Short Note

Bifurcation Analysis of Genetically Engineered Pacemaking in Mammalian Heart

WING CHIU TONG and ARUN V. HOLDEN

Computational Biology Laboratory, Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK; e-mail: a.v.holden@leeds.ac.uk

Abstract. Genetically engineered pacemaking in ventricular cells has been achieved by down-regulation of the time independent inward rectifying current (I_{K1}), or insertion of the hyperpolarisation-activated funny current (I_f). We analyse the membrane system (i.e. ionic concentrations clamped) of an epicardial Luo-Rudy dynamic cell model using continuation algorithms with the maximum conductance ($\bar{g}$) of I_{K1} and I_f as bifurcation parameters. Pacemaker activity can be induced either *via* Hopf or homoclinic bifurcations. As $\bar{g}_{K1}$ is decreased by $\approx 74\%$, autorhythmicity emerged *via* a homoclinic bifurcation, i.e., the periodicity first appear with infinitely large periods. In contrast, the insertion of $\bar{g}_f$ induced periodicity *via* a subcritical Hopf bifurcation at $\bar{g}_f \approx 0.25 \text{ mS}\mu\text{F}^{-1}$. Stable autorhythmic action potentials occurred at $\bar{g}_f > 0.329 \text{ mS}\mu\text{F}^{-1}$.

Key words: bifurcation analysis, pacemaking, I_{K1} , I_f , inward rectifying current, funny current, ventricular cell model

Failure of cardiac pacemaking or AV conduction are corrected by implanted electronic pacemakers. Ventricular cells are normally excitable and non-autorhythmic. However, pacemaking in ventricular cells has been genetically engineered by either loss of function (I_{K1} [1, 2]) or gain of function (I_f [3, 4]) interventions. The change from excitability to rhythmicity is a qualitative change in system behaviour, i.e. a bifurcation.

Bifurcations in models can be identified numerically by changes in the stability of solutions as a control parameter is varied. A Hopf bifurcation occurs when a stable equilibrium lose its stability into small amplitude oscillations with a defined frequency. A homoclinic bifurcation occurs suddenly with an infinitely long period at the bifurcation point, as the oscillatory region touches a limit point (LP in Figure 1) or a fold in the equilibrium solutions [5]. The analysis of bifurcations summarises the interactions among equilibrium, periodic and chaotic solutions, and describes the dynamics of the system as parameters change. Here we apply continuation algorithms [6, 7] to a biophysical detailed guinea-pig ventricular cell model (LRd00) [8], with the maximum conductance of I_{K1} and I_f as bifurcation parameters.

Figure 1. Bifurcation diagrams of the membrane system (LRd00, clamped ionic concentrations) for $\bar{g}_{K1}$ down-regulation (left box) and $\bar{g}_f$ insertion (right box). The right panels of $\bar{g}_f$ insertion are a magnified portion of the left panels. Stable equilibria (solid line), unstable equilibria (dotted line), stable periodic (black solid circles) and unstable periodic (grey empty circles) solutions are tracked. Upper panels show the membrane potential (V) at which $dV/dt = 0$. Lower panels show the corresponding periods. Physiological $\bar{g}_{K1} = 0.75 \text{ mS}\mu\text{F}^{-1}$; $\bar{g}_f = 0 \text{ mS}\mu\text{F}^{-1}$. LP – Limit point; HB – Hopf bifurcation

LRd00 is a system of ordinary differential equations which describe mammalian ventricular cell electrophysiology. For a cell,

$$C_m \frac{dV}{dt} = -I_{\text{ion}}, \quad (1)$$

where V is the membrane potential (mV); I_{ion} is the sum of membrane currents through ion channels, pumps and exchangers ($\mu\text{A cm}^{-2}$); $C_m = 1 \mu\text{F cm}^{-2}$, is the membrane specific capacitance. There are 21 differential equations for 16 ionic currents and buffer systems in LRd00. These equations, including I_{K1} , and the differential equation for I_f are in [8] and [3] respectively.

I_{K1} is a time independent current (with $\bar{g}_{K1} = 0.75 \text{ mS}\mu\text{F}^{-1}$) that contributes to the maintenance of the resting membrane potential. Expression of non-functional I_{K1} channels decreases its maximum conductance [1, 2]. I_f is a voltage-gated pace-making current not normally found in ventricular cells. I_f is inserted in LRd00 here and it consists of a sodium and a potassium components with a maximal conductance ratio of 0.3833:0.6167 [3]. We assumed this ratio is independent of $\bar{g}_f$.

We consider the membrane system (i.e. all intra- and extra-cellular ionic concentrations clamped) of an epicardial LRd00 cell model. The numerical solutions, equilibrium and periodic states are computed using the package XPPAUT and its

AUTO library [6, 7], with the fourth-order Runge-Kutta method and a fixed time-step of 0.002 ms.

Autorhythmicity can be induced either *via* Hopf or homoclinic bifurcations (Figure 1). In both cases (reducing $\bar{g}_{K1}$ or increasing $\bar{g}_f$) the resting membrane potential depolarised as the parameter moves away from the initial standard equilibrium ($\bar{g}_{K1} = 0.75 \text{ mS}\mu\text{F}^{-1}$ and $\bar{g}_f = 0 \text{ mS}\mu\text{F}^{-1}$).

As $\bar{g}_{K1}$ is reduced by $\approx 74\%$, large amplitude oscillations emerge *via* a homoclinic bifurcation (Figure 1, *LP1*), i.e., action potentials appear with infinitely large ($>10 \text{ s}$) periods. The rate increases as $\bar{g}_{K1}$ decreases further (at $\bar{g}_{K1} = 0$, period $\approx 0.36 \text{ s}$). These are consistent with the experimental results [1, 2].

In contrast, autorhythmicity emerge *via* a subcritical Hopf bifurcation at $\bar{g}_f \approx 0.25 \text{ mS}\mu\text{F}^{-1}$ into unstable small-amplitude oscillations, i.e. equilibrium and periodic solutions coexist over a range of $0.23 < \bar{g}_f < 0.25 \text{ mS}\mu\text{F}^{-1}$. Stable autorhythmic action potentials occurred at $\bar{g}_f > 0.329 \text{ mS}\mu\text{F}^{-1}$, with period $\approx 0.377 \text{ s}$. The period decreases as $\bar{g}_f$ increases (at $\bar{g}_f = 1.785 \text{ mS}\mu\text{F}^{-1}$, period $\approx 0.187 \text{ s}$).

Neither $\bar{g}_{K1}$ down-regulation nor $\bar{g}_f$ insertion alone offer a simple solution for genetically engineered pacemakers. The extremely low rate pacing due to the homoclinic bifurcation with $\bar{g}_{K1}$, and the irregularity within the range $0.25 < \bar{g}_f < 0.329 \text{ mS}\mu\text{F}^{-1}$ prior the stable autorhythmic action potentials, are both clinically undesirable for a functional pacemaker.

Nevertheless, bifurcation analysis shows that stable autorhythmic action potential can be induced within physiological periods (0.158–0.260 s for guinea-pig). A combination of $\bar{g}_{K1}$ down-regulation and $\bar{g}_f$ insertion (Figure 2) may offer better design for bioengineering cardiac pacemakers. It has been shown *in silico* that I_{K1} and I_f together can modulate the pacing rate [3].

We have considered an idealised and simplified situation where ionic concentrations are kept constant. Numerical integration of the full system shows more

Figure 2. Two-parameter bifurcation diagrams for $\bar{g}_f$ and $\bar{g}_{K1}$. The right panel is a magnified portion of the left panel. Standard $\bar{g}_{K1} = 0.75 \text{ mS}\mu\text{F}^{-1}$ (dotted line). *LP1*, *LP2* and *HB* are limit points and the Hopf bifurcation from Figure 1. *LP1* and *LP2* are connected on a fold (dashed line) within this state space. A cusp point is found in the unphysiological range (not shown). The Hopf bifurcation (solid line) separates the oscillation states (*OS*) (shaded area) and the steady states (*SS*). Below $\bar{g}_f \approx 0.00648 \text{ mS}\mu\text{F}^{-1}$ and $\bar{g}_{K1} \approx 0.223 \text{ mS}\mu\text{F}^{-1}$, *OS* and *SS* are separated by the fold.

complex behaviour including bursting and termination of pacing due to accumulation of intracellular sodium and calcium [9, 10]. Isolated cells are integrated systems, and changes in a single conductance designed to produce pacemaking require compensatory changes in other ion transport and sequestration processes to maintain cell homeostasis. Continuation algorithms applied in the multi-parameter bifurcation spaces of virtual cardiac tissues offer a tool for engineering the dynamical behaviours of the heart.

Acknowledgements

This work was supported by the European Union through the Network of Excellence BioSim, Contract No. LHSB-CT-2004-005137. WCT is supported by a British Heart Foundation research studentship (FS/03/075/15914).

References

1. Miake, J., Marban, E., and Nuss, H.B., Biological Pacemaker Created by Gene Transfer, *Nature* **419** (2002), 132–133
2. Miake, J., Marban, E., and Nuss, H.B., Functional Role of Inward Rectifier Current in Heart Probed by Kir2.1 Over Expression and Dominant-Negative Suppression, *J. Clin. Invest.* **111** (2003), 1529–1536
3. Azene, E.M., Xue, T., Marban, E., Tomaselli, G.F., and Li, R.A., Non-equilibrium Behavior of HCN Channels: Insights into the Role of HCN Channels in Native and Engineered Pacemakers, *Cardiovasc. Res.* **67** (2005), 263–273
4. Qu, J., Barbuti, A., Protas, L., Santoro, B., Cohen, I.S., and Robinson, R.B., HCN2 Overexpression in Newborn and Adult Ventricular Myocytes: Distinct Effects on Gating and Excitability, *Circ Res.* **89** (2001), E8–14
5. Strogatz, S.H., *Nonlinear Dynamics and Chaos*. (1994), Westview Press, Cambridge, MA, US.
6. Doedel, E.J., AUTO: A Program for the Automatic Bifurcation and Analysis of Autonomous Systems, *Cong Num* **30** (1981), 265–284
7. Ermentrout, G.B., XPPAUT. <http://www.math.pitt.edu/~bard/xpp/xpp.html>
8. Faber, G.M., and Rudy, Y., Action Potential and Contractility Changes in $[Na^+]_i$ Overloaded Cardiac Myocytes: A Simulation Study, *Biophys. J.* **78** (2000), 2392–2404
9. Tong, W.C. and Holden, A.V., Induced Pacemaker Activity in Virtual Mammalian Ventricular Cells, *LNCS* **3504** (2005), 226–235
10. Zhang, H., Tong, W.C., Garratt, C.J., and Holden, A.V., Stability of Genetically Engineered Cardiac Pacemaker: Role of Intracellular Ca^{2+} Handling, *Comput. Cardiol.* (2005) (in press)