Mathematical model of the neonatal mouse ventricular action potential

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Wang LJ, Sobie EA. Mathematical model of the neonatal mouse ventricular action potential. Am J Physiol Heart Circ Physiol 294: H2565–H2575, 2008. First published April 11, 2008; doi:10.1152/ajpheart.01376.2007.—Therapies for heart disease are developed to treat electrophysiological or contractile abnormalities in adult hearts may be inappropriate for treating heart disease in children because of the differing physiology of the immature heart cells. Second, cultured neonatal rat and mouse myocytes are a popular experimental model and have been used in many studies examining electrical propagation and reentrant arrhythmias (17, 34). The specific physiological characteristics of these cells may influence whether results obtained in these studies are applicable to phenomena seen in adult hearts. Finally, the development of heart failure in mature myocardium is associated with the induction of a “fetal gene program,” suggesting that the complement of genes expressed in heart failure resembles the set expressed earlier in development (12, 19). All of these reasons illustrate the potential benefits of a greater understanding of the behavior of the neonatal heart cell.

Although much has been learned about differences in electrophysiology and ion transport between immature and mature heart cells, many of the observed changes can be understood only qualitatively. To synthesize data from diverse sources and develop quantitative predictions, computer modeling can be used; however, this technique has been used only infrequently in studies of neonatal myocytes, and a complete model of the neonatal action potential has not yet been developed. To address this gap, we created a computer model that describes the ionic currents and Ca2+ transport mechanisms in the neonatal (1 day old) mouse ventricular myocyte. To build this model, we began with a recently published description of the adult mouse AP (7) and then altered the density and/or function of ion transport mechanisms in accordance with experimental data obtained in neonatal cells. The model recapitulates the AP shape seen in neonatal cells and is broadly consistent with results on how experimental interventions affect electrical behavior and intracellular Ca2+ transients. This new model can be used to understand, in quantitative terms, how altered expression and function of channels, pumps, and transporters contributes to changes observed during development.

METHODS

Bondarenko et al. (7) have presented a computer model of the AP of the adult mouse ventricular myocyte. The model includes ionic currents, transmembrane pumps, ion exchangers, and a system for intracellular Ca2+ cycling. Beginning with the adult mouse model, we modified the densities of ionic currents and/or their formulations on the basis of experimental data obtained in immature cells. We used data obtained in day 1 neonatal mouse ventricular myocytes wherever possible; when these were not available, we used results from embryonic mouse or neonatal rat cells, as noted. The main changes made to the model are summarized in the text; the complete set of model equations and parameters is developed.
provided in the supplementary material. (Supplemental data for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.)

**Geometry.** In a variety of species, including the mouse, neonatal cells are significantly smaller than adult myocytes (21, 42, 49). We assumed that the neonatal cell was a cylinder 50 µm in length and 9 µm in diameter. The 3.2-pl volume is divided into four compartments: network SR (NSR; 6.6% of total cellular volume), junctional SR (JSR; <1%), subsarcolemmal space (1.0%), and the bulk cytosolic space (71.8%). The remainder (20.6%) is assumed to consist of mitochondria and nuclei. Because of a lack of quantitative data, Ca\(^{2+}\) cycling into and out of these latter organelles was not explicitly considered in the simulations. Any effects of mitochondria or nuclei on intracellular Ca\(^{2+}\) homeostasis can only occur indirectly through the overall cytosolic Ca\(^{2+}\) buffering. We assumed that a larger relative proportion of the total cell volume was occupied by cytosol because of structural studies showing a decreased density of mitochondria in immature myocardium (31). Compartment volumes are listed in Supplementary Table S1.

Electron micrographs of adult mouse myocytes reveal invaginations of the cell membrane, known as transverse or T tubules, that greatly increase the cell’s surface area. In many species, including the mouse, T tubules are not yet present in day 1 neonatal cells (45). For this reason, we assumed that the capacitive surface area of the cell was equal to the physical surface area of the cylindrical cell (1.541 µm\(^2\)). In addition, because neonatal cells lack the close couplings between T tubule and JSR membranes seen in adult cells, we assumed that the model “subspace,” which is important for Ca\(^{2+}\) signaling (see below), included the entire region directly underneath the cell membrane, with a depth of 20 nm.

To achieve consistency with experiments performed on homogenates prepared from developing rat ventricles (6), we reduced the cytosolic buffering capacity in the neonatal cell by a factor of two compared with the adult myocyte. The resulting total cytosolic buffering is very similar to that measured in the experiments: an increase in free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) from 0.1 to 0.5 µM requires an increase in total [Ca\(^{2+}\)] of 25 µM in the model, compared with 27 µM as estimated by Bassani et al. (6). We kept the formulation of Bondarenko et al. (7) in which buffering results from binding of Ca\(^{2+}\) to calmodulin and two sites on troponin; however, we should note that concentrations of these proteins have not been determined in neonatal cells. Since changes in SR buffering in neonatal cells have not been reported, the SR buffering capacity of the adult cell (15 mM) was maintained.

**Na\(^+\) current.** In the adult mouse model, Na\(^+\) current (I\(_{Na}\)) is described by the Markov scheme originally developed by Clancy and Rudy (10), with some parameters modified. This model consists of three closed states, an open state, a fast inactivated state, two intermediate inactivated states, and two closed inactivated states. When we reduced the density of outward K\(^+\) currents in the adult model to reproduce data from neonatal cells (see below), we found that a persistent, noninactivating Na\(^+\) current (2–3 pA/pF) greatly extended the AP duration (APD; >250 ms). This resulted from the relatively large “window current” seen in this model compared with other models of the cardiac Na\(^+\) current (see Supplementary Fig. S1). Since experimental data on mouse I\(_{Na}\) are relatively sparse and an important role for late Na\(^+\) current in neonatal myocytes has not been established, we described I\(_{Na}\) using the more conventional Hodgkin-Huxley-type equation given in Luo and Rudy (26) as

\[
I_{Na} = G_{Na}m^3h(V - E_{Na})
\]

where \(E_{Na}\) is the reversal potential for Na\(^+\) and \(m, h, \) and \(j\) are the activation, fast inactivation, and slow inactivation gating variables, respectively. With this model, sustained I\(_{Na}\) was not present, and this current had little effect on the APD. The maximum conductance (G\(_{Na}\)) was adjusted to fit the maximum overshoot, and maximum upstroke velocity (dV/dt\(_{max}\)) values were measured in neonatal mouse myocytes.

**K\(^+\) currents.** The neonatal model contains all of the K\(^+\) currents that are present in the Bondarenko model of the adult AP, but maximal conductances and, in some cases, gating variables have been altered in accordance with experimental data, as described below.

Inward rectifier (I\(_{K1}\)) density increases with age in both rabbit (23) and rat (27), consistent with the more negative resting membrane potential observed in adult compared with neonatal cells (51). We therefore reduced the maximum I\(_{K1}\) conductance (G\(_{K1}\)) by 20% compared with the adult value.

The Bondarenko model includes both rapid and slow delayed rectifier currents (I\(_{Ks}\) and I\(_{Ks}\), respectively), although each current is small and plays little role in repolarization of the adult AP. These currents, however, have been shown to be relatively larger in day 1 mouse myocytes compared with adult cells (50). We increased G\(_{Ks}\) and G\(_{Ks}\), the maximum conductances for I\(_{Ks}\) and I\(_{Ks}\), by factors of 15 and 8, respectively.

Wang and Duff (49) showed that transient outward current (I\(_{to}\)) density increases greatly during development in the mouse, suggesting that these currents play a smaller role in repolarization in neonatal hearts than in adult hearts. I\(_{to}\) was also shown to inactivate faster, and with simpler kinetics, in day 1 neonatal compared with adult cells. To reproduce both the smaller amplitude and altered kinetics (see Fig. 2), we decreased G\(_{Ks}\), G\(_{Ks}\), and G\(_{Ks}\), the respective maximal conductances for fast transient outward current (I\(_{Kos}\)), ultrarapid delayed rectifier current (I\(_{Kos}\)), and steady-state K\(^+\) current (I\(_{Kos}\)) by 75, 97, and 70%. Slow transient outward current (I\(_{Kos}\)) has a maximum conductance of zero in simulations of cells from the ventricular apex in the model of Bondarenko et al. model (7). Since inactivation kinetics of transient outward current in neonatal mouse cells are more consistent with I\(_{Kos}\) than I\(_{Kos}\), the latter current was not included in the neonatal model.

In addition to a change in current density, Wang and Duff (49) observed a shift in the steady-state inactivation of transient outward current in neonatal cells. To reproduce this behavior (see Supplementary Fig. S2), we modified the equations governing the state variable \(i_{tof}\) as follows:

\[
\frac{d\tau_{tof}}{dr} = \frac{i_{tof} - i_{tof}}{\tau_{tof}}
\]

\[
\alpha_t = 0.000152e^{-V-3.81/15.75} + 0.067083e^{-V+12.05/15.75} + 1
\]

\[
\beta_t = 0.00095e^{V+12.05/15.75} + 0.051335e^{V+33.5/7.0} + 1
\]

\[
i_{tof} = \alpha_t / (\alpha_t + \beta_t)
\]

\[
\tau_{tof} = \left(0.000152e^{-V+13.5/0.7} + 0.067083e^{-V+33.5/7.0} + 0.00095e^{V+12.05/15.75} + 0.051335e^{V+33.5/7.0} + 1\right)^{-1}
\]

**Sarcolemmal Ca\(^{2+}\) fluxes.** In the developing myocardium, sarcolemmal Ca\(^{2+}\) channels are essential for supporting myocyte contraction. Several studies have demonstrated an increase in L-type Ca\(^{2+}\) current (I\(_{Ca,L}\)) density with increasing age in the rabbit (51), but results in rodent myocytes are more mixed. Cohen and Lederer (11) measured increased I\(_{Ca,L}\) in neonatal rat myocytes that had been cultured for 2 days, whereas Vornanen (48) observed relatively constant current I\(_{Ca,L}\) density at different developmental stages in freshly dissociated rat myocytes. Consistent with the latter study, we choose to increase G\(_{Ca,L}\), the maximum conductance of I\(_{Ca,L}\), by 10%.

T-type Ca\(^{2+}\) current (I\(_{Ca,T}\)) is generally not detected in adult mouse ventricular cells but has been observed in cells from neonatal rats (18) and fetal mice (13). We therefore incorporated...
The maximal conductance \((G_{CaT})\) was selected so that the peak of the \(I_{CaT}\) current-voltage \((IV)\) relation was \(\sim 3\) pA/pF. This is consistent with several studies performed on neonatal rat \((18, 24)\) and embryonic mouse \((13, 29)\) myocytes.

NCX has been shown to be upregulated in neonatal heart cells from a number of species and is thought to play a much greater role in excitation-contraction coupling in the neonate than in the adult \((1)\). Accordingly, we increased the maximal NCX current density by a factor of 3.1 in the neonatal model. The maximum sarcolemmal \(Ca^{2+}\) pump current was decreased by 80% so that the model more closely matched the relative contribution of each \(Ca^{2+}\) transport system to relaxation \(\text{(see Fig. 6)}\). To maintain diastolic \(Ca^{2+}\) balance across the cell membrane, we also decreased the background \(Ca^{2+}\) conductance \((G_{CaB})\) by 32% compared with the value in the adult model.

\(Ca^{2+}\)-activated \(Cl^{-}\) current \((I_{Cl, Ca})\) is included in the Bondarenko model of the adult myocyte. However, the \(IV\) plots produced by the model equations do not match those recorded in neonatal rabbit ventricular myocytes \((51)\). We therefore chose to describe this current using a modified form of the equations presented by Verkerk et al. \((47)\). The equation describing \(I_{Cl, Ca}\) and \(IV\) plots at different levels of intracellular \([Ca^{2+}]\) are displayed in Supplementary Fig. S3.

**SR \(Ca^{2+}\) handling.** We preserved the SR \(Ca^{2+}\) handling system used in the adult model but modified model variables dramatically to reproduce the much smaller contribution of SR \(Ca^{2+}\) release to excitation-contraction coupling in neonatal cells. The rate constant \((\nu_1)\) controlling \(Ca^{2+}\) uptake from the cytosol via SR \(Ca^{2+}\) -ATPase (SERCA) was reduced by 80% to match the slower decay of \(Ca^{2+}\) transients seen in neonatal rat cells \((5, 42)\). The volume of the JSR was decreased by a factor of 100 to simulate a dearth of close couplings between the T tubule and JSR membranes in the neonatal cell. Since neonatal myocytes largely lack T tubules, a much smaller percentage of the SR volume can be considered junctional compared with the adult cell. The SR \(Ca^{2+}\) release rate constant \((\nu_1)\) was reduced by 90%. Together, these two changes ensured that, consistent with experiments, release amplified the \(Ca^{2+}\) transient amplitude only slightly \(\text{(see Fig. 5)}\). In addition, recent immunocytochemical studies in rabbit suggest that the close couplings between L-type \(Ca^{2+}\) channels and SR \(Ca^{2+}\) release channels \(\text{(ryanodine receptors, RyRs)}\) seen in adult cells may be less pronounced in neonatal myocytes \((14, 38)\). We therefore assumed that all transmembrane \(Ca^{2+}\) fluxes, rather than only \(I_{CaT}\), enter the model subspace, and \(Ca^{2+}\) flux through any pathway can trigger SR \(Ca^{2+}\) release. Finally, to prevent diastolic SR \(Ca^{2+}\) content from becoming unrealistically large, the rate constant controlling \(Ca^{2+}\) leak from NSR to cytosol \((\nu_2)\) was increased by 20%.

**Model summary.** Schematics of the Bondarenko et al. \((7)\) adult mouse model and the model of the neonatal cell are shown in Fig. 1, A and B, respectively. Where the flux through a particular channel, pump, or transporter is increased compared with the adult model, the corresponding symbol is larger in Fig. 1B, and vice versa. Model parameters that are unchanged compared with the adult are shown the same size in A and B.

**Fig. 1.** Schematic diagrams of the adult and neonatal models. Arrows point in the predominant direction of each ionic flux \((f)\) or transmembrane current \((I)\). A: adult cell. Only L-type \(Ca^{2+}\) current \((I_{CaL})\) and \(Ca^{2+}\) released from the junctional sarcoplasmic reticulum \((JSR)\) enter into the subspace, as denoted by the dashed line. B: neonatal cell. Channels and pumps are resized, depending on whether a given current/flux was increased or decreased, relative to the adult, in the neonatal model. Because T tubules are absent in day 1 neonatal cells and structures responsible for SR \(Ca^{2+}\) release are less well defined, we assumed that all \(Ca^{2+}\)-dependent transmembrane currents communicated with the subspace. Consistent with experimental data, the neonatal cell is smaller than the adult cell by a factor of \(-10\). Cell volumes are displayed as equally sized to allow for comparison. Compartment volumes in the neonatal model are listed in Supplementary Table S1. CMDN, calmodulin; CSQN, calsequestrin; NSR, network SR; TRPN, troponin; \(I_{NaK}\), \(Na^{+}\) current; \(I_{NaB}, Na^{+}\) background current; \(I_{NaK}, Na^{+}-K^{+}\) pump current; \(I_{Kd}, K^{+}\) fast transient outward \(K^{+}\) current; \(I_{Ks}, slow\) delayed rectifier \(K^{+}\) current; \(I_{Ko}, rapid\) delayed rectifier \(K^{+}\) current; \(I_{Kur}, ultrarapid delayed rectifier \(K^{+}\) current; \(I_{Ks}, steady-state \(K^{+}\) current; \(I_{K1}, inward\) rectifier \(K^{+}\) current; \(I_{Cl, Ca}\), \(Ca^{2+}\) -activated \(Cl^{-}\) current; \(I_{Cl, Ca}, \beta\), \(Ca^{2+}\) current; \(I_{Cl, Ca}, \alpha\), \(Ca^{2+}\) current; \(I_{NaCa}, \beta\), \(Na\)-\(Ca\) exchange current; \(I_{NaCa}, Na^{+}/Ca^{2+}\) exchange current; \(I_{Na}, SR\) \(Ca^{2+}\) release; \(I_{Na}, transmembrane \(Ca^{2+}\) influx; \(I_{Na}, SR\) \(Ca^{2+}\) uptake; \(I_{Na}, SR\) \(Ca^{2+}\) leak.
Overall, our formulation contains 15 ionic currents, 6 intracellular \(\text{Ca}^{2+}\) fluxes, and 37 state variables. The model was implemented in MATLAB R2006a (MathWorks, Natick, MA) and solved using the program’s variable order stiff differential equation solver (ode15s).

**RESULTS**

We begin by presenting simulation results that illustrate important differences between neonatal and adult electrophysiology as predicted by the new model. We then show simulations that can be compared directly with experiments previously performed on hearts or myocytes from newborn animals. We conclude with novel predictions generated by the model that can be tested in subsequent experiments in neonatal heart cells. These predictions illustrate the potential strengths of the computational approach and suggest avenues for further research.

Simulated ionic currents under conditions of whole cell voltage-clamp are shown in Fig. 2. Figure 2, A and B, displays for the adult and neonatal models, respectively, fast-activating outward \(K^+\) currents (sum of \(I_{\text{ktof}}, I_{\text{Kur}},\) and \(I_{\text{Kss}}\)) produced by steps from \(-80\) mV to more positive potentials (see Fig. 2 legend for details). The plots illustrate two important characteristics of this composite \(K^+\) current in neonatal cells compared with adult cells: 1) transient outward currents are much smaller in magnitude (note scale bars), and 2) currents inactivate more quickly and with a simpler time course. This model behavior is consistent with the experimental results obtained by Wang and Duff (49), who measured transient outward \(K^+\) currents in day 1 mouse myocytes (see e.g., their Fig. 2). These authors reported increases in peak and steady-state current densities of \(-4\) and \(-2.5\) times, respectively, in adult compared with newborn myocytes. The complex inactivation time course (2 time constants) measured by Wang and Duff in adult cells is consistent with the idea that multiple \(K^+\) currents contribute to the overall “transient outward” current that is measured, as subsequent studies have demonstrated (8, 52). The simple and fast decay seen in neonatal cells suggests that this inactivating current consists primarily of fast transient outward current \(I_{\text{ktof}}\). In addition, Wang and Duff (49) reported slower recovery from inactivation of transient outward current in adult cells compared with neonatal cells (see their Fig. 5). This behavior is also reproduced by our model simulations (see Supplementary Fig. S4). These observations on \(K^+\) current in day 1 mouse cells dictated the percentages by which we reduced the maximum conductances \(G_{\text{ktof}}, G_{\text{Kur}},\) and \(G_{\text{Kss}}\) when formulating the neonatal model.

\(I/V\) relations of inward \(\text{Ca}^{2+}\) currents in the adult and neonatal models, respectively, are displayed in Fig. 2, C and D. In each model, inward currents were simulated under voltage-clamp conditions with holding potentials of \(-90\) and \(-40\) mV. Since \(I_{\text{CaT}}\) is present in the neonatal but not the adult model, \(I/V\) plots produced by the former show significantly more current at potentials negative to \(-20\) mV, as well as a greater dependence of peak current on holding potential. These model results are qualitatively consistent with experiments recently performed in cells isolated from neonatal rat (42) and embryonic mouse hearts (37).

The AP produced by the model of the neonatal myocyte and the underlying currents upon steady-state pacing at 0.5 Hz are shown in Fig. 3. Compared with the adult, early repolarization is slowed significantly in the neonatal model, leading to a much longer AP. APD, measured from the maximum upstroke ve-
Figure 4 displays simulations that mimic effects of drugs on APs. In addition to illustrating differences between the adult and neonatal responses to pharmacological perturbations, these results demonstrate the consistency of the neonatal model with published experimental data. Figure 4, A and B, shows the effects of a moderate dose (0.5 mM) of 4-aminopyridine (4-AP) on adult and neonatal APs, respectively. According to Xu et al. (52), this dose blocks $I_{\text{Ktof}}$ and $I_{\text{Kr}}$ by 54% and 78%, respectively; the maximal conductance of each current was therefore reduced by the appropriate amount to perform these simulations. The results show that this dose of 4-AP causes modest prolongation of the AP in either model. However, the percentage increase in APD was much greater in the adult than in the neonatal cell due to the shorter baseline APD in the adult. The effects of dofetilide, which was assumed to block $I_{\text{Kr}}$ completely, are shown in Fig. 4, C and D. The model results predict that dofetilide has virtually no effect on APD in adult cells but can cause slower phase 3 repolarization and significant lengthening of APD in the neonate. Both sets of simulations are consistent with the experimental results presented by Wang et al. (50).

Because numerous studies have investigated excitation-contraction coupling in neonatal cells (3, 21, 32, 40, 42), we also examined factors influencing Ca$^{2+}$ cycling in the neonatal model. Results are shown in Figs. 5 and 6. Intracellular Ca$^{2+}$ transients produced under conditions of steady-state pacing (0.5 Hz) in the adult and neonatal models are shown in Fig. 5A. In the neonatal model, diastolic [Ca$^{2+}$] is considerably higher than in the adult model (205 vs. 100 nM), consistent with the experimental results obtained in rabbit myocytes (21). The amplitude of the Ca$^{2+}$ transient, measured as peak minus diastolic [Ca$^{2+}$], is similar in the neonatal model. Figure 5B shows that [Ca$^{2+}$] reaches a higher peak, and displays much faster kinetics, in the region directly underneath the cell membrane, corresponding to the model “sub-space,” than in the cell interior. This is consistent with measurements made using confocal microscopy in newborn rat (40) and rabbit myocytes (21). Figure 5, C and D, shows the effects of disabling SR Ca$^{2+}$ release on Ca$^{2+}$ transients in the adult and neonatal models, respectively. In the adult mouse, most of the Ca$^{2+}$ that activates contraction is released from the SR, and inhibiting this process decreases the Ca$^{2+}$ transient amplitude profoundly. In contrast, inhibition of SR Ca$^{2+}$ release in the neonatal model causes only a modest (12.4%) reduction, consistent with the 19% decrease recently observed in acutely dissociated day 1 rat myocytes upon application of 10 μM ryanodine (42). Inhibition of SR function in the model also slows the rate of decay of the Ca$^{2+}$ transient, consistent with experimental results (5).

The contributions to relaxation are examined quantitatively in Fig. 6. Similar to the analyses presented by Bassani and colleagues (4, 5), these plots display the amounts of Ca$^{2+}$ carried by various transport pathways, computed by integrating each flux beginning at the peak of the Ca$^{2+}$ transient. The percentage of the total [Ca$^{2+}$] transported by each pathway is

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**NEONATAL ACTION POTENTIAL MODEL**

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that play a lesser role in shaping the simulated AP are shown in Fig. 3C. The model predicts that “reverse mode” NCX, whereby Ca$^{2+}$ is imported and Na$^+$ is exported, will supply repolarizing current during most of the AP. $I_{\text{Kt}}$ and $I_{\text{Kr}}$ are small in magnitude but become important during phase 3 repolarization after $I_{\text{Ktof}}$ has largely inactivated.

Figure 4 displays simulations that mimic effects of drugs on APs. In addition to illustrating differences between the adult and neonatal responses to pharmacological perturbations, these results demonstrate the consistency of the neonatal model with published experimental data. Figure 4, A and B, shows the effects of a moderate dose (0.5 mM) of 4-aminopyridine (4-AP) on adult and neonatal APs, respectively. According to Xu et al. (52), this dose blocks $I_{\text{Ktof}}$ and $I_{\text{Kr}}$ by 54% and 78%, respectively; the maximal conductance of each current was therefore reduced by the appropriate amount to perform these simulations. The results show that this dose of 4-AP causes modest prolongation of the AP in either model. However, the percentage increase in APD was much greater in the adult than in the neonatal cell due to the shorter baseline APD in the adult. The effects of dofetilide, which was assumed to block $I_{\text{Kr}}$ completely, are shown in Fig. 4, C and D. The model results predict that dofetilide has virtually no effect on APD in adult cells but can cause slower phase 3 repolarization and significant lengthening of APD in the neonate. Both sets of simulations are consistent with the experimental results presented by Wang et al. (50).

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indicated to the right of the plots. In the adult model (Fig. 6A), SR uptake via SERCA pumps is responsible for >90% of the \( \text{Ca}^{2+} \) decay, consistent with the dominant role played by SR \( \text{Ca}^{2+} \) release in excitation-contraction coupling. Surprisingly, the model predicts that the integrated flux through the sarcolemmal \( \text{Ca}^{2+} \) pump is roughly twice the \( \text{Ca}^{2+} \) efflux via NCX, contrary to what has been observed in experiments (25). Because of this, we reduced the transport rate of the sarcolemmal \( \text{Ca}^{2+} \) pump when constructing the neonatal myocyte model. The flux analysis in the neonatal model (Fig. 6B) shows the following notable features: 1) the total quantity of \( \text{Ca}^{2+} \) cycled through the cytoplasm with each beat is less than in the adult cell; 2) the percentage taken up into the SR (70%) is less than in the adult but still greater than any other pathway; and 3) the percentage transported by the NCX (24%) is considerably greater than in the adult. These characteristics are consis-

Fig. 4. Effects of \( \text{K}^{+} \) current block on AP morphology. A and B: effect of 4-aminopyridine (4-AP) on APs simulated using the adult (A) and neonatal models (B). Application of 0.5 mM 4-AP was assumed to reduce \( I_{Kur} \) and \( I_{Kt} \) by 54% and 78%, respectively. Consistent with experimental data (49), the percentage of AP prolongation increased with increasing age: neonate AP duration (APD), 104%; adult APD, 130%. B and C: effect of dofetilide on APs simulated using the adult (C) and neonatal models (D). Application of dofetilide was assumed to block \( I_{Kur} \) completely. Consistent with experimental data (50), dofetilide has virtually no effect on the adult APD as indicated by the superimposed curves in C. In the neonatal model, dofetilide increased APD by 25%. APD was measured from the upstroke (maximum \( \text{d}V/\text{d}t \)) to the −60-mV crossing.

Fig. 5. Cellular \( \text{Ca}^{2+} \) transients, kinetics, and effects of SR block. APs were evoked at a pacing frequency of 0.5 Hz for 200 s. A: \( \text{Ca}^{2+} \) transients produced in the neonatal (dashed line) and adult models (solid line). B: subcellular differences in neonatal \([\text{Ca}^{2+}] \) transients: subspace \( \text{Ca}^{2+} \) transient (solid line) and bulk cytosolic \( \text{Ca}^{2+} \) transient (dashed line). Consistent with experimental data (21), the \( \text{Ca}^{2+} \) transient in the cell interior rises more slowly, decays more slowly, and is smaller in amplitude than intracellular \([\text{Ca}^{2+}] \) at the cell periphery. C and D: effect of ryanodine on \( \text{Ca}^{2+} \) transients simulated using the adult and neonatal models, respectively. Application of 10 \( \mu \text{M} \) ryanodine was assumed to block release from the SR completely. Consistent with experimental data (42), the SR plays a smaller role in the neonate than in the adult, as shown by the relatively small decrease in the amplitude of the neonatal \( \text{Ca}^{2+} \) transient when SR release is blocked.

Fig. 6. Effects of K current block on AP morphology. A and B: effect of 4-aminopyridine (4-AP) on APs simulated using the adult (A) and neonatal models (B). Application of 0.5 mM 4-AP was assumed to reduce \( I_{Kur} \) and \( I_{Kt} \) by 54% and 78%, respectively. Consistent with experimental data (49), the percentage of AP prolongation increased with increasing age: neonate AP duration (APD), 104%; adult APD, 130%. B and C: effect of dofetilide on APs simulated using the adult (C) and neonatal models (D). Application of dofetilide was assumed to block \( I_{Kur} \) completely. Consistent with experimental data (50), dofetilide has virtually no effect on the adult APD as indicated by the superimposed curves in C. In the neonatal model, dofetilide increased APD by 25%. APD was measured from the upstroke (maximum \( \text{d}V/\text{d}t \)) to the −60-mV crossing.
tent with results obtained in day 1 neonatal rat myocytes by Bassani and Bassani (5), who calculated contributions of 72, 24, and 4%, respectively, for SERCA, NCX, and slow pathways. We should note, however, that our model predicts reduced flux through the sarcolemmal Ca\textsuperscript{2+}/H\textsuperscript{+} pump in neonatal compared with adult myocytes, in contrast to the increased role of slow pathways that has been seen in experiments (5). On the basis of the good quantitative match shown in Fig. 6B, however, we feel that this difference results from a maximum pump rate that is quite high in the original model of the adult myocyte (7).

To gain insight into the behavior of the neonatal myocyte and generate novel predictions, we implemented “action potential clamp” versions of the adult and neonatal models. Simulated APs obtained during steady-state pacing at 0.5 Hz were recorded in both models, and these were used as input waveforms to the voltage-clamp versions of either model. These simulations can illustrate which neonatal behaviors are due solely to the changes in ionic fluxes and which depend specifically on the neonatal AP morphology. Figure 7, A and B, shows Ca\textsuperscript{2+} transients obtained in the adult and neonatal AP clamp models, respectively, using either AP as a command waveform. For these simulations, the initial conditions of all state variables besides voltage were set to the values obtained upon steady-state pacing at 0.5 Hz. Figure 7A shows that, in the adult model, replacing the adult AP with the neonatal AP causes a large increase in the Ca\textsuperscript{2+} transient amplitude. Conversely, the Ca\textsuperscript{2+} transient in the neonatal model using the adult AP is much smaller than the one that results when the neonatal AP is the clamp waveform (Fig. 7B). Thus the longer AP in neonatal cells appears critical for maintaining a significant Ca\textsuperscript{2+} transient.

We observed in our simulations that, after steady-state pacing, intracellular [Na\textsuperscript{+}] ([Na\textsuperscript{+}]i) was greater in the neonatal than in the adult model. We used AP clamp simulations to gain insight into the mechanisms underlying this difference, as shown in Fig. 8. When the adult model was paced in current-clamp mode at 0.5 Hz, or when a long train of adult APs was used as an input waveform, [Na\textsuperscript{+}]i increased from 14 to 15.6 mM over the course of 2,000 s (Fig. 8A). When a train of neonatal APs was used instead as the clamp waveform, the resulting steady-state value of [Na\textsuperscript{+}]i was slightly less (15.5 mM). In contrast, [Na\textsuperscript{+}]i in the neonatal model increased to over 20.2 mM when the model was clamped with a train of either neonatal or adult APs. The increased [Na\textsuperscript{+}]i during pacing in the neonatal model is therefore primarily a consequence of altered ion transport pathways rather than the AP shape. To determine the mechanisms underlying this predicted altered [Na\textsuperscript{+}] homeostasis, we systematically changed parameters affecting [Na\textsuperscript{+}] and [Ca\textsuperscript{2+}] balance back to their values in the adult model and then repeated the simulations. The results (Fig. 8B) show that the factor most responsible for increased [Na\textsuperscript{+}]i is the decreased sarcolemmal Ca\textsuperscript{2+} pump in

![Fig. 6. Integrated Ca\textsuperscript{2+} fluxes simulated using the adult (A) and neonatal models (B). Each trace shows running integrals of the fluxes responsible for the decay of the Ca\textsuperscript{2+} transient, beginning from its peak. Total (solid line) and individual fluxes (broken lines) are indicated, and the percentage of Ca\textsuperscript{2+} carried by each protein is indicated at right. Consistent with experimental data (5), the SR plays a smaller role, whereas NCX has a greater contribution, in the neonate compared with the adult. SL pump, sarcolemmal Ca\textsuperscript{2+} pump.](http://ajpheart.physiology.org/)

![Fig. 7. Effects of AP shape on Ca\textsuperscript{2+} transients. Simulations were performed with AP clamp versions of the adult and neonatal models. A: in the adult model, the Ca\textsuperscript{2+} transient amplitude is considerably larger when the neonatal AP (dashed line), rather than the adult AP (solid line), is the clamp waveform. B: in the neonatal model, Ca\textsuperscript{2+} transient amplitude is also much larger when the model cell is clamped with the neonatal AP (dashed line), suggesting that the longer AP seen in neonatal cells is required to maintain contraction strength.](http://ajpheart.physiology.org/)
the neonatal model. The changes in NCX, SR Ca²⁺ release, and Ca²⁺ currents all contribute to the increase in [Na⁺], but to a somewhat lesser extent. Changing the characteristics of all four pathways back to their values in the adult model eliminated the increase in [Na⁺], compared with the adult model, indicating that other changes in the neonatal model do not influence [Na⁺] balance substantially.

DISCUSSION

We have presented a new mathematical model of ionic currents and intracellular Ca²⁺ handling in the neonatal mouse ventricular myocyte. Simulations performed with this model successfully reproduce both the AP morphology and important characteristics of the Ca²⁺ transient in the immature cell. The model predicts that the longer AP seen in neonatal compared with adult cells is primarily due to reduced outward K⁺ currents, specifically the fast-activating outward currents I_{Ksfl}, I_{Kur}, and I_{Kca} (Fig. 3). Ca²⁺ transients in the neonatal model are similar in amplitude to those in the adult model, but diastolic [Ca²⁺] is higher and Ca²⁺ transients result primarily from Ca²⁺ influx through the cell membrane rather than SR Ca²⁺ release (Fig. 5). In addition to reproducing these experimentally observed features of normal neonatal cellular physiology, the model can recapitulate the effects of pharmacological interventions, such as block of transient outward currents (Fig. 4B), block of rapid delayed rectifier currents (Fig. 4D), and inhibition of SR Ca²⁺ release (Fig. 5D). These validations suggest that the model assumptions are reasonable, although, as mentioned below, several specific issues remain somewhat unresolved.

In addition to reproducing previously obtained experimental results, this new model can generate novel predictions (Figs. 7 and 8). The model predicts that the longer AP in neonatal compared with adult myocytes is necessary to maintain an adequate Ca²⁺ transient amplitude in these cells. Because the Ca²⁺ transient in neonatal cells relies on Ca²⁺ transport across the cell membrane, the short plateau phase of the neonatal AP is critical since it allows additional time for Ca²⁺ influx through both Ca²⁺ channels and the NCX. This idea can now be explored quantitatively using computer simulations. An additional factor favoring Ca²⁺ entry via reverse-mode NCX in the neonatal model is the increased [Na⁺], seen upon steady-state pacing. The model predicts that this result depends on altered ion transport pathways rather than the difference in the neonatal AP shape per se (Fig. 8). To our knowledge this is a novel prediction that has not been examined experimentally in developing rodent myocytes. The model also predicts that during steady-state pacing, neonatal cells will exhibit elevated diastolic [Ca²⁺] compared with adult myocytes. Although this has indeed been seen in cells isolated from newborn rabbits (21), we should note that a study on isolated rat myocytes (5) did not observe differences in diastolic [Ca²⁺]. This may therefore represent a model prediction that warrants additional experimental scrutiny.

Although many studies have examined electrophysiology and excitation-contraction coupling in neonatal cells and hearts (see Refs. 1 and 51 for review), few attempts have been made to synthesize results using mathematical modeling. For instance, an important investigation by Haddock et al. (21) simulated diffusion of [Ca²⁺] within the newborn rabbit myocyte, but these computations did not consider the ionic currents responsible for the neonatal AP. Our study therefore represents an initial attempt to apply the techniques that have proven successful for understanding normal and pathological adult cellular physiology (36). These types of studies may, by leading to a greater understanding of the unique characteristics of immature hearts, suggest therapies that are especially effective in the treatment of pediatric heart disease. It is also important to investigate developing hearts because heart failure (HF) is associated with the induction of a “fetal gene program,” meaning that many of the genes expressed in HF are similar to those seen during development (12, 19). Indeed, the neonatal...
cell shares some characteristics with the failing myocyte, including a longer AP, reduced K+ currents, increased NCX, and reduced SR Ca2+ release compared with healthy adult myocytes (22). A better quantitative understanding of neonatal heart function may therefore provide insight into changes observed in HF, particularly as the signaling pathways activated during normal development and in disease states continue to become better understood.

Studies of neonatal cardiac physiology have been performed in a number of different species, including rat (42), mouse (50), and rabbit (21). We chose to develop a model of the neonatal mouse AP for a number of reasons. One is the popularity of this species for transgenic studies. Several genetic modifications that should conceivably produce interesting cardiac phenotypes also lead to embryonic or neonatal lethality (15, 35, 39). Understanding heart function in these mouse strains will therefore require a comparison with the appropriate age-matched control. In addition, neonatal mouse and rat myocytes are frequently cultured to form confluent monolayers. These cell networks are a popular experimental model for examining tissue-level aspects of cardiac electrophysiology and ion transport (17, 34). However, translating results obtained in these systems to adult hearts requires a detailed, quantitative understanding of the unique physiology of the cultured cells. We anticipate that our new model can provide a general framework for such efforts, although modifications will have to be made to account for physiological differences between neonatal mice and rats and for changes that occur to cells after several days in culture (42).

One of the benefits of constructing computer models is that the process clarifies assumptions and reveals the limits of one’s understanding, and this is true in the present case. For instance, we altered the conductances of the ionic currents $I_{Kol}$, $I_{Kur}$, and $I_{KCa}$ to match the data of Wang and Duff (49), who observed a smaller and more rapidly inactivating “transient outward” K+ current in day 1 neonatal mouse myocytes. However, these experiments were performed before the molecular entities responsible for the different current components had been identified, and the ionic current whose characteristics we attempted to reproduce (e.g., Fig. 2 and Supplementary Fig. S4) was therefore a composite. It was reassuring to discover an ionic current whose characteristics we at-

late $I_{Na}$ in neonatal mouse myocytes has not been observed, it seemed reasonable to change this formulation. It is possible, however, that such a current is present in neonatal cells but is counterbalanced by additional changes in outward currents not considered in our model. Additional experiments are required to resolve this issue.

Questions also exist regarding the Ca2+ currents in the neonatal mouse myocyte model. Many studies have shown that $I_{CaL}$ is more prominent in developing than in mature ventricles (see Refs. 46 and 53 for review), but we were unable to find quantitative data obtained in day 1 mouse myocytes. The voltage dependence and peak magnitude (~3 pA/pF) of $I_{CaT}$ in our model are consistent with currents measured in developing rats and mice in several studies (13, 18, 24, 29), but our formulation should not be considered unique, and parameters may change as more data become available. Similarly, for $I_{CaL}$, we could not find a direct comparison between day 1 mouse and adult ventricular myocytes. We increased $I_{CaL}$ by a mere 10% on the basis of a study by Vornanen (48) that observed little change in $I_{CaL}$ density with development in freshly dissociated rat myocytes. Some recent studies have suggested that $I_{CaL}$ in developing rodent hearts may have a different voltage dependence (42) and perhaps result from a different gene product (37, 44) than in adult myocytes. Because these observations are still preliminary, we maintained the $I_{CaL}$ gating parameters of the Bondarenko model, but it is possible that this formulation will be modified as additional studies are published.

Perhaps the cellular process about which the most uncertainty exists is the system for intracellular Ca2+ handling. Several studies in a number of species have shown that the SR in immature heart cells can store Ca2+ (3, 32, 40). However, inhibiting SR Ca2+ release pharmacologically tends to cause only slight reductions in Ca2+ transient amplitude in cells from neonatal rabbits (21) and rats (32, 42). In addition, the elementary units of SR Ca2+ release, Ca2+ sparks (9), are infrequently observed in myocytes from immature rat hearts (40, 42), tending to not be seen until transverse tubules develop several days after birth. In our new model of the neonatal mouse myocyte, we kept the adult formulation for SR Ca2+ release but changed the relevant rate constants and reduced the JSR volume to diminish the contribution of SR release to the Ca2+ transient. In addition, since it is not clear whether L-type Ca2+ channels have privileged access to RyRs in neonatal cells (38, 45), we altered the model so that all transmembrane Ca2+ fluxes can contribute equally to SR Ca2+ release. It should be emphasized, however, that the formulation for release is phenomenological rather than mechanistic, and the equations may change as SR Ca2+ release in neonatal cells becomes better understood.

Looking at the role of SR function more quantitatively, it appears at first glance difficult to reconcile two sets of results recently obtained in day 1 neonatal rat myocytes. Snapko et al. (42) observed a roughly 20% reduction in Ca2+ transient amplitude upon inhibition of release, whereas Bassani and Bassani (5) estimated that SR uptake accounts for ~75% of the decline in [Ca2+] during relaxation. One would expect that, unless the cell is accumulating or losing Ca2+ with each beat, the SR contributions to Ca2+ transient amplitude and to relaxation should be equivalent. Our computational results, which are consistent with both studies, provide a possible resolution
to this paradox. When we initially decreased the maximal Ca\(^{2+}\) release rate (\(v_1\)) to reduce the quantity of Ca\(^{2+}\) released with each beat, we observed a dramatic increase in the SR Ca\(^{2+}\) load, similar to the results of experiments in which this is accomplished pharmacologically (16). To make the steady-state SR Ca\(^{2+}\) load in the model more consistent with experimental measurements (3, 32, 40), we then increased the rate of passive Ca\(^{2+}\) leak from SR to cytosol (\(v_2\)). Thus the SR contribution to Ca\(^{2+}\) transient decay calculated by integrating the SERCA flux (Fig. 6B) is an overestimate, because some of the Ca\(^{2+}\) pumped into the SR leaks back into the cytosol later. As a direct consequence of these parameter choices, the model predicts that diastolic SR Ca\(^{2+}\) leak will be higher in neonatal than in adult myocytes. This represents another novel prediction that can be tested in future studies using experimental protocols to measure Ca\(^{2+}\) leak as a function of SR load (41). What makes this prediction especially interesting is the fact that Ca\(^{2+}\) sparks are rarely seen in newborn cells. Thus, if increased leak is indeed present, it must occur in an “invisible” mode (43).

In conclusion, we have presented a novel computational model of the action potential and Ca\(^{2+}\) transient in the day 1 mouse ventricular myocyte. The model, which is generally consistent with experimental data obtained in a number of studies, can be used to generate novel predictions regarding differences between adult and neonatal cellular physiology. This model should prove to be a useful tool for understanding changes in heart function that occur during development.

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GRANTS

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