Adaptation of Rabbit Ventricular Cell Model to Reproduce Action Potentials in Isolated Papillary Muscle

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Abstract

Aims: We sought to establish a computational model of the rabbit ventricular action potential (AP) suitable for investigation of drug effects on the AP of the isolated rabbit papillary muscle.

Methods: Data consisted of transmembrane AP recordings from isolated right ventricular papillary muscles from 21 rabbits. An existing model of the rabbit ventricular AP was adapted to reproduce experimental AP amplitude and AP duration (APD_{90}, APD_{60}, APD_{30},) at multiple pacing rates by reduction of fast sodium current and overall depolarizing current. The resulting model was validated.

Results: At 2.0 and 0.5 Hz pacing respectively, the experimentally recorded APD_{90} was 113.9 ± 11.2 ms and 150.2 ± 13.1 ms. The adapted model produced an APD_{90} of 130.5 ms and 142.5 ms. Validation of the adapted model showed that, while there was a substantial adaptation of the model APD to experimental data, model stability was maintained, and internal Ca dynamics and responses to stimuli were not substantially affected.

Conclusion: A model of the rabbit ventricular AP was adapted in order to reproduce experimental AP recordings from the isolated right ventricular rabbit papillary muscle. This model is useful for analysis of drug effects on the rabbit papillary AP.

1.   Introduction

The isolated papillary muscle is a standard preparation for investigation of drug induced effects on the ventricular AP. To facilitate model based analysis of mechanisms underlying drug effects in this preparation, we adapted the rabbit ventricular AP model published by Shannon et al. [1] to reproduce parameters of our baseline transmembrane AP recordings. The adapted model will be applied in future investigation of drug effects.

2.   Methods and materials

Data collection was previously described in detail [2]. APs were recorded in right ventricular papillary muscles isolated from 21 female New Zealand white rabbits. For all rabbits, APs were recorded at baseline without any drugs at pacing rates of 0.5 and 2.0 Hz. Sequences of baseline recordings were obtained from approximately eight different cells from each rabbit, and a median AP was calculated for each cell. We used a current MatLab implementation of the Shannon rabbit ventricular AP model published online. Adaptation of the model was done by changes in the maximal current conductance of membrane currents. The major transmembrane currents investigated were: the fast inward sodium current (I_{Na}), the rapid and slow components of the delayed rectifier potassium current (I_{Kr}, I_{Ks}), the fast and slow components of the transient outward potassium current (I_{tof}, I_{tos}), the inward rectifier potassium current (I_{K1}), the L-type calcium current (I_{CaL}), the sodium-potassium pump (I_{NaK}), and the sodium-calcium exchanger (I_{NaCa}).

2.1.   Baseline adaptation

The default model AP displayed longer APD_{90} and greater amplitude compared to experiment. The mean and 95 % confidence interval of measured AP amplitude (defined as the maximal value of the upstroke) was 21.4 ± 2.5 mV and 23.8 ± 2.4 mV at 2.0 and 0.5 Hz pacing respectively. The default model AP amplitude was 42.7 mV and 43.7 mV. Before adaptation to APD, the model AP amplitude was reduced by reduction in I_{Na} current conductance determined by minimization of the error between model and measured AP amplitude.

The mean and 95 % confidence interval of measured APD_{90} was 113.9 ± 11.2 ms and 150.2 ± 13.1 ms at 2.0 and 0.5 Hz pacing respectively. The model APD_{90} was 190 ms and 221.5 ms and was adapted to experiment by reduction of the I_{CaL}, I_{NaK}, and I_{NaCa} currents by multiplication of the current conductances with an identical factor to minimize the error between model and measured APD_{90} across both frequencies. This approach was based in part on the results of the sensitivity analysis as described in the discussion.
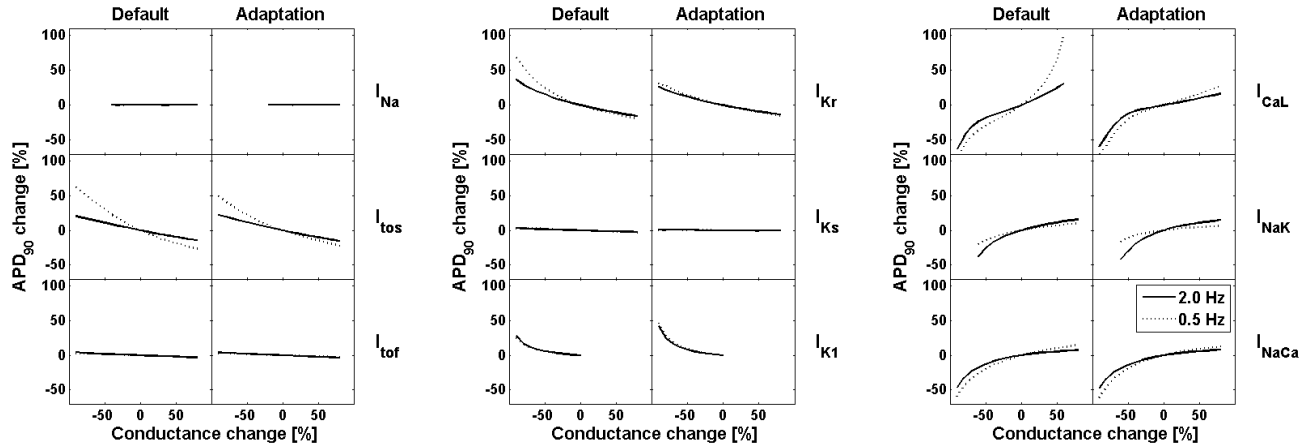
Figure 1: sensitivity analysis of APD_{90} change relative to baseline caused by reduction or increase in the conductance of major ion currents. Left subcolumns show the sensitivity analysis for default parameters, while the right subcolumns show results after adaptation of the model to experimental baseline measurements. Solid lines indicate changes at 2.0 Hz pacing while dotted lines indicate changes at 0.5 Hz pacing. Values are expressed as a percentage of baseline APD_{90}.
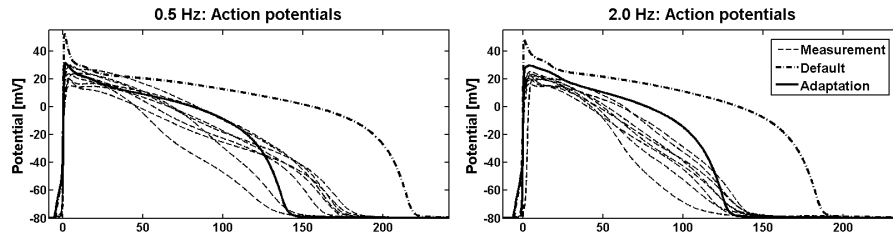

Figure 2: steady state model AP before and after baseline adaptation at 0.5 and 2.0 Hz. Also shown are example AP recordings from eight different rabbits.

The adapted model was validated using tests similar to those used to validate the original model as reported by Shannon et al. [1]. This was done in comparison with the default model and literature data. The frequency dependence of APD_{90}, cytosolic Na concentration (Na_i), and total Ca concentration in the sarcoplasmic reticulum (Ca_{SRT}, SR) was investigated. We also investigated the time-course of SR Ca release and uptake, and the balance between mechanisms competing to remove Ca from the cytoplasm following Ca release. These mechanisms were: I_{NaCa}, the SR Ca pump (I_{CaSR}), and the sarcolemmal Ca pump (I_{CaSL}). In order to test the stability of the model we also investigated the response in Ca_{SRT} and cytosolic Ca (Ca_i) transients to either a bolus of 0.4 mM free Ca added to the SR or an instantaneous removal of 0.4 mM free Ca from the SR. The change in APD induced by change in pacing rate was compared with our measurements.

3. Results

The adaptation of the model was evaluated. A minimal difference between model and measured AP amplitude was obtained by a 50 % reduction in I_{Na} conductance. This fit to baseline signals reduced the amplitude of the

model AP from 42.7 to 23.8 mV at 2.0 Hz pacing and from 47.3 to 26.0 mV at 0.5 Hz pacing. In both cases this was within the 95 % confidence interval, and no change in model APD_{90} occurred, see Figure 1.

The model was adapted to baseline APD_{90} by a reduction in I_{CaL}, I_{NaK}, and I_{NaCa} conductance. The minimal difference between model and measured APD_{90} was achieved by 40 % reduction in these conductances. This adaptation reduced APD_{90} from 190.0 to 130.5 ms at 2.0 Hz pacing and from 221.5 to 142.5 ms at 0.5 Hz pacing. The model baseline APD_{90} resulting from this adaptation was within the 95 % confidence interval of experimental measurement at 0.5 Hz pacing, but at 2.0 Hz pacing the modeled APD_{90} was slightly longer. The effect of the adaptation on the APD is shown in Figure 2.

3.1. Validation

The results of the validation are shown in Figure 3. The time-course and magnitude of Ca release from the SR was not strongly affected. The fractional release of Ca as a percentage of total Ca content changed from 27 to 24% at 0.5 Hz pacing. The balance between mechanisms competing to remove Ca from the cytoplasm was also
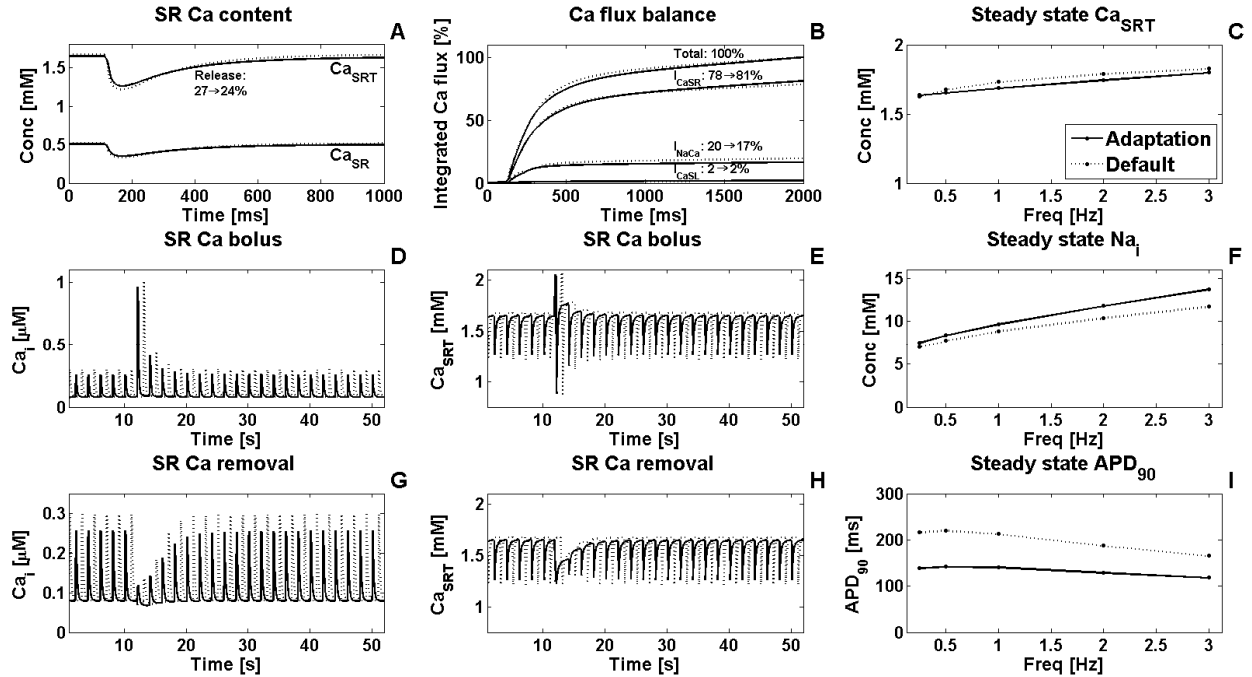
Figure 3: Results of validation. A: Ca release from SR following excitation. B: Ca removal fluxes. C: pacing rate dependence of total SR Ca in steady state. D: cytosolic Ca transient response to Ca bolus. E: total SR Ca response to Ca bolus. F: pacing rate dependence of cytosolic Na in steady state. G: cytosolic Ca transient response to Ca removal. H: total SR Ca response to Ca removal. I: pacing rate dependence of APD_{90} in steady state. In parts D, E, G, and H the results of the default model are shifted by 1 s to improve legibility.

almost unchanged at 0.5 Hz pacing. The fraction of total Ca removal attributable to the SR pump uptake changed from 78 to 81 %. The Ca removal attributable to I_{NaCa} changed from 20 to 17 %. The steady state rate dependence of total SR Ca content was highly similar between models. The steady state level of cytosolic Na was increased by the adaptation: from 10.33 to 11.65 mM (12.7 %) at 2 Hz pacing and from 7.79 to 8.41 mM (8.0 %) at 0.5 Hz. The APD_{90} was shortened as intended, and the steady state rate dependence of APD_{90} became slightly less steep. The amplitude of cytosolic Ca following excitation at 0.5 Hz pacing decreased from 0.30 to 0.26 µM (-13.6 %). After instantaneous addition or removal of free Ca in the SR, cytosolic Ca transients and SR Ca levels quickly returned to steady state over a time course identical to that for the default model.

The effects of the adaptation were also evaluated through sensitivity analysis, see Figure 1. The adaptation did not substantially change the sensitivity of APD_{90} towards change in the I_{Na}, I_{tof}, and I_{Ks} currents. The prolongation caused by high degrees of I_{K1} inhibition increased. The impact of I_{tos} and I_{Kr} at 2 Hz pacing did not change greatly, but the reverse rate dependence of prolongation due to I_{tos} inhibition was reduced moderately, and the reverse rate dependent prolongation due to I_{Kr} inhibition was almost completely abolished. The sensitivity to moderate I_{CaL} changes was reduced

slightly, and the reverse rate dependent prolongation due to great increases in I_{CaL} was dramatically reduced. The direct rate dependence of changes due to I_{NaK} was slightly increased, while the impact of I_{NaCa} changed little.

4. Discussion

Experimentally recorded APs were found to have baseline durations shorter than the default model. However, reported values of baseline rabbit APD span a relatively large range, as the APD depends on factors including age, gender, tissue type, anatomical region, and experimental methods and conditions. Romero et al. reports examples of baseline APD_{90} values ranging between 123 and 190 ms [3]. It was sought to adapt the model to baseline APD through minimal changes of the transmembrane currents, resulting in APs with a duration and rate dependence agreeing with experiment and the literature. In addition it was sought to maintain model stability and to avoid introducing unexpected behaviors. As the APD depends on the balance between repolarizing and depolarizing currents, reduction in model APD could be attained though either increasing the repolarizing current or decreasing the depolarizing current. These currents were selected based on the outcome of the sensitivity analysis. This analysis indicated that the repolarizing currents I_{tof} and I_{Ks} had only negligible

impact on APD, see Figure 1. The I_{K1} current had some impact on APD, but increased transient I_{K1} current during initial depolarization abolished AP triggering. The main repolarizing currents, I_{tos} and I_{Kr}, both displayed a substantial degree of rate dependent APD prolongation when inhibited. However, APD_{90} response to increases in these currents was relatively modest, such that substantial increase would be required to impact the adaptation to baseline. The I_{Kr} current was not increased, as the model APD_{90} prolongation due to I_{Kr} inhibition was very similar to that recorded experimentally for a pure I_{Kr} blocker [4]. The I_{tos} current was also determined to be unsuitable due to the strongly reverse rate dependent impact on APD_{90}. Increase in I_{tos} would decrease the rate dependence of APD at baseline contrary to measurements, and the rate dependence of prolongation due to inhibition would become unrealistically great. Consequently, an increase in repolarizing current was evaluated to be unsuitable for APD adaptation, and instead the adaptation was carried out by reduction in overall depolarizing current by reduction of I_{CaL}, I_{NaK}, and I_{NaCa} conductances.

The validation of the model indicated that, despite the relatively substantial change in APD_{90}, the inner mechanisms of the model behave very similarly to the default model. The release of Ca from the SR was only affected slightly by the adaptation and agrees well in time course and magnitude with experimental data [5], similarly to the default model [1]. With regards to Ca transport, the balance between Ca removal fluxes is one of the most important aspects and critical to the models predictive power, as the balance in steady state essentially determines the gain of the excitation-contraction coupling (the amount of Ca released from SR relative to the amount introduced by I_{CaL}) [1]. The validation showed that despite changes to I_{CaL} and I_{NaCa}, the balance changed very little. In addition, it is also in very good agreement with experimental data, similarly to the default model [1]. The adaptation also induced changes in the SR Ca load and cytosolic Na concentration. However, relative to the magnitude of APD changes, these changes in ionic concentration were small. Model stability was maintained, as the adapted model reliably arrived at a history-independent steady state under physiologically relevant conditions. Perturbation of the steady state by sudden addition or subtraction of Ca in the SR resulted in a rapid return to steady state identical to the default model. These results indicate that the feedback loops which ensure the stability of the real myocardial cell and which are described by the Shannon model are also maintained in the adapted model.

The changes induced by adaptation of the model to baseline recordings were investigated. The APD_{90} of the adapted model was similar to measurement, although the response of model APD_{90} to rate change was somewhat smaller than in experiment. The model AP shape had more pronounced plateau with a rapid final repolarization compared to AP recordings. However, a less pronounced plateau is commonly found in the literature for recordings of rabbit APs in isolated multicellular preparations, examples include [6]. The sensitivity analysis indicated that sensitivity of APD_{90} to the conductances of transmembrane currents was similar between the default and adapted models. Thus, the adaptation did not drastically alter the impact of current inhibitions, and no new unexpected behaviors were identified. The greatest difference was that the model became less vulnerable towards excessive reverse rate-dependent prolongation as observed for an increase in I_{CaL} current in the default model. The adaptation also reduced the reverse rate dependency of APD prolongation due to I_{Kr} inhibition. This may have been related to the short baseline APD.

5. Conclusion

The Shannon model of the rabbit ventricular AP was adapted to measured baseline AP features. The adapted model produces APs with duration similar to experiments while also maintaining the stability and key behaviors of the original model. The behavior of its internal mechanisms are in good agreement with experimental data. The adapted model serves as a tool to analyze experimentally measured drug effects.

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