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Mathematical model of the neonatal mouse ventricular action potential

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Abstract

Therapies for heart disease are based largely on our understanding of the adult myocardium. The dramatic differences in action potential (AP) shape between neonatal and adult cardiac myocytes, however, indicate that a different set of molecular interactions in neonatal myocytes necessitates different treatment for newborns. Computational modeling is useful for synthesizing data to determine how interactions between components lead to systems-level behavior, but this technique has not been used extensively to study neonatal heart cell function. We created a mathematical model of the neonatal (day 1) mouse myocyte by modifying, based on experimental data, the densities and/or formulations of ion transport mechanisms in an adult cell model. The new model reproduces the characteristic AP shape of neonatal cells, with a brief plateau phase and longer duration than the adult (APD_{80} =60.1 vs. 12.6 ms). The simulation results are consistent with experimental data, including: 1) decreased density, and altered inactivation, of transient outward K^+ currents, 2) increased delayed rectifier K^+ currents, 3) Ca^{2+} entry through T-type as well as L-type Ca^{2+} channels, 4) increased Ca^{2+} influx through Na^+ - Ca^{2+} exchange, and 5) Ca^{2+} transients resulting from transmembrane Ca^{2+} entry rather than release from the sarcoplasmic reticulum (SR). Simulations performed with the model generated novel predictions, including increased SR Ca^{2+} leak and elevated intracellular $[Na^+]$ in neonatal compared with adult myocytes. This new model can therefore be used for testing hypotheses and obtaining a better quantitative understanding of differences between neonatal and adult physiology.

Keywords

ionic currents; excitation-contraction coupling; cardiac development; cardiac myocyte

INTRODUCTION

Developmental changes in heart morphology and function occur in all species. A number of studies performed in recent years have shed light on the changes in electrophysiology and ion transport that take place in myocytes as hearts develop. These studies have generally found that cells from immature ventricles, compared with adult myocytes, display: 1) a reduction in the density of outward K^+ currents (28;51); 2) greater activity and expression of Na^+ - Ca^{2+} exchange (NCX) (2); and 3) intracellular Ca^{2+} transients that depend less on sarcoplasmic reticulum (SR) Ca^{2+} release and more on transmembrane Ca^{2+} influx (21;32). In addition, action potentials (APs) recorded in myocytes from neonatal mouse and rat hearts have a brief plateau phase and are longer in duration than the extremely short spike-like APs seen in adult cells from these species (30;42;50).

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It is important to understand these developmental changes in heart cell function for a number of reasons. First, therapies developed to treat electrophysiological or contractile abnormalities in adult hearts may be inappropriate for treating heart disease in children due to 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.

Though 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 Ca^{2+} 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), 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 Ca^{2+} 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 Ca^{2+} 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 here; the complete set of model equations and parameters is provided in the online Supplementary Material.

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 of length 50 μm and diameter 9 μm . The 3.2 pL volume is divided into four compartments: network sarcoplasmic reticulum (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 Table S1 in the online Supplementary Material.

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 ($1541 \mu\text{m}^2$). 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 2 compared with the adult myocyte. The resulting total cytosolic buffering is very similar to that measured in the experiments: an increase in free $[\text{Ca}^{2+}]$ from 0.1 to 0.5 μM requires an increase in total $[\text{Ca}^{2+}]$ of 25 μM in the model, compared with a 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, non-inactivating Na^+ current (2–3 pA/pF) greatly extended the AP duration (> 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 Figure 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):

$$I_{\text{Na}} = G_{\text{Na}} m^3 h j (V - E_{\text{Na}})$$

With this model, sustained I_{Na} was not present, and this current had little effect on the AP duration. The maximum conductance G_{Na} was adjusted to fit the maximum overshoot and dV/dt_{max} values 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_{Kr} and I_{Ks} , respectively), even though 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_{Kr} and G_{Ks} , the maximum conductances for I_{Kr} 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 Figure 2), we decreased G_{Ktof} , G_{Kur} , and G_{Kss} , the maximal conductances, respectively, for fast transient outward current (I_{Ktof}), ultrarapid delayed rectifier current (I_{Kur}) and steady-state K^+ current (I_{Kss}) by 75%, 97%, and 70 %. Slow transient outward current, I_{Ktos} , has a maximum conductance of zero in simulations of cells from the ventricular apex in the Bondarenko et al. model (7). Since inactivation kinetics of transient outward current in neonatal mouse cells are more consistent with I_{Ktof} than I_{Ktos} , 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 Figure S2), the equations governing the state variable i_{tof} were modified as follows:

$$\begin{aligned} \frac{di_{tof}}{dt} &= \frac{i_{tof,ss} - i_{tof}}{\tau_{i_{tof}}} \\ \alpha_i &= \frac{0.000152e^{-(V+3.81)/15.75}}{0.067083e^{-(V+132.05)/15.75} + 1} \\ \beta_i &= \frac{0.00095e^{(V+132.05)/15.75}}{0.051335e^{(V+132.05)/15.75} + 1} \\ i_{tof,ss} &= \alpha_i / (\alpha_i + \beta_i) \\ \tau_{i_{tof}} &= \left(\frac{0.000152e^{-(V+13.5)/7.0}}{0.067083e^{-(V+33.5)/7.0} + 1} + \frac{0.00095e^{(V+33.5)/7.0}}{0.051335e^{(V+33.5)/7.0} + 1} \right)^{-1} \end{aligned}$$

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_{CaL}) density with increasing age in the rabbit (51), but results in rodent myocytes are more mixed. Cohen and Lederer (11) measured increased I_{CaL} in neonatal rat myocytes that had been cultured for two days whereas Vornanen (48) observed relatively constant current I_{CaL} density at different developmental stages in freshly dissociated rat myocytes. Consistent with the latter study, we choose to increase G_{CaL} , the maximum conductance of I_{CaL} , by 10%.

T-type current Ca^{2+} (I_{CaT}) 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 I_{CaT} , computing this current using the equations of Puglisi and Bers (33):

$$\begin{aligned}
 I_{CaT} &= G_{CaT} b g (V - E_{CaT}) \\
 \frac{db}{dt} &= \frac{(b_{\infty} - b)}{\tau_b} \\
 \frac{dg}{dt} &= \frac{(g_{\infty} - g)}{\tau_g} \\
 b_{\infty} &= \frac{1}{1 + e^{-(V+48)/6.1}} \\
 \tau_b &= 0.1 + \frac{5.4}{1 + e^{(V+100)/33}} \\
 g_{\infty} &= \frac{1}{1 + e^{(V+66)/6.6}} \\
 \tau_g &= 8 + \frac{32}{1 + e^{(V+65)/5}}
 \end{aligned}$$

The maximal conductance G_{CaT} was selected so that the peak of the I_{CaT} current-voltage (IV) relation is approximately 3 pA/pF. This is consistent with several studies performed on either neonatal rat (18;24) and embryonic mouse (13;29) myocytes.

Na^+ - Ca^{2+} exchange (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 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 (see Figure 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_{CaCl}) 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_{CaCl} and IV plots at different levels of intracellular $[Ca^{2+}]$ are displayed in Supplementary Figure S3.

Sarcoplasmic Reticulum 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 (v_3) 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 (v_1) was reduced by 90%. Together these two changes ensured that, consistent with experiments, release amplifies the Ca^{2+} transient amplitude only slightly (see Figure 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 (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_{CaL} , 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 (v_2) was increased by 20%.

Summary

Schematics of the Bondarenko et al. (7) adult mouse model and the model of the neonatal cell are shown in Figures 1A and 1B, 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 the two panels.

Overall, our formulation contains 15 ionic currents, 6 intracellular Ca^{2+} fluxes, and 37 state variables. The model was implemented in MATLAB r2006a (The 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 Figure 2. Figs. 2A and 2B display, for the adult and neonatal models respectively, fast-activating outward K^+ currents (sum of I_{tof} , I_{Kur} , and I_{Kss}) produced by steps from -80 mV to more positive potentials (see figure 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 Figure 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 (two 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_{tof} . In addition, Wang and Duff (49) reported slower recovery from inactivation of transient outward current in adult cells compared with neonatal cells (see their Figure 5). This behavior is also reproduced by our model simulations (see Supplementary Figure S4). These observations on K^+ current in day 1 mouse cells dictated the percentages by which we reduced the maximum conductances G_{Ktof} , G_{Kur} , and G_{Kss} when formulating the neonatal model.

Current-voltage (IV) relations of inward Ca^{2+} currents in the adult and neonatal models, respectively, are displayed in Figs. 2C and 2D. In each model inward currents were simulated under voltage-clamp conditions with holding potentials of -90 mV (closed symbols) and -40 mV (open symbols). Since T-type Ca^{2+} current is present in the neonatal but not the adult model, IV 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 (37) hearts.

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 Figure 3. Compared with the adult, early repolarization is slowed significantly in the neonatal model, leading to a much longer AP. AP duration, measured from the maximum upstroke velocity to 80% repolarization, is 60.1 ms, which compares favorably with measurements made in day 1 mouse ventricular myocytes (63.2 ms; (50)). The main inward and outward ionic currents responsible for the neonatal AP are displayed in Figs. 3B and 3C, on different scales so that their time courses can be more easily compared. As in most cardiac cell types, I_{Na} is an order of magnitude larger than most other currents and is responsible for the rapid AP upstroke. The primary inward currents that sustain depolarization are I_{CaL} and I_{CaT} , with the magnitude of the former roughly 5 times greater than the latter. The model predicts that the slower repolarization in neonatal cells is mainly due to reduced outward K^+ currents. The primary outward current early in the AP is fast transient outward current, I_{Ktof} , but its magnitude (~ 7 pA/pF) is considerably reduced compared with the contribution it can make in the adult model (> 20 pA/pF; compare with Figure 16 in (7)). The peak current supplied by I_{Kss} is not great (~ 1.5 pA/pF), but since this current activates quickly and does not inactivate, its cumulative repolarizing effect is significant. Ionic currents that play a lesser role in shaping the simulated AP are shown in Fig. 3C. The model predicts that “reverse mode” Na^+ - Ca^{2+} exchange, whereby Ca^{2+} is imported and Na^+ is exported, will supply repolarizing current during most of the AP. I_{K1} and I_{Kr} are small in magnitude but become important during phase 3 repolarization after I_{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. Figs. 4A and 4B show 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_{tof} and I_{Kur} 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 much shorter baseline APD in the adult. The effects of dofetilide, which was assumed to block I_{Kr} completely, are shown in Figs. 4C and 4D. 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).

As 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 Figures 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 Figure 5A. In the neonatal model diastolic $[Ca^{2+}]$ is considerably higher than in the adult model (205 versus 100 nM), consistent with experimental results obtained in rabbit myocytes (21). The amplitude of the Ca^{2+} transient, measured as peak $[Ca^{2+}]$ minus diastolic $[Ca^{2+}]$, is similar in the neonatal model. Fig. 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). Figs. 5C and 5D show 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 Figure 6. Similar to the analyses presented by Bassani and Bassani (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 $[\text{Ca}^{2+}]$ transported by each pathway is indicated to the right of the plots. In the adult model (Fig. 6A), SR uptake via SERCA pumps is responsible for over 90% of the Ca^{2+} decay, consistent with the dominant role played by SR Ca^{2+} release in EC coupling. Surprisingly, the model predicts that the integrated flux through the sarcolemmal Ca^{2+} pump is roughly twice the Ca^{2+} efflux via NCX, contrary to what has been observed in experiments (25). Because of this, we reduced the transport rate of the SL 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 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; 3) the percentage transported by the Na^+ - Ca^{2+} exchanger (24%) is considerably greater than in the adult. These characteristics are consistent 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, Na^+ - Ca^{2+} exchange, and slow pathways. We should note, however, that our model predicts reduced flux through the sarcolemmal Ca^{2+} pump in neonatal compared with adult myocytes, in contrast to the increased role of slow pathways that has been seen in experiments (5). Based on the good quantitative match shown in Figure 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 model behaviors are due solely to the changes in ionic fluxes, and which depend specifically on the neonatal AP morphology. Figures 7A and 7B show Ca^{2+} transients obtained, respectively, in the adult and neonatal AP clamp models, 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. Fig. 7A shows that, in the adult model, replacing the adult AP with the neonatal AP causes a large increase in the Ca^{2+} transient amplitude. Conversely, the Ca^{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^{2+} transient.

We observed in our simulations that, after steady-state pacing, intracellular $[\text{Na}^+]$ ($[\text{Na}^+]_i$) 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 Figure 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, $[\text{Na}^+]_i$ increased from 14 to 15.6 mM over the course of 2000 s (Fig. 8A, lower solid line). When a train of neonatal APs was used instead as the clamp waveform, the resulting steady-state value of $[\text{Na}^+]_i$ was slightly less (15.5 mM; lower dashed line). In contrast, $[\text{Na}^+]_i$ in the neonatal model increased to over 20.2 mM when the model was clamped with a train of either neonatal (upper dashed line) or adult (upper solid line) APs. The increased $[\text{Na}^+]_i$ 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 $[\text{Na}^+]_i$ homeostasis, we systematically changed parameters affecting $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ balance back to their values in the adult model, then repeated the simulations. The results (Fig. 8B) show that the factor most responsible for increased $[\text{Na}^+]_i$ is the decreased sarcolemmal Ca^{2+} pump in the neonatal model (blue line). The changes in NCX (red), SR Ca^{2+} release (green), and Ca^{2+} currents (magenta), and all contribute to the increase in $[\text{Na}^+]_i$, but to a somewhat lesser extent. Changing the characteristics of all four pathways back to their values in the adult model (cyan line) eliminates the increase in $[\text{Na}^+]_i$ compared with the adult model, indicating that other changes in the neonatal model do not influence $[\text{Na}^+]_i$ balance substantially.

DISCUSSION

We have presented a new mathematical model of ionic currents and intracellular Ca^{2+} handling in the neonatal mouse ventricular myocyte. Simulations performed with this model successfully reproduce both the AP morphology and important characteristics of the Ca^{2+} 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_{Ktof} , I_{Kur} , and I_{Kss} (Fig. 3). Ca^{2+} transients in the neonatal model are similar in amplitude to those in the adult model, but diastolic $[\text{Ca}^{2+}]_i$ is higher and Ca^{2+} transients result primarily from Ca^{2+} influx through the cell membrane rather than SR Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} transient amplitude in these cells. Because the Ca^{2+} transient in neonatal cells relies on Ca^{2+} transport across the cell membrane, the short plateau phase of the neonatal AP is critical, as it allows additional time for Ca^{2+} influx through both Ca^{2+} channels and the Na^+ - Ca^{2+} exchanger. This idea can now be explored quantitatively using computer simulations. An additional factor favoring Ca^{2+} entry via reverse-mode NCX in the neonatal model is the increased $[\text{Na}^+]_i$ 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 $[\text{Ca}^{2+}]_i$ compared with adult myocytes. While 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 $[\text{Ca}^{2+}]_i$. 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 (1;51) for review), few attempts have been made to synthesize results using computational modeling. For instance, an important investigation by Haddock et al. (21) simulated diffusion of $[\text{Ca}^{2+}]_i$ within the newborn rabbit myocyte, but these computations did not consider the ionic currents responsible for the neonatal action potential. 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 Na^+ - Ca^{2+} exchange, and reduced SR Ca^{2+} 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 cellular 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 $I_{K_{to}}$, $I_{K_{ur}}$, and $I_{K_{ss}}$ 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. Figs. 2 and S4) was therefore a composite. It was reassuring to discover a paper published very recently, after our model had been constructed, that measured separately $I_{K_{to}}$, $I_{K_{ur}}$, and $I_{K_{ss}}$ in developing mouse myocytes (20). The observed differences in the currents between adult and day 1 cells were very similar to the conductance scaling factors we chose on the basis of different data, with extremely large reductions in $I_{K_{ur}}$ and smaller, but still substantial, changes in $I_{K_{to}}$ and $I_{K_{ss}}$. This gives us confidence that the K^+ current parameters chosen for our neonatal AP model are reasonable.

On the other hand, with respect to differences in inward currents between neonatal and adult myocytes, several issues remain unresolved. For instance, we reverted to a simple Hodgkin-Huxley type formulation of I_{Na} because the more complex scheme in the Bondarenko model (7) produced considerable late, non-inactivating Na^+ current at negative potentials. This “window current” is not apparent in simulations of the adult AP because strong outward K^+ currents rapidly repolarize the membrane, but it lengthened the AP considerably when we reduced K^+ conductances to construct the neonatal model (Fig. S1). Since a prominent 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 it is counterbalanced by additional changes in outward currents not considered in our model. Additional experiments will be required to resolve this issue.

Questions also exist regarding the Ca^{2+} currents in the neonatal mouse myocyte model. Many studies have shown that T-type Ca^{2+} currents are more prominent in developing than in mature ventricles (see (46;53) for review), but we were unable to find quantitative data obtained in day 1 mouse myocytes. The voltage-dependence and peak magnitude ($\sim 3 \text{ pA/pF}$) 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 L-type Ca^{2+} currents we could not find a direct comparison between day 1 mouse and adult ventricular myocytes. We increased L-type Ca^{2+} current by a mere 10% based on 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 L-type Ca^{2+} current 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 Ca^{2+} handling. Several studies in a number of species have shown that the SR in immature heart cells can store Ca^{2+} (3;32;40). However, inhibiting SR Ca^{2+} release pharmacologically tends to cause only slight reductions in Ca^{2+} transient amplitude in cells from neonatal rabbits (21) and rats (32;42). In addition, the elementary units of SR Ca^{2+} release, Ca^{2+} 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 Ca^{2+} release but changed the relevant rate constants and reduced the JSR volume to diminish the contribution of SR release to the Ca^{2+} transient. In addition, since it is not clear whether L-type Ca^{2+} channels have privileged access to RyRs in neonatal cells (38;45), we altered the model so that all transmembrane Ca^{2+} fluxes can contribute equally to SR Ca^{2+} release. It should be emphasized, however, that the formulation for release is phenomenological rather than mechanistic, and the equations may change as SR Ca^{2+} 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. Snopko et al. (42) observed a roughly 20% reduction in Ca^{2+} transient amplitude upon inhibition of release, whereas Bassani and Bassani (5) estimated that SR uptake accounts for approximately 75% of the decline in $[\text{Ca}^{2+}]$ during relaxation. One would expect that, unless the cell is accumulating or losing Ca^{2+} with each beat, the SR contributions to Ca^{2+} 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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Supplementary Material

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Figure 1.

Schematic diagrams of the adult and neonatal models. Arrows point in the predominant direction of each ionic flux (J) or transmembrane current (I). **A:** Adult cell. Only L-type Ca^{2+} current and Ca^{2+} released from the junctional SR (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 approximately 10. Cell volumes are displayed as equally sized to allow for comparison. Compartment volumes in the neonatal model are listed in Table S1 in the Online Supplementary Materials.

Figure 2.

Differences in ionic currents in adult versus neonatal models. **A** and **B**: Simulated voltage-clamp recordings of outward K^+ currents. Currents were evoked by 1 s pulses from a holding potential of -80 mV to test potentials ranging from -70 to $+50$ mV in 10 mV increments. Each plot shows the sum of three K^+ currents: I_{Ktof} , I_{Kur} , and I_{Kss} . Consistent with experimental data (49), neonatal cells (**B**) display considerably less outward current than adult cells (**A**). The inactivation kinetics also differed, with the adult having two clear components and the neonate exhibiting characteristics of a monoexponential decay. **C** and **D**: Peak Ca^{2+} current versus membrane potential (V_m) in the adult (**C**) and neonatal (**D**) models. I_{Ca} consists of I_{CaL} and I_{CaT} in the neonatal model but only I_{CaL} in the adult. Compared with simulated adult myocytes, neonatal cells show more current at negative V_m and a greater effect of the holding potential on the IV peak.

Figure 3.

Simulated action potential (AP) and membrane currents. The simulated neonatal cell was paced at 0.5 Hz for >500 seconds to reach steady state. **A:** Membrane potential versus time. Electrical stimulus delivered at t=20 ms. **B** and **C:** Currents corresponding to the AP shown in **A**. Note that the right axis in panel **B** refers to the scale for the large Na⁺ current I_{Na} . All other currents are scaled to the axis on left.

Figure 4.

Effects of K^+ current block on action potential morphology. A and B: Effect of 4-aminopyridine (4-AP) on action potentials simulated using the adult (A) and neonatal (B) models. Application of 0.5 mM 4-AP was assumed to reduce $I_{K_{to,f}}$ and $I_{K_{ur}}$ by 54% and 78%, respectively (52). Consistent with experimental data (49), the percentage of action potential prolongation increased with increasing age: neonate (APD, 104%) and adult (APD, 130%). B and C: Effect of dofetilide on action potentials simulated using the adult (C) and neonatal (D) models. Application of dofetilide was assumed to block I_{K_r} completely. Consistent with experimental data (50), dofetilide has virtually no effect on the adult APD as indicated by the superimposed curves in panel C. In the neonatal model, dofetilide increased APD by 25%. APD was measured from the upstroke (maximum dV/dt) to the -60 mV crossing.

Figure 5.

Cellular Ca^{2+} transients, kinetics, and effects of SR block. APs were evoked at a pacing frequency of 0.5 Hz for 200 seconds. **A:** Ca^{2+} transients produced in the neonatal (dashed line) and adult (solid line) models. **B:** Sub-cellular differences in neonatal $[Ca^{2+}]_i$ transients. Subspace Ca^{2+} transient (black solid line) and bulk cytosolic Ca^{2+} transient (dashed line). Consistent with experimental data (21), the Ca^{2+} transient in the cell interior rises more slowly, decays more slowly, and is smaller in amplitude than $[Ca^{2+}]_i$ at the cell periphery. **C** and **D:** Effect of ryanodine on Ca^{2+} transients simulated using the adult and neonatal models, respectively. Application of 10 μ 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 Ca^{2+} transient when SR release is blocked.

Figure 6.

Integrated Ca^{2+} fluxes simulated using the adult (A) and neonatal (B) models. Each trace shows running integrals of the fluxes responsible for the decay of the Ca^{2+} transient, beginning from its peak. Total (bold, solid line) and individual fluxes are indicated, and the percentage of Ca^{2+} carried by each protein is indicated to the right. Consistent with experimental data (5), the SR plays a smaller role, and $\text{Na}^{+}\text{-Ca}^{2+}$ exchange has a greater contribution, in the neonate compared with the adult.

Figure 7.

Effects of AP shape on Ca^{2+} transients. Simulations were performed with AP clamp versions of the adult and neonatal models. **A.** In the adult model, the Ca^{2+} transient amplitude is considerably larger when the neonatal AP (dashed line), rather than the adult AP (solid line), is the clamp waveform. **B.** Neonatal model. Ca^{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.

Figure 8.

Changes in $[Na^+]_i$ observed during steady-state pacing. Simulations were performed with AP clamp versions of the adult and neonatal models, using a train of action potentials delivered at 0.5 Hz as the clamp waveform. **A.** In the neonatal cell (upper plots), $[Na^+]_i$ increases to just over 20 mM when either the adult AP (solid line) or the neonatal AP (dashed line) is used as the command waveform. $[Na^+]_i$ in the adult cell (lower plots) increases to only 15.5 mM, with either AP command. **B.** Simulations performed as in (A), but with particular parameters in the neonatal model changed back to the values in the adult model. Specific changes made were as follows: 1) red line: decrease Na^+-Ca^{2+} exchange scaling factor (k_{NaCa}) from 907.68 to 292.8 pF/pF; 2) magenta line: change parameters

controlling SR Ca^{2+} cycling, v_1 , v_2 , v_3 , from 0.45, 2.088e^{-5} , and 0.09 ms^{-1} , respectively, to 4.5, 1.74e^{-5} , and 0.45 ms^{-1} , and change V_{JSR} from 1.2e^{-9} to $1.2\text{e}^{-8}\text{ }\mu\text{L}$; 3) magenta line: decrease maximal L-type and T-type conductances, G_{CaL} and G_{CaT} , from 0.1902 and 0.055 mS/mF to 0.1729 and 0 mS/mF, respectively; 4) blue line: increase maximum sarcolemmal Ca^{2+} pump current from 0.2 to 1 pA/pF; 5) cyan line: all four of the above changes. Black lines are same results as in (A), re-plotted for comparison.