dishes were stored in a humidified, gassed (5% CO<sub>2</sub>) incubator at 37 °C until use. Coverslips of cells were placed in a recording chamber mounted on the stage of an inverted microscope. Whole-cell recordings were made using the conventional ruptured patch configuration. Patch pipettes (1.5 – 2.5 MΩ) were filled with a solution containing (in mM): 110 KCl, 20 NaCl, 5 Mg-ATP, 5 EGTA, 10 HEPES, pH 7.2 corrected with KOH. For recordings from K<sub>i3.1/3.4</sub>-transfected cells, 0.9 mM GTPγS was included in the pipette solution to activate the GIRK channel currents. Experiments were performed using a HEKA EPC-10 amplifier controlled by Pulse Software (Ver8.7, HEKA GmbH, Germany). During recordings, cells were continuously superfused with bath solution containing (in mM): 150 NaCl, 10 KCl, 3 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES, pH 7.4 corrected with NaOH.
Experiments were performed at room temperature. Only those cells with an inward current at -140 mV larger than -1 nA were used for experiments. During experiments, the series resistance (2 – 6 MΩ) was typically compensated by 90%. The inwardly rectifying current density was monitored using a descending voltage ramp protocol as follows: Cells were held at a voltage of -60 mV from which a depolarising voltage step to +60 mV (100 ms) was applied prior to a descending ramp-repolarisation (0.4 V.s⁻¹) to -140 mV (100 ms) before returning to -60 mV. This waveform was repeated every 10 s throughout the experiment. The voltage protocol was repeatedly applied to monitor the mean current density at -140 mV. Once a stable current baseline was achieved in control bath solution, the cell was superfused with glibenclamide (10⁻⁵ M) and allowed to equilibrate for at least 3 minutes (at this concentration, glibenclamide completely inhibits Kᵢᵣ6.2/SUR2A Kᵦ₅ channel currents in <30 s, data not shown). On reaching steady-state inhibition of the current, the cell was superfused with K⁺-free bath solution (isotonic replacement with NaCl), resulting in the complete inhibition of Kᵢᵣ current at -140 mV⁶,⁷. Thus, Kᵢᵣ currents were measured as the K⁺-sensitive component of the inward current at -140 mV. The percentage inhibition was calculated according to equation S2.

Equation S2:  
\[ \% \text{inhibition} = \frac{(I_{\text{control}} - I_{\text{GLIB}})}{I_{\text{control}}} \times 100, \]

where \( I_{\text{control}} \) and \( I_{\text{GLIB}} \) are the K⁺-sensitive currents in control solution and in the presence of 10⁻⁵ M glibenclamide, respectively.

**Whole-cell voltage-clamp recording from left atrial myocytes.** Left atrial myocytes were isolated by Langendorff perfusion of hearts from adult male Wistar rats with a collagenase-containing solution using a protocol described previously¹. Isolated atrial myocytes were stored in Kraftbrühe (KB) solution containing (in mM), 70 L-glutamic acid, 30 KCl, 10 HEPES,
1 EGTA, 5 MgCl₂, 5 Na-pyruvate, 20 taurine, 10 D-glucose, 5 succinic acid, 5 creatine, 2 Na₂ATP, and 5 β-hydroxybutyric acid (pH 7.2) in a refrigerator (~4°C). Prior to recording, cells were transferred to a perfusion chamber and superfused with Tyrode’s solution containing (in mM), 140 NaCl, 4 KCl, 1 MgCl₂, 2.5 CaCl₂, 10 D-glucose, 5 HEPES (pH 7.45 with NaOH) at 36 °C. Patch pipettes were pulled from borosilicate glass and had a tip resistance of 1.5 – 2.0 MΩ when filled with internal solution containing (in mM), 110 KCl, 10 NaCl, 0.4 MgCl₂, 5 D-glucose, 10 HEPES, 5 BAPTA, 5 K₂ATP, 0.5 TrisGTP (pH 7.3 with KOH). Voltage-clamp recordings of whole-cell current were made from a holding potential of -80 mV. A modified voltage ramp protocol suitable for the survey of background K⁺ conductance was applied every 1/10 s⁹. A pre-pulse to -40 mV was used to inactivate voltage-gated Na⁺ currents. This was followed by a step depolarization to +20 mV (100 ms) and a descending voltage-ramp from +20 mV to -120 mV (500 ms) (Fig. 7A). Voltage commands were created and currents were recorded to the hard drive of a PC using an EPC-9 amplifier with Pulse (v8.3) software (HEKA Gmbh, Germany). Currents were analyzed using IgorPro vs3.6B (Wavemetrics Inc, USA).

**Measurement of myocardial metabolites.** Hearts from a separate group of adult male Wistar rats were used to investigate the effect of β-adrenoceptor agonism on myocardial metabolite concentrations. Hearts were excised under general anesthesia and mounted on a Langendorff apparatus, as described above. Following a stabilization period (30 minutes), eight hearts were perfused with control KH solution and six hearts were perfused with KH solution containing 10⁻⁶ M isoproterenol (ISO) for a further 30 minutes, which is sufficient time for the effect of ISO on heart rate to reach a steady-state. Hearts were then removed from the apparatus, biopsies were collected from the left atrium and left ventricle,
immediately frozen and stored in liquid nitrogen for analysis of metabolites. Frozen tissue samples were crushed to powder in liquid nitrogen, transferred to ice-cold 4.8% perchloric acid, mixed, and centrifuged at 2,800 X g for 10 minutes at 4° C. The supernatant was then neutralized using 0.44 M K₂CO₃ and was centrifuged again at 2,800 X g for 10 minutes at 4° C, after which the supernatant was collected for high-performance liquid chromatography (HPLC) analysis. The concentrations of adenine nucleotides were measured using a Beckman HPLC machine as described previously.

**Drugs.** Isoproterenol (ISO), glibenclamide (GLIB) and tolbutamide (TOLB) were purchased from Sigma-Aldrich (Poole, Dorset, UK). ISO solutions (10⁻⁹-10⁻⁶ M) were made by dilution to the final concentration in Krebs’ Henseleit solution from a stock solution of 10⁻² M in deionised water made fresh on each day of experimentation. Glibenclamide solutions, at concentrations previously shown to be effective in reducing the incidence of ischemia-induced ventricular tachyarrhythmia in isolated, perfused hearts (10⁻⁷-10⁻⁵ M)¹¹, ¹², were made by dilution to the final concentration in Krebs’ Henseleit solution from a stock solution of 10⁻² M in dimethylsulfoxide (DMSO). Glibenclamide stock solutions were stored at -20 °C until use. Tolbutamide-containing solutions, at a concentration that has been shown to be effective against atrial Kₐ₅ channels (10⁻³ M)¹³, were made on the day of experiment, by addition to the final concentration in Kreb’s Henseleit solution. Note that in order to avoid the effects of possible desensitization of β-adrenoceptors to repeated exposures to ISO, each heart was perfused with ISO at just one concentration.

**Statistics.** Data are presented as mean±SEM. Tests were performed, as appropriate, using Prism 5.03 (GraphPad Software Inc, San Diego, CA). Data in Supplemental Table 3 were analyzed by either paired Student’s t-test or one-way ANOVA with repeated measures with
Bonferroni multiple comparisons test (3 comparisons). $P<0.05$ was considered statistically significant.
Metabolic stress and substrate delivery in the Langendorff-perfused rat heart

The induction of metabolic stress in the left atrium but not the left ventricle (Fig. 6) is likely to be due to differences in O\textsubscript{2} and substrate delivery to the two tissues: In the Langendorff-perfused rat heart, the perfusion of the left atrium arises from a branch of the circumflex artery, itself a branch of the left coronary artery, whereas the perfusion of the left ventricle arises from the left coronary artery itself\textsuperscript{14}. A limitation of the Langendorff-perfusion technique is the low oxygen carrying capacity of crystalloid-based solutions, which requires that a high oxygen tension (gassing with 95% O\textsubscript{2}/5% CO\textsubscript{2} can be expected to achieve pO\textsubscript{2} of >500 mmHg) and perfusion rate be used\textsuperscript{14}. While under control conditions, perfusion was clearly adequate to meet the metabolic demand of both the left ventricle and left atrium, the increase in metabolic demand with ISO appears to have resulted in significant metabolic stress in the left atrium but not the ventricle.
REFERENCES


**Supplemental Table 1.** Fitted parameters to the ISO concentration-dependent inhibition of AERP and CV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CL = 75 ms</th>
<th>CL = 100 ms</th>
<th>CL = 150 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AERP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>47.97±5.573</td>
<td>51.55±4.789</td>
<td>54.61±4.895</td>
</tr>
<tr>
<td>LogEC\textsubscript{50} (M)</td>
<td>-7.724±0.507</td>
<td>-7.810±0.377</td>
<td>-7.568±0.386</td>
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<tr>
<td>EC\textsubscript{50} (M)</td>
<td>1.887×10\textsuperscript{-8}</td>
<td>1.549×10\textsuperscript{-8}</td>
<td>2.706×10\textsuperscript{-8}</td>
</tr>
<tr>
<td>r\textsuperscript{2}</td>
<td>0.452</td>
<td>0.549</td>
<td>0.576</td>
</tr>
<tr>
<td><strong>CV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>20.86±4.670</td>
<td>27.02±6.347</td>
<td>27.64±5.959</td>
</tr>
<tr>
<td>LogEC\textsubscript{50} (M)</td>
<td>-7.725±0.631</td>
<td>-7.954±0.586</td>
<td>-7.191±0.593</td>
</tr>
<tr>
<td>EC\textsubscript{50} (M)</td>
<td>1.885×10\textsuperscript{-8}</td>
<td>1.111×10\textsuperscript{-8}</td>
<td>6.448×10\textsuperscript{-8}</td>
</tr>
<tr>
<td>r\textsuperscript{2}</td>
<td>0.369</td>
<td>0.321</td>
<td>0.338</td>
</tr>
<tr>
<td>Degrees of Freedom</td>
<td>23</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>
### Supplemental Table 2. Fitted parameters to the concentration-dependent inhibition by glibenclamide of ISO-induced AERP and CV changes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CL = 75 ms</th>
<th>CL = 100 ms</th>
<th>CL = 150 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AERP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>44.67±2.060</td>
<td>45.55±2.082</td>
<td>48.15±1.751</td>
</tr>
<tr>
<td>LogIC\textsubscript{50} (M)</td>
<td>-6.505±0.313</td>
<td>-6.416±0.238</td>
<td>-6.270±0.185</td>
</tr>
<tr>
<td>IC\textsubscript{50} (M)</td>
<td>3.124×10\textsuperscript{-7}</td>
<td>3.834×10\textsuperscript{-7}</td>
<td>5.369×10\textsuperscript{-7}</td>
</tr>
<tr>
<td>Bottom</td>
<td>-5.229±4.484</td>
<td>3.919±3.931</td>
<td>2.063±3.760</td>
</tr>
<tr>
<td>r\textsuperscript{2}</td>
<td>0.813</td>
<td>0.733</td>
<td>0.812</td>
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<tr>
<td><strong>CV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>17.63±4.301</td>
<td>19.74±3.156</td>
<td>22.63±3.041</td>
</tr>
<tr>
<td>LogIC\textsubscript{50} (M)</td>
<td>-5.963±0.546</td>
<td>-6.011±0.330</td>
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<tr>
<td>IC\textsubscript{50} (M)</td>
<td>1.090×10\textsuperscript{-6}</td>
<td>9.745×10\textsuperscript{-7}</td>
<td>1.156×10\textsuperscript{-6}</td>
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<tr>
<td>Bottom</td>
<td>-27.08±15.52</td>
<td>-28.23±9.378</td>
<td>-29.95±9.762</td>
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<tr>
<td>r\textsuperscript{2}</td>
<td>0.481</td>
<td>0.540</td>
<td>0.584</td>
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<tr>
<td>Degrees of Freedom</td>
<td>25</td>
<td>47</td>
<td>47</td>
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**Supplemental Table 3.** Effects of tolbutamide and ISO on ECG parameters.

<table>
<thead>
<tr>
<th>Condition Parameter</th>
<th>Control</th>
<th>ISO (10^{-6} M)</th>
<th>+ Tolbutamide (10^{-3} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval (ms)</td>
<td>231.5±10.5</td>
<td>173.7±4.2*</td>
<td>189.0±1.4†</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>100.2±4.4</td>
<td>85.1±1.7‡</td>
<td>89.5±3.8§</td>
</tr>
<tr>
<td>QT_c interval (ms)</td>
<td>100.9±6.0</td>
<td>94.4±1.3</td>
<td>95.1±2.4</td>
</tr>
</tbody>
</table>

* P=0.0013; † P=0.0096; ‡ P=0.0030; § P=0.0130; one-way ANOVA with Bonferroni multiple comparisons test vs control.
Figure Legends

Supplemental Figure 1. Representative ECG and atrial electrogram recordings during perfusion with ISO (10^{-6} M) showing the spontaneous termination of a paroxysm of AT. Data correspond to that shown in Figure 2A, but are shown here with a faster time scale to allow inspection of electrograms and the assessment of rhythm. Complexes labeled ‘a’ and ‘v’ in channel 13 correspond to atrial and ventricular electrograms detected via the atrial electrode array. For purposes of clarity, not all complexes are labeled. Note the lack of a 1:1 correspondence between atrial and ventricular depolarization during the AT due to the filtering effect of the atrioventricular node.