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Modeling transmural heterogeneity of $K_{\text{ATP}}$ current in rabbit ventricular myocytes

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Models and mechanisms regulating excitation-metabolic coupling in various myocardial sublayers have been extensively studied in recent years. The cardiac myocyte is a unique cell type characterized by a complex ionic environment and a high degree of metabolic activity. The $K_{\text{ATP}}$ channel, a key component in excitation-contraction coupling, is known to play a crucial role in cardiac function.

In this study, we aimed to model the transmural heterogeneity of $K_{\text{ATP}}$ current in rabbit ventricular myocytes, focusing on the epicardial, endocardial, and midmyocardial regions. We incorporated equations for the $K_{\text{ATP}}$ channel and other relevant ion channels, using a mathematical model to simulate the electrical activity of ventricular myocytes under normal and ischemic conditions.

Results indicate that the ATP-sensitive $K_{\text{ATP}}$ channel is activated by the smallest reduction in intracellular ATP in epicardial cells and largest in endocardial cells when cytosolic ADP, AMP, PCR, Cr, P, total Mg$^{2+}$, Na$^+$, K$^+$, Ca$^{2+}$, and pH diastolic levels are normal. The model predicts that only $K_{\text{ATP}}$ ionophore (Kir6.2 subunit) and not the regulatory subunit (SUR2A) might differ from endocardium to epicardium.

During hypoxia and ischemia, the inhomogeneous accumulation of the metabolites in the tissue sublayers may alter in a very irregular manner the $K_{\text{ATP}}$ channel properties. The model predictions are in qualitative agreement with experimental data measured under normal and ischemic conditions in rabbit ventricular myocytes.

A NUMBER OF EXPERIMENTAL STUDIES have focused on the physiological distinctions between the ventricular subepicardium and subendocardium (5, 6, 18, 28, 34, 57, 64). Recently, a unique subpopulation of cells (M cells) has also been identified in the deep subepicardial to midmyocardial layers with physiological features intermediate between those of myocardial and conducting cells (3, 5, 7, 57, 83). These studies report that under normal conditions the enzymatically isolated cells from the epicardial, endocardial, and midmyocardial regions differ primarily with respect to their repolarization characteristics, with the epicardial myocytes displaying the shortest and mid-myocardial cells the longest action potential duration.

It has been demonstrated that the observed differences in action potential configuration and duration are due to the differences of various ion currents: fast Na$^+$ current, transient outward K$^+$ current, rapid-activating delayed rectifier K$^+$ current, slow-activating delayed rectifier K$^+$ current, and plateau K$^+$ current (5, 36, 64).

The epicardial, endocardial, and midmyocardial surfaces also respond differently to pharmacological agents and pathophysiological states (35, 38, 50, 51, 75, 88). The observations suggest that despite the greater susceptibility of endocardium to metabolic effects of ischemia, the electrophysiological changes evoked in epicardium are greater, and that this phenomenon may facilitate reentrant arrhythmias (35, 38, 50, 51, 72, 75). It has been demonstrated experimentally that during ATP depletion, the shortening in action potential duration is significantly greater in epicardial cells than in endocardial (35, 75).

Various explanations have been offered from in vivo studies and from multicellular ventricular preparations, including effects of cavity blood (33, 54), thebesian blood flow (30), greater capacity of the subendocardium for anaerobic metabolism (4, 40), and resistance of Purkinje fibers to the effects of hypoxia and ischemia (38). However, it is impossible to derive an understanding for the inherent regional electrophysiological cell properties from tissue preparations, which have extracellular ionic and electrotonic influences.

In this context, several reports (35, 50, 51, 75) add a further twist to the story by revealing a new role for $K_{\text{ATP}}$ channels when ATP is depleted and these channels are activated. These reports suggest that a differential sensitivity of $K_{\text{ATP}}$ channels to ATP among the cell subtypes might be partially responsible for the greater action potential shortening in epicardial myocytes during ischemia. To test the above hypothesis, Fukuwaka et al. (35) measured the effects of ATP depletion on the $K_{\text{ATP}}$ channel activity in single cells isolated from epicardial and endocardial surfaces. They demonstrated that the reduction in intracellular ATP evoked currents through $K_{\text{ATP}}$ channel to a greater magnitude in epicardial myocytes than in endocardial. However, the physiological basis for the site-related differential sensitivity of $K_{\text{ATP}}$ channels to ATP remains contradictable and unclear.

The contribution of active ion transport and the physiological distinctions between ventricular epicardial, midmyocardial, and endocardial myocytes to the development of the

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action potential has been established in cell modeling as well (32, 45, 59, 62, 70, 73, 74, 78, 80, 87, 91). In these models the empirical functions and parameters are, in general, fitted from data obtained under normal physiological conditions when ATP is high and KATP channels are closed. However, little attention has been paid to the electrophysiological effects of anoxia (fall in [ATP]tot/[ADP]tot ratio and opening of ATP-sensitive K+ channels), acidosis (intra- and extracellular pH drop), and hyperkalemia (accumulation of extracellular K+) among the cell types, despite their obvious importance in understanding pathologies such as ischemia (10, 17, 21, 39, 63, 65, 67, 81).

In the present study we used the modeling approach to investigate the mechanisms regulating excitation-metabolic coupling in rabbit epicardial, midmyocardial, and endocardial ventricular myocytes under normal conditions and during 20 min of simulated ischemia. Here we extended the LabHEART model (74) by incorporating equations for Ca2+ and Mg2+ buffering by ATP and ADP (65) and equations describing the nucleotide regulation of several ion channels and transporters [KATP channel, L-type Ca2+ channel, Na+/K+-ATPase, sarcolemmal Ca2+-ATPase, SR Ca2+-ATPase] (20, 67). In the model, creatine and adenylyl kinase (CK and AK, respectively) reactions, known to communicate the intracellular ATPases flux changes, were also included (16, 17). To simulate pH regulation in control conditions and during ischemia, we used the approaches of Iotti et al. (43) and Shaw and Rudy (81).

In agreement with experiments of Furukawa et al. (35) and Light et al. (56), our studies revealed that in isolated membrane patches the KATP channels are activated by smallest reduction in ATP in epicardial and largest in endocardial myocytes at constant for ATP (\(k_{\text{ATP}}\)). This in turn may cause differential action potential shortening among the cell subtypes. Preliminary results of (41) our studies also suggest that during ischemia, the inhomogeneous accumulation of metabolites in the sarcolemma may alter in a very irregular manner the normal channel sensitivity to ATP (i.e., epicardial and endocardial \(k_{\text{ATP}}\) values) through metabolic interactions with the endogenous lipid phosphoinositide (PI) cascade. This in turn may cause differential action potential shortening among the cell subtypes. Preliminary results of this work have been presented to the Biophysical Society in abstract form (58).

GLOSSARY

Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>EC coupling</td>
<td>excitation-contraction coupling</td>
</tr>
<tr>
<td>epi</td>
<td>epicardium (epicardial cell)</td>
</tr>
<tr>
<td>endo</td>
<td>endocardium (endocardial cell)</td>
</tr>
<tr>
<td>M</td>
<td>midmyocardium (M cell)</td>
</tr>
<tr>
<td>PIP</td>
<td>phosphatidylinositol phosphates (PIP2, PIP)</td>
</tr>
<tr>
<td>1-PC</td>
<td>1-palmitoylcarnitine</td>
</tr>
<tr>
<td>Cr</td>
<td>creatine</td>
</tr>
<tr>
<td>PCr</td>
<td>phosphocreatine</td>
</tr>
<tr>
<td>CK</td>
<td>creatine kinase reactions</td>
</tr>
<tr>
<td>AK</td>
<td>adenylyl kinase reaction</td>
</tr>
<tr>
<td>pHi</td>
<td>intracellular pH</td>
</tr>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>APD90</td>
<td>action potential duration at 90% repolarization</td>
</tr>
<tr>
<td>V</td>
<td>membrane potential</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
</tr>
<tr>
<td>SERCA2a</td>
<td>sarcoplasmic reticulum Ca2+-ATPase</td>
</tr>
<tr>
<td>TnC</td>
<td>troponin C</td>
</tr>
<tr>
<td>KATP</td>
<td>ATP-sensitive K+ channel</td>
</tr>
<tr>
<td>Kir6.2</td>
<td>inwardly rectifying K+ channel subunit</td>
</tr>
<tr>
<td>SUR2A</td>
<td>regulatory sulfonylurea receptor subunit</td>
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Volumes, Areas, and Capacity

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<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>(V_{\text{myo}})</td>
<td>myoplasmic volume</td>
</tr>
<tr>
<td>(A_{\text{cap}})</td>
<td>capacitative membrane area</td>
</tr>
<tr>
<td>(C_{\text{sc}})</td>
<td>specific membrane capacity</td>
</tr>
<tr>
<td>(F)</td>
<td>Faraday constant</td>
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Concentrations

<table>
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<td>[ATP]_tot</td>
<td>total ATP</td>
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<tr>
<td>[ATP]_i</td>
<td>free ATP</td>
</tr>
<tr>
<td>[MgATP]_i</td>
<td>myoplasmic concentration of Mg2+-bound ATP</td>
</tr>
<tr>
<td>[CaATP]_i</td>
<td>myoplasmic concentration of Ca2+-bound ATP</td>
</tr>
<tr>
<td>[ADP]_tot</td>
<td>total ADP</td>
</tr>
<tr>
<td>[ADP]_i</td>
<td>free ADP</td>
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<td>[MgADP]_i</td>
<td>myoplasmic concentration of Mg2+-bound ADP</td>
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<td>[CaADP]_i</td>
<td>myoplasmic concentration of Ca2+-bound ADP</td>
</tr>
<tr>
<td>[creatinine]_i</td>
<td>total creatine</td>
</tr>
<tr>
<td>[Cr]_i</td>
<td>free creatine</td>
</tr>
<tr>
<td>[PCr]_tot</td>
<td>total phosphocreatine</td>
</tr>
<tr>
<td>[AMP]_tot</td>
<td>total AMP</td>
</tr>
<tr>
<td>[phosphate]_i</td>
<td>total phosphate</td>
</tr>
<tr>
<td>[Pi]_i</td>
<td>free phosphate</td>
</tr>
<tr>
<td>[Mg2+]_tot</td>
<td>total Mg2+</td>
</tr>
<tr>
<td>[Mg2+]_i</td>
<td>free Mg2+</td>
</tr>
<tr>
<td>[K+]_i</td>
<td>extracellular K+ concentration</td>
</tr>
<tr>
<td>[K+]_o</td>
<td>intracellular K+ concentration</td>
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<tr>
<td>[Na+]_i</td>
<td>extracellular Na+ concentration</td>
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<tr>
<td>[Na+]_o</td>
<td>intracellular Na+ concentration</td>
</tr>
<tr>
<td>[Ca2+]_i</td>
<td>extracellular Ca2+ concentration</td>
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<tr>
<td>[Ca2+]_o</td>
<td>intracellular Ca2+ concentration</td>
</tr>
<tr>
<td>[Ca2+]_ISR</td>
<td>SR Ca2+ concentration</td>
</tr>
<tr>
<td>(J_{\text{rpn}})</td>
<td>buffering of Ca2+ by troponin C</td>
</tr>
<tr>
<td>(\beta,\beta')</td>
<td>rapid buffering approximation factors</td>
</tr>
<tr>
<td>[CAL]_tot</td>
<td>total calmodulin</td>
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Membrane Currents, SR Fluxes, and Parameters

\[ I_{\text{Na}} \quad \text{fast Na}^+ \text{ current} \]
\[ G_{\text{Na}} \quad I_{\text{Na}} \text{ density} \]
\[ I_{\text{Ca,b}} \quad \text{Na}^+ / \text{Ca}^{2+} \text{ exchanger current} \]
\[ I_{\text{Ca}} \quad \text{L-type Ca}^{2+} \text{ current} \]
\[ k_{\text{Mg}ATP(2-)} \quad \text{constant at which half of MgATP binding sites on } I_{\text{Ca}} \text{ are occupied} \]
\[ I_{\text{Ca,b}} \quad \text{Ca}^{2+} \text{ background current} \]
\[ I_{\text{NaCa}} \quad \text{Na}^+ / \text{Ca}^{2+} \text{ exchange current} \]
\[ I_{\text{o}} \quad I_{\text{o}} \text{ density} \]
\[ I_{\text{Kr}} \quad \text{rapid-activating delayed rectifier K}^+ \text{ current} \]
\[ I_{\text{Ks}} \quad \text{slow-activating delayed rectifier K}^+ \text{ current} \]
\[ I_{\text{Kp}} \quad \text{plateau K}^+ \text{ current} \]
\[ I_{\text{K1}} \quad \text{time-independent K}^+ \text{ current} \]
\[ I_{\text{NaK}} \quad \text{Na}^+ / \text{K}^+ \text{ pump current} \]
\[ k_{\text{NaK}} \quad \text{ATP half-saturation constant for Na}^+ / \text{K}^+ \text{ pump} \]
\[ k_{\text{MgADP}(--)}/(\text{Ca}^{2+}) \quad \text{ADP inhibition constant for Na}^+ / \text{K}^+ \text{ pump sarcolemmal Ca}^{2+} \text{ pump current} \]
\[ K_{\text{Ca}^{2+}}/P_{\text{Ca}} \quad \text{first ATP half-saturation constant for sarcolemmal Ca}^{2+} \text{ pump} \]
\[ k_{\text{MgATP}2(2-)}/P_{\text{Ca}} \quad \text{second ATP half-saturation constant for sarcolemmal Ca}^{2+} \text{ pump} \]
\[ k_{\text{MgADP}(--)}/P_{\text{Ca}} \quad \text{ADP inhibition constant for sarcolemmal Ca}^{2+} \text{ pump} \]
\[ J_{\text{KATP}} \quad \text{ATP sensitive K}^+ \text{ current} \]
\[ k_{\text{ATP}(-)} \quad \text{constant at which half of the ATP binding sites are occupied} \]
\[ k_{\text{MgADP}(-)} \quad \text{constant at which half of the MgADP binding sites are occupied} \]
\[ g_{\text{o}} \quad \text{relative conductance in the absence of nucleotides} \]
\[ g_{\text{d}} \quad \text{relative conductance with two molecules MgADP bound to one SUR2A subunit} \]
\[ G_{\text{K(ATP)}} \quad \text{maximum } K_{\text{ATP}} \text{ channel conductance at 0 mM [ATP]} \]
\[ E_{\text{K}} \quad \text{K}^+ \text{ reversal potential} \]
\[ IC_{50} \quad \text{half-maximal inhibition of } K_{\text{ATP}} \text{ channel} \]
\[ J_{\text{up}} \quad \text{Ca}^{2+} \text{ flux via SR Ca}^{2+} \text{-ATPase pump} \]
\[ k_{\text{MgATP}2(2-)} \quad \text{ATP half-saturation constant for SERCA2a} \]
\[ k_{\text{MgADP}(-)} \quad \text{ADP first inhibition constant for SERCA2a} \]
\[ k_{\text{MgADP}(-)} \quad \text{ADP second inhibition constant for SERCA2a} \]

Dissociation, Rate, and Equilibrium Constants

\[ K_{\text{nuc}} \quad \text{on and off rate constants} \]
\[ K_{\text{CaATP}2(2-)} \quad \text{Ca}^{2+} \text{-ATP dissociation constant} \]
\[ K_{\text{MgATP}2(2-)} \quad \text{Mg}^{2+} \text{-ATP dissociation constant} \]
\[ K_{\text{MgADP}(-)} \quad \text{Mg}^{2+} \text{-ADP dissociation constant} \]
\[ k_{d} \quad \text{calmodulin dissociation constant} \]
\[ K_{\text{CK}} \quad \text{apparent equilibrium constant of creatine kinase reaction} \]

METHODS

Ion-ionic-metabolic model in rabbit epicardial, endocardial, and mid-myocardial myocytes. The overall scheme of the model is shown in Fig. 1. In this article, the equations describing ion channel currents and Ca$^{2+}$ dynamics in rabbit ventricular myocytes were the same as in the original paper by Puglisi and Bers (74). The relative current densities ($G_{\text{Na}}, G_{\text{Ca}}, G_{\text{Ks}}, G_{\text{Kp}}, G_{\text{K1}}$) in each region were introduced into our whole-cell model as well (see Table 1).

Here we provide only the modified equations of Michailova and McCulloch (65), which describe the buffering of Ca$^{2+}$ and Mg$^{2+}$ by ATP and ADP and the time changes in free intracellular Ca$^{2+}$ and Mg$^{2+}$ levels during cell excitation:

\[ [\text{ATP}]_{i0} = [\text{ATP}]_{i0} + [\text{ADP}]_{i} + [\text{MgATP}^{2-}], \]
\[ \frac{d[MgATP^{2-}]}{dt} = k_{\text{MgATP}^{2-}/[\text{ATP}]^{4}_{i}}, \]
\[ \frac{d[CaATP^{2-}]}{dt} = k_{\text{CaATP}^{2-}/[\text{CaATP}]^{4}_{i}}, \]
\[ \frac{d[CaADP]}{dt} = k_{\text{CaADP}^{2-}/[\text{CaATP}]^{4}_{i}}, \]
\[ \frac{d[MgADP]_{i}}{dt} = k_{\text{MgADP}^{2-}/[MgADP]_{i}}, \]
\[ \frac{d[CaATP]_{i}}{dt} = k_{\text{CaATP}^{2-}/[Ca^{2+}]_{i}}, \]
\[ \frac{d[CaADP]}{dt} = k_{\text{CaADP}^{2-}/[Ca^{2+}]_{i}}, \]
\[ \frac{d[MgADP]_{i}}{dt} = k_{\text{MgADP}^{2-}/[MgADP]_{i}}, \]
\[ \frac{d[ATP]^{3}_{i}}{dt} = -\frac{d[CaATP]_{i}}{dt} + \frac{d[CaADP]}{dt} \]
\[ \frac{d[ADP]^{3}_{i}}{dt} = -\frac{d[MgADP]_{i}}{dt} + \frac{d[CaADP]}{dt} \]
\[ \frac{d[Mg^{2+}]_{i}}{dt} = -\frac{d[CaATP]_{i}}{dt} + \frac{d[MgADP]_{i}}{dt} \]
\[ \frac{d[Ca^{2+}]_{i}}{dt} = \beta \left[ -J_{\text{up}} - J_{\text{up}} - (I_{\text{Ca}} + I_{\text{Na}} + 2I_{\text{NaCa}} + I_{\text{Ko}}) \right] \]
\[ \times \frac{A_{\text{m}}}{2V_{\text{myoc}}F} \]
(20) for Na⁺-K⁺ pump current, sarcolemmal Ca²⁺ pump current, and SR Ca²⁺-ATPase pump current, assuming $I_{\text{NaK}}$, $I_{\text{p(Ca)}}$, and $J_{\text{up}}$ are regulated by MgATP²⁻ and MgADP⁻:

$$I_{\text{NaK}}(t) = \left(1 + \frac{K_{\text{NaATP}}}{[\text{MgATP}^2^-]}\right)^{-1} \left(1 + \frac{[\text{MgADP}^+]}{K_{\text{NaADP}}^{-1}}\right) I_{\text{NaK}}(t)$$  \hspace{1cm} (12)

$$I_{\text{p(Ca)}}(t) = \left(1 + \frac{K_{\text{CaATP}}}{[\text{MgATP}^2^-]}\right)^{-1} \left(1 + \frac{[\text{MgADP}^+]}{K_{\text{CaADP}}^{-1}}\right)^{-1} I_{\text{p(Ca)}}(t)$$  \hspace{1cm} (13)

To simulate the differential sensitivity of the cardiac KATP channel to free ATP ([ATP⁴⁻]) and MgADP⁻ among the cell subtypes, we used the KATP channel model of Michaelova et al. (67) that contains four pore-forming subunits (Kir6.2) and four regulatory subunits (SUR2A) (see APPENDIX A). Our formulation for MgATP²⁻ regulation of L-type Ca²⁺ current originally described in Ref. 67 is shown in APPENDIX A as well.

Here it is important to emphasize that model agreement with experiment will be strongly dependent on the use of realistic ligand (ATP, ADP, AMP, P₃, PCr, Cr) and extracellular and intracellular ionic (Na⁺, K⁺, Mg²⁺, H⁺) concentrations under normal conditions and during ischemia (14, 15, 43, 47, 52, 61, 77). However, experimental data for the nucleotide, PCr, Cr, P₃, and ionic concentration changes in rabbits during ischemia are limited (48, 61, 75). Thus, to be able to calculate, if experimental data were not available, the resting nucleotide, metabolite, or Mg²⁺ levels, we included in the model the basic reactions (ATP hydrolysis, CK and AK catalysis) known to communicate the intracellular ATPase fluxes:

Table 1. Ion current densities among cell types

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$G_{\text{Na}}$, ms/µF</th>
<th>$G_{\text{Ca}}$, ms/µF</th>
<th>$G_{\text{K}}$, ms/µF</th>
<th>$G_{\text{K}}$, ms/µF</th>
<th>$G_{\text{p}}$, ms/µF</th>
<th>$G_{\text{p}}$, ms/µF</th>
</tr>
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<tbody>
<tr>
<td>Endocardium</td>
<td>7.0</td>
<td>0.025</td>
<td>0.004</td>
<td>0.03</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Mid-myocardium</td>
<td>8.0</td>
<td>0.06</td>
<td>0.006</td>
<td>0.015</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Epicardium</td>
<td>6.0</td>
<td>0.06</td>
<td>0.008</td>
<td>0.04</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

Ion current densities in the three regions estimated in rabbits by Saucerman et al. (78).
ATP → ADP + P_i
PCr + ADP ⇄ ATP + Cr
2ADP ⇄ ATP + AMP

Subsequently this allowed us to derive the key expressions for CK and AK equilibrium reactions and for total intracellular adenine, creatine, and phosphate levels:

\[
\text{[adenine]} = [\text{ATP}]_i + [\text{ADP}]_i + [\text{AMP}]_i + \text{[PCr]}_i
\]

\[
\text{[creatinine]} = [\text{Cr}_i] + [\text{PCr}]_i
\]

\[
\text{[phosphate]} = [\text{PCr}]_i + [\text{P}_i] + 3[\text{ATP}]_i
\]

\[
K'_\text{CK}[\text{ADP}]_i[\text{PCr}]_i = [\text{ATP}]_i[\text{Cr}_i] + 2[\text{ADP}]_i + [\text{AMP}]_i
\]

\[
K''_{\text{AK}}[\text{ADP}]_i^2 = [\text{ATP}]_i[\text{AMP}]_i
\]

Recent studies also suggest that the exact knowledge of the apparent equilibrium constant of creatine kinase reaction (K'\text{CK}) is essential for accurate in vivo quantification of the total nucleotide, phosphate, and total Mg\textsuperscript{2+} levels (43). Therefore, in the present study, we used the model of CK reaction from Iotti et al. (43) to estimate K'\text{CK} in control conditions when diastolic [K\textsuperscript{+}]\textsubscript{i}, [Na\textsuperscript{+}]\textsubscript{i}, [Mg\textsuperscript{2+}]\textsubscript{i}, and pH\textsubscript{i} levels are known:

\[
\log K'_{\text{CK}} = a + bx + cy + dx^2 + exy + fy^2 + gx^3 + hx^2y + ixy^2 + jy^3
\]

where: a–j are coefficients, x is pH\textsubscript{i}, and y is pMg = −log [Mg\textsuperscript{2+}]\textsubscript{i}.

The coefficient equations (a–j) describing K'\text{CK} in the pH\textsubscript{i} and pMg range 5–8 and 2–4, respectively, are shown in Table 2. Our pH\textsubscript{i} and pMg values are within that range (see Table 3, column 2). The using the accurate model for the creatine kinase reaction in normoxia was essential, because subsequently this allowed evaluating the nucleotide and free Mg\textsuperscript{2+} concentrations as well the apparent K'\text{CK} constant at 20 min of ischemia (see Table 3).

In the model, we also assumed that among the cell subtypes the total ligand (ATP, ADP, AMP, PCr), ionic (Mg\textsuperscript{2+}, H\textsuperscript{+}, K\textsuperscript{+}, Na\textsuperscript{+}, Ca\textsuperscript{2+}), and phosphatidylinositol phosphate (PIP) levels are equal, spatially uniform, and remain approximately constant during a single beat.

Finally, we need to add that in this study the effects of acidosis among the cell subtypes were simulated using the approach of Shaw and Rudy (81): 1) the maximum conductance of L-type Ca\textsuperscript{2+} was reduced by 50%; and 2) the maximum conductance of Ih\textsubscript{Na} was reduced by 25%, and its voltage-current curve was shifted to the right by 3.4 mV.

Rapid equilibrium for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} buffering by ATP and ADP.

Our previous studies have demonstrated that the Eqs. 1–11 are biologically accurate but complicated computationally (65). In this article we sought to minimize the computational complexity by assuming that Ca\textsuperscript{2+} and Mg\textsuperscript{2+} buffering by ATP and ADP occur on much faster time scales than other excitation-contraction coupling processes included in the new ionic-metabolic model. Thus the following equations can be written:

\[
[\text{CaATP}^2] = \frac{[\text{Ca}^{2+}]}{K_{\text{d}}[\text{ATP}^2]}
\]

\[
[\text{ADP}^2] = \frac{[\text{ATP}]}{[\text{ATP]}_i[\text{ADP}]}[\text{ATP}^2] + [\text{ADP}]_i[\text{ADP}]_i[\text{ADP}^2]/[\text{ATP}]_i[\text{ADP}]
\]

The validation studies (see Fig. 2) indicate that the simulated action-potential time courses under normal conditions with the Eqs. 1–11 or 21–27 are quite similar in shape among the cell subtypes. Since all subsequent simulations yielded very similar results with either approximation, only the simulations using the rapid buffering approximation are shown in the RESULTS.

Unless specified otherwise in the legends to Figs. 1–6 or in the text, the standard set of parameters used in the calculations is listed in the Tables 1–4. All initial conditions and values of the parameters that are not included in the present paper correspond to those used in Puglisi and Bers (74), Cortassa et al. (20), Michailova and McCulloch (65), and Michailova et al. (67).

RESULTS

ATP dose-response relationships in epicardial, endocardial, and midmyocardial membrane patches. Sarcolemmal K\textsubscript{ATP} channels have been identified and investigated in many regions of the heart, including the atria, ventricles, and AV and SA nodes (6, 18, 29, 51, 56, 90). However, experimental data describing macroscopic and single-channel properties among the tissues sublayers are scarce. In 1991, Furukawa et al. (35) compared the sensitivity of ATP-regulated K\textsuperscript{+} channel to the reduction of free ATP in single myocytes isolated from cat ventricular endocardial and epicardial regions. In these experiments the intracellular surface of inside-out membrane patches was superfused with high-K\textsuperscript{+} solution ([K\textsuperscript{+}]\textsubscript{i} ~ 140 mM).

Table 2. K'\text{CK} parameter values in normal conditions

<table>
<thead>
<tr>
<th>[Na\textsuperscript{+}]\textsubscript{i}</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM</td>
<td>−0.621</td>
<td>3.2245</td>
<td>−0.9316</td>
<td>−0.5517</td>
<td>0.1347</td>
<td>−0.0341</td>
<td>0.0175</td>
<td>0.03869</td>
<td>−0.1098</td>
<td>0.07188</td>
</tr>
</tbody>
</table>

The Iotti et al. (43) K'\text{CK} coefficient values obtained at [Na\textsuperscript{+}]\textsubscript{i} = 10 mM, [K\textsuperscript{+}]\textsubscript{i} = 150 mM, and pH\textsubscript{i} and pMg ranges of 5-8 and 2-4, respectively.

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containing 10, 25, 50, 100, 250, 500, and 1,000 μM free ATP while total intracellular Mg2+, ADP, AMP, PCr, Pi, extra- and intracellular K+, Na+, Ca2+, and pH remained at normal diastolic levels. The data from Furukawa et al. (35) (see diamonds and triangles in Fig. 3A) suggest that the open probability of KATP channels was reduced in a dose-response fashion among the cell subtypes. Figure 3A also shows that the concentration of ATP that produced half-maximal inhibition of the channel was ~23.6 μM in endocardial and ~97 μM in epicardial patches. In 1999, Light et al. (56) recorded the ATP concentration-dependent relationship for KATP channel in isolated rabbit ventricle myocytes without regard to the cell location (see circles in Fig. 3B). Note that the data from Light et al. (56) show that the rabbit dose-response curve (IC50 ~ 21 μM) is quite similar to the ATP dose-response relation in cat endocardial myocytes (IC50 ~ 23.6 μM). Therefore, this was our justification for using cat data to fit the ionic-metabolic model in rabbits among the cell subtypes. Figure 3 shows our attempt to create simulations that quantitatively approximate the reported experimental data in epicardial and endocardial cat and rabbit myocytes. During this experiment, in each point on the simulated curves, the relative KATP current (I_{KATP}/I_{KATP=0}) was computed in steady state at fixed total ATP while total Mg2+ and ADP, [PCr]_i, [Cr], [AMP]_i, [Pi], and H+, K+, Na+, and Ca2+ diastolic concentrations were kept normal (see Table 3, column 2).

Under the above conditions, we were able to fit the data of Furukawa et al. (35) in epicardial cells by using the original parameter values of Michailova et al. (67) for k_{KATP(-)}, k_{MgADP(-)}, g_{o}, g_{d}, and G_{KATP} in guinea pig myocytes (see
The greater sensitivity of $I_{\text{KATP}}$ to ATP changes in the endocardium was modeled by decreasing only the Kir2.6 half-maximal saturation constant ($k_{\text{ATP}}$) (see Fig. 3A and B, and Table 4). Here, it is important to mention that an interesting model prediction is that the calculated relative $I_{\text{KATP}}$ current remained almost unaffected by changes in SUR2A half-maximal saturation constant ($k_{\text{MgADP}}$) and changes in the relative channel conductance ($g_o$) in a wide region ($k_{\text{MgADP}} = 0.001–10$, $g_o = 0–1$, $g_d = 0–1$) (data not shown). To simulate the ATP dose-dependent effects in midmyocardial cells (see Fig. 3A), we used the assumption of Gima and Rudy (39) that the midmyocardium half-maximal saturation constant ($k_{\text{ATP}}$) is ~50% that in epicardial cells (see Table 4). In addition, the results in Fig. 3A also indicate that the simulated relative currents in response to rhythmically applied pulses (1 Hz, 19–20 s) approached 1 at ~0.1 μM intracellular free ATP in the three myocyte subtypes but were close to 0 at ~1 mM [ATP$^4^-$]$_i$ in epicardial, at ~650 μM [ATP$^4^-$]$_i$ in midmyocardial, and at ~300 μM [ATP$^4^-$]$_i$ in endocardial cells.

**Modeling normoxia in rabbit endocardial, midmyocardial, and epicardial ventricular myocytes.** Many experimental protocols indicate that in normal conditions the $K_{\text{ATP}}$ channel activity is inhibited throughout the cell subtypes (see Fig. 1A) (8, 31, 35, 69, 71). To test whether our whole-cell ionic-metabolic model is able to predict that $I_{\text{KATP}}$ current is almost inactive in healthy ventricular tissue, we calculated this current during a single beat. Graphs (see Fig. 4A) demonstrate that the channel activity in the three regions was low during the cell excitation. These results also indicate that the predicted epicardial, midmyocardial, and endocardial steady-state $I_{\text{KATP}}$ current after ~20 ms reached peaks of ~0.12, ~0.027, and ~0.004, respectively, and that the current duration was shortest in epicardial and longest in midmyocardial myocytes. In addition, these $K_{\text{ATP}}$ site-related currents had minimal contribution to the action potential shape when [ATP$^4^-$]$_i$ was high (390 μM) and [MgADP$^-$]$_i$, low (67 μM) (see Fig. 4B). The channel parameter values and resting ligand and ionic concentrations used in this numerical experiment are shown in Tables 1 and 2, 4, and Table 3 (column 2).

**Modeling simulated ischemia in rabbit endocardial and epicardial ventricular myocytes.** Experimental data for the differential electrophysiological transmural responses of the myocytes and for the changes in resting normoxic ligand and ionic levels in rabbit ventricular tissue are shown. We found such data for endocardium and epicardium after 20 min of simulated ischemia in Qi et al. (75). These data suggest, under normal conditions (see gray graphs in Fig. 5A), the following: 1) the action potential duration recorded from subepicardial myocytes was shorter; 2) the resting potentials recorded from the two cell subpopulations had no significant difference; and 3) the density of steady-state outward K$^+$ current in subepicardial myocytes was greater than that in subendocardial myocytes. In addition, the data of Qi et al. (75) demonstrate, under simulated ischemic conditions (see black graphs in Fig. 5A), the following: 1) the action potential duration in subepicardial cells was shortened more compared with that in subendocardial myocytes; 2) the resting potentials recorded from the two cell subpopulations had no significant difference; and 3) the relative increase of steady-state outward K$^+$ current in subepicardial cells was greater than that in subendocardial myocytes. In addition, in the experiment by Qi et al. (75), when myocytes were perfused with ischemia solution for 20 min, showed the following: pH decreased to 6.8; extracellular K$^+$ was kept normal (5.4 mM) while extracellular Na$^+$ decreased (from 137 to 117 mM); and the changes in ATP, ADP, AMP, PCR, [K$^+$], [Ca$^{2+}$], [Ca$^{2+}$]$_{si}$, [Na$^+$], and [K$^+$] normoxic resting levels were not reported. Thus, these measurements reflect some combined contributions of two ischemic conditions, acidosis and anoxia. Figure 5B shows our attempt to reproduce qualitatively the data of Qi et al. (75) in normoxia and under acidosis-anoxia conditions. The channel parameter values and resting ligand and ionic concentrations used in this numerical experiment are shown in Tables 1 and 2, 4, and Table 3 (column 4).

**Table 4. $K_{\text{ATP}}$ channel parameters under normal conditions**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>$k_{\text{ATP}}$ [mM]</th>
<th>$k_{\text{MgADP}}$ [mM]</th>
<th>$g_o$</th>
<th>$g_d$</th>
<th>$G_{\text{KATP}}$ [mS/μF]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardium</td>
<td>0.131*</td>
<td>0.4†</td>
<td>0.08†</td>
<td>0.89†</td>
<td>0.05</td>
</tr>
<tr>
<td>Mid-myocardium</td>
<td>0.275*</td>
<td>0.4†</td>
<td>0.08†</td>
<td>0.89†</td>
<td>0.05</td>
</tr>
<tr>
<td>Epicardium</td>
<td>0.6†</td>
<td>0.4†</td>
<td>0.08†</td>
<td>0.89†</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Gima and Rudy (39). †Michailova et al. (67).
The results indicate that the fall in [ATP]_o/[ADP]_o ratio (~33 times) with [Mg^{2+}]_o constant and the drop in pH; 1) decreased APD_{90} in epicardium from 146 to 71 ms, i.e., ~2 times (see solid gray and dashed black lines in Fig. 5B(a)); 2) did not affect the normal resting potentials; and 3) increased the steady-state outward K^+ current in subepicardial cell more sensitively than in subendocardial cell (see Fig. 5B(b) and c)). However, it is important to stress that according to the data of Qi et al. (75), the endocardial APD_{90} decreased ~1.32 times while the epicardial APD_{90} drop was much more pronounced, i.e., ~2.6 times. Thus, questions arising here were the following: 1) What might be the physiological reason(s) for the observed different subendocardial and subepicardial APD_{90} shortening? and 2) Does our ionic-metabolic model help to explain (at least partially) this phenomenon that has puzzled people for years?

To further explore the ionic basis for the differential electrophysiological responses to ischemia at the two sites, Qi et al. (75) used specific I_{KATP} channel blockers. They demonstrated that, although the changes in steady-state outward K^+ current were the sum of the changes of many K^+ currents (% I_{Na}, % I_{Ko}, % I_{Ks}, % I_{K1}, % I_{NaK}, % I_{KATP}), the observed increases in the total outward K^+ current among the cell types were mainly due to K_{ATP} channel opening (see Fig. 5A, b and c). However, why ischemia evoked a greater increase in the epicardial outward K^+ current and a greater decrease in the epicardial APD_{90} remained unclear. Here, we hypothesized that ischemia may alter the normal ATP sensitivity of K_{ATP} channels among the cell subtypes in a very irregular manner, contributing to the differential response of those myocytes. To test this hypothesis we performed another set of calculations searching for new epicardial and endocardial k_{ATP4-}, k_{MgADP4-}, g_o, and g_d parameter values. Surprisingly, our model predicted that in epicardial myocytes, by varying only the Kir6.2 half-maximal saturation constant (i.e., increasing k_{ATP4-} from 0.6 mM up to infinity), we could be shortening the APD_{90} more significantly (~2.43 times) (see solid black line in Fig. 5B(a)). Furthermore, we searched for values of epi k_{ATP4-} which are reasonably close to 600 μM and that yield action potential shortening sufficiently close to the theoretical maximum. Thus, the model predicts that APD_{90} with k_{ATP4-} = 8 mM is quite similar to that with k_{ATP4-} → ∞. The results also revealed that the increase in steady-state outward K^+ current in subepicardial cells was significantly greater with k_{ATP4-} = 8 mM than predicted with k_{ATP4-} at 600 μM (see solid black and dashed black lines in Fig. 5B(b)). Furthermore, our studies in the endocardium revealed that the normal APD_{90} dropped from 195 to 127 ms (~1.54 times) when k_{ATP4-} decreased from 131 μM to zero. The simulations also showed that APD_{90} remained at 127 ms with k_{ATP4-} at 20 μM and that there was little change in APD_{90} with lower values of k_{ATP4-} (see Fig. 5Bc, dotted black and dash-dotted black lines). In addition, our analysis suggests that changes in k_{MgADP4-}, g_o, and g_d alone (k_{MgADP4-} = 0.001–10, g_o = 0–1, g_d = 0–1) do not seem to account for the experimental findings in either region (data not shown).

The predicted ionic currents and Ca^{2+} time courses in the myoplasm and sarcoplasmic reticulum in each of the cell subtypes during normal and ischemic action potentials (see solid and dotted lines in Fig. 5B) are shown in Fig. 6.

The simulations revealed that in control conditions (see gray graphs in Fig. 6, A and F–I), due to the higher K^+ conductance in epicardium, the I_{Ko}, I_{Kp}, I_{Kr}, and I_{Ks} currents were enhanced, whereas the converse was true for I_{Na} current (epicardial G_{Na} < endocardial G_{Na}). Model results also demonstrate that under normal conditions, the differing outward K^+ current densities among the tissue sublayers (see gray graphs in Fig. 5B, b and c) had a differential effect on the epicardial and endocardial I_{Ca}, I_{NaCa}, I_{NaK}, I_{K1}, I_{p(Ca)}, I_{Ca,b}, I_{Na,b}, [Ca^{2+}], and [Ca^{2+}]_{SR} time courses (see gray graphs in Fig. 6, B–D and J–O). Finally, Fig. 6E shows that in normoxia the K_{ATP} channel activity was low in both regions.

At 20 min simulated ischemia, the epicardial and endocardial I_{Na} values were reduced due to the effects of acidosis (see Fig. 6A). The combined effects of acidosis and anoxia had a significant influence on I_{Ca} peak and duration throughout the cell subtypes (see Fig. 6B). Anoxia also resulted in activation of I_{KATP} current that was much more pronounced in epicardial myocytes (Fig. 6E). Our analysis also suggests that
changes in \([\text{MgATP}^2^-]\) and \([\text{MgADP}^-]\), most significantly affected the SR \(\text{Ca}^{2+}\)-ATPase pump activities that in turn depleted the SR \(\text{Ca}^{2+}\) content and decreased the myoplasmic \(\text{Ca}^{2+}\) peaks in both cell subtypes (see Fig. 6, N and O). In addition, the ischemic changes in global \([\text{Ca}^{2+}]\) affected the efficiency of the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger (see Fig. 6C). Figure 6D shows that in both regions, the anoxic changes in \(\text{MgATP}^2^-\) and \(\text{MgADP}^-\) significantly shortened \(I_{\text{NaK}}\) duration, increased the rest current, and decreased \(I_{\text{NaK}}\) peak while not significantly affecting \(I_{\text{Ca}}\) time course (see Fig. 6K). The results in Fig. 6 also indicate that the changes in the ligand, \([\text{Na}^+]_o, [\text{Mg}^{2+}]_o, \text{pH}\), and \([\text{Pi}]\) levels and the drop in \(K_C^v\) value (~1.8 times) at 20 min of simulated ischemia had marked effects on \(I_{\text{Na}}, I_{\text{Kp}}, I_{\text{Kr}}, I_{\text{Ks}}, I_{\text{Ca,k}}, I_{\text{Ca,b}},\) and \(I_{\text{Na,b}}\) time courses throughout the cell subtypes. The model predictions for reduced SR content, decreased systolic \(\text{Ca}^{2+}\) peak, and activated \(I_{K(\text{ATP})}\) current during ischemia are in qualitative agreement with experiment (15, 44, 46).

### DISCUSSION

Regional ionic-metabolic models in rabbit ventricular myocytes. In 2001, we extended the model of Winslow et al. (87) in dog ventricular myocytes to investigate how \(\text{Ca}^{2+}\) and \(\text{Mg}^{2+}\) buffering and transport by ATP and ADP regulate cardiac excitation-contraction coupling (65, 87) and how the fall in \([\text{ADP}]_{\text{out}}/[\text{ATP}]_{\text{out}}\) ratio may affect the intracellular \(\text{Ca}^{2+}\), \(\text{Mg}^{2+}, \text{Na}^+,\) and \(\text{K}^+\) concentrations, the free and bound ATP and ADP diastolic and systolic levels, and the ionic currents in the myoplasm, submembrane space, and sarcoplasmic reticulum. The important limitation of this model was that the \(K_{\text{ATP}}\) current, known to be inactive in healthy ventricular tissue and increasingly outward with decreasing levels of ATP, was not included. For this reason recently, using a model approach aimed at simulating the underlying molecular nature of the \(K_{\text{ATP}}\) channel rather than taking a phenomenological approach, we formulated a new model for \(I_{K(\text{ATP})}\) regulation by intracel-
Fig. 6. A–O: the calculated ionic currents and Ca\(^{2+}\) concentrations in epicardial cell (solid lines) and endocardial cell (dotted lines) in response to 1-Hz periodic pulse (19–20 s). Normal conditions (gray graphs): epicardial \(k_{ATP} = 600 \mu M\), endocardial \(k_{ATP} = 131 \mu M\). Ischemia 20 min (black graphs): epicardial \(k_{ATP} = 8 \text{ mM}\), endocardial \(k_{ATP} = 20 \mu M\).
lular free ATP and MgADP and incorporated this model into our whole-cell dog ionic-metabolic model (67). The updated model was able to reproduce quantitatively or qualitatively a sequence of events that corresponds well with published experimental data under normal conditions and during ischemia (2, 55, 69). However, we need to acknowledge that both models (65, 67) have limitations: 1) over a longer time period ($t > 15$ s) these models failed to achieve steady state; 2) the effects of acidosis on the ionic currents and concentrations were not included; 3) some unrealistic ligand ([ATP]$_{m}$, [ADP]$_{m}$) and extra- and intracellular ionic concentrations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$) under normal conditions and for the duration of ischemia were used. To overcome the above limitations, in this article we extended the LabHEART model in rabbits (74), which is reasonably stable over a long time period, by incorporating equations for $Ca^{2+}$ and $Mg^{2+}$ buffering by ATP and ADP, and for the nucleotide regulation of K$_{ATP}$ and L-type $Ca^{2+}$ channels. Here, to further investigate and better understand how the changes in ATP and ADP during ischemia regulate the complex cellular dynamics, we updated the flux equations from Michailova et al. (65) for SERCA2a, $I_{\text{CaL}}$, and $I_{\text{NaK}}$ ATPases. For this purpose, we modified the Cortassa et al. (20) ATP/ADP kinetics for these cytosolic ATP-consuming transporters, assuming dependence on MgATP$^{2-}$ and MgADP$^{-}$. In addition, because we could not find experimental data for [CaATP$^{2-}$], [ADP$^{3-}$], [MgADP$^{-}$], [CaADP$^{-}$], [AMP$_{m}$], [Cr$_{i}$], [phosphate], or total Mg$^{2+}$ levels in normoxia, we included in the model expressions for total adenine, creatine, and phosphate, and CK and AK equilibrium reactions (16, 17), which allowed us to calculate these model parameters. To maximize the advantages of biophysically based modeling while minimizing computational complexity, we assumed also that $Ca^{2+}$ and $Mg^{2+}$ are buffered by ATP and ADP on much faster time scales than other excitation-contraction coupling processes. Finally, we used the approaches of Shaw and Rudy (81) and Iotti et al. (43) to simulate the pH$_{i}$ regulation in normoxia and during ischemia. To further test the assumptions made about the kinetic mechanisms of nucleotide actions on the K$_{ATP}$ channel subunits, we investigated the mechanisms regulating excitation-metabolic coupling in cardiac cells of epicardial, endocardial, and midmyocardial origin. Every attempt has been made to create simulations that quantitatively or qualitatively approximate published experimental data in normoxia or during ischemia or the excited patch method of voltage-clamp? Recent attempts to model the possible regulation of the cell is disrupted by the excited patch method of voltage-clamp? Recently, the a solution to the puzzle has been suggested (9, 12, 24, 26, 27, 42, 60, 82). It has been demonstrated that this phenomenon (known as channel run-down) is induced by wash out of phospholipids, including phosphatidylinositol-4,5-bisphosphates (PIP$_{2}$) or phosphatidylinositol-4-phosphates (PIP) and is accompanied by a marked increase in the ATP sensitivity of the channel (12, 27, 37). Thus, it has been assumed that in vivo, MgATP$^{2-}$ might serve as a substrate maintaining the membrane concentrations of PIPs critical for channel activation. On the basis of these findings, a molecular/physical model describing how the channel activity might be regulated by the PIPs has been proposed (9, 22, 23, 26). This model suggests that the membrane-incorporated PIPs can bind to the positive charges in the cytoplasmic region of the channel’s Kir6.2 subunits, stabilizing the open state of the channel and antagonizing the inhibitory effect of ATP. However, important questions still remain to be answered: What is the resting concentration of PIPs in the cell membrane and is it sufficient to account for the difference in the ATP sensitivity of the channel between the normal intact cell and excited patch? Why are K$_{ATP}$ channels activated by a smaller reduction in intracellular ATP in epicardial cells than in endocardial cells in the excited patches?
In this paper, we used the model approach to address some of these questions. Our first goal was to estimate as accurately as possible the normal $K_{C_{\text{K}}}$ value, taking into account realistic intracellular ionic concentrations ($Na^+$, $K^+$, $Mg^{2+}$, $H^+$) reported in rabbit myocytes in control conditions (43, 74, 75). Here, we need to mention that we calculated normal $K_{C_{\text{K}}}$ as follows: 1) assuming $pHi$ 7.1, not 7.4 (as reported in the study of Qi et al., Ref. 75), because the former value was the most widely reported (14, 15, 48); and 2) using a value of 1 mM for free normal $Mg^{2+}$ because this value is reported in the experiment by Qi et al. (75) and is widely cited (1, 16, 17, 80, 84, 86). Furthermore, we assumed that $K_{C_{\text{K}}}$ at $[K^+]_i$ of 150 mM (see Ref. 43), which we used in our simulations, is approximately equal to $K_{C_{\text{K}}}$ at $[K^+]_i$ of 145 mM, the value widely reported in normal rabbit myocytes (14, 15, 74, 80). Our studies revealed that the predicted normoxic $[\text{ATP}]_{\text{tot}}$, $[\text{ADP}]_{\text{tot}}$, $[\text{ATP}^4^-]$, and $[\text{MgATP}^2^-]$ concentrations are comparable to experimentally measured values when $pHi$ was 7.1, $[Mg^{2+}]_i$ was 1 mM, and $[K^+]_i$ was 150 mM (see Table 3, column 2).

An interesting theoretical result was that the model predictions were in a good agreement with experiment for intact epicardial, endocardial, and midmyocardial cells (15, 35, 77) despite use of $k_{\text{ATP}(4^-)}$, $k_{\text{MgADP}^2}$, $g_\text{at}$, and $g_\text{o}$ values estimated in isolated patches. The simulations demonstrated that $K_{\text{ATP}}$ channel activity was inhibited in the endocardial patch at free ATP concentration of 390 $\mu$M and that the predicted endocardial $K_{\text{ATP}}$ current was approximately zero in normal conditions. Although there was some channel activity in the epicardial and midmyocardial membrane patches in normal conditions, the $K_{\text{ATP}}$ currents in all three populations of myocytes had minimal contributions to the normal action potential configuration when $[\text{ATP}^4^-]$ was 390 $\mu$M. In addition, our model predictions in normoxia were in good qualitative agreement with the experimental data of Qi et al. (75), suggesting shorter action potential duration and greater relative increase of the outward $K^+$ current in the subepicardial myocytes and no differences in the epi and endo resting potentials. However, these model predictions presented a new question: how could we solve or explain the apparent dilemma that both in normal intact cells (where no alterations in normal MgATP$^2$ and membrane PIPs levels occur) and in excited patches (where the gradual loss of PIPs has been suggested in the MgATP$^2$ free solution), our model predictions were in good quantitative agreement with experiment (35, 75)? The answer is that in this study, in agreement with the experiment of Furukawa et al. (35) in excited patches, we estimated the $K_{\text{ATP}}$ channel parameters ($k_{\text{ATP}(4^-)}$, $k_{\text{MgADP}^2}$, $g_\text{at}$, $g_\text{o}$) in the different cell types assuming $[\text{MgATP}^2^-]_i \neq 0$ (~4.49 mM). In addition, we concluded that our $K_{\text{ATP}}$ model may still lack important channel structure or function details to account for the channel run-down, including how $[\text{MgATP}^2^-]$ directly regulates the $K_{\text{ATP}}$ channel activity.

Another interesting model prediction, which was in good agreement with experimental data of Furukawa et al. (35) and Light et al. (56), was that in isolated membrane patches, $K_{\text{ATP}}$ channels are activated by a smaller reduction in free ATP in epicardial and larger in endocardial cells at normal resting ligand (ADP, AMP, PCR, Cr), ionic ($Na^+$, $K^+$, $Ca^{2+}$), $P_i$, total $Mg^{2+}$, and $pHi$ levels. Here, we found that variations only in the Kir6.2 half-saturation constant ($k_{\text{ATP}(4^-)}$) among the isolated membrane cell patches, and not in SUR2A half-saturation constant ($k_{\text{MgADP}^2}$) or in the relative channels conductance ($g_\text{at}$, $g_\text{o}$), are able to quantitatively fit these measurements (35, 56). Therefore, we concluded that only the $K_{\text{ATP}}$ ionophore (i.e., Kir6.2 subunit and not the regulatory subunit (i.e., SUR2A) differs among the cell subtypes. However, this new result yielded a new question: what could be the physiological reason(s) for the predicted regional differences in the $K_{\text{ATP}}$ channel ionophore? Could it be differences in Kir6.2 subunit structure or some other mechanism regulating the channel function? Note that in the model, we assume that PIP levels are equal and spatially uniform and that there is not the inhomogeneous accumulation of metabolites throughout the regions in normoxia. New experiments should be suggested to test above hypotheses and the correctness of our model predictions. In addition, we need to emphasize that because the data in control conditions from Qi et al. (75) were incomplete, we did not attempt to reproduce these data quantitatively (see Table 5).

**Modeling $K_{\text{ATP}}$ channel heterogeneity during 20 min simulated ischemia.** The contribution of the physiological distinctions between ventricular epicardial, midmyocardial, and endocardial myocytes to the development of the action potential and intracellular $Ca^{2+}$ transient under normal conditions has been the focus of numerous studies (5, 6, 18, 28, 32, 34, 35, 64, 75). These studies revealed that the observed regional differences in the action potential configuration and duration are due to differences of variant ionic currents ($I_{Na}$, $I_{K_{s}}$, $I_{K_{r}}$, $I_{f_{\text{lo}}}$, $I_{K_{p}}$). Recently, the differences in the normal $Ca^{2+}$ homeostasis and mechanical function throughout the three regions has also been reported (18, 64). However, little attention has been directed to the regional mechanisms regulating cardiac excitation-contraction coupling during ischemia (35, 75).

The advantage of this model is its ability to examine and predict how the fall of $[\text{ATP}]_{\text{tot}}/[\text{ADP}]_{\text{tot}}$ ratio regulates action potential development, outward $K^+$ current, myoplasmic and SR $Ca^{2+}$ transients, $I_{K_{\text{ATP}}}$, and many other ionic currents in the different cell subtypes. To estimate as accurately as possible the initial ligand, $[P_i]$, and $[Mg^{2+}]$, concentrations at 20 min of ischemia, we searched the literature for such data measured in rabbits, and when such data could not be found, we calculated some values with the model (see Table 3, column 4). Note that Table 3 shows that predicted free $Mg^{2+}$ concentration (~2.24 mM) is comparable with the experimentally reported value (2–6 mM) (15). Here we simulated the effects of 20 min ischemia assuming $[K^+]_r$, $[Na^+]_r$, $[Ca^{2+}]_r$, $[Ca^{2+}]_{\text{SR}}$, and $[Ca^{2+}]_i$, normal diastolic levels. The main reasons for this were as follows: 1) in the experiment of Qi et al. (75), $[Na^+]_r$ decreased by ~20 mM; however, the changes in

<table>
<thead>
<tr>
<th>APD$\text{\textsubscript{90}}$, ms</th>
<th>Experiment</th>
<th>Model</th>
<th>Model</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>754</td>
<td>146</td>
<td>797</td>
<td>195</td>
</tr>
<tr>
<td>AP$\text{\textsubscript{m}}$, mV</td>
<td>16</td>
<td>35</td>
<td>7</td>
<td>36</td>
</tr>
<tr>
<td>Outward $K^+$ current (−30 mV, A/F)</td>
<td>0.5</td>
<td>1.4</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Outward $K^+$ current (0 mV, A/F)</td>
<td>2</td>
<td>2.4</td>
<td>0.75</td>
<td>1.25</td>
</tr>
<tr>
<td>Outward $K^+$ current (+30 mV, A/F)</td>
<td>5.5</td>
<td>9.8</td>
<td>3.75</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table 5. Quantitative discrepancies between the model predictions and the measured regional changes in action potential characteristics and outward $K^+$ current in normal conditions.
diastolic [K\(^{+}\)], [Na\(^{+}\)], [Ca\(^{2+}\)], [Ca\(^{2+}\)]\(_{SR}\), and [Ca\(^{2+}\)]\(_{L}\) levels were not reported; and 2) by assuming normal [K\(^{+}\)]; while increasing [Na\(^{+}\)], up to 50 mM (see the fourth table in Lotti et al., Ref. 43), the predicted ischemic epicardial and endocardial action potentials were quite different in shape compared with those shown in the article by Qi et al. (75) (data not shown). To calculate \(K_{\text{AK}}\) and total ADP and AMP ischemic values, we used Eqs. 15–19. In addition, we assumed \(K_{\text{AK}} \sim K_{\text{AK}}^{\text{endocardial}}\) because changes in the apparent \(K_{\text{AK}}\) constant during ischemia are not reported (79).

Our study clearly demonstrates that models, including the individual components that retain details of underlying molecular processes regulating the channel kinetics, are of great importance in better understanding and explaining the electrical and contractile functions of the cell. Thus, our whole-cell model predicts that in endocardial myocytes at 20 min ischemia, the APD\(_{90}\) decreased \(\sim 1.82\) times when \(k_{\text{ATP}}\) was at the normal level of 131 \(\mu\)M. Note that in the experiment by Qi et al. (75), the reported decrease in the endo APD\(_{90}\) was \(\sim 1.32\) times. In addition, experiment suggests that in epicardial myocytes at 20 min ischemia, the APD\(_{90}\) decreased \(\sim 2.6\) times while the predicted APD\(_{90}\) drop in the epicardium was \(\sim 2\) times when \(k_{\text{ATP}}\) was at the normal level of 600 \(\mu\)M. For this reason we searched for new epicardial and endocardial \(k_{\text{ATP}}\), \(k_{\text{MgADP}}\), \(g_{a}\) and \(g_{o}\) parameter values. Surprisingly, the model predicted again that by varying only the Kir6.2 half-maximal saturation constant (i.e., increasing \(k_{\text{ATP}}\) from 600 \(\mu\)M to 8 \(\mu\)M and decreasing endocardial \(k_{\text{ATP}}\) from 131 \(\mu\)M to 20 \(\mu\)M), the degree of APD\(_{90}\) shortening during ischemia could more closely approximate the experiment, with epicardial APD\(_{90}\) shortening by \(\sim 2.43\) times and endocardial APD\(_{90}\) shortening by \(\sim 1.54\) times. The analysis also suggests that the changes in \(k_{\text{MgADP}}\), \(g_{a}\) and \(g_{o}\) alone (\(k_{\text{MgADP}} = 0.001–10, g_{o} = 0–1, g_{a} = 0–1\)) did not account for the experimental findings in either myocyte layer at 20 min ischemia (data not shown). In addition, results revealed that the increase in outward epicardial K\(^{+}\) current was now significantly greater than that predicted with \(k_{\text{ATP}}\) 600 \(\mu\)M while endocardial outward K\(^{+}\) current decreased slightly when \(k_{\text{ATP}}\) was 20 \(\mu\)M (75). However, these new findings yielded a new question: What could be the physiological reason for the predicted dramatic changes in epicardial and endocardial \(k_{\text{ATP}}\) values during ischemia? A reasonable explanation has been suggested by Haruna et al. (41). They demonstrated experimentally that during ischemia, l-palmitoylcarnitine (a fatty acid metabolite) accumulates in the sarcolemma, deranging in a very irregular manner the membrane lipid environment, including the endogenous PI cascade (PIP\(_{3}\), PIP). Thus, we hypothesize here that during ischemia the inhomogeneous accumulation of the metabolites in the different tissue sublayers may alter differently the normal epicardial and endocardial ATP sensitivity of the K\(_{\text{ATP}}\) channel (i.e., \(k_{\text{ATP}}\) values) via the interactions with the membrane lipid environment (or PIPs) that in turn may cause differential transmural action potential shortening. New experiments must be performed to test the correctness of this hypothesis.

However, it is important to stress that 1) in epicardium the experiment suggest \(\sim 2.6\) times APD\(_{90}\) decrease during ischemia while the model predicted \(\sim 2.43\) times decrease at \(k_{\text{ATP}}\) of 8 \(\mu\)M; 2) in endocardium, APD\(_{90}\) dropped \(\sim 1.54\) times at \(k_{\text{ATP}}\) of 20 \(\mu\)M while the experiment suggests a decrease of \(\sim 1.32\) times; and 3) the predicted increases in the endocardial outward K\(^{+}\) current were insignificant at both \(k_{\text{ATP}}\) of 131 or 20 \(\mu\)M. Possible reasons for these differences might be that our K\(_{\text{ATP}}\) model may be incomplete or that during ischemia there are some other factors not yet included in our whole-cell model, such as intracellular acidosis (21), the activation of K\(_{\text{ATP}}\) channels by arachidonic acids (15, 49), or the regional variations in the other ionic currents during ischemia (53, 75), which might additionally affect the outward K\(^{+}\) current and subsequently the regional action potential configuration. Furthermore, because the data from Qi et al. (75) at 20 min of ischemia were also incomplete, in this study we did not attempt to reproduce quantitatively these data (see Table 6).

Finally, we should mention that in this article the effects of 20 min simulated ischemia in the midmyocardial cells have not been simulated since we could not find experimental data in the literature. In addition, the reported regional effects in normal Ca\(^{2+}\) homeostasis (18, 64) have not been investigated, as this is beyond the scope of the present study.

**Conclusion**

In the present study we developed a detailed biochemical model that connects Ca\(^{2+}\) signaling and cell electrophysiology with the main interactions between the phosphorylated species (ATP, ADP, AMP, PCr, Cr, P) and the cytosolic Lewis acids (Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), H\(^{+}\)). This comprehensive ionic-metabolic model was able to reproduce qualitatively a sequence of events in the epicardium, midmyocardium, and endocardium that corresponds well with experimental data under normal conditions and during 20 min of simulated ischemia. New and more precise experiments must be performed to test the model predictions. This model provides a good basis for further investigation of how cell electrophysiology and cytosolic metabolism might regulate the ATP consumption by ATPases and contraction, the cell respiration and glycolysis, and the progressive changes during ischemia across the different cell subtypes of the myocardium.

**APPENDIX A**

Equations describing K\(_{\text{ATP}}\) current regulation by [ATP\(^{4-}\)], and [MgADP\(^{2-}\)]:

\[
I_{\text{K_{ATP}}} = G_{\text{K_{ATP}}}f_{\text{K_{ATP}}}
\]

\[
\left[\frac{[K^{+}]}{[K^{+}]_{\text{normal}}}\right]^{0.24}
\]

\[
(\text{V-E K})
\]

\[
f_{\text{K_{ATP}}} = \left(1 - \frac{[\text{ATP}^{4-}]}{[\text{ATP}^{4-}] + k_{\text{ATP}}^{\text{endocardial}}}\right) \times (g_{o}f_{\text{MgADP}} + g_{a}(1-f_{\text{MgADP}}))
\]

### Table 6. Quantitative discrepancies between the model predictions and the measured regional changes in action potential characteristics and outward K\(^{+}\) current at 20 min of ischemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Epicardium</th>
<th>Endocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD(_{90}), ms</td>
<td>291</td>
<td>60</td>
</tr>
<tr>
<td>AP(_{90}) current ((\pm)0.001–10), A/F</td>
<td>14</td>
<td>12.5</td>
</tr>
<tr>
<td>Outward K(^{+}) current ((\pm)0.001–10), A/F</td>
<td>0.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Outward K(^{+}) current ((\pm)0.001–10), A/F</td>
<td>3.2</td>
<td>6</td>
</tr>
<tr>
<td>Outward K(^{+}) current ((\pm)0.001–10), A/F</td>
<td>8.6</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Significance: *p < 0.05; **p < 0.01; ***p < 0.001.
\begin{equation}
\frac{f_{Mg_{ADP}}}{t} = \left(1 - \left(\frac{[MgADP]^*}{[MgADP]^* + k_{MgADP}}\right)^2\right)^4
\end{equation}

Equation 34 describes \([MgATP]^2\) regulation of L-type \(Ca^{2+}\) current:

\begin{equation}
I_{Ca}(t) = \frac{1}{1 + \left(\frac{k_{MgATP}^2}{[MgATP]^2}\right)^2} I_{Ca}(t)
\end{equation}

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