

Determining the effects of memory and action potential duration alternans on cardiac restitution using a constant-memory restitution protocol

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Abstract

Restitution, the dependence of action potential duration (APD) on diastolic interval, may be causally linked to the vulnerability of cardiac tissue to certain types of arrhythmias. While a number of pacing protocols are commonly used to quantify the restitution relation, one of these, the dynamic protocol, may result in the occurrence of APD alternans. However, the effects of APD alternans, and the concomitant alternation in cardiac memory, on the restitution curve are currently not well understood. Alternans preceding a given action potential may cause that action potential to have a different duration from one preceded by action potentials of identical duration. This interaction of alternans and memory can result in a dynamic restitution curve that is not unique. To address this, we have developed a constant-memory restitution protocol that enables the experimenter or modeller to obtain unique, constant-memory restitution curves at all diastolic intervals. Using this protocol, we obtained unique restitution curves for two ionic models of the cardiac action potential in the absence of alternans at all diastolic intervals. A comparison of the unique constant-memory and non-unique dynamic restitution curves for the two models shows that the presence of alternans can significantly alter the shape of the restitution curve compared to when alternans is absent.

Keywords: APD alternans, restitution curve, ionic model

1. Introduction

Action potential duration (APD) alternans is a sequence of action potentials characterized by a long–short–long–short alternation in APD. It has recently been shown, both in experiments (Tachibana *et al* 1998, Pastore *et al* 1999) and modelling studies (Qu *et al* 2000, Watanabe *et al* 2001), that the presence of alternans can lead to the initiation of reentrant wave propagation in cardiac tissue. Given the complexity of studying and understanding alternans either *in vivo* or *in vitro*, mathematical models of cardiac myocytes exhibiting alternans have been studied in an attempt to gain insight into the mechanisms underlying alternans. Alternans has been studied in ionic models, ‘reduced’ ionic models (Fenton and Karma 1998, Fenton *et al* 2002) and low-dimensional discrete map models (Otani and Gilmour 1997, Hall *et al* 1999, Fox *et al* 2002a). Of these approaches, the ionic models that incorporate ion channels, pumps and exchangers most faithfully represent physiological reality. Four ionic models in particular have been widely used to investigate alternans or alternans-related phenomena: (i) the Beeler–Reuter model (Beeler and Reuter 1977), in both its original and modified forms (Zemlin *et al* 2002); (ii) the original Luo–Rudy model (Luo and Rudy 1991), modified to reproduce stable alternans (Cao *et al* 1999, Yehia *et al* 1999, Arce *et al* 2000); (iii) the dynamic Luo–Rudy model (Luo and Rudy 1994), with a modified calcium handling system resulting in quasi-periodic alternans (Chudin *et al* 1999); and (iv) the Fox–McHarg–Gilmour model (Fox *et al* 2002b).

The nonlinearities inherent to cardiac myocyte electrophysiology contribute significantly to the difficulties involved in identifying the root cause of alternans. Apart from the nonlinear relationships between membrane potential and ion-channel gating activity that characterize many ionic currents, a number of these currents also possess time-dependent gating properties. In addition, dynamic intracellular ion concentrations feed back to influence channel gating. These voltage- and time-dependent currents, along with the variable intracellular ionic concentrations, endow the action potential with complex rate-dependent properties. One such property is so-called ‘memory.’ Memory represents a slowly accumulating and dissipating (on a time course of seconds to minutes) influence of excitation rate on APD (Koller *et al* 1998). The fact that the action potential often takes a number of beats to adjust to a new steady morphology following an abrupt change in excitation rate is due to the presence of memory (Elharrar and Surawicz 1983, Watanabe and Koller 2002).

In an attempt to distil these complex rate-dependent properties into a simple framework, the APD restitution relation is widely used to characterize the rate dependence of cardiac tissue. The restitution relation, expressed mathematically as $APD_{n+1} = f(DI_n)$, relates the duration of an action potential solely to its preceding diastolic interval (DI) through an experimentally derived function f (Nolasco and Dahlen 1968). An example restitution curve is shown in figure 1. Because $APD + DI = BCL$, where BCL is the basic cycle length (the time between successive excitations), the restitution relation may also be thought of as relating the next APD (APD_{n+1}) to the previous APD (APD_n) through f . In such a one-dimensional mapping relationship, the restitution function ‘remembers’ the previous action potential only. Thus, such a one-dimensional map ignores slowly accumulating and dissipating memory.

It has been suggested that the restitution behaviour of cardiac tissue, and in particular the slope of the restitution curve, is a major determinant of the tissue’s vulnerability to arrhythmias. This so-called ‘restitution hypothesis,’ which stems from the one-dimensional restitution relation, is based on the nonlinear dynamics of such one-dimensional maps. The hypothesis states that when the excitation rate is slow enough that the slope of the restitution curve is less than one, a single APD occurs at that BCL (corresponding to the period-one fixed point of the one-dimensional map at that BCL). However, when the excitation rate is fast

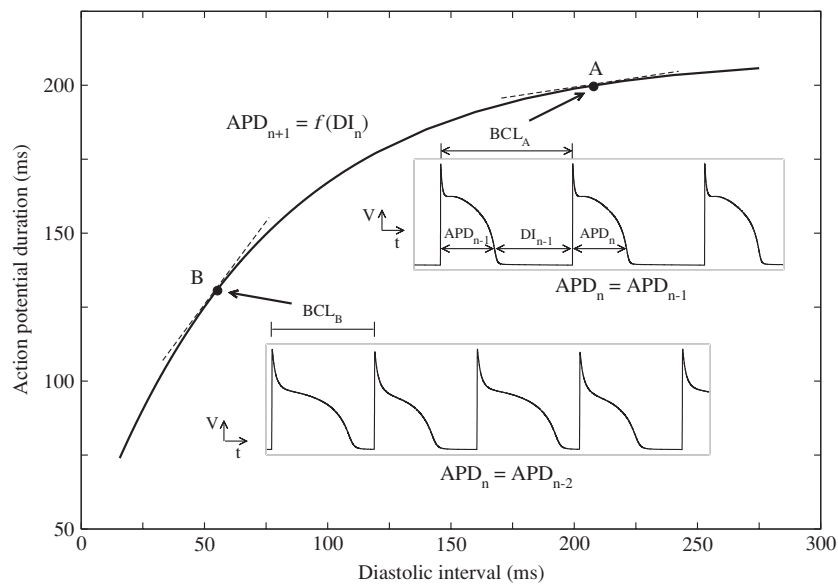


Figure 1. A typical cardiac APD restitution curve. Action potential duration APD_{n+1} is related to the preceding diastolic interval DI_n through the experimentally or computationally determined function f . The restitution hypothesis proposes that if the BCL corresponds to a region where the slope of f is less than 1 (point A; dashed line indicates slope), a sequence of identical action potentials results (upper inset). However, if the BCL corresponds to a region where the slope of f is greater than 1 (point B; dashed line indicates slope), a long–short–long–short sequence of action potentials known as APD alternans results (lower inset).

enough that the slope of the restitution curve is greater than one, the period-one fixed point becomes unstable and a bifurcation to the period-two fixed point occurs, resulting in a long–short–long–short APD alternans rhythm at that BCL (figure 1) (Nolasco and Dahlen 1968, Guevara *et al* 1984). In whole tissues, the slope of the restitution curve, and the corresponding presence or absence of alternans, has been linked to the stability of spiral waves (Gilmour 2003). A number of studies have implicated tissue restitution properties in wave break (Karma 1994, Qu *et al* 1999, Weiss *et al* 1999), while others have shown that flattening the slope of the restitution relation prevents the development of ventricular fibrillation (Riccio *et al* 1999, Garfinkel *et al* 2000).

Given the emphasis that has been placed on the restitution relation, and in particular on the slope of the restitution curve, it is essential that the factors influencing the shape of the restitution curve be understood. One well-known factor that significantly influences the shape of the restitution curve is the pacing protocol with which it is developed (Gilmour *et al* 1997, Watanabe and Koller 2002). A number of different pacing protocols have been used to quantify the restitution relation in cardiac myocytes, with each one resulting in a slightly different curve. Clinically, the S1–S2 restitution protocol (Elharrar and Surawicz 1983), which involves pacing the tissue for a set number of beats at cycle length S1 before providing a single premature stimulus at a variable cycle length S2, is widely used. Experimentally, both the S1–S2 and dynamic (which involves pacing the cell at a constant cycle length for a set number of beats, recording the APD and DI after all transients have died out, then reducing the cycle length and repeating) restitution protocols are commonly used (Koller *et al* 1998). An experimental comparison of the S1–S2 and dynamic restitution protocols applied to canine Purkinje fibres

showed that the dynamic protocol consistently produced a steeper restitution curve than the S1–S2 protocol, with the difference between the two restitution curves possibly attributable to differing contributions of memory (Koller *et al* 1998). The authors of that study also found that the restitution relation obtained using the dynamic restitution protocol was able to account for the APD dynamics encountered during ventricular fibrillation while that obtained via the S1–S2 protocol was not. Another less widely used restitution curve, known as the constant-BCL curve, describes the transient APD response as the action potential approaches steady state following a change in BCL (Otani and Gilmour 1997, Tolkacheva *et al* 2003). The results of a recent simulation study comparing the three restitution curves suggest that the constant-BCL curve may be a better predictor of the onset of APD alternans than the S1–S2 and dynamic curves (Tolkacheva *et al* 2003).

The dynamic restitution protocol, which utilizes pacing at constant cycle lengths to obtain the dynamic restitution curve, is well suited to characterizing cardiac restitution at slow excitation rates. At any such long cycle length, constant-BCL pacing produces a train of action potentials of identical duration, each being preceded by the same DI (i.e., $DI_n = DI_{n-1}$). In this situation, the contribution of memory to each action potential is identical. However, at faster pacing rates, constant-BCL pacing may result in the occurrence of alternans (figure 1). During alternans, the contribution of memory to each action potential alternates on a beat-to-beat basis (figure 2), due to the fact that the DI alternates (i.e. $DI_n = DI_{n-2}$). This means that the dynamic restitution protocol may combine action potential data obtained in the presence of two intrinsically different memory dynamics into a single restitution curve. As will be shown later, this can cause the dynamic restitution curve to be non-unique in some situations.

To address this issue, we have developed a restitution pacing protocol that avoids alternans at short cycle lengths. Incorporating a DI-control feedback technique described recently (Patwardhan and Moghe 2001, Wu and Patwardhan 2003), our protocol can be used to obtain restitution curves where, at any given DI, the preceding action potentials have identical duration, and therefore the memory contribution to every consecutive action potential is identical. Our protocol allows the experimenter or modeller to obtain a truly steady-state, constant-memory restitution curve over the entire range of diastolic intervals of interest, without concern for the occurrence of alternans at rapid excitation rates. Using two well-known ionic models of the ventricular action potential, we show how this restitution curve (which we will refer to henceforth as the *constant-memory* restitution curve) compares to the dynamic restitution curve. As a result, we are able to demonstrate that fluctuations in the contribution of memory during alternans can influence the shape of the restitution curve, and can lead to a non-unique relationship between APD and DI.

2. Methods

2.1. Models of the ventricular action potential

Modelling was performed with a modified version of the original Luo–Rudy (LR1) model (Luo and Rudy 1991) and the Fox–McHarg–Gilmour (FMG) model (Fox *et al* 2002b). Using the notation proposed by Qu *et al* (1999), replacing the notation ' I_{si} ' with ' I_{Ca} ', the equation describing conservation of current across the membrane of a LR1 model cell is

$$C_m \frac{dV}{dt} = -(I_{Na} + I_{Ca} + I_K + I_{K1} + I_{Kp} + I_b + I_{stim}).$$

Here, C_m is the membrane capacitance, V is the transmembrane potential, t is time, I_{Na} is the sodium current, I_{Ca} is the slow inward calcium current, I_K is the time-dependent potassium current, I_{K1} is the time-independent potassium current, I_{Kp} is the plateau potassium current

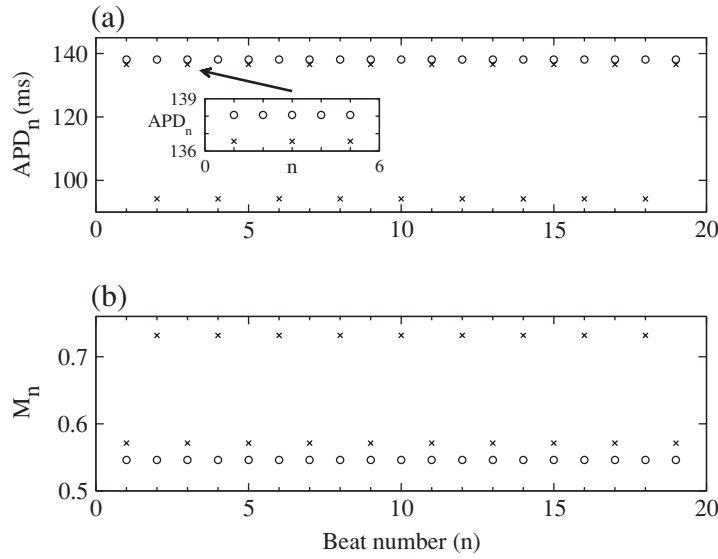


Figure 2. The contribution of memory to APD. In both (a) and (b), crosses signify the presence of alternans, while open circles signify the absence of alternans. Simulations were performed with the memory map model of Fox *et al* (2002a). The equations describing the evolution of APD_n and the memory variable M_n are $APD_{n+1} = (1 - 0.35M_{n+1})(88 + 122/[1 + \exp\{(-DI_n + 40)/28\}])$ and $M_{n+1} = [1 + (M_n - 1)\exp\{-APD_n/150\}]\exp\{-DI_n/150\}$. Unlike ionic models, where memory is an intrinsic behavioural element of the model that cannot be easily separated from the other parts of the system, the map model of Fox *et al* (2002a) incorporates memory through an explicit memory variable M_n . Pacing at a constant cycle length of 155 ms was used to obtain action potentials during alternans, while constant-DI pacing (which involves adjusting the BCL during every beat to keep DI constant) was used to obtain action potentials in the absence of alternans. The DI preceding the long action potential during alternans ($DI = 60.815$ ms) was used as the target DI during constant-DI pacing. As (a) shows, even though the diastolic intervals preceding beats 1, 3, 5, ... are identical, the presence of alternans changes the duration of the long action potential compared to when alternans is absent (see inset for detail). This may be directly attributed to the difference in the contribution of memory (shown in (b)) occurring between the presence and absence of alternans.

and I_b is the background current. In this model, I_{Na} , I_{Ca} and I_K possess both voltage- and time-dependent gating properties, while I_{K1} , I_{Kp} and I_b are only voltage dependent. I_{Ca} is also a function of the intracellular calcium concentration. As in Cao *et al* (1999), we set $g_{Na} = 16.0 \mu S cm^{-2}$, $g_{Si} = 0.06 \mu S cm^{-2}$ and $g_K = 0.432 \mu S cm^{-2}$. All other parameter values were identical to those of the original model formulation (Luo and Rudy 1991). C_m was set to be $1.0 \mu F cm^{-2}$, and the cell was stimulated with an injected current (I_{stim}) of $100 \mu A cm^{-2}$ applied for 0.5 ms.

The equivalent conservation of current equation for the FMG model is

$$C_m \frac{dV}{dt} = -(I_{Na} + I_{NaK} + I_{NaCa} + I_{Nab} + I_{Kr} + I_{Ks} + I_{K1} + I_{Kp} + I_{to} + I_{Ca} + I_{CaK} + I_{Cab} + I_{pCa} + I_{stim}).$$

Here, I_{NaK} is the sodium–potassium pump current, I_{NaCa} is the sodium–calcium exchanger current, I_{Nab} is the background sodium current, I_{Kr} is the rapid component of the delayed rectifier potassium current, I_{Ks} is the slow component of the delayed rectifier potassium current, I_{to} is the transient outward potassium current, I_{CaK} is the potassium current through the L-type calcium channel, I_{Cab} is the background calcium current and I_{pCa} is the sarcolemmal calcium

pump current. The FMG model also incorporates dynamic intracellular and sarcoplasmic reticulum calcium ion concentrations. The cell was stimulated with an injected current (I_{stim}) of $80 \mu\text{A cm}^{-2}$ applied for 1 ms.

Custom code was written in the C programming language to numerically solve the current conservation equations and the related gating differential equations. All numerical integration was performed using forward Euler schemes, with a fixed time step of 0.01 ms for the LR1 model and 0.0025 ms for the FMG model.

2.2. Restitution protocols

The dynamic restitution curves for each model were obtained using a pacing protocol almost identical to that described in Koller *et al* (1998). Briefly, the model cell was paced at a constant BCL of 400 ms until steady state was reached. The action potential durations at 95% of repolarization (APD_{95}) and the durations of the preceding diastolic intervals of the last two action potentials were measured, and then the BCL was reduced by 10 ms. After steady state was reached at the new BCL, APD_{95} and DI were measured again, and the BCL was again reduced by 10 ms. This procedure was repeated until a BCL of 210 ms was reached, after which the BCL was reduced in 1 ms increments. Pacing was stopped in both models when 2:1 block occurred. At each BCL, a sufficient number of pacing stimuli were applied to ensure that steady-state behaviour was achieved before the action potential durations and diastolic intervals were measured.

The constant-memory restitution curve is constructed using a pacing protocol that incorporates a DI-control feedback stimulation technique described recently (Patwardhan and Moghe 2001, Wu and Patwardhan 2003). In our protocol, the goal is to vary the time at which the stimulus current is applied (i.e. to vary the BCL) in order to control the DI to a desired target value on a beat-to-beat basis. This is accomplished by measuring the duration of every action potential on-the-fly, and then adjusting the timing of the next stimulus to maintain the DI at the target value. If a sufficient number of pacing stimuli are applied at any given DI value, the action potential eventually settles down to a constant morphology and duration that repeats indefinitely; that is only one steady-state APD occurs for any given DI. By varying the target DI, unique steady-state (APD , DI) pairs can be obtained over the complete range of possible diastolic intervals. Plotting the APD values as a function of the DI values results in the constant-memory restitution curve.

The particular pacing protocol we used is as follows. The model cell was initially paced at a BCL of 400 ms (where alternans was absent), and APD and DI were measured at steady state at a threshold voltage of -75 mV. After steady state had been reached, DI control was switched on, such that the target DI was equivalent to that occurring during pacing at a BCL of 400 ms. The control DI was then changed to a new target value by reducing the DI by 1 ms. The subsequent timing of each stimulus (i.e. the BCL) was then adjusted on a beat-to-beat basis in order to keep the DI at this new value until identical action potentials of constant duration occurred. At steady state, APD_{95} and DI were measured. Following measurement, the control DI was again reduced by 1 ms. This procedure of reducing DI by 1 ms and measuring the resulting steady-state APD was repeated until 2:1 block occurred, thus allowing APD and DI values to be obtained over a wide range of diastolic intervals in the absence of alternans.

3. Results

Figure 3 illustrates the bifurcation diagrams for the LR1 and FMG models. Both diagrams were obtained using the dynamic pacing protocol described in the methods section. The

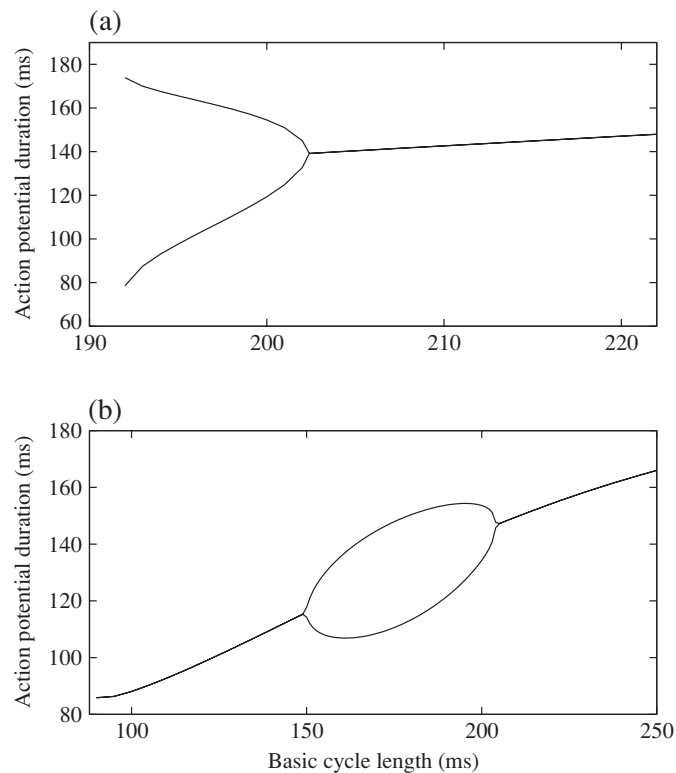


Figure 3. Bifurcation diagrams for the (a) LR1 and (b) FMG models obtained using the dynamic pacing protocol described in section 2.

figure clearly illustrates how different ionic models may produce markedly different alternans behaviour: the magnitude of alternans increases monotonically (leading to 2:1 block) as BCL is reduced in the LR1 model, while alternans initially increases in magnitude, then decreases and finally disappears in the FMG model. Alternans also occurs over a much broader range of excitation rates in the FMG model than in the LR1 model.

Figure 4 shows these bifurcation diagrams redrawn as dynamic restitution curves. Here, the same APD values as shown in figure 3 are plotted against their preceding DI values. In figure 4(a), which illustrates the dynamic restitution curve for the LR1 model, the lower arm of data at diastolic intervals greater than 63 ms corresponds to action potentials obtained in the absence of alternans (that is, at basic cycle lengths above 202 ms). Alternans in the LR1 model occurred at basic cycle lengths between 202 ms and 192 ms, resulting in the action potentials corresponding to the upper arm of data for diastolic intervals greater than 63 ms and all of the data points for diastolic intervals less than 63 ms. 2:1 block occurred for basic cycle lengths shorter than 192 ms.

Figure 4(b) is the dynamic restitution curve for the FMG model. At a BCL of approximately 205 ms, when the bifurcation occurs, the long and short action potentials (seen clearly as the upper and lower arms of the bifurcation diagram in figure 3(b)) follow the two arms of the restitution curve as shown. These arms recombine to form the remainder of the dynamic restitution curve when alternans disappears at cycle lengths below approximately 140 ms.

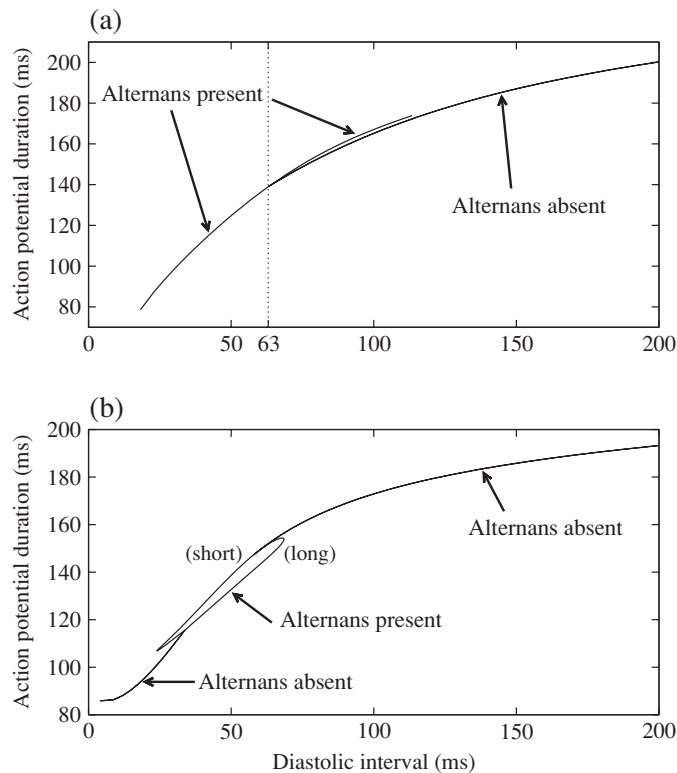


Figure 4. The dynamic restitution curves for the (a) LR1 and (b) FMG models. The data in these curves are identical to that in figure 3. In both models, regions of the dynamic restitution curve exist where two (or three) APD values occur for any one DI value.

The two curves in figure 4 clearly illustrate how the presence of alternans can influence the dynamic restitution curve. In both restitution curves, instances exist where one DI maps to two (or three) different action potential durations, clearly violating the assumption of a one-dimensional restitution relation. In such instances, the only plausible explanation for the occurrence of multiple action potential durations for one DI is that there are different memory contributions for each APD, based on the history of the preceding action potentials (as depicted in figure 2).

Figure 5 shows a typical action potential response in the LR1 model during DI control. Figure 5(a) illustrates a train of action potentials, where the action potentials preceding those numbered 1–4 are steady-state action potentials corresponding to a BCL of 500 ms (a DI of approximately 300 ms). When the target DI was changed from 300 ms to 30 ms, the resulting action potentials (1–4 and beyond) rapidly adjusted to the new DI, with the APD quickly settling to a new steady-state value. Figure 5(b) illustrates this evolution of APD following the change of target DI. After initially over-shortening (beat number 1), the action potential gradually relaxed to its new steady-state value after approximately 10 beats.

The steady-state values of APD and DI occurring during the constant-DI pacing protocol were used to construct the constant-memory restitution curves for the LR1 and FMG models. Figure 6(a) compares the dynamic (dashed line) and constant-memory (solid line) restitution curves for the LR1 model. As expected, the two restitution curves are identical when alternans is absent in the dynamic restitution protocol, but the curves differ when alternans is present.

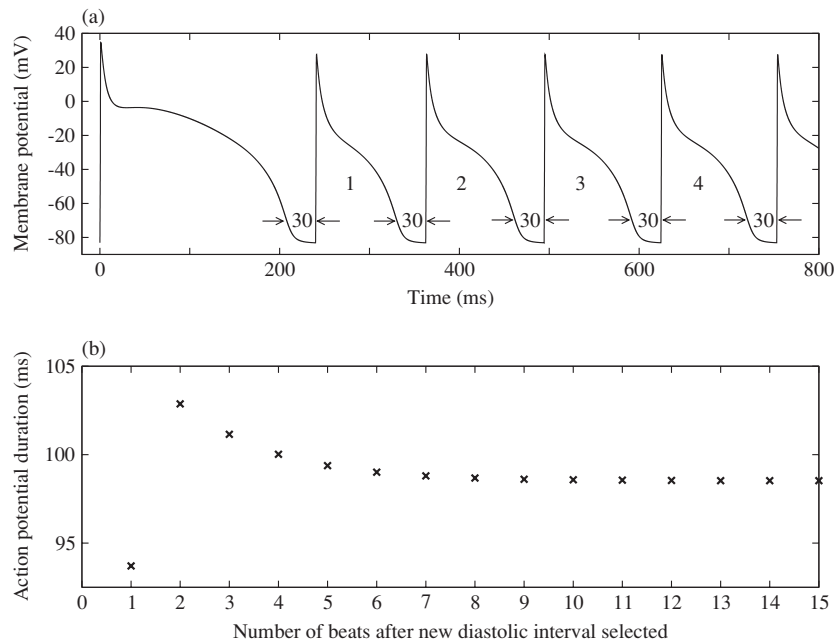


Figure 5. A typical action potential response during DI control for the LR1 model. In (a), the target DI was reduced from approximately 300 ms (preceding the first action potential shown) to 30 ms (preceding the action potentials numbered 1–4). The corresponding action potential durations at the new target DI are shown in (b). After approximately ten beats, the APD has settled to its new steady-state value. Note that no alternans is present at steady state during DI control.

The long action potentials during alternans are longer than the steady-state action potentials generated by the constant-DI protocol, while the short action potentials are shorter than the corresponding steady-state action potentials at the same DI.

Figure 6(b) compares the dynamic (dashed line) and constant-memory (solid line) restitution curves for the FMG model. As for the LR1 model, the two curves are identical when alternans is absent in the dynamic restitution protocol, but the curves differ when alternans is present. However, the variation between the two restitution curves for the FMG model is opposite to that seen in the LR1 model. For the FMG model, the long action potentials during alternans are shorter than action potentials with the same preceding DI obtained with the constant-DI protocol, while the short action potentials during alternans are longer than action potentials with the same preceding DI obtained with the constant-DI protocol. Similar results were also obtained when the dynamic and constant-memory restitution curves of the memory map model of Fox *et al* (2002a) were compared (data not shown).

The constant-memory restitution curve could also have been obtained by using a nonlinear-dynamical alternans-control algorithm (Hall *et al* 1997, Hall and Christini 2000, Hall and Gauthier 2002). While the DI-control algorithm adjusts the BCL to obtain the target DI, nonlinear-dynamical alternans-control algorithms attempt to stabilize the period-one fixed point by adjusting the BCL based on the measured APD values. A typical adjustment to the BCL is made according to the following rule (Hall and Christini 2000):

$$BCL_n = \begin{cases} BCL^* + \Delta BCL_n & \text{if } \Delta BCL_n < 0 \\ BCL^* & \text{if } \Delta BCL_n \geq 0 \end{cases}$$

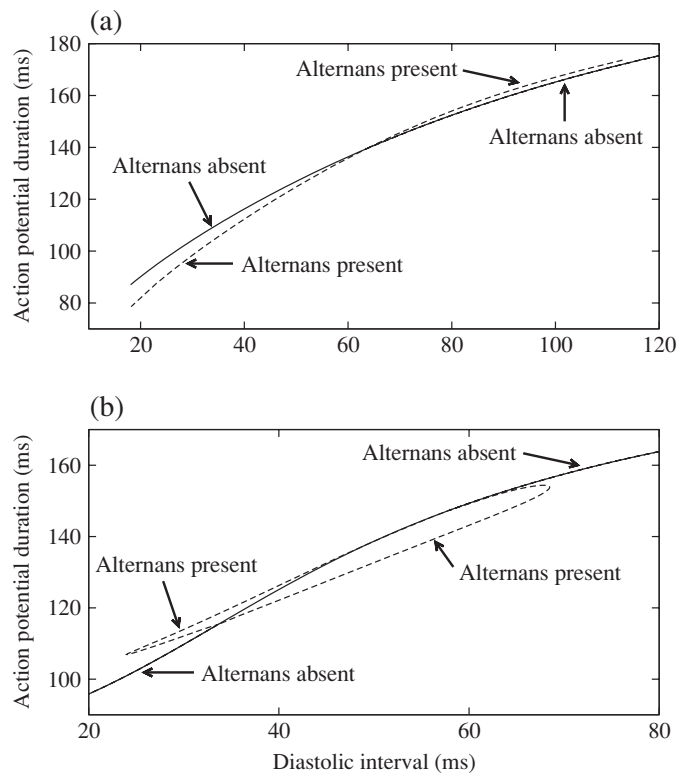


Figure 6. Constant-memory (solid) and dynamic (dashed) restitution curves for the (a) LR1 and (b) FMG models. In the LR1 model, the presence of alternans makes the long action potentials during alternans longer, and the short action potentials shorter, than if alternans was absent in the preceding action potentials. In the FMG model, the presence of alternans makes the long action potentials during alternans shorter, and the short action potentials longer, than if alternans was absent in the preceding action potentials. In panel (a), the constant-memory and dynamic restitution curves overlap for the region marked ‘alternans absent’ at diastolic intervals greater than 63 ms. In panel (b), the two restitution curves overlap in both regions marked ‘alternans absent’.

where

$$\Delta BCL_n = (\lambda/2)(APD_n - APD_{n-1}).$$

Here, BCL^* is the pacing period without control, λ is the feedback gain and n is the interval number. After initiating control (with $\lambda/2 = 0.5$), we obtained constant-memory restitution curves for the LR1 and FMG models that were identical to those shown in figure 6, as control established a period-one rhythm at every pacing period.

4. Discussion

For some time, memory has been acknowledged as an important factor in cardiac dynamics and restitution (Boyett and Jewell 1980, Franz *et al* 1988, Gilmour *et al* 1997, Koller *et al* 1998, Watanabe and Koller 2002, Fox *et al* 2002a). While the restitution hypothesis predicts that APD alternans will occur when the slope of the restitution curve is equal to one, it has been demonstrated that the presence of memory can affect the dynamics such that the slope of the restitution curve is no longer predictive of the onset of alternans (Hall *et al* 1999, Fox *et al*

2002a). The mechanism by which memory accumulation and dissipation modulates alternans behaviour has received little attention in the literature, partly due to the difficulty in separating memory from alternans. Recently, Li and Otani (2003) developed a mathematical 'eigenmode' method to separate memory and alternans, which they applied to the Beeler–Reuter model (Beeler and Reuter 1977); however, the authors of that study note that a shortcoming of their method is the difficulty in directly observing or measuring eigenmodes in experiments.

The constant-memory restitution curve that we have developed allows the experimenter to directly determine how the tissue behaves at all diastolic intervals in the absence of alternans. While our method does not explicitly separate memory and alternans, it does allow the experimenter to determine whether memory affects the duration of the action potential during alternans, as it provides a method for obtaining steady-state, non-alternating reference data at diastolic intervals for which other pacing techniques (such as the dynamic restitution protocol) fail to suppress alternans. It therefore enables the experimenter to account for the difference in memory dynamics, and corresponding differences in APD, between alternating and non-alternating action potentials that may occur when constructing the restitution curve.

It has been shown experimentally that a non-unique relationship between DI and time to full repolarization (analogous to APD) can exist during rapid pacing in Purkinje fibres (Gilmour *et al* 1997). This non-uniqueness appeared in the case of a single stimulus (S3) applied after a S1–S2 pacing protocol. We found that for a given DI, action potentials preceded by alternans can have different durations from those preceded by action potentials of constant duration, which can lead to the dynamic restitution curve being non-unique. This non-uniqueness can be attributed to differences in the contribution of memory based on the preceding action potential histories. Further experimental studies in other types of cardiac tissue are needed to explore both the transient non-uniqueness seen previously in Purkinje fibres (Gilmour *et al* 1997), and the steady-state non-uniqueness seen in our simulations. Further experimental studies are also required to determine the applicability of this protocol to spatially extended whole tissue preparations, in which the APD and DI resulting from pacing may not be spatially homogeneous.

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